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Efficacy of Some Selected Indigenous Plant Extracts against Two Urban Mosquitoes *Culex quinquefasciatus* Say and *Aedes aegypti* L. (Diptera: Culicidae): An Update

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ABSTRACT

Efficacies of the chloroform and aqueous extracts of the leaf, stem and root of three indigenous plants *viz. Calotropis procera*, *Polygonum hydropiper* and *Thevetia neriifolia* against the larval mortality and reproductive potential of two urban mosquitoes *Culex quinquefasciatus* Say and *Aedes aegypti* L. are reported here. Results show that the chloroform extracts of *C. procera* leaf (LC_{50} = 167.48 ppm), *P. hydropiper* stem (LC_{50} = 341.79 ppm) and *T. neriifolia* leaf (LC_{50} = 209.45 ppm) had excellent larvicidal effect on *Cx. quinquefasciatus*. The aqueous extracts of *C. procera* stem (LC_{50} = 207.18 ppm), *P. hydropiper* stem (LC_{50} = 634.92 ppm) and *T. neriifolia* leaf (LC_{50} = 453.34 ppm), however, had relatively milder larvicidal effect on the mosquitoes under study. Both the extracts significantly reduced percentage of egg-hatch and adult emergence per raft, lengthened immature duration and increased immature mortality in all the treatment groups. The chloroform extracts had a significantly negative effect on the number of eggs per raft, but both chloroform and aqueous extracts failed to induce any appreciable effect on the female ratio. Compared to the aqueous extracts, chloroform extracts are much more efficient against *Cx. quinquefasciatus* productivity. Results on *Ae. aegypti* indicated that the leaf extracts of *C. procera* (LC_{50} =696.14 ppm), the stem extract of *P. hydropiper* (LC_{50} =1164.36 ppm), and the leaf extract of *T. neriifolia* (LC_{50} =872.91 ppm) had significant larvicidal effects compared to the respective controls. In general, the extracts significantly reduced egg-laying, decreased egg-hatch, lengthened immature duration, and increased immature mortality culminating in reduced adult emergence. A comparison of the larvicidal efficacy of the extracts against the two mosquito species reveals that *Cx. quinquefasciatus* is more sensitive to the plant extracts than *Ae. aegypti.* However, further research is solicited to evaluate the impact, persistence and effectiveness of these extracts against the vector mosquitoes under indoor and field conditions.

Introduction

Plant extracts contain botanical insecticides or phytochemicals that could be used to limit reproduction and survival of various pest species including mosquitoes[1-5]. Mosquitoes are of much concern to public health and well-beings of the global human population[2,6-8]. Since these mosquitoes transmit a number of dreadful diseases like filaria, elephantiasis and dengue, control measures using non-conventional insecticides like botanicals and/or phytochemical derivatives are gaining much attention in recent days due to a number of favourable reasons[9-10].

A though review of major literature on the impacts of phytochemicals on mosquito control strategies reveals some encouraging information. Thus, the larvicidal activities of five Philippino plant species against *Aedes aegypti* and *Culex quinquefasciatus*[11], effectiveness of the flower, bud and root extracts of *Calotropis procera* against a chloroquine-sensitive strain of *Plasmodium falciparum* in the malaria vector mosquito *Anopheles* species[12], histochemistry of *Cx. pipiens* ovaries after treating them with oil extracts of *Thevetia peruviana*[13], larvicidal and adult mosquito repellent actions of *Delbergia sisso* oil and *Mentha piperita* oil, respectively against *Anopheles stephensi*, *Cx. quinquefasciatus* and. *Ae. aegypti*[14-15], acetone extracts of *Feronia limnia* leaves as potent larvicides for *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti*[16], foliar extracts of neem *Azadirachtin indica* against the larvae of *Cx. quinquefasciatus*[17], chloroform extracts of *Az. indica* against the 4th instar larvae of *Cx.* quinquefasciatus[18], extracts of *Quereus lusitania* against the 2nd and 4th instar larvae of *Cx. quinquefasciatus*[19], *Argemone mexicana* seed extracts in petroleum ether having larvicidal and growth inhibiting activities against the 2nd instar larvae of *Ae. aegypti*[20] and larvicidal activities of three Thai plant species against *Aedes* and *Culex* mosquitoes[21] were note worthy.

Promising advances made in phytochemical research have been reviewed where mosquito larvicidal plant species, extraction procedures, growth and reproduction inhibiting phytochemicals, botanical ovicides and screening method logies were addressed against *Anopheles*, *Culex* and *Aedes* mosquitoes[22-23]. Recently, a number of workers reported the larvicidal and toxic effects of the leaf extracts[3,24] and latex constituents[25-26] from *Calotropis* on *Culex*, *Aedes* and *Anopheles*; bioefficacies of the essential oils from the leaves

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of *P. hydropiper* against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*[27-29]; and the toxicity of the leaf extracts of *T. peruviana* was evaluated against the larvae of *Ae. aegypti and An. stephensi* mosquitoes[30]. The present investigation reports the efficacies of the aqueous and chloroform extracts of the leaves, stems and roots of three selected plant species *viz*., *C. procera*, *P. hydropiper* and *T. neriifolia* against such important reproductive potential as egg-laying, egg hatchability, immature duration and mortality, adult emergence and female ratio of the two common urban mosquitoes *Cx. quinquefasciatus* and *Ae. aegypti* (Diptera: Culicidae) under laboratory conditions.

Materials and Methods

The experiments were conducted from January 2017 to December 2017 in the Genetics & Molecular Biology Laboratory, Department of Zoology, Rajshahi University (RU), Rajshahi 6205, Bangladesh. Brief protocol and the experimental design are described below.

Collection and rearing of mosquitoes

Egg rafts of *Cx. quinquefasciatus* were collected from the open drains of RU Campus and brought to laboratory for rearing. Each raft was released into a 500 mL glass beaker with 400 mL tap water for hatching. The larvae were provided with the larval food that consisted of ground toast biscuit and yeast powder in the ratio of 3: 1 by weight. On the third day after hatching, the 1st- instar larvae moulted into the 2nd-instar larvae. On the fifth day, the 3rd-instar larvae were observed which moulted into the 4th-instar larvae on the seventh day. Larval food was supplied to the larvae until pupation. Actively swimming, non-feeding pupae were collected into separate 500 mL glass beakers with tap water but without food and placed in adult rearing cages where they were left to emerge as adults. Each rearing cage was of 45cm $\times$ 30cm $\times$ 25cm dimensions, made from wooden frame with sides of wire nettings. The base of the cages was made of wood and the front side was provided with sleeve for taking materials into and out of the cages. The adults were provided with 10% sugar solution soaked in cotton wool in small Petri dishes. After three days, the adult females were blood fed on restrained tender chicks twice a week. Finally, beakers with tap water were supplied to the females for egg-laying. Mosquitoes for plant extract treatments were chosen from F_2 progenies. Eggs of *Ae. aegypti*, on the other hand, were collected by offering earthen water pots in the gardens and were transferred into 500 mL glass beakers provided with pond water for hatching. The rearing protocol for *Ae. aegypti* larvae were the same as *Cx. quinquefasciatus* larvae. Adult females of *Ae. aegypti* were blood fed on restrained mice once a week. As in *Cx. quinquefasciatus*, however, the F_2 progenies of *Ae. aegypti* were also selected for the plant extract treatments.

Experimental plants

Initial screening for bioactive properties was made with seven plant species, *viz*. castor oil plant (*Ricinus communis* L.), custard apple (*Annona muricata* L.), marsh pepper (*Polygonum hydropiper* L.), milkweed (*Calotropis procera* (Aiton), long pepper (*Piper longum* L.), wood apple (*Aegle marmelos* (L.) and yellow oleander (*Thevetia neriifolia* Juss.). But considering the availability, handling convenience and efficacy, three species *viz*., *C. procera* (F. Asclepiadaceae), *P. hydropiper* (F. Polygonaceae) and *T. neriifolia* (F. Apocynaceae), collected from RU Campus and around Rajshahi Metropolitan City, were finally selected for the present work.

Identities of the plants were confirmed at the Department of Botany, RU, and voucher specimens have been preserved as herbarium sheets for future reference.

Extraction protocols

Prominent plant parts namely leaves, stems and roots of the three plant species were brought to the laboratory, rinsed in tap water, cut into small pieces, and dried in the shade at room temperature (28$\pm$2° C) and uncontrolled relative humidity (75$\pm$5%) for about three weeks. The dried specimens were ground with the help of an electric blender to form fine powder, sieved, sealed in reagent bottles and refrigerated at 4° C until extraction. Crude extracts were made in chloroform ($CHCl_3$) and distilled water as follows.

(a)Extractions in chloroform: Ground plant parts were extracted in chloroform as per the Soxhlet extraction method[8] with some modifications. For each extraction, 100g of the dust was extracted in 800 mL of chloroform for 8 hrs over a mantle heater at 62° C (equivalent to the boiling point of the solvent). Then the filtrates were concentrated in a rotary vacuum evaporator under reduced pressure (22-26 mmHg) to remove the solvent completely and to yield gummy extracts. The extracts were weighed ((Table 1), sealed, refrigerated at 4° C and protected from light until use. When needed for use, the crude extracts were re-dissolved in 0.04% Dimethyl sulphoxide (DMSO) that acted as an emulsifier. By further dilution with required amount of water, four different concentrations *viz.* 62.5, 125, 250 and 500 parts per million (ppm) were prepared (Table 2). Chloroform extracts, however, were used only against *Cx. quinquefasciatus.*

(b)Extractions in water: For each plant part, 10% water solution was prepared by taking 10g of the plant dust in a 250 mL conical flask to which 100 mL of distilled water was added, soaked, and kept in an electric shaker for 24 hrs. The aqueous extract thus obtained was filtered, sealed and refrigerated at 4° C until use. The extracts were diluted volumetrically to obtain the test solutions of 250, 500, 1000 and 2000 ppm (Table 3). Aqueous extracts were used against both species of the mosquitoes.

Table 1. Recovery of crude plant extracts with chloroform.

Plant species	Parts used	Weight of dry dust (g)	Vol. of $CHCl_3$ (mL)	Final yield of crude extracts (mg)
C. procera	Leaf	100	800	2625.5
	Stem	100	800	4412.5
	Root	100	800	3335.0
P. hydropiper	Leaf	100	800	2025.0
	Stem	100	800	2537.5
	Root	100	800	1975.0
T. neriifolia	Leaf	100	800	2845.0
	Stem	100	800	8413.0
	Root	100	800	5037.0

Table 2. Reconstitution of the chloroform extracts in distilled water used for larvicidal bioassays.

Crude extracts* (mg)	DMSO (µL)	Distilled water (mL)	Final concentrations (ppm)
12.5	50	200	62.5
25	100	200	125
50	200	200	250
100	400	200	500

*See Table 1 for detail; DMSO= Dimethyl sulphoxide; ppm= parts per million.

Table 3. Reconstitution of aqueous extracts in distilled water used for larvicidal bioassays.

Plant extracts* (µL)	Distilled water (mL)	Final concentrations (ppm)
62.5	199.9375	250
125	199.8750	500
250	199.7500	1000
500	199.5000	2000

*10% aqueous solution of each plant part; ppm= parts per million

Treatment protocols

(a)Treatments of chloroform extracts: The larvicidal bioassays of the chloroform extracts of the selected plant parts against *Cx. quinquefasciatus* at ambient temperature (28±2° C) was evaluated as per the standard procedure[31]. The crude extracts were volumetrically diluted with distilled water to obtain the test solutions of desired concentrations (Table 2).Since even traces of chloroform do not support the development of mosquito larvae, untreated water served as control (0 ppm). Twenty-five 4th-instar larvae were introduced to each 250 mL glass beaker with 200 mL tap water. For each concentration, four replicates were run at a time. The larvicidal effects of each extract were monitored by counting the number of dead larvae at 24 hr intervals up to three days (72 hrs) of exposures. The dead larvae were identified when they failed to move after being probed by a needle in the siphon or cervical region. The larvae were also considered dead if they were unable to reach the water surface. Finally, the larvicidal effect was determined by the use of the median lethal concentration (LC_{50}) for each extract.

The effects of plant extracts on some vital reproductive potential, for example, eggs per raft, percent egg-hatch, immature duration ((*i.e*. larval and pupal developmental periods in days), immature mortality (% larval and pupal deaths), adult emergence per raft and female ratio (*i.e.* number of females ÷ total number of adults per raft) was determined by the use of the lowest LC_{50} values obtained for each extract. Glass beakers (500 mL) containing 400 mL tap water, in which the LC_{50} dose of the extract was mixed, were offered to the blood-fed females for the collection of egg-rafts. Control groups were set up for each extract in a similar fashion. The number of emerged adults and their sexes were recorded in all the treatment groups until adult emergence was completed in the control beakers.

(b)Treatments of aqueous extracts: Same as chloroform extract treatments except that control (0), 250, 500, 1000 and 2000 ppm concentrations of aqueous extracts of the plant parts were used (Table 3). Unlike chloroform extracts, however, aqueous extracts were applied against both species of the mosquitoes. Larvicidal bioassays and evaluations of reproductive potential were the same as that described for the chloroform extracts.

Statistical procedures

Cumulative larval mortality data recoded up to 72 hrs post treatment were loaded on to the *GWBASIC* Probit Analysis software to determine LC_{50} values, 95% fiducial limits (lower and upper) and regression equations[32]. In addition, one-way ANOVA, followed by the least significant difference (LSD) tests using SPSS (Version 16.0) were performed to analyze the significance of the data on reproductive potential[33].

Results

Larvicidal efficacy of plant parts against *C. quinquefasciatus*

(a)Chloroform extracts: Compared to the control (0% larval mortality), chloroform extracts of the three parts of *C. procera* induced mortalities in the 4th-instar larvae of *Cx. quinquefasciatus* in a dose-dependent manner. The cumulative 72-hr mortalities at the highest concentration (500 ppm) in the leaf, stem and root extracts were 97%, 89% and 78%, respectively (Table 4). The estimated LC_{50} values were 167.48 ppm, 189.73 ppm and 248.67 ppm respectively, suggesting that the leaf extract of *C. procera* was most effective against *Cx. quinquefasciatus* larvae. Larval mortalities with *P. hydropiper* at the highest concentration were 66% (LC_{50} = 345.31 ppm), 67% (LC_{50} = 341.79 ppm), and 55% (LC_{50} = 485.15 ppm), and those with *T. neriifolia* were 88% (LC_{50} = 209.45 ppm), 73% (LC_{50} = 249.66 ppm) and 67% (LC_{50} = 310.69 ppm), respectively for the leaf, stem and root extracts (Table 4). These results indicate that the leaf extract of *C. procera*, the stem extract of *P. hydropiper* and the leaf extract of *T. neriifolia* induced the highest larval mortalities against *Cx. quinquefasciatus* under laboratory conditions. Thus, the larval efficacies of the three plant species were in the order of *C. procera* leaf > *T. neriifolia* leaf > *P. hydropiper* stem (Fig. 1).

(b)Aqueous extracts: In comparison with the control treatments, aqueous extracts of leaf, stem and root produced progressively increased larval mortalities. The highest larval mortalities at 2000 ppm were 79%, 83% and 56%; 37%, 47% and 26%; and 52%, 49% and 38%, respectively for the three parts of *C. procera*, *P. hydropiper* and *T. neriifolia* (Table 5). The calculated LC_{50} values of the experimental extracts (Fig. 2) indicate that the stem extract of *C. procera* (207.18 ppm) was the most effective compared to the leaf extract of *T. nerrifolia* (453.34 ppm) and stem extract of *P. hydropiper* (634.92 ppm). A comparison of the LC_{50} values between the chloroform and aqueous extracts revealed that the former required much less concentration (*e.g*. 167.48 ppm, 341.79 ppm and 209.45 ppm for the three plant species, respectively) than the latter (*e.g*. 207.18 ppm, 634.92 ppm and 453.34 ppm, respectively) to kill 50% of the treated 4th-instar larvae of *Cx. quinquefasciatus.*

Table 4. Larvicidal efficacy of the chloroform extracts derived from three plant species against the 4th-instar larvae of *Cx. quinquefasciatus* after 72 hrs post treatment.

Plant species/ Conc. in ppm	[1]Percentage of larval mortalities in plant part extracts		
	Leaf	Stem	Root
C. procera			
0 (control)	0	0	0
62.5	17	14	10
125	30	30	25
250	63	58	46
500	97	89	78
LC_{50} (in ppm)*	**167.48**	**189.73**	**248.67**
95% CL (lower-upper)	128.78-217.81	167.12-215.40	214.80-287.89

Regression equations	Y=-1.2683+2.8185X	Y=-0.7538+2.5413X	Y=-0.3370+2.2324X
P. hydropiper			
0 (control)	0	1	0
62.5	10	11	8
125	17	18	14
250	36	35	26
500	66	67	55
LC_{50} (in ppm)*	**345.31**	**341.79**	**485.15**
95% CL (lower-upper)	283.11-421.17	279.09-418.58	367.85-639.86
Regression equations	Y=0.2294+1.8802X	Y=0.3390+1.8404X	Y=0.4723+1.6809X
T. neriifolia			
0 (control)	0	0	1
62.5	12	16	7
125	26	32	26
250	51	45	40
500	88	73	67
LC_{50} (in ppm)*	**209.45**	**249.66**	**310.69**
95% CL (lower-upper)	184.01-238.40	206.02-302.56	260.30-370.83
Regression equations	Y=-0.9385+2.5745X	Y=0.9176+1.7075X	Y=-0.2632+2.1128X

[1]Cumulative counts for 72 hrs; *Median lethal concentration to kill 50% larvae; CL= Confidence limits

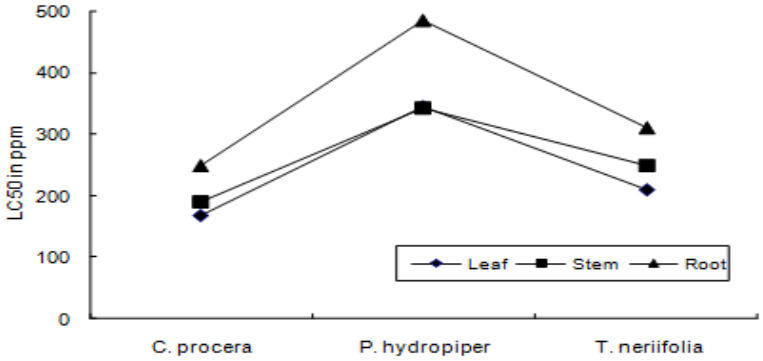

Fig 1. A comparison of LC_{50} values in ppm derived from three plant species and their parts extracted in chloroform and used in bioassays against the 4th-instar larvae of *Cx. quinquefasciatus.*

Table 5. Larvicidal efficacy of the aqueous extracts derived from three plant species against the 4th-instar larvae of *Cx. quinquefasciatus* after 72 hrs post treatment.

Plant species/ Conc. in ppm	**[1]Percentage of larval mortalities in plant part extracts**		
	Leaf	**Stem**	**Root**
C. procera			
0 (control)	0	2	0
250	13	11	9
500	22	23	13
1000	58	67	32
2000	79	83	56
LC_{50} (in ppm)*	**223.53**	**207.18**	**443.94**
95% CL (lower-upper)	194.32-257.14	183.55-233.85	344.44-572.19
Regression equations	Y=-0.2918+2.2556X	Y=-1.2373+2.6875X	Y=0.4725+1.7042X
P. hydropiper			
0 (control)	0	0	1
250	9	12	6
500	14	18	9
1000	18	27	14
2000	37	47	26
LC_{50} (in ppm)*	**1249.53**	**634.92**	**2294.48**
95% CL (lower-upper)	562.86-2773.91	400.05-1007.68	728.90-7222.64
Regression equations	Y=1.7009+1.0597X	Y=1.6073+1.1992X	Y=1.4059+1.0597X
T. neriifolia			
0 (control)	0	0	0
250	17	14	10
500	29	27	18
1000	38	35	32
2000	52	49	38
LC_{50} (in ppm)*	**453.34**	**535.63**	**841.57**
95% CL (lower-upper)	298.55-688.41	337.10-851.07	450.56-1571.93
Regression equations	Y=2.1411+1.0763X	Y=1.9565+1.1195X	Y=1.7465+1.1162X

[1]Cumulative counts for 72 hrs; *Median lethal concentration to kill 50% larvae; CL= Confidence limits

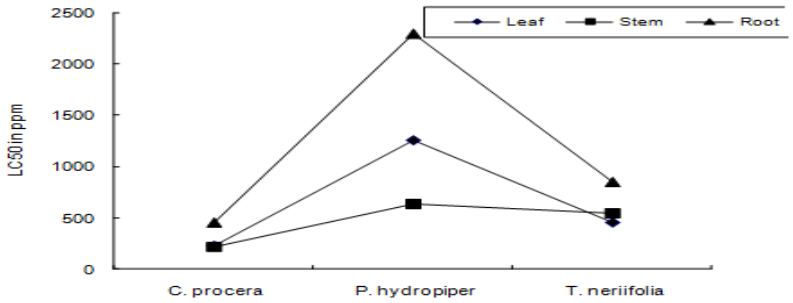

Fig 2. A comparison of LC_{50} values in ppm derived from three plant species and their parts extracted in distilled water and used in bioassays against the fourth-instar larvae of *Cx. quinquefasciatus.*

Effects of plant extracts on reproductive potential in *Cx. quinquefasciatus*

(a) Chloroform extracts: Data on the effects of the chlorofom extracts on the reproductive potential in *Cx. quinquefasciatus* are presented in Table 6. In comparison with the control, LC_{50} values derived from the larvicidal bioassays (Table 4) for *C. procera* leaf extract and *T. neriifolia* leaf extract resulted in significant reduction in eggs per raft. However, *P. hydropiper* stem extract induced a greater number of eggs per raft. Percent egg-hatch and adult emergence per raft were significantly reduced, immature duration was lengthened, and immature mortalities were increased, but the female ratio was unaffected by the application of plant extracts against *Cx. quinquefasciatus* (Fig. 3; Table 8).

(b) Aqueous extracts: Almost similar to the chloroform extracts, aqueous extracts of *C. procera* stem, *P. hydropiper* stem and *T. neriifolia* leaf (Table 7) significantly reduced percent egg-hatch and adult emergence per raft, lengthened immature duration and increased immature mortality in the experimental mosquitoes. But these extracts did not have any appreciable effects on the eggs per raft as well as on the female ratio (Fig. 4; Table 8).

Table 6. Efficacy of the chloroform extracts derived from three plant parts against some reproductive potential in *Cx. quinquefasciatus.*

Plant parts (LC_{50} in ppm)[1]	Eggs per Raft	Percent Hatch	Immature Duration (d)	Immature Mortality (%)	Adults per Raft	Female Ratio*
Control (0.0)	135.2±17.2^{a}	97.4±0.9^{a}	10.1±0.2^{a}	2.1±1.7^{a}	97.9±1.7^{a}	0.49±0.04^{a}
C. procera Leaf (167.48)	122.6±11.5^{b}	46.7±10.8^{b}	12.8±0.3^{b}	51.9±1.6^{b}	27.8±8.5^{b}	0.52±0.06^{a}
P. hydropiper Stem (341.79)	145.8±16.8^{a}	52.4±4.1^{c}	10.5±0.6^{a}	44.8±6.3^{c}	41.8±3.5^{c}	0.53±0.08^{a}
T. neriifolia Leaf (209.45)	110.2±9.7^{b}	55.6±4.3^{c}	13.2±0.6^{b}	47.6±11.3^{bc}	31.8±6.1^{b}	0.54±0.20^{a}

[1]Estimates are shown in Table 4; *Number of females/total number of adults; All values are mean ±SD of 5 replicates; Dissimilar superscripts indicate significant differences by LSD tests at P<0.05

Table 7. Efficacy of the aqueous extracts derived from three plant parts against some reproductive potential in *Cx. quinquefasciatus.*

Plant parts (LC_{50} in ppm)[1]	Eggs per Raft	Percent Hatch	Immature Duration (d)	Immature Mortality(%)	Adults per Raft	Female Ratio*
Control(0.0)	130.2±16.3^{a}	98.1±0.4^{a}	10.2±0.3^{a}	1.7±0.9^{a}	125.6±15.9^{a}	0.52±0.03^{a}
C. procera Stem (207.18)	123.2±14.7^{a}	53.5±6.9^{b}	10.9±0.4^{a}	48.6±5.7^{bc}	34.2±8.6^{b}	0.52±0.04^{a}
P. hydropiper Stem (634.92)	112.6±9.9^{b}	50.3±3.2^{b}	11.4±0.4^{b}	51.0±8.6^{c}	27.4±3.0^{c}	0.50±0.10^{a}
T. neriifolia Leaf (453.34)	115.0±13.3^{b}	52.9±4.2^{b}	11.4±0.4^{b}	46.8±8.8^{b}	32.4±7.6^{bc}	0.53±0.12^{a}

[1]Estimates are shown in Table 5; *Number of females/total number of adults; All values are mean ±SD of 5 replicates; Dissimilar superscripts indicate significant difference by LSD tests at P<0.05

Table 8. ANOVA table showing the efficacy of plant extracts on some reproductive potential in *Cx. quinquefasciatus.*

Sources of variance	Extracts	Degrees of freedom	F-values	Probabilities
Eggs per raft	Chloroform	3,16	5.91	0.007
	Aqueous	3,16	1.71	Ns
Percent hatch	Chloroform	3,16	70.06	<0.001
	Aqueous	3,16	140.72	<0.001
Immature duration	Chloroform	3,16	60.20	<0.001
	Aqueous	3,16	10.75	<0.001
Immature mortality	Chloroform	3,16	62.13	<0.001
	Aqueous	3,16	60.20	<0.001
Adults per raft	Chloroform	3,16	131.93	<0.001
	Aqueous	3,16	113.74	<0.001
Female ratio	Chloroform	3,16	0.28	Ns
	Aqueous	3,16	0.12	Ns

Ns = not significant

These data suggest that the chloroform extracts of the selected plant species had much pronounced effects than the corresponding aqueous extracts on the reproductive potential in *Cx. quinquefasciatus* under present experimental conditions. However, it should be borne in mind that compared to the easy and almost inexpensive aqueous extracts, chloroform extracts are expensive, require a series of time-consuming preparations and the use of Soxhlet apparatus and rotary vacuum evaporation.

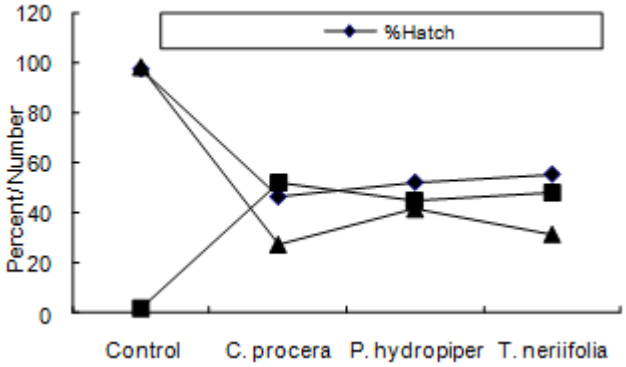

Fig 3. Efficacy of chloroform extracts of selected plant parts against egg-hatch (%), immature mortality (days) and adult emergence per raft in *Cx. quinquefasciatus.*

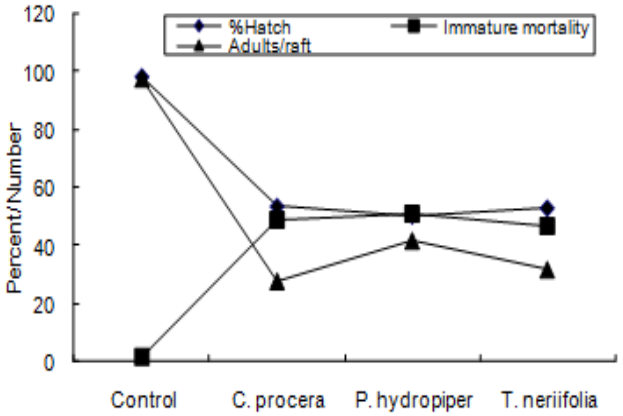

Fig 4. Efficacy of aqueous extracts of selected plant parts against egg-hatch (%), immature mortality (days) and adult emergence per raft in *Cx. quinquefasciatus.*

Larvicidal efficiency of plant parts against *Ae. aegypti*

Estimated LC_{50} values of the leaf, stem and root extracts of *C. procera* against *Ae. aegypti* were 696.14 ppm, 1048.01 ppm and 2078.32 ppm; those of *P. hydropiper* were 1297.85 ppm, 1164.36 ppm and 5340.02 ppm; and those of *T. neriifolia* were 872.91 ppm, 1509.69 ppm and 4288.20 ppm, respectively (Table 9; Fig. 5).

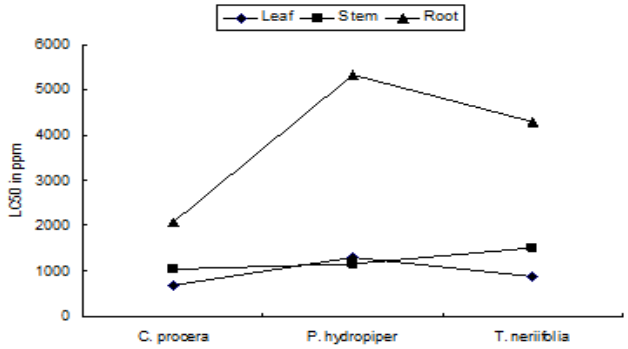

Fig 5. A comparison of LC_{50} values in ppm derived from three plant species and their parts extracted in water and used in bioassays against the 4th-instar larvae of *Ae. aegypti.*

Mortalities of 88%, 72% and 49%; 58%, 62% and 32%; and 78%, 52% and 35% were induced respectively by the leaf, stem and root extracts of *C. procera*, *P. hydropiper* and *T. neriifolia* in the experimental *A. aegypti* larvae. These data suggest that the leaf extracts of *C. procera* (696.14 ppm), the stem extracts of *P. hydropiper* (1164.36 ppm) and the leaf extracts of *T. neriifolia* (872.91 ppm) resulted in the highest larval mortalities of 80%, 62% and 78% in the three plant species under study (Fig. 6).

Effects of plant extracts on reproductive potential in *Ae. aegypti*

Results on the aqueous extracts of plant parts against the reproductive potential in *Ae. aegypti* are presented in Table 10. Compared to the control, the leaf extract of *C. procera* significantly reduced egg-laying, decreased egg-hatch, increased immature mortality and decreased adult emergence in the mosquitoes.

Table 9. Larvicidal efficacy of the aqueous extracts derived from three plant species against the 4th-instar larvae of *Ae. aegypti* after 72 hrs post treatment.

Plant species/ Conc. in ppm	**[1]Percentage of larval mortalities in plant part extracts**		
	Leaf	**Stem**	**Root**
C. procera			
0 (control)	0	0	0
250	17	13	10
500	31	23	12
1000	66	50	34
2000	88	72	49
LC_{50} (in ppm)*	696.14	1048.01	2078.32
95% CL (lower-upper)	**611.12-792.99**	**883.14-1243.67**	**1425.18-3030.76**
Regression equations	Y=-1.8750+2.4250X	Y=-0.8789+1.9499X	Y=0.4870+1.3653X
P. hydropiper			
0 (control)	0	0	0
250	11	18	5
500	28	32	10
1000	45	48	12
2000	58	62	32
LC_{50} (in ppm)*	**1297.85**	**1164.36**	**5340.02**
95% CL (lower-upper)	1028.60-1637.59	900.89-1504.88	2535.38-11247.14
Regression equations	Y=0.0078+1.5746X		Y=0.4404+1.3009X

T. neriifolia			
0 (control)	0	0	0
250	16	13	9
500	28	29	17
1000	55	47	29
2000	78	52	35
LC_{50} (in ppm)*	**872.91**	**1509.69**	**4288.20**
95% CL (lower-upper)	746.84-1020.25	1100.51-2070.99	2050.49-8967.95
Regression equations	Y=-0.8375+1.9899X	Y=0.7767+1.3321X	Y=0.9247+1.1328X

[1]Cumulative counts for 72 hrs; *Median lethal concentration to kill 50% larvae; CL= Confidence limits

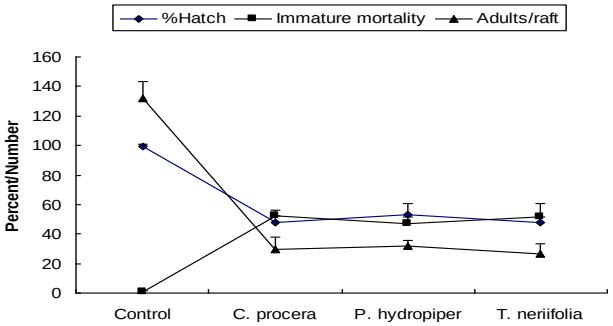

Fig 6. Efficacy of aqueous extracts of selected plant parts against egg-hatch (%), immature mortality (days) and adult emergence in *Ae. aegypti.*

However, immature duration and female ratio were not affected by the treatments. The stem extracts of *P. hydropiper*, on the other hand, significantly lowered egg-hatch, lengthened immature duration, increased immature mortality and decreased adult emergence in *Ae. aegypti*, but the number of eggs and female ratio were unaffected.

Again, the leaf extracts of *T. neriifolia* significantly decreased egg-laying accompanied by reduced egg-hatch, increased immature mortality and reduced adult emergence in the experimental mosquitoes, although the extracts had no appreciable effect on the immature duration and female ratio.

The overall effects of the aqueous plant parts' extracts on the reproductive potential in *Ae. aegypti* have been shown in Table 10. Data indicate that egg-laying, egg-hatch percentage, immature duration and mortality as well as adult emergence are significantly affected, while only the female ratio remained unaffected following the plant extract treatments (Table 11).

A comparison of LC_{50} values estimated for the aqueous extracts demonstrate that *Cx. quinquefasciatus* is more sensitive to the three plant extracts than *Ae. aegypti* under study. Thus, 207.18 ppm stem extract of *C. procera*, 634.92 ppm stem extract of *P. hydropiper* and 453.34 ppm leaf extract of *T. neriifolia* brought about the highest larval mortalities of 79%, 47% and 52%, respectively in *Cx. quinquefasciatus* in comparison with 696.14 ppm leaf extract of *C. procera*, 1164.36 ppm of stem extract of *P. hydropiper* and 872.91 ppm leaf extract of *T. neriifolia* that induced the highest larval mortalities of 88%, 62% and 78%, respectively in *Ae. aegypti*.

Discussion

In an attempt to limit reproduction and longevity of mosquito species in natural habitats, recent research on the use of phytochemicals has increased tremendously. For example, blends of botanical insecticides+IGRs (Fenitrothion, delta-cypermethrin, methoprene) and phytochemicals from seeds of *Callitris glaucophylla*, *Daucus carota* and *Khaya senegalensis* extracted in acetone, ethanol and hexane were effective against *Culex* and *Aedes* mosquito species[22]. Ethanolic leaf extracts of *Centella asiatica* were found effective against *Cx. quinquefasciatus* larvae where LC_{50} values ranged between 6.84-1.12 ppm and inhibition of adult emergence was more pronounced at increased temperature, suggesting use of these extracts in small volume aquatic habitats or breeding sites of limited size around human dwellings[34]. Aqueous extracts of nine medicinal plants were effective against the larvae of *Cx. quinquefasciatus* and *Ae aegypti*[35].

Table 10. Efficacy of the aqueous extracts derived from three plant parts against some reproductive potential in *Ae. aegypti* .

Plant parts (LC_{50} in ppm)[1]	Eggs per Female	Percent Hatch	Immature Duration (d)	Immature Mortality(%)	Number of Adults	Female Ratio*
Control(0.0)	134.0 ± 7.6^{a}	99.0 ± 1.6^{a}	10.0 ± 0.2^{a}	0.9 ± 0.8^{a}	132.1 ± 11.4^{a}	0.51 ± 0.02^{a}
C. procera Leaf (696.14)	118.2 ± 11.9^{b}	47.4 ± 4.7^{b}	11.1 ± 0.7^{a}	52.6 ± 3.4^{b}	29.6 ± 8.3^{bc}	0.52 ± 0.06^{a}
P. hydropiper Stem (1164.36)	122.4 ± 12.1^{a}	52.8 ± 7.5^{b}	12.2 ± 1.0^{b}	46.8 ± 4.9^{c}	31.8 ± 4.2^{b}	0.49 ± 0.08^{a}
T. neriifolia Leaf (872.91)	113.6 ± 8.5^{b}	48.0 ± 3.8^{b}	11.4 ± 0.3^{a}	51.2 ± 9.1^{b}	26.2 ± 6.9^{bc}	0.52 ± 0.10^{a}

[1]Estimates are shown in Table 9; *Number of females/total number of adults; All values are mean±SD of 5 replicates; Dissimilar superscripts indicate significant differences by LSD tests at P<0.05

Table 11. ANOVA table showing the efficacy of aqueous plant extracts on some reproductive potential in *Ae. aegypti.*

Sources of variance	Degrees of freedom	F-values	Probabilities
Eggs per female	3,16	3.30	<0.05
Percent hatch	3,16	137.93	<0.001
Immature duration	3,16	5.98	<0.01
Immature mortality	3,16	148.48	<0.001
Adult emergence	3,16	65.63	<0.001
Female ratio	3,16	1.18	Ns

Ns = not significant

Larvicidal and adult mosquito repellent action of pine oil *Pinus longifolia* against *An. culicifacies* and *Cx. quinquefasciatus* was reported where LC_{50} values varied between 82.0-112 ppm and 100% protection against *An. culicifacies* for 11 hrs and 97% against *Cx. quinquefasciatus* for 9 hrs, suggesting that pine oil could be used as an effective mosquito repellent than larvicidal agents[36].

Relevant to the present plant extracts, acetone and petroleum ether extracts of *C. procera* had their larvicidal action on the 4th-instar larvae of *Cx. quinquefasciatus*[37]. Toxicity of the aqueous extracts of *C. procera* leaves against larvae of *Cx. pipiens* was estimated where acute LC_{50} of 322 ppm indicated that *C. procera* extracts applied to mosquito larval breeding sites may well provide an environmentally safe method for control of mosquito populations[38]. Fresh leaf extract of *C. procera* showed larvicidal properties against mosquito larvae of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*[3]. These results indicate the utility of *C. procera* as potential technology for control of mosquito larvae, and the present findings are quite consistent with the above phytochemicals against mosquitoes. Extracts from *P. hydropiper* were used as indoor fumigant in the form of smoke or spray for mosquito control[39]. Further blossoms of *P. equisetiforme* attracted adult *Cx. pipiens*[40]. Reduced vitellogenesis, synthesis of proteins, carbohydrates and lipid contents and nucleic acid materials of *Cx. pipiens* ovaries were induced by the oil extracts of *T. peruviana*[13] and larvicidal activity of this plant was most potent against *Ae. aegypti* and *Cx. quinquefasciatus* larvae[21].

Tests with *C. procera* latex showed 99% mortality at 64 ppm for *An. stephensi*, 44% mortality against *Cx. quinquefasciatus* and 67% in 256 ppm[26]. Methanol leaf extracts of *Calotropis gigantea* were tested against the 1st to 4th-instar larvae and pupae of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* where LC_{50} values were 73.77, 89.64, 121.69, 155.49, and 213.79 ppm for *An. stephensi*; 92.27, 106.60, 136.48, 164.01, and 202.56 ppm for *Ae. aegypti* and 104.66, 127.71, 173.75, 251.65, and 314.70 ppm for *Cx. quinquefasciatus*, respectively indicating the mosquitocidal property of the extracts[24]. Essential oil extracts from the leaves of *P. hydropiper* against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* demonstrated that the extracts could be utilized in an eco-friendy mosquito control programme[27-29]. Petroleum ether, chloroform, acetone and methanol extracts of the leaf of *Thevetia peruviana* against the larvae of *An. stephensi* and *Ae. aegypti* had mean LC_{50} values of 0.045, >0.05, 0.026, 0041 and 0.038, >0.05, 0.021 and 0.036%, respectively[30]. These findings corroborate nicely with the present results which clearly demonstrated that the chloroform and aqueous extracts of the leaf, stem and root of *C. procera*, *P. hydropiper* and. *T. neriifolia* are capable of inducing pronounced larvicidal effect on *Cx. quinquefasciatus* as well as *Ae. aegypti*. Moreover, the reproductive potential of these mosquitoes may be reduced significantly with the aqueous extracts of the three plant species. These findings are quite encouraging with regard to any integrated strategies for controlling *Cx. quinquefasciatus* and *Ae. aegypti*, the notorious vector species of public health importance.

Conclusion

Efficacies of the leaf, stem and root extracts of *C. procera*, *P. hydropiper* and *T. neriifolia* on the larvae and some reproductive attributes of two mosquito species *Cx. quinquefasciatus* and *Ae. aegypti* revealed that the chloroform extracts of *C. procera* leaf, *P. hydropiper* stem and *T. neriifolia* stem had excellent larvicidal effect on *Cx. quinquefasciatus*. The aqueous extracts of the above plant parts, however, had a relatively milder larvicidal effect on the mosquito. Both the extracts significantly reduced egg-hatch and adult emergence, lengthened immature duration and increased immature mortality. Owing to expensive and time-consuming procedures, only aqueous extracts were used against *Ae. aegypti* in which the larvicidal effects of the leaf extracts of *C. procera*, stem extracts of *P. hydropiper* and leaf extracts of *T. neriifolia* were effective. Compared to the control, these extracts significantly lowered egg-hatch, lengthened immature duration, increased immature mortality and decreased adult emergence. The present results therefore indicated that *Cx. quinquefasciatus* is more sensitive than *Ae. aegypti* to the three plant species under study. In view of the cost and environmental pollution incurred by the synthetic insecticides, use of the botanical derivatives in mosquito control programmes is quite commendable. Further work is therefore solicited to evaluate the impact, persistence and effectiveness of these extracts against the mosquitoes under out-door and field conditions.

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