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Investigation of Plants Extracts as Potential Larvicidal Agents against Culex quinquefasciatus and Anopheles stephensi

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Abstract

To eradicate insects chemical insecticides have been used for long time. Synthetic insecticides have created havoc in the environment by altering the environment as well proved toxic no non-target animals/organisms. Hence, the focus to control the insects is now shifted to naturally occurring plant based insecticides which are eco-friendly. Among the insects, mosquitoes form important vectors as they transmit diseases like malaria and lymphatic filariasis etc. The present study is carried out to investigate the larvicidal activity of four different plants against Culex quinquefasciatus (vector of lymphatic filariasis) and Anopheles stephensi (malaria). There are many studies which state that the plant crude extracts act against mosquito as larvicidal, pupicidal, and adulticidal agents. As plants have antioxidant, toxic and bioactive properties they are used extensively in mosquito control. The chosen plants in this study are Ipomoea carnea (Leaves), Commiphora caudate (Leaves), Euphorbia ant quorum (Latex) and Acapulco senna-alata (Leaves). Ethanol, Methanol and Acetone extracts from four plants were tested against late third instar larvae of Culex quinquefasciatus and Anopheles stephensi. The larvae were exposed to different concentrations of plant crude extracts. After 24 hours the mortality rate of each dose was observed. There was no mortality in control. Among the plants screened, Ipomoea carnea (Leaves), crude extract showed highest mortality (100%), followed by Commiphora caudate (Leaves) which is 97%. The other two plants such as Euphorbia antiquorum and Acalpulco senna-alata showed 86% and 78% mortality respectively. The chemical composition of plant crude extract were analyzed by GC-MS and given herewith. The plant extracts needs to be tested for further as an effective larvicidal under field conditions.

Keywords: Plants; Mosquito Larvicidal activity; GC-MS; Lethal concentration

Introduction

Vector borne diseases account for 17% of all the diseases among the various illnesses that kills the people. Blood sucking insects usually act as vectors to transmit the pathogens from human to human or from animals to humans. Mosquitoes form the major part of vector species others being the ticks, mites, sandflies, flea and bugs [1].Mosquitoes transmit a number of diseases, such as filariasis, dengue, malaria, yellow fever, chikungunya, Japanese encephalitis [2, 3]. Mosquitoes are also described as most dangerous animal in the world because of its ability to transmit malaria. There are 3,500 species of mosquitoes in earth [3]. Mosquitoes that transmit malaria are grouped as Anopheles. There are about 40 different species of Anopheles mosquitoes which transmit malaria [4]. Malaria parasites Plasmodium falciparum and Plasmodium vivax are carried by Anopheles stephensi important mosquito vector. This species of mosquito is abundant in Middle East, Indian subcontinent and also china [5]. Malaria is a serious global health problem that affect millions of people particularly young children (below 5 years old), pregnant ladies, and patients with conditions –like HIV/AIDS [6]. Lymphatic filariasis is a global health problem found mainly in tropical region of the world [6]. About 1.4 billion people in 73 countries of the world are threatened by the disease [7]. Lymphatic filariasis is called as Elephantiasisis a painful and disfiguring disease. Wuchereria bancrofti, Brugia malayi and Brugia timori, are the three types of nematode worms which cause the disease filariasis [8]. The vectors of genus Culex carry Wuchereria Bancroft, in urban and semi-urban areas, Aedes in pacific islands, Anopheles in Africa and elsewhere. Culex quinquefasciatus (Diptera Culicidae) is a predominant house-resting mosquito in most tropical countries [9]. This mosquito's breed in polluted waters such as septic tanks, blocked drains, soakage pools close to human habitations [10]. There are scientific reports which say that the larvicidal activity can be seen in the plant extract from leaves and latex [11]. Repellent plant crude extract in mosquito control programs due to their excellent larvicidal, pupicidal and adulticidal properties have been carried out by various groups of scientists in recent past [12]. Plants have bioactive phytochemical compounds which act as source of alternative agents that control mosquito [13]. In our study, we have chosen Commiphora caudate, Ipomoea carnea, Euphorbia antiquorum, Acalpulco senna-alata as potential bioactive plants which act against Anopheles stephensi and Culex quinquefasciatus. However, to be noted is 80% of the words population use plant as their primary and secondary source of medication [14]. Ipomoea carnea (Convolvulaceae family) is the large diffuse shrub and it is grown in wetland areas and river beds [15]. Ipomoea carnea leaves contain seven compound such as stearic acid, 3-diethylamino-1-propanol, hexatriacontane, 1,2diethylphthalate, n-octadecanol, octacosane, hexadecanoic acid, tetracontane. This plant is used in Ayurveda traditional medicinal systems [16]. Euphorbia antiquorum (Euphorbiaceous family) is a medium size annual herb common to the tropical and sub-tropical areas. It grows 12 cm tall. Euphorbia antiquorum leaves and stem produce milky juice or white latex when cut. It's grown in grassland, road sides and pathways etc. [17].Euphorbia antiquorum exhibit the antifungal, antimutagenic, antibacterial, insecticidal, molluscicidal, antitumor activity [18]. It generally grows in dry forest. Acalpulco senna-alata is a shrub. The

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leaves include chrysophanic acid [19]. It is commonly used for Skin disease, Tinea, Ringworm and athlete's foot diseases. This plant is grown in many areas in south India [20]. Based on the above said information; the objective of the present study was to examine larvicidal activity of different plant extracts against Culex quinquefasciatus and Anopheles stephensi.

1. To identify and select the toxic plants and to find out the active compounds in the plants chosen

2. To rear the Anopheles stephensi and Culex quinquefasciatus mosquitoes in lab

3. To evaluate the larvicidal properties of different plant extracts against Anopheles stephensi and Culex quinquefasciatus.

Material and Methods

Plants Collection

The leaves of Commiphora caudate, Ipomoea carnea, Acalpulco senna-alata and latex of Euphorbia antiquorum were collected from the rural area of Maruthappatinum in Thiruvarur District, Tamil Nadu, given in [Figure 1a, 1b] and [Figure 1c, 1d].

Preparation of Ipomoea carnea extract

The Ipomoea carnea plant leaves were collected and the leaves were dried 7-10 days. The dried leaves (100g) were powdered using electrical blender. Powder was dissolved in 250ml of ethanol into 500ml of conical flask for 24 hrs. The mixture was filtered through whatmanNo.1 filter paper using Buchner funnel and then concentrated vacuum using rotary evaporator (400C) [Figure 2a]. The obtained extracts were kept at 40C until further use [13, 11].

Preparation of Commiphora caudate extract

The Commiphora caudate plant leaves were collected and then dried 7-10 days under room temperature. Then the dried leaves (100g) were powdered using electrical blender. The powdered plant materials were soaked in the extracting 500ml methanol solvents for 24 hrs. The mixture was filtered through whatmanNo.1 filter paper using Buchner funnel and then concentrated vacuum using rotary evaporator (400C) [Figure 2b]. The obtained extracts were kept at 40C until further use [4, 9].

Figure 1 (a): Ipomoea carnea .

Figure 1 (b): Commiphora caudate.

Figure 1(c): Euphorbiaantiquorum.

Figure 1 (d): Acalpulcosenna-alata.

Preparation of Euphorbia antiquorum extract

The Euphorbia antiquorum latex were collected. The collected latex was mixed with methanol in the ratio of 1:9 (10%) and centrifuged at 3500rpm for 5 minutes. The supernatant was collected in a glass vial and stored at 40C till further use [12, 18] shown in [Figure 2c].

Preparation of Acalpulcosenna-alata extract

The Acalpulcosenna-alata plant leaves were collected. The leaves of plant (100g) were air dried and ground to powder using grinder. The powdered plant material was extracted by maceration with shaking for 24 hrs, in 70% acetone with 10:1 solvent to dry weight ratio. The extract

Figure 2 (a): Ipomoea carnea leaf extraction.

Figure 2 (b): Commiphora caudate leaf extract.

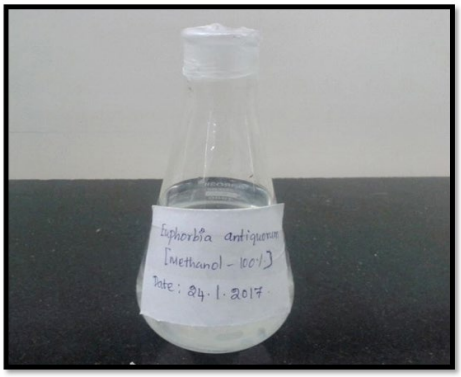

Figure 2 (c): Euphorbia antiquorum latex extract.

was the filtered through WhatmanNo.1 filter paper using Buchner funnel, and the acetone removed under steam of air. The acetone extract was dried in a rotary evaporator under reduced pressure and kept temperature 400C [30, 21] shown in [Figure 2d]. Buchner Funnel & Rotary Evaporator was used for all the plant extracts [Figure 3a, 3b].

Rearing of larvae

The Mosquito eggs were collected from Centre Research in Medical Entomology (CRME), Madurai, Tamil Nadu State and Eggs were reared under laboratory condition from the Central University of Tamil Nadu, Thiruvarur. The larvae of Anopheles stephensi and Culex quinquefasciatus were feed with dog biscuit mixed with yeast powdered in 3:1 ratio were floated on the water surface after the larvae turned into the late third instar they were used for the bioassay [22].

Larvicidal Bioassay:

The larvicidal plant crude extract was evaluated as per the protocol previously described by WHO (2005). From the plant crude extract stock solutions six different test concentrations. (50,100,150,200,250 and 300 ppm) were prepared and tested against the freshly (24 hrs.) late third instar larvae of Anopheles stephensi and Culex quinquefasciatus as shown in [Figure 4]. The test medium (350ml paper cups) were used by adding 500µl of plant extract mixed with 99.5ml of distilled water to make up (50 ppm) of test sample.1000µl of plant crude extract mixed with 99.0ml of distilled water to make up (100 ppm) of test sample.1500µl of plant extract mixed with 98.5ml of distilled water to make up (150ppm) of test sample. 2000µl of plant crude extract mixed with 98.0ml of distilled water to make up (200ppm) of test sample.2500µl of plant crude extract mixed with 97.5ml of distilled water to make up (250ppm) of test sample.3000µl of plant crude extract mixed with 97.0ml of distilled water to make up (300ppm) of test sample. The control (with the plant extracts) experiments were also run parallel with each replicate. Four experiment; four replicates were maintained at a time. A minimum number of 25 larvae mortality were observed and recorded after 24hrs post treatment, percentage of mortality were calculated using Abbott's formula. There is no mortality found in the control [23].

Figure 2(d): Acalpulcosenna-alata leaf extraction

Figure 3 (a): Buchner Funnel

Figure 3(b): Rotary Evaporator

Figure 4: Larvicidal Bioassay.

Abbots formula= % test mortality - %control mortality ×100

100 - %control mortality

Gas chromatography Mass spectrometry for plant compounds identification

All plant extracts (500ul) were derivatized by adding 90µl of O-methoxyamine hydrochloride solution in anhydrous pyridine and mixed vigorously for 1 min, and incubated at 65 °C for 30 min in a heating block. Subsequently, 14oµl of MSTFA was added and the extracts were incubated at 60 °C for 60 min. Samples were then made up to a volume of 800µl with hexane prior to GC-MS analysis. GC coupled with mass spectrometer (Thermo Scientific, FL, USA) was used to determine the metabolites. TG-5MS fused-silica capillary column (30 m × 250 µm) was utilized to separate the derivatives. The injector temperature was set at 250 °C. Helium, the carrier gas, with constant flow rate of 1.1 ml/min and the column temperature was initially kept at 67 °C for 3min, then ramped to 230 °C at a rate of 5°C/min and then finally increased to 290 °Cat for 20 min. Electron ionization (EI+) mode used for mass detection. The Mass spectra were acquired at the scan ranged between m/z of 45-800. The sample volume of 1 µl was injected for GC-MS analysis [24].

Statistical Analysis

The average larvicidal test mortality data were subjected to log probit analysis for calculating LC50 and LC90, and other statistics at 95% fiducially limits of upper confidence limit and lower confidence limit, and chi-square values were calculated by using the IBM SPSS Statistics 21.0 software. The result with $p<0.005$ were considered to be statistically significant [25, 26].

Results

Phytochemicals in plants have been widely used as an alternative to synthetic pesticides/insecticides as a part of integrated vector management against mosquitoes. Cost effective, novel plant based insecticides. Phytochemicals and their efficacy against the mosquito larvae have been reviewed extensively. Botanical Name, Tamil Name, parts used and methods used for preparation of plant crude extract used in the present study are given in [Table 1]. Mortality of Anopheles stephensi and Culex quinquefasciatus after the treatment of ethanol extract of Ipomoea carnea(leaf) extract, methanol extract of Commiphora caudate (leaf), methanol extract of Euphorbia antiquorum (latex) extract and acetone extract of Acalpulco senna-alata(leaf) extract were observed. The LC50 and LC90 values after 24hrs of exposure were calculated using probit regression analysis (95% log probit confident limit) with IBM SPSS 21.0 Software. The larval mortality of Anopheles stephensi [late third instar larvae] after the treatment of four different medicinal plants at different

Table 1: Botanical Name, Tamil Name, parts used and methods used for preparation of plant crude extract.

S.No	Botanical Name	Tamil Name	Parts Used	Method of Extraction
1	Ipomoea carnea	Neyveli kattamanakku	Leaves	Maceration method
2	Commiphora caudate	Kiluvai	Leaves	Maceration method
3	Euphorbia antiquorum	Sadhurakalli	Latex	Maceration method
4	Acalpulcosenna-alata	Vandukadi	Leaves	Maceration method

Table 2: Larvicidal activity of different plant extracts in Anopheles stephensi.

S. No	Plants name	Ethanol	Methanol	Acetone
1	Ipomoea carnea	100%	_	_
2	Commiphora caudate	_	97%	_
3	Euphorbia antiquorum	_	86%	_
4	Acalpulco senna-alata	_	_	78%

Table 3: Larvicidal activity of different plant extracts in Culex quinquefasciatus.

S. No	Plants name	Ethanol	Methanol	Acetone
1	Ipomoea carnea	100%	_	_
2	Commiphora caudate	_	100%	_
3	Euphorbia antiquorum	_	80%	_
4	Acalpulco senna-alata	_	_	78%

concentrations (50 to300) is shown in [Table 2]. The larval mortality of Culex quinquefasciatus (late third instar larvae) after treatment of four different plants at different concentrations (50 to 300 ppm) is given in [Table 3]. 21% mortality was noted in late third instar larvae after treatment of Ipomoea carnea at 50ppm, whereas it has been increased to 100% at 300 ppm of Ipomoea carnea leaf extract treatment. 13% mortality was noted in late third instar larvae after treatment of Commiphora caudate leaf extract at 50ppm, whereas it has been increased to 97% at 300 ppm with Commiphora caudate leaf extract treatment [Table 5]. 14% mortality was noted in late third instar larvae after treatment of Euphorbia antiquorum at 50ppm, whereas it has been increased to 80% at 300 ppm with Euphorbia antiquorum latex extract. 5% mortality was noted in late third instar larvae after treatment of Acalpulco senna-alata at 50ppm, whereas it has been increased to 78% at 300ppm of Acalpulco senna-alata leaf extract treatment. All the values were in four replicates given in [Table 4, 6]. 18% mortality was noted in late third instar larvae after treatment of Ipomoea carnea at 50 ppm, whereas it has been increased to 100% at 300 ppm of Ipomoea carnea leaf extract treatment.15% mortality was noted in late third instar larvae Culex quinquefasciatus after treatment of Commiphora caudate leaf at 50ppm, whereas it has been increased to 100% at 300ppm of Commiphora caudate leaf extract treatment. 11% mortality was noted in late third instar larvae of Culex quinquefasciatus after treatment of Euphorbia antiquorum at 50ppm; whereas it has been increased to 80% at 300 ppm of Euphorbia antiquorum latex extract treatment. 7% mortality was noted in late third instar larvae of Culex quinquefasciatus after treatment of Acalpulco senna-alata at 50 ppm, whereas it has been increased to 78% at 300 ppm of Acalpulco senna-alata leaf extract treatment. All values are in four replicates given from [Table 7-11]. The LC50 and LC90 values for Anopheles stephensi are represented as follows: LC50 value of Ipomoea carnea is

Table 4: Larvicidal activity of Commiphora caudate against Anopheles stephensi.

Doses in ppm	Total no of larvae	Mortality (%)
50	100	13%
100	100	20%
150	100	45%
200	100	63%
250	100	78%
300	100	97%
Control	100	0%

Table 5: Larvicidal activity of Ipomoea carnea against Anopheles stephensi.

Doses in ppm	Total no of larvae	Mortality (%)
50	100	21%
100	100	51%
150	100	67%
200	100	76%
250	100	82%
300	100	100%
Control	100	0%

Table 6: Larvicidal activity of Euphorbia antiquorum against Anopheles stephensi.

Doses in ppm	Total no of larvae	Mortality (%)
50	100	14%
100	100	28%
150	100	48%
200	100	67%
250	100	77%
300	100	86%
Control	100	0%

Table 7: Larvicidal activity of Acalpulco senna-alata against Anopheles stephensi.

Doses in ppm	Total no of larvae	Mortality (%)
50	100	5%
100	100	20%
150	100	35%
200	100	57%
250	100	68%
300	100	78%
Control	100	0%

Table 8: Larvicidal Activity of Commiphora caudate against Culex quinquefasciatus.

Doses in ppm	Total no of larvae	Mortality (%)
50	100	15%
100	100	27%
150	100	59%
200	100	76%
250	100	85%
300	100	100%
Control	100	0%

26.970, LC50 value of Commiphora caudate is 47.854, LC50 value of Euphorbia antiquorum is 37.579, and LC50 value of Acalpulco senna-alata is 55.581. LC90 value of Ipomoea carnea is 34.382, LC90 value of Commiphora caudate is 58.761, LC90 value of Euphorbia antiquorum is 48.012, and LC90 value of Acalpulco senna-alata is 69.085. All values are in four replicates in [Table 12]. Likewise, in this study, the larvicidal activity of Culex quinquefasciatus was tested at different concentrations of four different plants mentioned above. The LC50 and LC90 value are represented as follows: LC50 value of Ipomoea carnea is 35.973, LC50 value of Commiphora caudate is 42.775, LC50 value of

Table 9: Larvicidal activity of Ipomoea carnea against Culex quinquefasciatus.

Doses in ppm	Total no of larvae	Mortality (%)
50	100	18%
100	100	38%
150	100	63%
200	100	75%
250	100	89%
300	100	100%
Control	100	0%

Table 10: Larvicidal activity of Euphorbia antiquorum against Culex quinquefasciatus.

Doses in ppm	Total no of larvae	Mortality (%)
50	100	11%
100	100	27%
150	100	43%
200	100	63%
250	100	74%
300	100	80%
Control	100	0%

Table 11: Larvicidal activity of Acalpulcosenna-alata against Culex quinquefasciatus.

Doses in ppm	Total no of larvae	Mortality (%)
50	100	7%
100	100	20%
150	100	32%
200	100	56%
250	100	69%
300	100	78%
Control	100	0%

Euphorbia antiquorum is 40.082, and LC50 value of Acalpulco senna-alata is 52.794. LC90 value of Ipomoea carnea is 44.275, LC90 value of Commiphora caudate is 51.937, LC90 value of Euphorbia antiquorum was 51.501, and LC90 value of Acalpulco senna-alata is 66.284. . Present study showed that highest mortality rate was found in the ethanol leaf extract of Ipomoea carnea (100% mortality) p value is ($p \leq 0.001$) followed by methanol leaves extract of Commiphora caudate (97%) p value is (0.008) given in [Table 13]. Chemical components present in Ipomoea carnea plant analysed by Gas Chromatography –Mass Spectrometry is cited in [Table 14]. Chemical components present in Commiphora caudate plant analyzed by Gas Chromatography –Mass Spectrometry is listed in [Table15]. Chemical components present in Euphorbia antiquorum plant analysed by Gas Chromatography –Mass Spectrometry is given in [Table16]. Chemical components present in Acalpulco senna-alata plant analyzed by Gas Chromatography –Mass Spectrometry is listed in [Table 17].

Discussion

Plant extracts are recognized as cheap and eco-friendly bio pesticides (68, 69). For a given plant species to be classified as an effective insecticide, the secondary metabolites and plant metabolism are indispensable. Such metabolites can vary with the area, season, method of collection and time of collection etc [27, 28]. In the present study, four different plant extract of Ipomoea carnea, Commiphora caudate, Euphorbia antiquorum, Acalpulco senna-alata were tested against Anopheles stephensi and Culex quinquefasciatus. For example, in line with our observations, a study reported, Ipomoea cairica as an effective larvicidal agent against dengue vector such as Aedes Aegyptus and Aedes albopictus [13]. Further, the authors observed 100% mortality Ipomoea cairica. Further studies using this plant by

Table 12: LC50 Values of plant extract after 24 hrs against Anopheles stephensi.

S.NO	Name of the Plant extracts	Concentration on in ppm	% Mortality	LC_{50} (LCL±UCL)	LC_{90} (LCL±UCL)	X2 df=4
1	Ipomoea carnea	50ppm 100ppm 150ppm 200ppm 250ppm	21%			
		300ppm	51%	26.97	34.382	13.794
			67%	(7.839±45.615)	(11.860±54.472)	
			76%			
			82%			
			100%			
2	Commiphora caudate	50ppm 100ppm 150ppm 200ppm 250ppm	13%			
		300ppm	20%			
			45%	47.854	58.761	
			63%	(10.498±77.537	(16.385±89.548)	
			78%			22.244
			97%			
3	Euphorbia antiquorum	50ppm 100ppm 150ppm 200ppm 250ppm	14%			
		300ppm	28%			
			48%	55.581	69.085	5.124
			67%	(43.371±66.448	(50.308±80.345)	
			77%			
			86%			
4	Acalpulco senna-alata	50ppm 100ppm 150ppm 200ppm 250ppm	5%			
		300ppm	20%			
			35%	37.576	48.012	1.769
			57%	(27.990±46.655	(37.400±57.768)	
			68%			
			78%			

Control nil mortality. LC50 lethal concentration that kills 50% of the exposed larvae, LC90 lethal concentration that kills 90% of the exposed larvae, UCL upper confidence limit, LCL lower confidence limit, $\chi 2$ chi-square, df degree of freedom, $P<0.001$.

Table 13: LC_{50} Values of plant extract after 24 hrs against Culex quinquefasciatus.

S. No	Name of the Plant extracts	concentration in ppm	% Mortality	LC_{50} (LCL±UCL)	LC_{90} (LCL±UCL)	X2 df=4
1	Ipomoea carnea	50ppm 100ppm 150ppm 200ppm 250ppm	18%			
		300ppm	38%	35.973	44.275	15.308
			63%	(13.687±55.532)	(19.267±64.850)	
			75%			
			89%			
			100%			
2	Commiphora caudate	50ppm 100ppm	15%			21.077
			27%	42.775	51.936	
		150ppm 200ppm 250ppm	59%	(14.264±66.242)	(20.303±76.333)	
		300ppm	76%			
			85%			
			100%			
3	Euphorbia antiquorum	50ppm 100ppm 150ppm 200ppm 250ppm	11%			
		300ppm	27%	40.082	51.501	2.672
			43%	(29.675±49.858)	(39.998± 61.982)	
			63%			
			74%			
			80%			
4	Acalpulcosenna alata	50ppm 100ppm 150ppm 200ppm 250ppm	7%			
		300ppm	20%	52.794	66.284	5.37
			32%	(40.909±63.619)	(53.590 ±77.567)	
			56%			
			69%			
			78%			

Table14: List of chemical components present in Ipomoea carnea plant analysed by Gas Chromatography –Mass Spectrometry.

S.No.	Compounds	Peak Value	Mol.Formula	Mol. Weight
1	1,2,4 Butanetriol	106	C4H10O3	75
2	Bensa 4 methyl, O-methyloxime	149	$C_9H_{11}NO$	91
3	Succinimide	99	$C_4H_5NO_2$	99
4	Benzoic acid	122	$C_7H_6O_2$	105
5	Benzene 1 methyl 2 (methylthio)	138	$C_8H_{10}S$	138
6	Phenol 2 ethoxy	138	C8H10O2	110
7	Cyclohexasiloxane, dodecamethyl	444	C12H36O6Si6	73
	Trimethylsiloxy-6 hexadecenoic acid,			
8	emthyester	356	C20H40O3Si	73
9	1H Isoindole-1,3 (2H) dione,2methyl	161	$C_9H_7NO_2$	161
10	Dichoroacetic acid, tetradecylester	324	C16H30Cl2O2	43
11	2 propenoic acid 3phenyl	148	$C_9H_8O_2$	147
12	Cycloheptasilaxane,tetra decamethyl	518	C14H42O7Si7	73
13	D-ribose, 2 deoxy-bis (thio hepty)-dithioacetal	380	C19H40O3S2	117
14	Cyclooctasilaxane, hexadecamthyl	592	C16H48O8Si8	355
15	1H Indene,3 butyl 1 methyl	186	C14H18	143
16	n- Hexadecanoic acid	256	C16H32O2	43
17	cis- Vaccenic acid	282	C18H34O2	55
18	Octadecanoic acid	284	C18H36O2	43
19	Pyridine	79	C_5H_5N	79
20	Cyclopentasiloxane, decamethyl	370	C10H30O5Si5	355
21	1,2, Propanediol 3 chloro	110	$C_3H_7ClO_2$	43
22	N- methoxy – N methylacetamide	103	$C_4H_9NO_2$	43

Table 15: List of chemical components present in Commiphora caudate plant analyzed by Gas Chromatography Mass Spectrometry.

S. No	Compounds	Peak Value	Mol.Formula	Mol. Weight
1	Hexadecanoic acid, methyl ester	74	$C_{17}H_{34}O_2$	270
2	Pyridine	79	C_5H_5N	79
3	Benzaldehyde 4 methyl	91	C_8H_8O	120
4	Levoglucosenone	98	$C_6H_6O_3$	126
5	Cyclooctasilaxane, hexadecamthyl	355	$C_{10}H_{30}O_5Si_5$	370
6	Pentasiloxane, dodecamethyl	281	$C_{12}H_{36}O_4Si_5$	384
7	Benzaldehyde 4 methyl, O-methyloxime	91	$C_9H_{12}NO$	149
8	2,4,4,7 Tetramethyl- Octa-5,7- dien-3-one	109	$C_{12}H_{20}O$	180
9	Cyclohexasiloxane, dodecamethyl	73	$C_{12}H_{36}O_6Si_6$	444
10	Acetic acid, trifluro- dodecyl ester	69	$C_{14}H_{25}F_3O_2$	282
11	Cycloheptasiloxane, tetradecamethyl	73	$C_{14}H_{42}O_7Si_7$	518
12	Phenol 2,4-bis(1,1-dimethylethyl)	191	$C_{14}H_{22}O$	206
13	3,7,11,15,Tetramethyl 2-hexadecen-1-ol	81	$C_{20}H_{40}O$	296
14	n-Hexadecanoic acid	43	$C_{16}H_{32}O_2$	256
15	Methyl 9-cis, 11-trans- octadecadienoate	67	$C_{19}H_{34}O_2$	294
16	10-Octadecenoic acid, methyl ester	55	$C_{19}H_{36}O_2$	296
17	Methyl stearate	74	$C_{19}H_{38}O_2$	298
18	cis- Vaccenic acid	55	$C_{18}H_{34}O_2$	282

Table 16: List of chemical components present in Euphorbia antiquorum plant analysed by Gas Chromatography –Mass Spectrometry.

S.No.	Compounds	Peak Value	Mol.Formula	Mol. Weight
1	Pyridine	79	C_5H_5N	79
2	Benzaldehyde 2 methyl	91	C_8H_8O	120
3	Cyclopentasiloxane, decamethyl	355	$C_{10}H_{30}O_5Si_5$	370
4	Benzaldehyde 4 methyl, O-methyloxime	91	$C_9H_{11}NO$	149
5	Acetic acid, trifluro- dodecyl ester	73	$C_{12}H_{36}O_6Si_6$	282
6	Phenol 2,4-bis(1,1-dimethylethyl)	69	$C_{14}H_{42}O_7Si_7$	206
7	3,7,11,15,Tetramethyl 2-hexadecen-1-ol	191	$C_{14}H_{22}O$	296
8	n-Hexadecanoic acid	81	$C_{20}H_{40}O$	256
9	Hexadecanoic acid, methyl ester	88	$C_{18}H_{36}O_2$	284
10	trans -13-Octadecenoic acid	55	$C_{18}H_{34}O_2$	282
11	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	55	$C_{18}H_{34}O_2$	294
12	Ethyl 9,12,15-octadecatrienoate	79	$C_{20}H_{34}O2$	306

Table 17: List of chemical components present in Acalpulco senna-alata plant analyzed by Gas Chromatography –Mass Spectrometry.

S.No.	Compounds	Peak Value	Mol.Formula	Mol. Weight
1	Pyridine	52	C_5H_5N	79
2	Cyclotetrasiloxane, octamethyl	281	C8H24O4Si4	296
3	Silicic acid, diethyl bis(trimethylsilyl) ester	207	C10H28O4Si3	296
4	Benzaldehyde, 4-methyl	91	C_8H_8O	120
5	Benzene, 1-(chloromethyl)-4-methyl	150	C_8H_9Cl	140
6	Cyclopentasiloxane, decamethyl	355	C10H13O5Si5	370
7	Pentasiloxane, dodecamethyl	281	C12H36O4Si5	384
8	Benzaldehyde, 2,4-dimethyl-	133	$C_9H_{10}O$	134
9	Tricyclo[4.3.1.1 (3,8)]undecane, 1-methoxy	109	C12H2OO	180
10	Cyclohexasiloxane, dodecamethyl	73	C24H50O4Si2	444
11	Dodecanedioic acid, bis(tert-butyldimethylsilyl) ester	401	C24H50O4Si2	458
12	Acetic acid, trifluoro-, dodecyl ester	69	C14H2F3O2	282
13	Nonane, 1-chloro	43	$C_9H_{19}CL$	162
14	Cycloheptasiloxane, tetradecamethyl	73	C14H42O7SI7	444
15	Phenol, 2,4-bis(1,1-dimethylethyl)	191	C14H22O	206
16	Oleic acid, eicosyl ester	57	C38H74O2	562
17	n-Hexadecanoic acid	43	C16H32O2	256
18	Cyclodecasiloxane, eicosamethyl	73	C20H60O16SI10	740

a different group showed that the highest Mortality was observed in acetone extract of Ipomoea cairica leaf with LC50 of 101.94 and 105.59 and LC90 of 447.78 and 321.56 against dengue vector respectively [11]. Several research groups have characterized, the mosquitocidal activity of different plant extracts, in one such study, repellent activity of leaf Commiphora caudate extract against malarial, dengue, filariasis vector mosquitoes was reported (4). Very recently, highest mortality was found in the ethanol extract of Cadaba indicawith LC50115.70, 96.04, 144.50, 145.75ppm; LC90value of 215.46, 204.98, 233.82, 26.86ppm against dengue vector. Also, Pinus sylvestris and Syzgium aromaticum oils were tested against mosquito and the authors found the LC50 92.56 the LC90 value of 137.80 respectively. Further, the high mortality rate was found in the Syzgium.aromaticum plant. Hexane extract of Citrus sinensis against dengue vector was tested and it showed LC50 value of 446.84mg/L; LC90value of 370.96 mg/L respectively [29]. Not only crude extracts and oils but flowers were also used as insecticidal agents. In one such study the repellent activity of flowering extract of Nerium oleander against filarial vector was tested [10]. In this experiment, hexane flower extract was used and the LC50and LC90 value of 102.54 and 7731.80 were reported. All parts of plants can be used as insecticidal agents, interestingly, in one study it was seen that fruit extract was tested as a larvicidal agent against dengue vector [21]. In the present study, our result showed that similar trend of plants as effective larvicidal agents. We tested the late third instar of Anopheles stephensi and Culex quienquefasciatus by using different concentrations of four different plants. The mortality rate was observed after 24hrs which is followed by LC50 and LC 90 values. Based on the values, it was observed that the plant Ipomoea carnea possess high mortality rate. Based on the plants larvicidal activity, GC-MS was carried out to find the active compounds. It was found that cyclo octasilaxane, hexadecamethyl, cyclopentasiloxane, decamethyl, dodecanedioic acid, bis (tert-butyldimethylsilyl ester are the major chemicals present in the plants. It was also seen that the larvae of Anopheles stephensi were more susceptible to the plant extracts than the larvae of Culex quinquefasciatus. In a nutshell, the results in the present suggest that Ipomoea carnea shows that highest mortality rate and can be used as a larvicidal agent for mosquito vector control programmes.

Conclusion

In the present study larvicidal activity of different plants against Culex quinquefasciatus and Anopheles stephensi were tested. The chosen plants were subjected to crude extraction. Different parts of the plants were used for this purpose and the extraction was done by using methanol (100%), ethanol (100%), and, acetone (70%). Ipomoea carnea leafs were subjected to ethanol extraction. Latex of Euphorbia antiquorum and leaves of Commiphora caudate were subjected to methanol extraction, and Acalpulco senna-alata were subjected to acetone extraction. After 24hrs of exposure plants crude extracts displayed high mortality rates. Amongst the plants tested, highest mortality rate was found in the ethanol extract of Ipomoea carnea and lowest mortality rate was found in acetone extract of Acalpulco senna-alata. In order to find the active components in the plant crude extract, GC-MS was carried out and different compounds were reported. The major active component in Ipomea cornea based on the retention time is cyclooctasilaxane, hexadecamethyl. This plant could be utilized for developing a cost effective and environment friendly new type of larvicidal for mosquito control. This plant can be used as a good alternative for synthetic pesticides. The results reported here open the possibility of further investigations of field trails.

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