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Laboratory evaluation of Asteraceae species *Tagetes erecta* Linnaeus and *Tridax procumbens* Linnaeus for their toxicity against the larvae of *Culex quinquefasciatus* Say 1823 (Diptera: Culicidae)

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Abstract
Mosquitoes are insects renowned as a major health problem since they are important vectors of several diseases like malaria, yellow fever, dengue, chikungunya, filariasis, encephalitis, West Nile virus, Zika virus fever, etc., in tropical and subtropical countries of the world. *Culex quinquefasciatus* is the principal vector of lymphatic filariasis and additionally, it can transmit Japanese encephalitis virus. Application of chemical insecticides, though undesirable, is still the major tool adopted globally for mosquito control but has reflected in various drawbacks. In the current era, research is focused on natural products to combat these disease transmitting vectors and a recent emphasis has been placed on plant material. Plants enriched with phytochemicals are reported to possess insecticidal properties particularly mosquitocidal. Therefore, in the present study, the larvicidal efficacy of crude petroleum ether, chloroform, ethyl acetate and methanol extracts of *Tagetes erecta* flowers and *Tridax procumbens* aerial parts against *Culex quinquefasciatus* was studied at concentrations of 62.5, 125, 250, 500 and 1000mg/L. Larval mortality was assessed 24, 48 and 72 hours after treatment. The results revealed that the crude petroleum ether extract of *Tagetes erecta* flowers and *Tridax procumbens* aerial parts showed the highest larvicidal activity than the other extracts tested. One hundred per cent larval mortality was observed in petroleum ether flower extract of *Tagetes erecta* at 500mg/L and at 1000mg/L in *Tridax procumbens* aerial parts after 24 hours. The LC_{50} values of crude petroleum ether flower extract of *Tagetes erecta* was 128.33 and 91.58 and for aerial extract of *Tridax procumbens* it was 70.51 and 52.70mg/L after 24 and 48 hours, respectively. Among the two plant species tested, *Tagetes erecta* was found to show higher activity as its LC_{50} value was marginally less than the LC_{50} value of *Tridax procumbens* after 24 and 48 hours treatment. Further investigations are needed to elucidate this activity against a wide range of all stages of mosquito species and also the active ingredient(s) of the extract responsible for larvicidal activity should be identified.

Keywords: *Tagetes erecta* flowers, *Tridax procumbens* aerial parts, *Culex quinquefasciatus*, larvicidal efficacy

1. Introduction
Despite several decades of control effort, mosquito-borne diseases are still regarded as a major public health problem in the tropical and subtropical regions of the world. Mosquitoes (Diptera: Culicidae) are not only a nuisance to humans as they bite them to get their blood meal but, also transmit disease causing parasites and viruses in the process. Female mosquitoes are responsible for the transmission of a number of human vector-borne diseases. Mosquitoes belonging to the genera *Anopheles*, *Culex* and *Aedes* act as a vector for most of the life threatening diseases like malaria, filariasis, encephalitis, West Nile virus infection, dengue, chikungunya, yellow fever, Zika virus fever, etc., in tropical and subtropical countries and also in other parts of the world [1, 2]. *Culex* mosquito is probably the most abundant house mosquito in towns and cities of the tropical countries. *Culex* mosquitoes develop in standing water such as polluted ponds, marshes, tanks, street gutters, tin cans, barrels, ornamental ponds, puddles, creeks, ditches, etc. [3]. *Culex quinquefasciatus*, the principal vector of Lymphatic filariasis (LF) also known as elephantiasis represents a major vector-borne public health problem

worldwide. *Culex quinquefasciatus* is the most significant vector for the transmission of human LF worm, *Wuchereria bancrofti* [4] and additionally, it can transmit Japanese encephalitis virus (JEV) [5] and West Nile virus (WNV) [6]. Thus, evidently this cosmopolitan mosquito is a potential vector of pathogens causing concern to public health.

To prevent proliferation of mosquito-borne diseases and to improve quality of environment and public health, mosquito control is essential. Application of chemical insecticides, though undesirable, is still the major tool adopted globally for mosquito control. Since the mid of the 20th century, a number of insecticides from different chemical groups (i.e., organochlorines, organophosphates and pyrethroids) were recommended and applied for outdoor and indoor control of both adult and larval stages of mosquitoes [7-10]. This long journey of extensive chemicals has been reflected in various drawbacks exemplified largely by; decline of insecticides activities due to emergence of resistant mosquito strains, contamination of environment and mortality of non-target organisms, biomagnification in ecosystem, and the increasing prices of chemicals coupled with high cost of control [11-16]. Thus, ecologically safe and potentially effective control alternatives are being sought carefully everywhere. Despite the fact that such alternatives are slowly growing, appreciable efforts were devoted to biocontrol and natural biocides including botanical products [17-21]. In the current era, research is focused on natural products to combat these disease transmitting vectors and a recent emphasis has been placed on plant material and various reports on the use of natural plant products against mosquito vectors have been documented [22-37]. Therefore, in the present study, two plant species belonging to the Asteraceae family *viz*., the flowers of *Tagetes erecta* Linnaeus and aerial parts of *Tridax procumbens* Linnaeus were evaluated for their larvicidal efficacy against the lymphatic filarial vector, *Culex quinquefasciatus* Say.

Tagetes erecta a common aromatic herb and a popular stout [38, 39], annual garden herbaceous plant [40] originated in North and South America and are widely cultivated in other Asian countries *viz*., Bhutan, China, Nepal and India [41]. It is known as 'marigold' in English [42], 'thulukka samanthi' in Tamil and 'genda' in Hindi. *Tagetes erecta* is a medicinal plant which has a high therapeutic value in the field of medicine [43] for treating hiccups, dermatitis, athlete's food, colitis and wound burns [44]. The plant has been used to treat stomachache, diarrhoea, liver problems, vomiting, indigestion, toothache, chest pain, rheumatic pains, cold, bronchitis, ulcer, diseases of the eye and uterus and to expel worms from the body [45]. The leaves are used for treating kidney problems, muscular pain and as an application on boils. The infusion of this plant is used against rheumatism, cold and bronchitis. In unani, the tender leaves of *Tagetes erecta* are prescribed for kidney problems [46]. The flower of this plant is used for treating fever, epileptic fits, stomachache, scabies, liver complaints and eye diseases. Besides these, they even purify blood and the flower juice is taken as a remedy for bleeding piles [47]. The decoction of its flowers is very effective for cold, conjunctivitis, mumps and eye sore [48]. The plant possesses phytochemical constituents *viz*., thiophenes, flavonoids, carotenoids, triterpenoids [47], glycosides, terpenoids [49], alkaloids, quinines, phenols, coumarins [50], carbohydrates, tannins, steroids [51], terpenes and salicylic acid [50]. Some of the phytocompounds extracted from *Tagetes erecta* include quercetagetin, syringic acid, methyl-3, 5-dihydroxy-4-methoxy benzoate, quercetin, thienyl, ethyl gallate [47], piperitone and D-limonene [52]. Further, the plant is used as a poison for invertebrates and in plant pest control [53]. The plant possesses antibacterial [54], antimicrobial [55], antioxidant [56], analgesic [57], nematicidal [58], wound healing [59] and hepatoprotective [60] property. The plant is reported to possess insecticidal activity against *Tribolium castaneum* [61] and mosquito species *viz*., *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus* [62-69].

Tridax procumbens is commonly known as 'coat button' in English, 'vettukaaya-thalai' in Tamil and 'ghamra' in Hindi [70]. It is an annual herb, native of tropical America and naturalized in tropical Africa, Asia, Australia and widely distributed throughout India [71]. Traditionally, the plant has been extensively used in ayurvedic medicine for liver disorders [72] and is also employed to treat jaundice [73]. Traditionally, the leaves of this plant as a remedy against conjunctivitis [74], fever, typhoid, cough, asthma, epilepsy and diarrhoea [75], bronchial catarrh, dysentery [76], hair loss and to check haemorrhage from cuts, bruises and wounds [77]. The entire plant is used for treating malaria, leishmaniasis, vaginitis and gastrointestinal disorder [78]. The plant possess antifungal, anticoagulant [79], immunomodulating [80, 81], antioxidant, antidiabetic [80, 82, 83], antimicrobial [84-87], antiinflammatory [74], hepatoprotective [88], antibacterial [89] and anticancer [90] properties. Phytochemical constituents of this plant include alkaloids, tannins, flavonoids, saponins, carotenoids, anthraquinones, steroids, terpenes, glycosides, phenols, and flavanols [91]. Besides these, the phytocompounds extracted are fucosterol, sitosterol, arachidic, behenic, lauric, linoleic, linolenic, myristic, palmitic, stearic acids [92], luteolin, glucoluteolin [93], fumaric acid, oleanolic acid [94], quercetin and oxoester [95]. Rajkumar and Jebanesan [96] reported the leaves of *Tridax procumbens* for repellency against *Anopheles stephensi*. The plant is also reported for larvicidal activity against *Anopheles subpictus* and *Culex tritaeniorhynchus* [97], and its toxic effect on *Aedes aegypti* [98]. The crude leaf extracts of this plant and also the whole plant were reported for larvicidal activity against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* [63, 99].

2. Materials and Methods

2.1. Plant collection and extraction

Mature fresh and healthy plants of *Tagetes erecta* and *Tridax procumbens* collected from Chennai, Tamil Nadu, India, were brought to the laboratory. Taxonomical identity was confirmed at the Department of Plant Biology and Plant Biotechnology, Madras Christian College, Chennai, Tamil Nadu, India. The dried flowers of *Tagetes erecta* and aerial parts of *Tridax procumbens* were washed with dechlorinated tap water and shade dried at room temperature. Dried flowers of *Tagetes erecta* were powdered with the aid of an electric blender. The powdered leaves (1 Kg) were sequentially extracted with solvents (3 L) each *viz*., petroleum ether, chloroform, ethyl acetate and methanol using a Soxhlet apparatus [100]. The crude leaf extracts were filtered through a Buchner funnel with Whatman number 1 filter paper and were then evaporated to dryness in a rotary vacuum evaporator to obtain crude petroleum ether, chloroform, ethyl acetate and methanol extracts of *Tagetes erecta* flowers. Likewise, the same methodology was adopted to obtain the crude petroleum ether, chloroform, ethyl acetate and methanol aerial extracts of *Tridax procumbens*. One per cent stock solution from the

crude extracts of each plant was prepared by adding adequate volume of acetone and was refrigerated at 4°C until testing for larvicidal bioassay.

2.2. Test mosquitoes

Culex immatures collected from various places in Chennai, Tamil Nadu, India were transported to the laboratory where, the immature mosquitoes were transferred to enamel larval trays until adult emergence. After emergence, the adult mosquitoes were identified upto species level and confirmed before rearing. Cyclic generations of *Culex quinquefasciatus* were maintained separately in mosquito cages (2'x2'x2') in an insectary with a mean room temperature of 27±2 °C and a relative humidity of 70-80%. The adult mosquitoes were fed on ten per cent glucose solution in water. The eggs laid in ovitraps placed inside the mosquito cages were then transferred to enamel larval trays maintained in the larval rearing chamber. The larvae were fed with larval food (dog biscuits and yeast in the ratio 3:1). The larvae on becoming pupae were collected, transferred to plastic bowls and kept inside another mosquito cage for adult emergence.

2.3. Larvicidal bioassay

Standard WHO [101] protocol with minor modifications was adopted for the study. The tests were conducted in glass beakers. *Culex quinquefasciatus* immatures particularly early third instar larvae from laboratory colonized mosquitoes of F_1 generation were used for the study. Larvicidal activity at test concentrations of 62.5, 125, 250, 500 and 1000mg/L of each crude plant extracts was assessed. The required test concentrations and quantity of test solution was prepared by serially diluting one per cent stock solution of the crude extract. Twenty healthy larvae were released into each 250mL glass beaker containing 200mL of water and test concentration. Mortality was observed 24, 48 and 72 hours after treatment. A total of three trials with three replicates per trial for each concentration were carried out. Controls were run simultaneously. Treated control was prepared by the addition of acetone to distilled water. Distilled water served as untreated control.

Table 1: Larvicidal efficacy of *Tagetes erecta* crude flower extracts against *Culex quinquefasciatus*

Solvents	Concentration (mg/L)	Hours		
		24	48	72
Petroleum ether	UC	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)
	TC	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)
	62.5	6.33 ±0.57^{b} (31.6)	10.00 ±1.00^{b} (50.0)	12.66 ±1.52^{b} (63.3)
	125	10.00 ±1.00^{c} (50.0)	13.66 ±1.52^{c} (68.3)	17.33 ±1.15^{c} (86.6)
	250	18.33 ±2.08^{d} (91.6)	19.66 ±0.57^{d} (98.3)	19.66 ±0.57^{d} (98.3)
	500	20.00 ±0.00^{e} (100.0)	20.00 ±0.00^{d} (100.0)	20.00 ±0.00^{d} (100.0)
	1000	20.00 ±0.00^{e} (100.0)	20.00 ±0.00^{d} (100.0)	20.00 ±0.00^{d} (100.0)
LC_{50} (mg/L)		128.33	91.58	71.45
LC_{90} (mg/L)		223.32	162.39	129.15
Chloroform	UC	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)
	TC	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)
	62.5	3.00 ±1.00^{b} (15.0)	4.33 ±1.52^{b} (21.6)	6.66 ±2.08^{b} (33.3)
	125	5.00 ±1.73^{c} (25.0)	7.33 ±1.52^{c} (36.6)	10.00 ±1.00^{c} (50.0)
	250	12.00 ±1.00^{d} (60.0)	13.66 ±1.15^{d} (68.3)	15.66 ±0.57^{d} (78.3)
	500	17.33 ±1.52^{e} (86.6)	18.66 ±1.15^{e} (93.3)	19.66 ±0.57^{e} (98.3)
	1000	19.00 ±1.00^{e} (95.0)	20.00 ±0.00^{e} (100.0)	20.00 ±0.00^{e} (100.0)
LC_{50} (mg/L)		366.02	310.39	155.39
LC_{90} (mg/L)		772.72	612.00	296.26
Ethyl acetate	UC	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)
	TC	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)
	62.5	0.66 ±0.57^{ab} (3.3)	1.33 ±0.57^{a} (6.6)	1.33 ±0.57^{a} (6.6)
	125	2.00 ±1.00^{b} (10.0)	3.66 ±1.52^{b} (18.3)	6.33 ±2.51^{b} (31.6)
	250	5.66 ±1.52^{c} (28.3)	7.33 ±1.52^{c} (36.6)	8.66 ±0.57^{c} (43.3)
	500	10.33 ±1.15^{d} (51.6)	10.66 ±0.57^{d} (53.3)	12.00 ±1.00^{d} (60.0)
	1000	11.33 ±0.57^{d} (56.6)	13.00 ±1.00^{e} (65.0)	14.66 ±0.57^{e} (73.3)
LC_{50} (mg/L)		742.82	583.70	542.50
LC_{90} (mg/L)		1362.02	1235.83	1113.98
Methanol	UC	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)
	TC	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)
	62.5	0.00 ±0.00^{a} (0.0)	0.33 ±0.57^{a} (1.6)	2.33 ±0.57^{a} (11.6)
	125	0.66 ±0.57^{a} (3.3)	1.66 ±0.57^{ab} (8.3)	6.00 ±1.00^{b} (30.0)
	250	1.33 ±0.57^{a} (6.6)	2.66 ±0.57^{c} (13.3)	10.00 ±1.00^{c} (50.0)
	500	3.66 ±1.15^{b} (18.3)	7.00 ±2.00^{d} (35.0)	13.00 ±2.64^{d} (65.0)
	1000	5.33 ±1.52^{c} (26.6)	14.00 ±1.00^{e} (70.0)	15.66 ±1.52^{e} (78.3)
LC_{50} (mg/L)		1272.64	738.15	482.24
LC_{90} (mg/L)		2039.05	1250.22	1014.97

UC: Untreated control; TC: Treated control; Values are mean of three replicates of three trials ±standard deviation; Values in parenthesis denote per cent larval mortality; Different superscript alphabets indicate statistical significant difference in larval mortality between concentrations at $P<0.05$ level by one way ANOVA followed by Duncan multiple range test (DMRT); LC_{50}: Lethal concentration that kills 50% of the exposed larvae; LC_{90}: Lethal concentration that kills 90% of the exposed larvae.

The larval per cent mortality was calculated and when larval control mortality ranged from 5-20% it was corrected using Abbott's formula [102].

$$\text{Larval per cent mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

$$\text{Abbott's formula} = \frac{\text{Percentage of test mortality} - \text{Percentage of control mortality}}{100 - \text{Percentage of control mortality}} \times 100$$

2.4. Statistical analysis

Data from all replicates were pooled for statistical analysis. LC_{50} and LC_{90} values were calculated using SPSS software by probit analysis [103]. ANOVA was performed to determine the difference in larval mortality between concentrations. Results with $P<0.05$ level were considered to be statistically significant.

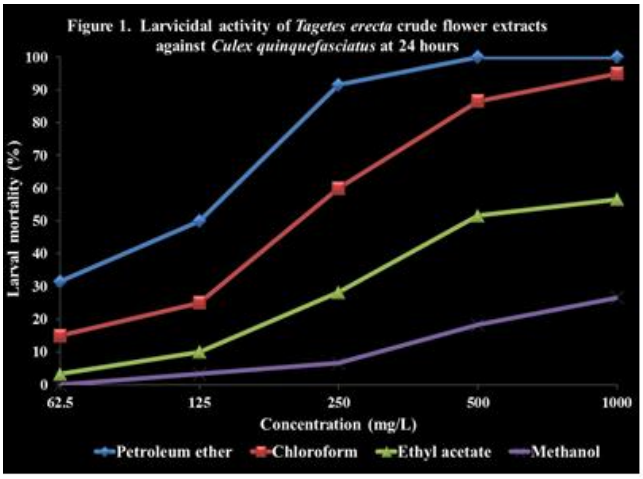
3. Results

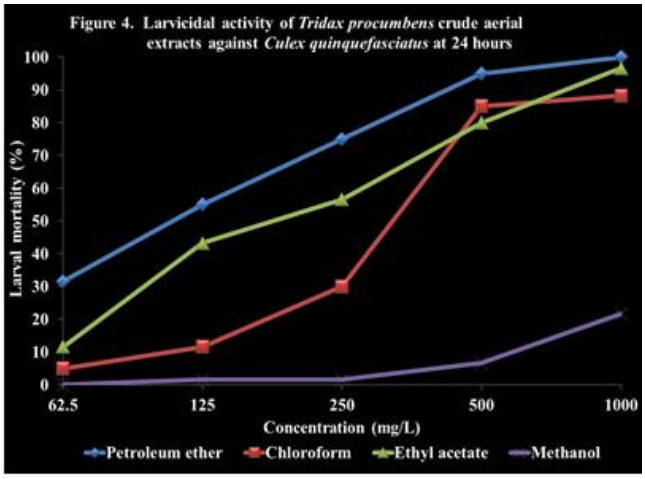
The results revealed that the crude petroleum ether flower extract of *Tagetes erecta* exhibited the highest larvicidal activity against *Culex quinquefasciatus* followed by chloroform after 24 hours (Figure 1). One hundred per cent larval mortality was observed in petroleum ether at 500mg/L after 24 hours and also in chloroform extract at 1000mg/L after 48 hours (Table 1; Figure 2 & 3). The LC_{50} values of the crude petroleum ether flower extract was 128.33 and 91.58mg/L after 24 and 48 hours, respectively (Table 1; Figure 7, 8 & 9). In the case of *Tridax procumbens*, the same trend was observed as the crude petroleum ether extract exhibited the highest larval mortality followed by ethyl acetate (Figure 4). One hundred per cent larval mortality was observed in petroleum ether extract at 1000mg/L after 24 hours (Table 2; Figure 5 & 6). The LC_{50} values of petroleum ether extract was 170.30 and 128.10 mg/L after 24 and 48 hours, respectively (Table 2; Figure 10, 11 & 12). Among the two plant species tested, the petroleum ether extract of *Tagetes erecta* was found to show higher activity as its LC_{50} value was slightly less than the LC_{50} value of *Tridax procumbens* petroleum ether extract after 24 and 48 hours treatment.

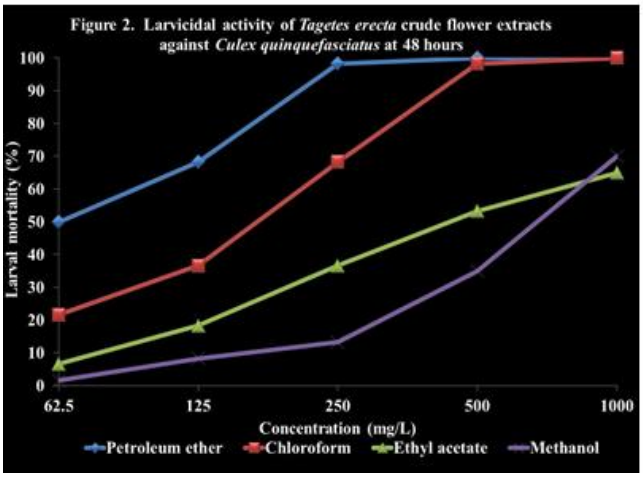
Table 2: Larvicidal efficacy of *Tridax procumbens* crude aerial extracts against *Culex quinquefasciatus*

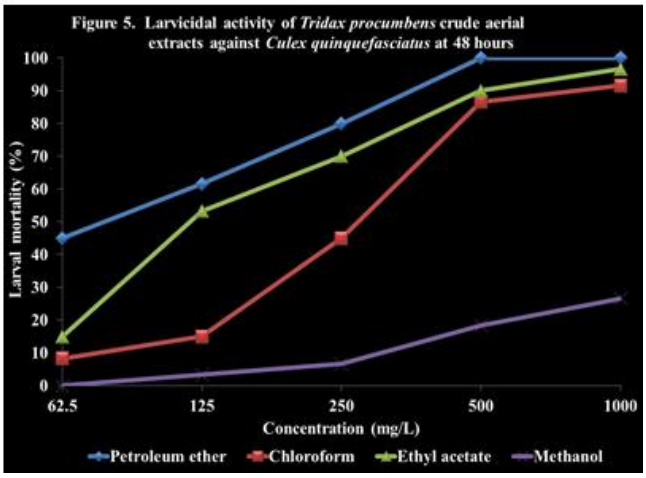
Solvents	Concentration (mg/L)	Hours		
		24	48	72
Petroleum ether	UC	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)
	TC	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)
	62.5	6.33 ±1.52^{b} (31.6)	9.00 ±2.00^{b} (45.0)	11.66 ±1.52^{b} (58.3)
	125	11.00 ±1.00^{c} (55.0)	12.33 ±1.52^{c} (61.6)	15.00 ±1.00^{c} (75.0)
	250	15.00 ±1.00^{d} (75.0)	16.00 ±0.00^{d} (80.0)	18.00 ±1.00^{d} (90.0)
	500	19.00 ±1.00^{e} (95.0)	20.00 ±0.00^{e} (100.0)	20.00 ±0.00^{e} (100.0)
	1000	20.00 ±0.00^{e} (100.0)	20.00 ±0.00^{e} (100.0)	20.00 ±0.00^{e} (100.0)
LC_{50} (mg/L)		170.30	128.10	96.54
LC_{90} (mg/L)		343.35	251.25	194.31
Chloroform	UC	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)
	TC	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)
	62.5	1.00 ±1.00^{a} (5.0)	1.66 ±1.15^{a} (8.3)	3.66 ±1.15^{b} (18.3)
	125	2.33 ±0.57^{a} (11.6)	3.00 ±1.00^{a} (15.0)	6.00 ±1.00^{b} (30.0)
	250	6.00 ±3.00^{b} (30.0)	9.00 ±3.00^{b} (45.0)	12.00 ±2.64^{c} (60.0)
	500	17.00 ±2.64^{c} (85.0)	17.33 ±2.51^{c} (86.6)	18.33 ±1.52^{d} (91.6)
	1000	17.66 ±2.08^{c} (88.3)	18.33 ±1.52^{c} (91.6)	19.00 ±1.00^{d} (95.0)
LC_{50} (mg/L)		443.46	381.10	287.82
LC_{90} (mg/L)		793.33	582.35	544.69
Ethyl acetate	UC	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)
	TC	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)
	62.5	2.33 ±2.08^{a} (11.6)	3.00 ±1.00^{b} (15.0)	3.66 ±0.57^{b} (18.3)
	125	8.66 ±3.05^{b} (43.3)	10.66 ±2.08^{c} (53.3)	11.33 ±2.30^{c} (56.6)
	250	11.33 ±1.52^{b} (56.6)	14.00 ±1.00^{d} (70.0)	15.00 ±1.00^{d} (75.0)
	500	16.00 ±1.00^{c} (80.0)	18.00 ±1.00^{e} (90.0)	19.00 ±1.00^{e} (95.0)
	1000	19.33 ±1.15^{d} (96.6)	19.33 ±1.15^{e} (96.6)	19.33 ±1.15^{e} (96.6)
LC_{50} (mg/L)		300.52	239.82	213.56
LC_{90} (mg/L)		608.67	512.92	466.91
Methanol	UC	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)
	TC	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)
	62.5	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)	0.00 ±0.00^{a} (0.0)
	125	0.33 ±0.57^{a} (1.6)	0.66 ±0.57^{a} (3.3)	0.66 ±0.57^{ab} (3.3)
	250	0.33 ±0.57^{a} (1.6)	1.33 ±0.57^{a} (6.6)	1.66 ±0.57^{b} (8.3)
	500	1.33 ±0.57^{a} (6.6)	3.66 ±1.15^{b} (18.3)	4.33 ±1.15^{c} (21.6)
	1000	4.33 ±2.08^{b} (21.6)	5.33 ±1.52^{c} (26.6)	5.66 ±1.15^{d} (28.3)
LC_{50} (mg/L)		1391.73	1272.64	1223.19
LC_{90} (mg/L)		2073.18	2039.05	1985.09

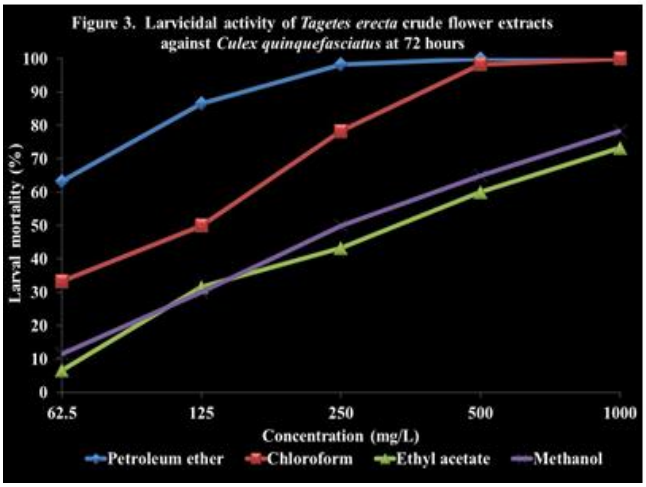
UC: Untreated control; TC: Treated control; Values are mean of three replicates of three trials ±standard deviation; Values in parenthesis denote per cent larval mortality; Different superscript alphabets indicate statistical significant difference in larval mortality between concentrations at $P<0.05$ level by one way ANOVA followed by Duncan multiple range test (DMRT); LC_{50}: Lethal concentration that kills 50% of the exposed larvae; LC_{90}: Lethal concentration that kills 90% of the exposed larvae.

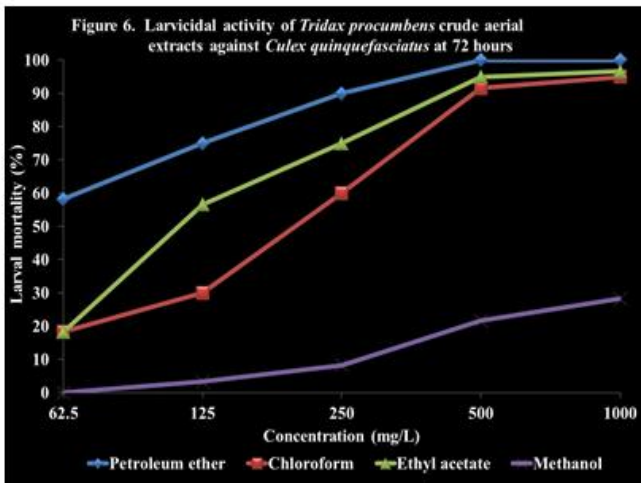










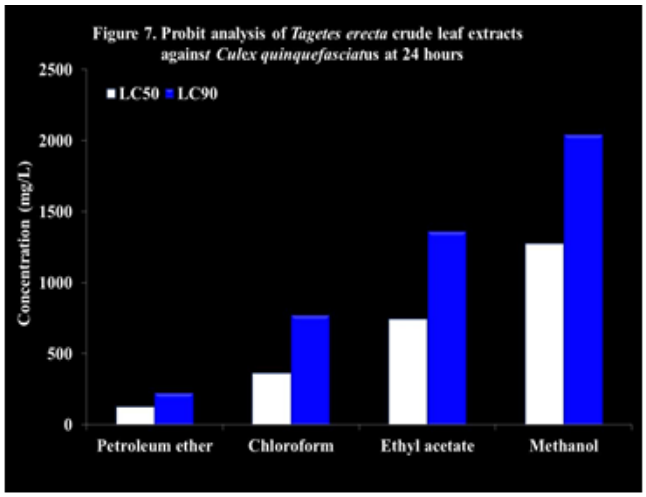


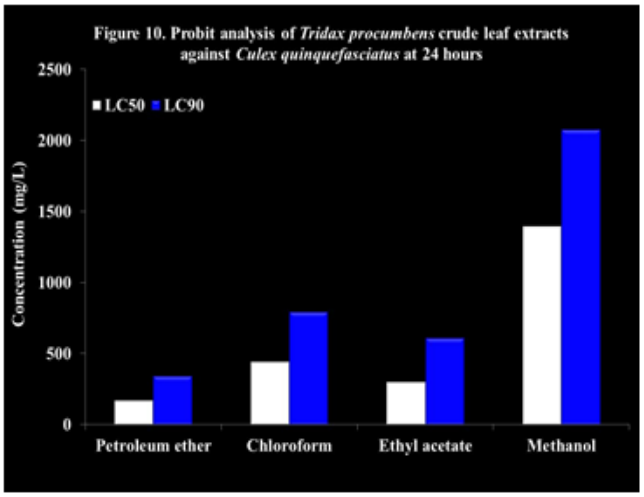
4. Discussion

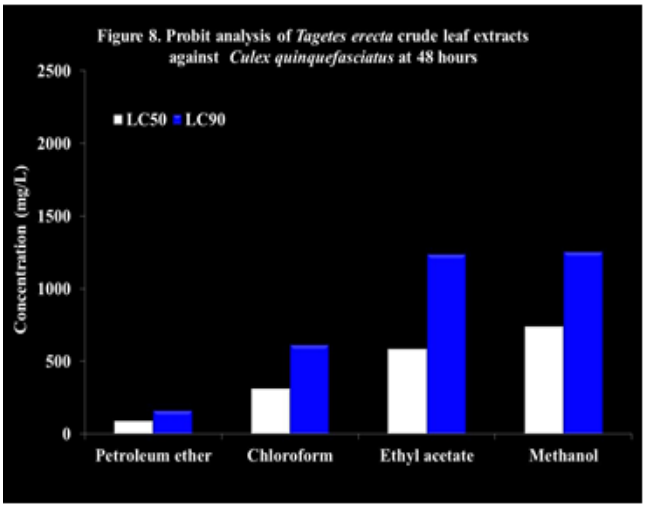
Plants are chemical factories of nature. Human have used plant parts, products and metabolites in pest control since early historical times. Plant metabolites have strong medicinal and insecticidal property and by using plant extracts in recent times, man has been able to control certain pests and vectors [104]. Employment of eco-friendly and biodegradable natural insecticides of plant origin has been recognized and given importance, for mosquito-borne disease control. Larval control is regarded as the best approach to diminish mosquito population at very early stage as imprisonment to water bodies and very stumpy rate of scattering make the mosquito larvae most susceptible. Hence, mosquito control is mostly aimed at wrigglers control and only against matures when necessary. Botanicals are proved to be efficient biopesticides not only as crude extract but as solvent extracts also [105]. Members of the plant families Asteraceae, Cladophoraceae, Lamiaceae, Meliaceae, Oocystaceae, Rutaceae and Solanaceae have various types of larval, adulticidal or repellent activities against different species of mosquitoes [106, 107].

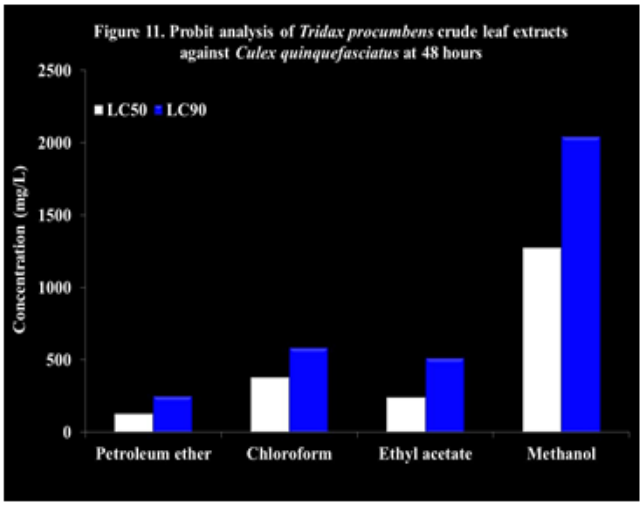
Plants belonging to the family Asteraceae have been extensively screened/studied for their larvicidal activity ever since the discovery of the larvicidal potential of the extract of *Chrysanthemum cinerariaefolium* [108]. In the present study, the larvicidal activity of the crude flower and aerial extracts of Asteraceae plants, *Tagetes erecta* and *Tridax procumbens* were studied against *Culex quinquefasciatus* respectively. The crude petroleum ether extract of both the plants were found to be effective and LC_{50} values were 128.33 and 170.30mg/L

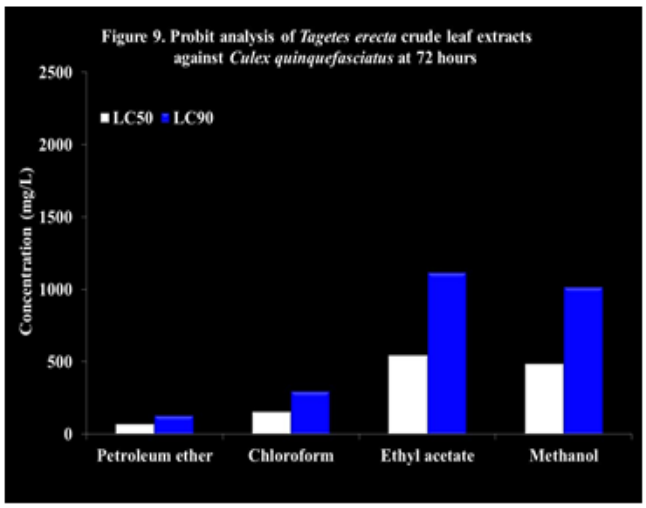
after 24 hours, respectively. However, among the two plant species tested, *Tagetes erecta* was found to show marginally higher activity than *Tridax procumbens* based on their LC_{50} values after 24 hours.

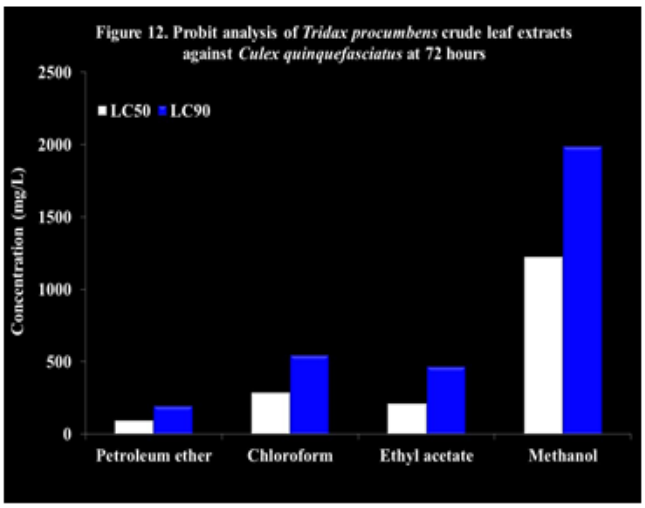












Macedo *et al*. [109] screened the ethanolic aerial parts of 83 plants belonging to Asteraceae family and found 27 of them to cause significant lethality to the fourth larvae of *Aedes fluviatilis* of which *Tagetes minuta* caused 100% mortality followed by *Eucalyptus paniculata* (98.8%) and *Vernonia ammophila* (93.3%) at 100 mg/L after 24 hours of exposure. Asteraceae plants that showed promising larvicidal activity are ethanolic extract of aerial parts of *Tagetes minuta* (LC_{50} 1.0ppm) against *Aedes fluviatilis* [109], *Artemisia annua* leaves (LC_{50} 1.76ppm) against *Anopheles stephensi* [110], aerial parts of *Eucalyptus paniculata* (LC_{50} 3.3ppm) against *Aedes fluviatilis* (Macedo *et al*., 1997) [109] and *Otanthus maritimus* methanolic stem extract (LC_{50} 7.0ppm) against *Culex quinquefasciatus* [111]. Other plant extracts that showed potential larvicidal activity are the petroleum ether leaf extract of *Artemisia annua* (LC_{50} 16.85 ppm) against *Anopheles stephensi* [112], *Artemisia campestris* methanolic stem extract (LC_{50} 23.0ppm) against *Culex quinquefasciatus* [113], ethanolic extract of *Vernonia ammophila* aerial parts (LC_{50} 40.0ppm) against *Aedes fluviatilis* [109], methanolic extracts of *Gleoonis coronarium* flowers (LC_{50} 53.0ppm), *Sonchus arvensis* stem (LC_{50} 68.0ppm), *Matricaria maritima* flowers (LC_{50}

72.0ppm) against *Culex quinquefasciatus* [111], ethyl acetate leaf extract of *Eclipta prostrata* (LC_{50} 78.28ppm) against *Anopheles subpictus* [114], *Tagetes patula* flower methanol extract against *Aedes albopictus* (LC_{50} 84.79 ppm) [115], petroleum ether leaf extract (LC_{50} 100.0pm) against *Culex quinquefasciatus* [63] and the LC_{50} values of the crude extract of these aforementioned plants were always below 100.0ppm. Crude extracts of many other plants showed moderate larvicidal activity and their LC_{50} values ranged between 100 to 200ppm. They include *Tagetes patula* flower methanol extract against *Culex quinquefasciatus* (LC_{50} 118.10 ppm) [115], ethyl acetate leaf extract of *Eclipta prostrata* (LC_{50} 119.89ppm) against *Culex tritaeniorhynchus* [114], *Achillea millefolium* methanolic stem extract (LC_{50} 120.0ppm) against *Culex quinquefasciatus* [111], dichloromethane extract of aerial parts of *Pterocaulon polystachyum* (LC_{50} 149.2ppm) against *Aedes aegypti* [116], petroleum ether extract of *Centratherum anthelmincticum* fruits (LC_{50} 162.60ppm) against *Anopheles stephensi* [117], methanolic extract of *Tanacetum vulgare* flowers (LC_{50} 178.0ppm) and *Otanthus maritimus* stem (LC_{50} 195.0ppm) against *Culex quinquefasciatus* [113] and LC_{50} values ranging between 100 and 200ppm were found in petroleum ether leaf extracts of *Artemisia nilagirica* against *Culex quinquefasciatus*, *Galinsoga quadriradiata* against *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*, *Tagetes erecta* against *Anopheles stephensi* and *Aedes aegypti* [63].

Recently, some Asteraceae plants were studied for their larvicidal activity against mosquito species. Kamaraj *et al*. [97] pointed out the leaves of *Chrysanthemum indicum* methanol extract and *Tridax procumbens* acetone extract to possess larvicidal activity against *Anopheles subpictus* with their respective LC_{50} values as 39.98 and 51.57mg/L while the ethyl acetate extract of both plants exhibited larvicidal activity against *Culex tritaeniorhynchus* and LC_{50} values were 42.29 and 69.16mg/L. Arivoli *et al*. [118] reported the ethyl acetate, chloroform, acetone and methanol leaf extract of *Vernonia cinerea* to be toxic to the larvae of *Culex quinquefasciatus* and LC_{50} values were 1.63, 1.84, 1.89 and 2.08mg/mL. Samuel and Arivoli [119] reported the larvicidal activity of *Sphaeranthus indicus* whole plant extracts against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. The ethyl acetate extract was found to be effective with LC_{50} value of 201.11ppm against *Aedes aegypti* and *Anopheles stephensi* (526.50ppm) while hexane against *Culex quinquefasciatus* (1007.18ppm). Further, Arivoli *et al*. [120] reported the fractions of ethyl acetate whole plant extract of *Sphaeranthus indicus* to show promising results against the larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* with LC_{50} values of 36.76, 26.85 and 32.60ppm respectively after 24 hours. Samuel *et al*. [121] indicated the chloroform and acetone extracts of *Ageratum houstonianum* leaves to possess larvicidal activity against *Culex quinquefasciatus* with LC_{50} values of 1.54 and 1.02 mg/mL. Sukhthankar *et al*. [122] reported the methanolic leaf extracts of *Chromolaena odorata* for larvicidal activity against *Culex quinquefasciatus* (43ppm), *Aedes aegypti* (138ppm) and *Anopheles stephensi* (1613ppm). Samuel *et al*. [123] screened the hexane, ethyl acetate and methanol leaf extracts of *Ageratum houstonianum* against the larvae of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* and found their respective LC_{50} values in ethyl acetate extract to be 3377.84, 1952.12 and 3558.32mg/L.

The susceptibility of larvae to botanical insecticides depends in general on the extract or fraction, the concentration applied, and the mosquito species tested in this study. As expected, the larvicidal activity varies considerably according to the species of mosquito and the plant/ plant part used. In order to get a potent extract, the solvents should be chosen with a thorough understanding and knowledge based on the phytochemical profile of the plant/plant part used as there exists a relationship between the extract effectiveness and solvent polarity [124]. The results of the present study were comparable with the earlier reports of *Tagetes erecta* and *Tridax procumbens* against mosquito larvae in view of the aforementioned factors. Sakthivadivel and Daniel [63] reported the crude petroleum ether leaf extract of *Tagetes erecta* to possess larvicidal activity against *Culex quinquefasciatus* and its LC_{50} was within 100ppm whereas for *Anopheles stephensi* and *Aedes aegypti* it was between 100 and 200ppm. Nikkon *et al*. [65] indicated the crude ethanolic extracts and petroleum ether and chloroform fractions of *Tagetes erecta* flowers to possess activity against the larvae of *Culex quinquefasciatus* after 24 and 48 hours with respective LC_{50} values of 918.62 and 256.67; 403.58 and 142.52; 75.20 and 36.88µg/mL.

Sakthivadivel and Daniel [63] reported the crude petroleum ether flower extract of *Tridax procumbens* to possess larvicidal activity against *Culex quinquefasciatus* with LC_{50} >200 ppm and no mortality against *Aedes aegypti* and *Anopheles stephensi*. Begum *et al*. [125] showed that the crude hexane, ethyl acetate, acetone and methanol leaf extract of *Tridax procumbens* exhibited larvicidal activity against *Aedes aegypti* and *Culex quinquefasciatus* and their respective LC_{50} values were 393.34, 174.06, 97.93, 52.87 and 450.62, 224.60, 195.03, 94.19ppm after 24 hours. However, Rajasekaran and Duraikannan [98] reported the crude petroleum ether, chloroform and aqueous leaf extracts of *Tridax procumbens* to exhibit 60%, 15.78% and no mortality against *Aedes aegypti* at 1000µg/mL after 24 hours. Kamaraj *et al*. [97] tested the crude hexane, chloroform, ethyl acetate, acetone, and methanol leaf extracts of *Tridax procumbens* against the larvae of *Anopheles subpictus* and *Culex tritaeniorhynchus* but only the acetone extract against the larvae of *Anopheles subpictus* (LC_{50} 51.57mg/L) and the ethyl acetate extract against *Culex tritaeniorhynchus* (69.16mg/L) to exhibit toxicity. Elumalai *et al*. [99] reported the crude aqueous, chloroform, ethanol, petroleum ether and methanol extract of *Tridax procumbens* leaf to possess larvicidal activity against *Aedes aegypti* (LC_{50} 83.40, 108.22, 55.67, 94.13 and 56.02ppm), *Anopheles stephensi* (LC_{50} 92.79, 104.73, 77.70, 117.09 and 66.66ppm) and *Culex quinquefasciatus* (LC_{50} 80.58, 97.93, 57.46, 111.48 and 60.31ppm).

The larvicidal property of the extract of the test species, *Tagetes erecta* and *Tridax procumbens* may be due to the presence of alkaloids, carotenoids, flavonoids and tannins [47, 49, 50, 94]. All terpenoids, alcohols, ketones and carboxylic esters show toxicity to mosquito species. Monoterpene alcohols were the most toxic compounds against mosquito species. *Tagetes erecta* and *Tridax procumbens* extracts caused mortality to *Culex quinquefasciatus* larvae which can also be attributed to the presence of terpenoids, an insecticidal compound. Reduction in the rate of mortality could be due to biodegradation of terpene in water as its resistance in water is reduced by its volatilization [126]. All these reports emphasize that *Tagetes erecta* and *Tridax procumbens* extracts possess lethal effects against *Culex quinquefasciatus*.

The choice of extraction solvent is also a critical factor that

affects larvicidal efficacy [127]. Of primary consideration is the type of solvent used since polar solvents will extract polar molecules and non-polar solvents will extract non-polar molecules. The purpose of a general screen for bioactivity is to extract as many potentially active constituents as possible. This is achieved by using solvents ranging from hexane, the least polar with a polarity index (P) of 0.1 to chloroform, ethyl acetate (relatively nonpolar; P=4.1) and water (P=10.2) the most polar including a number of intermediary solvents such as ethyl alcohol. If incomplete screening of botanical material is attempted, the solvents for phytochemical extractions should be carefully selected because different solvent types can significantly affect the potency of extracted plant compounds [29,128]. A relationship is said to exist between extract effectiveness and solvent polarity where efficacy increases with decreasing polarity [124]. This is not consistent due to differences between the characteristics of active chemicals among plants. Berry and Rodriguez [129] suggested the use of different solvents based on the type of molecules targeted for extraction. Petroleum ether (P=0.1) appears to have been the solvent of choice for some time which has been used in the present study. Successive extraction by solvents of increasing polarity showed that potency against the larvae and adults of *Culex* species was highly attributed to the non-polar fraction (e.g. petroleum ether) which corroborates with the results of the present study. Selection of mosquito species for testing is also of fundamental importance since great variations exist in responses between the genera and species. Since aedines are usually less susceptible to insecticides and *Aedes aegypti* being the most commonly colonized mosquito, it should be used for comparative screening. Obviously this species will not be the species of choice if a polluted environment involving decomposed leaf litter is being evaluated for activity. In this case, the use of the mosquito *Culex quinquefasciatus* or a close relative is preferable [107] which was used in the present study. Many plant species have been screened for their larvicidal activity against *Culex quinquefasciatus* in the recent years [24, 30, 34, 35, 118, 130-136]. The successful results of the present preliminary study on mosquitocidal potential of petroleum ether extracts of *Tagetes erecta* flowers and *Tridax procumbens* aerial parts encourage further efforts to investigate the bioactive compounds that might possess good larvicidal properties when isolated in pure form. Tehri and Singh [137] stressed that the activity of botanicals is generally attributed to some particular compounds but if a synergistic phenomenon is established among these metabolites it may result in an increased bioactivity compared to isolated components, thus enhancing the effectiveness. Identification, isolation and mass synthesis of bioactive compounds of plant origin against mosquito menace are imperative for the management of mosquito-borne diseases. In addition, novel drug delivery systems of plant based active substances are need of time. Identifying plant based insecticides that are efficient as well as suitable and adaptive to local ecological conditions, biodegradable and have the wide spread mosquitocidal property will work as a new weapon in the arsenal of insecticides and in the future may act as a suitable alternative product to fight against mosquito-borne diseases.

5. Acknowledgement

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