4

RESULTS AND DISCUSSION

PHASE I

4.1 OVICIDAL ACTIVITY

The efficacy of different plants may vary from one another on the basis of their toxic effects (Table 2 to 21). The egg rafts of *C. quinquefasciatus* were treated with selected doses of plant extracts at different concentrations. Throughout the experiment, egg hatchability was found to be 100% in the control.

Leaf extracts of *C. gigantea*

The maximum ovicidal activity was noted in ethanol extract of *C. gigantea* leaf. Egg hatchability was found to be totally inhibited by higher concentration of 300 ppm (Table 2 & Fig. 4). At 250 ppm zero percentage egg hatchability was recorded at 72 h and 96 h. However, minimum ovicidal activity was exhibited by petroleum ether extract, which induced total egg hatchability percentage of 45 at the concentration of 300 ppm.

Egg mortality

Maximum egg mortality was shown in ethanol extract which showed 100% egg mortality in higher concentration of 300 ppm (Fig 6c) which was followed by chloroform extract which recorded 65% egg mortality in 300 ppm (Fig 6b). Lowest egg mortality (55%) was recorded in petroleum ether extract (Fig 6a). Highest egg mortality was shown in ethanol extract and its LC_{50} value was 79.01 ppm and regression equation was Y=1.24+1.95X. The UCL LC_{50} of ethanol extract was 134.50 ppm and LCL LC_{50} value was 35.57 ppm (Table 7).

Leaf extracts of *T. peruviana*

Among the three soluble fractions tested chloroform extract of *T. peruviana* leaf was found to be more effective (Table 3) than other two extracts inducing zero percentage egg hatchability throughout the experiment period at the

concentration of 250 and 300 ppm (Fig. 3). Minimum ovicidal activity was shown in petroleum ether extract exhibiting a total egg hatchability percentage of 25 in 300 ppm (Fig. 5a).

Egg mortality

Chloroform extract of *T. peruviana* leaf recorded the highest egg mortality (100%) at the highest concentrations of 250 and 300 ppm (Fig. 6b). Minimum egg mortality (75%) was shown in petroleum ether extract at 300 ppm (Fig. 6a). LC_{50} values indicated that chloroform extract was most effective with LC_{50} of 65.52 ppm with the regression equation Y= 0.54+3.05X (Table 8). The UCL LC_{50} of chloroform extract was 93.71 ppm and LCL LC_{50} was 45.81 ppm.

Leaf extracts of *T. erecta*

Among the three solvents tested, the ethanol soluble fractions showed the highest toxicity at 300 ppm by inducing 25% total egg hatchability (Table 4 & Fig. 5c). However, in all the three solvent extracts egg hatchability of <7 is observed at 96h in 300 ppm. Minimum ovicidal activity was observed in petroleum ether extract with 28.33% total egg hatchability in 300 ppm concentration (Fig. 5a).

Egg mortality

Egg mortality (75%) was found to be higher in ethanol extract (Fig. 6c) at the higher concentration of 300 ppm. Following this 72.33% egg mortality was exhibited by chloroform extract at 300 ppm (Fig. 6b). Minimum egg mortality percentage of 71.67 was noted in petroleum ether extract (Fig 6a). The ethanol extract of *T. erecta* leaf is more effective exhibiting an LC_{50} value of 123.43 ppm with the regression equation of Y=2.41+1.23X. The UCL LC_{50} value was 166.47 ppm and LCL LC_{50} value was 91.51 ppm (Table 9).

Leaf extracts of *L. camara*

In petroleum ether extract at higher concentration of 300 ppm 10% egg hatchability was noted during 48 h of the study period. In lower concentrations of 100 ppm < 25% egg hatchability was observed. Petroleum ether extract showed the highest ovicidal activity inducing only 26.67% egg hatchability (Fig. 5a) which was followed by chloroform extract which induced 31.67% egg hatchability at

300 ppm (Table 5 & Fig. 5b). Minimum ovicidal activity was shown in ethanol extract with total egg hatchability percentage of 35 in 300 ppm concentration (Fig. 5c)

Egg mortality

Maximum egg mortality was observed in the petroleum ether extract (73.33%) at 300 ppm followed by chloroform extract which showed 68.33% total egg mortality. The lowest egg mortality (65%) was shown in ethanol extract in 250 and 300 ppm. The LC_{50} value of petroleum ether extract was 144.91 ppm with regression equation of Y=1.40+1.66X. UCL LC_{50} value was 174.04 ppm and LCL LC_{50} value was 120.65 ppm (Table 10 & Fig. 6).

Leaf extracts of *B. acuminata*

Maximum ovicidal activity was exhibited by petroleum ether extract at the higher concentration of 300 ppm (Table 6) which showed 15%, 11.67% and 6.67% egg hatchability at 48, 72 and 96 h respectively. The total egg hatchability percentage was 33.33% (Fig. 5a). Total egg hatchability of 38.33% and 41.67% was noted in chloroform extract and ethanol extract respectively in the higher concentration (Fig 5b & c). However, at lower concentration of 100 ppm moderate egg hatchability percentage was observed in all the extracts at 48 h (Fig. 2a, 3a & 4a).

Egg mortality

Maximum egg mortality of 66.67% was recorded in petroleum ether extract at higher concentrations of 300 ppm (Fig. 6a). Petroleum ether extract was followed by chloroform extract. Minimum egg mortality (58.33%) was induced in ethanol extract at 300 ppm (Fig. 6c). The LC_{50} value of petroleum ether extract was 179.21 ppm with the regression equation of Y=2.38+1.16X. UCL and LCL LC_{50} values were 207.76 ppm and 143.72 ppm respectively (Table 11).

Among the leaf extracts, maximum ovicidal activity (100% egg mortality) was noted at the concentration of 250 and 300 ppm in chloroform extract of *T. peruviana* followed by ethanol extract of *C. gigantea* at 300 ppm

Flower extracts of *C. gigantea*

Among the three solvents tested ethanol extract was found to be highly effective exhibiting zero percentage egg hatchability at concentrations ranging from 200 - 300 ppm (Table 12 & Fig. 9) throughout the experiment period. Only 5% eggs were found to be hatched which indicated that the ethanol extract of *C. gigantea* induced very high ovicidal activity in 100 ppm at 48 h. Minimum ovicidal activity was shown in chloroform extract inducing total egg hatchability of 26.67% in 300 ppm concentration (Fig. 10b).

Egg mortality

Maximum egg mortality was noted in the ethanol extract inducing 100% egg mortality in the concentrations ranging from 200 - 300 ppm (Fig. 11c). Moreover, 90% egg mortality was recorded at the concentration of 150 ppm. Effectiveness of ethanol extract was followed by petroleum ether and chloroform extract (Fig. 11a & b) which recorded 78.33% and 73.33% egg mortality respectively. The egg mortality data were subjected to probit analysis for calculating LC_{50}, LC_{70}, LC_{90}, upper confidence limit, lower confidence limit and Chi-square values (Table 17).

Flower extracts of *T. peruviana*

Among the three flower extracts, maximum ovicidal activity was displayed in petroleum ether extract which totally inhibited the egg hatchability at 300 ppm (Table 13 & Fig. 7). At 48 and 72 h egg hatchability was 3.33% and 1.67% in 250 ppm concentration and the egg hatchability was totally inhibited after 96 h. Minimum egg hatchability was induced in ethanol flower extract exhibiting total egg hatchability percentage of 25 in 300 ppm (Fig. 10c).

Table 2

Ovicidal activity of petroleum ether, chloroform and ethanol extracts of *C. gigantea* leaf against *C. quinquefasciatus*

Solvents used	Concentration in ppm	Egg hatchability %			Total egg hatchability	Total Egg mortality
		48 h	72 h	96 h		
Petroleum Ether	Control	43.33 ^{a}	31.67 ^{a}	25.00 ^{a}	100 ^{a}	-
	100	31.67 ^{b}	23.33 ^{bc}	18.33 ^{ab}	73.33 ^{bc}	26.67 ^{cd}
	150	30.00 ^{b}	25.00 ^{ab}	20.00 ^{ab}	75.00 ^{b}	25.00 ^{d}
	200	25.00 ^{bc}	23.33 ^{bc}	18.33 ^{ab}	66.67 ^{c}	33.33 ^{c}
	250	21.67 ^{bc}	16.67 ^{cd}	15.00 ^{b}	53.33 ^{d}	46.67 ^{b}
	300	16.67 ^{c}	13.33 ^{d}	15.00 ^{b}	45.00 ^{e}	55.00 ^{a}
	F	7.96**	8.58**	-	123.68**	123.69**
	SED	2.96	2.20	2.82	2.13	2.13
	CD (0.05)	6.46	4.79	6.14	4.63	4.63
	CD (0.01)	9.05	6.77	8.60	6.50	6.50
Chloroform	Control	46.67 ^{a}	30.33 ^{a}	23.00 ^{a}	100 ^{a}	-
	100	35.00 ^{b}	26.67 ^{b}	15.00 ^{a}	80.00 ^{b}	20.00 ^{e}
	150	33.33 ^{b}	21.67 ^{bc}	20.00 ^{a}	71.67 ^{c}	28.33 ^{d}
	200	18.33 ^{c}	18.33 ^{c}	21.67 ^{a}	58.33 ^{d}	41.67 ^{c}
	250	16.67 ^{c}	13.33 ^{d}	15.00 ^{a}	45.00 ^{e}	55.00 ^{b}
	300	10.00 ^{d}	13.33 ^{d}	15.00 ^{a}	35.00 ^{f}	65.00 ^{a}
	F	63.02**	19.65**	-	114.03**	114.04**
	SED	1.65	1.76	3.57	2.53	2.53
	CD (0.05)	3.59	3.83	7.78	5.51	5.51
	CD (0.01)	5.03	5.38	10.91	7.72	7.72
Ethanol	Control	41.67 ^{a}	30.00 ^{a}	28.33 ^{a}	100 ^{a}	-
	100	14.00 ^{b}	7.67 ^{b}	5.00 ^{b}	26.67 ^{b}	73.33 ^{b}
	150	13.34 ^{b}	3.33 ^{c}	3.33 ^{bc}	20.00 ^{b}	80.00 ^{b}
	200	3.33 ^{bc}	1.67 ^{c}	1.67 ^{c}	6.67 ^{c}	93.33 ^{a}
	250	1.67 ^{c}	-	-	-	98.33 ^{a}
	300	-	-	-	-	100 ^{a}
	F	46.33**	24.86**	15.71**	110.37**	110.37**
	SED	3.31	3.77	4.35	4.57	4.57
	CD (0.05)	7.21	8.21	9.49	9.96	9.96
	CD (0.01)	10.10	11.51	13.30	13.97	13.97

** - Significant at p = 0.01 * - Significant at p = 0.05 SED = Standard Error Deviation CD = Critical Difference

Table 3

Ovicidal activity of petroleum ether, chloroform and ethanol extracts of *T. peruviana* leaf against *C. quinquefasciatus*

Solvents used	Concentration in ppm	Egg hatchability %			Total egg hatchability	Total Egg mortality
		48 h	72 h	96 h		
Petroleum Ether	Control	43.33 [a]	31.67 [a]	25.00 [a]	100 [a]	-
	100	26.67 [b]	26.67 [ab]	20.00 [ab]	73.33 [b]	26.67 [c]
	150	25.00 [b]	18.33 [bc]	16.67 [ab]	60.00 [b]	40.00 [c]
	200	11.67 [c]	18.33 [bc]	13.33 [b]	43.33 [c]	56.67 [b]
	250	6.67 [c]	11.67 [c]	15.00 [b]	33.33 [c]	66.67 [b]
	300	10.00 [c]	8.33 [c]	6.67 [d]	25.00 [d]	75.00 [a]
	F	31.07**	17.81**	9.48**	86.23**	86.23**
	SED	3.15	3.63	4.00	3.95	3.95
	CD (0.05)	6.87	7.92	8.73	8.60	8.60
	CD (0.01)	9.63	11.10	12.23	12.06	12.06
Chloroform	Control	46.67 [a]	30.33 [a]	23.00 [a]	100 [a]	-
	100	10.67 [b]	9.33 [b]	1.67 [b]	21.67 [b]	78.33 [b]
	150	9.99 [b]	6.67 [b]	1.67 [b]	18.33 [b]	81.67 [b]
	200	6.00 [b]	5.66 [b]	1.67 [b]	13.33 [b]	86.67 [b]
	250	-	-	-	-	100[a]
	300	-	-	-	-	100[a]
	F	35.31**	127.88**	13.20**	264.48**	264.47**
	SED	3.49	1.62	3.94	2.81	2.81
	CD (0.05)	7.61	3.53	8.57	6.11	6.11
	CD (0.01)	10.67	4.95	12.02	8.57	8.57
Ethanol	Control	41.67 [a]	30.00 [a]	28.33 [a]	100 [a]	-
	100	23.33 [b]	20.00 [b]	18.33 [b]	61.67 [b]	38.33 [c]
	150	18.33 [bc]	13.33 [bc]	15.00 [bc]	46.67 [c]	53.33 [b]
	200	15.00 [bc]	10.00 [c]	11.67 [c]	36.67 [cd]	63.33 [ab]
	250	11.67 [c]	8.33 [c]	11.67 [c]	31.67 [d]	68.33 [a]
	300	1.67 [d]	1.67 [d]	3.33 [c]	6.67 [d]	93.33 [a]
	F	12.07**	12.87**	20.29**	87.31**	87.31**
	SED	3.29	2.62	1.85	3.33	3.33
	CD (0.05)	7.17	5.71	4.04	7.27	7.27
	CD (0.01)	10.06	8.01	5.67	10.19	10.19

** - Significant at p = 0.01 * - Significant at p = 0.05 SED = Standard Error Deviation CD = Critical Difference

Table 4

Ovicidal activity of petroleum ether, chloroform and ethanol extracts of *T. erecta* leaf against *C. quinquefasciatus*

Solvents used	Concentration in ppm	Egg hatchability %			Total egg hatchability	Total Egg mortality
		48 h	72 h	96 h		
Petroleum Ether	Control	43.33 [a]	31.67 [a]	25.00 [a]	100 [a]	-
	100	21.67 [b]	15.00 [bc]	16.67 [b]	51.67 [b]	48.33 d
	150	25.00 [b]	18.33 [b]	15.00 [b]	48.33 [bc]	51.67 d
	200	20.00 [b]	11.67 [c]	10.00 [bc]	41.67 [c]	58.33 c
	250	18.33 [b]	11.67 [c]	6.67 [c]	36.67 [d]	63.33 b
	300	11.67 [c]	10.00 [c]	6.67 [c]	28.33 [e]	71.67 a
	F	12.71**	14.63**	12.54**	535.50**	535.50**
	SED	2099	1.93	2.50	1.28	1.28
	CD (0.05)	6.52	4.21	5.45	2.79	2.79
	CD (0.01)	9.14	5.89	7.64	3.91	3.91
Chloroform	Control	46.67 [a]	30.33 [a]	23.00 [a]	100 [a]	-
	100	25.00 [b]	25.00 [b]	13.33 [bc]	63.33 [b]	36.67 [d]
	150	20.00 [bc]	18.33 [c]	15.00 [b]	53.33 [c]	46.67 [c]
	200	15.00 [cd]	16.67 [d]	10.00 [bcd]	41.67 [d]	58.33 [b]
	250	10.00 [d]	11.67 [d]	8.33 [cd]	30.00 [e]	70.00 [a]
	300	11.00 [d]	10.00 [d]	6.67 [d]	27.67 [e]	72.33 [a]
	F	22.85**	23.79**	14.64**	339.29**	339.33**
	SED	2.34	1.83	2.14	1.67	1.67
	CD (0.05)	5.11	3.98	4.67	3.64	3.64
	CD (0.01)	7.16	5.58	6.54	5.11	5.11
Ethanol	Control	41.67 [a]	30.00 [a]	28.33 [a]	100 [a]	-
	100	25.00 [ab]	21.67 [b]	13.33 [b]	60.00 [b]	40.00 [c]
	150	23.33 [abc]	20.00 [b]	10.00 [c]	53.33 [b]	46.67 [c]
	200	15.00 [abc]	15.00 [bc]	10.00 [c]	40.00 [c]	60.00 [b]
	250	13.33 [bc]	10.00 [c]	5.00 [d]	28.33 [d]	71.67 [a]
	300	10.00 [c]	10.00 [c]	5.00 [d]	25.00 [d]	75.00 [a]
	F	2.92[NS]	9.74**	54.44**	196.72**	196.72**
	SED	4.77	2.84	1.07	2.22	2.22
	CD (0.05)	10.39	6.18	2.32	4.83	4.83
	CD (0.01)	14.58	8.67	3.26	6.77	6.77

** - Significant at p = 0.01 * - Significant at p = 0.05 SED = Standard Error Deviation CD = Critical Difference

Table 5

Ovicidal activity of petroleum ether, chloroform and ethanol extracts of *L. camara* leaf against *C. quinquefasciatus*

Solvents used	Concentration in ppm	Egg hatchability %			Total egg hatchability	Total Egg mortality
		48 h	72 h	96 h		
Petroleum Ether	Control	43.33 ^{a}	31.67 ^{a}	25.00 ^{a}	100 ^{a}	-
	100	20.00 ^{b}	20.00 ^{b}	20.00 ^{a}	60.00 ^{b}	40.00 ^{d}
	150	15.00 ^{bc}	18.33 ^{bc}	18.33 ^{a}	51.67 ^{bc}	48.33 ^{cd}
	200	13.33 ^{bc}	15.00 ^{bcd}	15.00 ^{a}	43.33 ^{cd}	56.67 ^{bc}
	250	11.67 ^{bc}	13.33 ^{cd}	8.33 ^{b}	33.33 ^{de}	66.67 ^{ab}
	300	10.00 c	10.00 ^{d}	6.67 ^{b}	26.67 ^{e}	73.33 ^{a}
	F	24.96**	11.36**	12.45**	109.13**	109.13**
	SED	2.92	1.86	2.16	2.87	2.87
	CD (0.05)	6.36	4.05	4.71	6.26	6.26
	CD (0.01)	8.92	5.68	6.59	8.78	8.78
Chloroform	Control	46.67 ^{a}	30.33 ^{a}	23.00 ^{a}	100 ^{a}	-
	100	23.33 ^{b}	20.00 ^{b}	16.67 ^{b}	60.00 ^{b}	40.00 ^{d}
	150	20.00 ^{bc}	16.67 ^{b}	15.00 ^{b}	51.67 ^{c}	48.33 ^{c}
	200	16.67 ^{cd}	11.67 ^{c}	10.00 ^{c}	38.33 ^{d}	61.67 ^{b}
	250	13.33 ^{d}	11.67 ^{c}	8.33 ^{c}	33.33 ^{e}	66.67 ^{a}
	300	13.33 ^{d}	10.00 ^{c}	8.33 ^{c}	31.67 ^{e}	68.33 ^{a}
	F	41.33**	30.88**	19.83**	659.13**	659.12**
	SED	1.63	1.57	1.67	1.16	1.16
	CD (0.05)	3.57	3.29	3.64	2.52	2.52
	CD (0.01)	5.00	4.61	5.09	3.53	3.53
Ethanol	Control	41.67 ^{a}	30.00 ^{a}	28.33 ^{a}	100 ^{a}	-
	100	25.00 ^{b}	16.67 ^{b}	20.00 ^{b}	61.67 ^{b}	38.33 ^{c}
	150	25.00 ^{b}	11.67 ^{bc}	15.00 ^{b}	51.67 ^{c}	48.33 ^{b}
	200	21.67 ^{bc}	13.33 ^{bc}	10.00 ^{c}	45.00 ^{c}	55.00 ^{b}
	250	16.67 ^{c}	10.00 ^{bc}	8.33 ^{c}	35.00 ^{d}	65.00 ^{a}
	300	16.67 ^{c}	8.00 ^{c}	10.00 ^{c}	35.00 ^{d}	65.00 ^{a}
	F	16.13**	10.39**	21.22**	142.89**	142.89**
	SED	2.22	2.62	1.72	2.39	2.39
	CD (0.05)	4.83	5.71	3.75	5.22	5.22
	CD (0.01)	6.78	8.01	5.25	7.31	7.31

** - Significant at p = 0.01 * - Significant at p = 0.05 SED = Standard Error Deviation CD = Critical Difference

Table 6

Ovicidal activity of petroleum ether, chloroform and ethanol extracts of *B. acuminata* leaf against *C. quinquefasciatus*

Solvents used	Concentration in ppm	Egg hatchability %			Total egg hatchability	Total Egg mortality
		48 h	72 h	96 h		
Petroleum Ether	Control	43.33 [a]	31.67 [a]	25.00 [a]	100 [a]	-
	100	28.33 [b]	21.67 [b]	16.67 [ab]	66.67 [b]	33.33 [e]
	150	26.67 [bc]	20.00 [bc]	11.67 [bc]	58.33 [c]	41.67 [d]
	200	23.33 [c]	15.00 [cd]	8.33 [c]	46.67 [d]	53.33 [c]
	250	16.67 [d]	13.33 [d]	10.00 [bc]	40.00 [e]	60.00 [b]
	300	15.00 [d]	11.67 [d]	6.67 [c]	33.33 [f]	66.67 [a]
	F	38.88**	15.74**	8.40**	345.45**	345.45**
	SED	1.43	2.01	2.73	1.51	1.51
	CD (0.05)	3.11	4.37	5.94	3.29	3.29
	CD (0.01)	4.36	6.13	8.32	4.61	4.61
Chloroform	Control	46.67 [a]	30.33 [a]	23.00 [a]	100 [a]	-
	100	26.67 [b]	25.00 [b]	23.33 [a]	73.33 [b]	26.67 [c]
	150	25.00 [b]	20.00 [b]	21.67 [ab]	66.67 [bc]	33.33 [bc]
	200	23.33 [b]	20.00 [b]	18.33 [b]	61.67 [c]	38.33 [b]
	250	15.00 [c]	13.00 [c]	13.33 [c]	41.67 [d]	58.33 [a]
	300	15.00 [c]	13.33 [c]	10.00 [c]	38.33 [d]	61.67 [a]
	F	73.40**	17.72**	19.65**	86.39**	86.39**
	SED	1.06	1.79	1.45	2.84	2.84
	CD (0.05)	2.32	3.89	3.16	6.18	6.18
	CD (0.01)	3.25	5.46	4.43	8.66	8.66
Ethanol	Control	41.67 [a]	30.00 [a]	28.33 [a]	100 [a]	-
	100	23.33 [b]	21.67 [b]	18.33 [ab]	63.33 [bc]	36.67 [c]
	150	20.00 [bc]	20.00 [bc]	13.33 [bc]	53.33 [c]	46.67 [b]
	200	16.67 [cd]	16.67 [cd]	11.67 [c]	45.00 [d]	55.00 [a]
	250	16.67 [cd]	15.00 [d]	11.67 [c]	43.33 [d]	56.67 [a]
	300	15.00 [d]	13.33 [d]	13.33 [bc]	41.67 [d]	58.33 [a]
	F	57.83**	12.46**	6.81**	232.86**	232.86**
	SED	1.55	1.43	1.94	1.76	1.76
	CD (0.05)	3.38	3.12	4.23	3.84	3.84
	CD (0.01)	4.74	4.37	5.93	5.39	5.39

** - Significant at p = 0.01 * - Significant at p = 0.05 SED = Standard Error Deviation CD = Critical Difference

Table 7

Lethal concentration of leaf extracts of *C. gigantea* against eggs of *C. quinquefasciatus*

Solvents Used	Log LC_{50}	Log LC_{70}	Log LC_{90}	LC50 (ppm)	LC_{70} (ppm)	LC_{90} (ppm)	Regression Equation	95% Confidence Limits				χ^2	SE
								UCL (ppm)		LCL (ppm)			
								LC_{50}	LC_{90}	LC_{50}	LC_{90}		
Petroleum ether	2.47	2.78	3.23	295.38	608.44	1726.88	Y=0.87+1.67X	374.54	4354.94	232.95	684.76	5.89	0.69
Chloroform	2.35	2.55	2.83	226.76	357.16	688.17	Y=−1.26+2.65X	252.64	982.31	203.52	482.10	1.59	0.71
Ethanol	1.89	2.18	2.57	79.01	153.23	373.44	Y=1.24+1.95X	134.50	880.98	35.57	158.30	8.33	1.17

Table 8

Lethal concentration of leaf extracts of *T. peruviana* against eggs of *C. quinquefasciatus*

Solvents Used	Log LC_{50}	Log LC_{70}	Log LC_{90}	LC50 (ppm)	LC_{70} (ppm)	LC_{90} (ppm)	Regression Equation	95% Confidence Limits				χ^2	SE
								UCL (ppm)		LCL (ppm)			
								LC_{50}	LC_{90}	LC_{50}	LC_{90}		
Petroleum ether	2.21	2.36	2.56	164.60	229.49	370.80	Y=−3.05+3.63X	219.68	676.67	123.33	203.19	15.22	1.21
Chloroform	1.81	1.98	2.23	65.52	97.33	172.32	Y=0.54+3.05X	93.71	207.42	45.81	143.15	2.07	1.44
Ethanol	2.14	2.40	2.78	140.03	255.79	610.37	Y=0.69+2.00X	164.13	935.40	119.47	398.28	0.14	0.67

Table 9

Lethal concentration of leaf extracts of *T. erecta* against eggs of *C. quinquefasciatus*

Solvents Used	Log LC_{50}	Log LC_{70}	Log LC_{90}	LC50 (ppm)	LC_{70} (ppm)	LC_{90} (ppm)	Regression Equation	95% Confidence Limits				χ^2	SE
								UCL (ppm)		LCL (ppm)			
								LC_{50}	LC_{90}	LC_{50}	LC_{90}		
Petroleum ether	2.17	2.42	2.76	151.34	263.87	588.68	Y=0.26+2.17X	173.19	863.73	132.25	401.22	0.92	0.68
Chloroform	2.15	2.40	2.77	143.94	256.75	592.03	Y=0.49+2.08X	166.89	884.39	124.15	396.32	1.68	0.67
Ethanol	2.09	2.51	3.12	123.43	327.43	1338.97	Y=2.41+1.23X	166.47	3953.37	91.51	453.50	1.40	0.66

LCL = Lower Confidence Limit UCL= Upper Confidence Limit χ^2 = Chi Square SE= Standard Error

Table 10

Lethal concentration of leaf extracts of *L. camara* against eggs of *C. quinquefasciatus*

Solvents Used	Log LC_{50}	Log LC_{70}	Log LC_{90}	LC50 (ppm)	LC_{70} (ppm)	LC_{90} (ppm)	Regression Equation	95% Confidence Limits				χ^2	SE
								UCL (ppm)		LCL (ppm)			
								LC_{50}	LC_{90}	LC_{50}	LC_{90}		
Petroleum ether	2.16	2.47	2.93	144.91	299.62	855.04	Y=1.40+1.66X	174.04	1614.85	120.65	452.73	1.01	0.66
Chloroform	2.17	2.45	2.86	148.05	285.94	739.46	Y=1.01+1.83X	174.17	1253.86	125.85	436.09	0.87	0.67
Ethanol	2.20	2.53	3.02	158.80	346.11	1065.70	Y=1.58+1.55X	189.87	2298.91	132.83	494.02	0.56	0.66

Table 11

Lethal concentration of leaf extracts of *B. acuminata* against eggs of *C. quinquefasciatus*

Solvents Used	Log LC_{50}	Log LC_{70}	Log LC_{90}	LC50 (ppm)	LC_{70} (ppm)	LC_{90} (ppm)	Regression Equation	95% Confidence Limits				χ^2	SE
								UCL (ppm)		LCL (ppm)			
								LC_{50}	LC_{90}	LC_{50}	LC_{90}		
Petroleum ether	2.25	2.70	3.35	179.21	507.40	2279.39	Y=2.38+1.16X	207.76	9627.95	143.72	539.64	0.62	0.66
Chloroform	2.25	2.53	2.94	180.77	346.33	885.35	Y=0.80+1.85X	223.48	1581.96	157.28	495.49	0.31	0.67
Ethanol	2.35	2.61	2.98	226.26	408.57	958.88	Y=0.18+2.04X	260.14	1677.10	196.79	548.24	4.40	0.68

LCL = Lower Confidence Limit UCL= Upper Confidence Limit χ^2 = Chi Square SE= Standard Error

Table 12

Ovicidal activity of petroleum ether, chloroform and ethanol extracts of *C. gigantea* flower against *C. quinquefasciatus*

Solvents used	Concentration in ppm	Egg hatchability %			Total egg hatchability	Total Egg mortality
		48 h	72 h	96 h		
Petroleum Ether	Control	50.00 [a]	28.33 [a]	21.67 [a]	100 [a]	-
	100	21.67 [b]	18.33 [b]	18.33 [ab]	58.33 [b]	41.67 [c]
	150	16.67 [bc]	16.67 [b]	13.33 [b]	46.67 [c]	53.33 [b]
	200	13.33 [cd]	13.33 [bc]	13.33 [b]	40.00 [c]	60.00 [b]
	250	10.00 [de]	10.00 [cd]	8.33 [c]	28.33 [d]	71.67 [a]
	300	8.33 [e]	6.67 [d]	6.67 [c]	21.67 [d]	78.33 [a]
	F	57.57**	22.14**	10.56**	234.58**	234.58**
	SED	1.93	1.81	2.15	2.07	2.07
	CD (0.05)	4.19	3.93	4.69	4.51	4.51
	CD (0.01)	5.88	5.52	6.57	6.32	6.32
Chloroform	Control	40.00 [a]	31.67 [a]	28.33 [a]	100 [a]	-
	100	21.67 [b]	18.33 [b]	15.00 [b]	55.00 [b]	45.00 [c]
	150	16.67 [bc]	13.33 [bc]	13.33 [b]	43.33 [c]	56.67 [b]
	200	13.33 [bc]	11.67 [cd]	10.00 [bc]	35.00 [cd]	65.00 [ab]
	250	10.00 [c]	10.00 [cd]	10.00 [bc]	30.00 [d]	70.00 [a]
	300	10.00 [c]	8.33 [d]	8.33 [c]	26.67 [d]	73.33 [a]
	F	15.89**	24.08**	15.53**	138.90**	138.90**
	SED	2.84	1.85	2.01	2.65	2.65
	CD (0.05)	6.18	4.02	4.38	5.77	5.77
	CD (0.01)	8.67	5.64	6.15	8.08	8.08
Ethanol	Control	53.33 [a]	26.67 [a]	20.00 [a]	100 [a]	-
	100	5.00 [b]	6.67 [b]	6.67 [b]	18.33 [b]	81.67 [c]
	150	3.33 [bc]	5.00 [b]	1.67 [c]	10.00 [c]	90.00 [b]
	200	-	-	-	-	100 [a]
	250	-	-	-	-	100 [a]
	300	-	-	-	-	100 [a]
	F	39.97**	31.37**	24.82**	4189.27**	4189.36**
	SED	4.06	3.38	2.71	0.76	0.76
	CD (0.05)	8.84	7.38	5.91	1.66	1.66
	CD (0.01)	12.39	10.34	8.28	2.32	2.32

** - Significant at p = 0.01 * - Significant at p = 0.05 SED = Standard Error Deviation CD = Critical Difference

Table 13

Ovicidal activity of petroleum ether, chloroform and ethanol extracts of *T. peruviana* flower against *C. quinquefasciatus*

Solvents used	Concentration in ppm	Egg hatchability %			Total egg hatchability	Total Egg mortality
		48 h	72 h	96 h		
Petroleum Ether	Control	50.00 [a]	28.33 [a]	21.67 [a]	100 [a]	-
	100	16.67 [b]	18.33 [a]	3.33 [b]	38.33 [b]	61.67 [c]
	150	15.00 [b]	10.00 [ab]	1.67 [b]	26.67 [b]	73.33 [c]
	200	6.67 [bc]	3.33 [bc]	1.67 [b]	11.67 [c]	88.33 [b]
	250	3.33 [cd]	1.67 [c]	-	5.00 [d]	95.00 [a]
	300	-	-	-	-	100 [a]
	F	16.99**	12.07**	13.65**	87.25**	87.25**
	SED	5.16	4.79	4.31	4.85	4.85
	CD (0.05)	11.25	10.45	9.39	10.58	10.58
	CD (0.01)	15.77	14.66	13.17	14.83	14.83
Chloroform	Control	40.00 [a]	31.67 [a]	28.33 [a]	100 [a]	-
	100	20.00 [b]	13.33 [b]	6.67 [b]	43.33 [b]	56.67 [d]
	150	18.33 [b]	13.33 [b]	8.33 [b]	40.00 [bc]	60.00 [cd]
	200	15.00 [bc]	10.00 [bc]	6.67 [b]	31.67 [cd]	68.33 [bc]
	250	11.67 [cd]	10.00 [bc]	10.00 [b]	30.00 [de]	70.00 [ab]
	300	8.33 [d]	6.67 [c]	6.67 [b]	21.67 [e]	78.33 [a]
	F	25.72**	16.36**	5.54**	157.41**	157.42**
	SED	2.18	2.36	4.28	2.58	2.58
	CD (0.05)	4.75	5.15	9.32	5.61	5.61
	CD (0.01)	6.66	7.22	13.07	7.87	7.87
Ethanol	Control	53.33 [a]	26.67 [a]	20.00 [a]	100 [a]	-
	100	16.67 [b]	16.67 [b]	16.67 [b]	50.00 [b]	50.00 [c]
	150	16.67 [b]	16.67 [b]	15.00 [b]	48.33 [b]	51.67 [c]
	200	13.33 [bc]	10.00 [c]	11.67 [bc]	35.00 [c]	65.00 [b]
	250	8.33 [c]	8.33 [c]	8.33 [c]	25.00 [d]	75.00 [a]
	300	8.33 [c]	8.33 [c]	8.33 [c]	25.00 [d]	75.00 [a]
	F	22.95**	19.75**	9.29**	328.88**	328.93**
	SED	2.68	1.92	0.07	1.75	1.75
	CD (0.05)	5.83	4.19	0.16	3.82	3.82
	CD (0.01)	8.18	5.87	0.23	5.36	5.36

** - Significant at p = 0.01 * - Significant at p = 0.05 SED = Standard Error Deviation CD = Critical Difference

Table 14

Ovicidal activity of petroleum ether, chloroform and ethanol extracts of *T. erecta* flower against *C. quinquefasciatus*

Solvents used	Concentration in ppm	Egg hatchability %			Total egg hatchability	Total Egg mortality
		48 h	72 h	96 h		
Petroleum Ether	Control	50.00 [a]	28.33 [a]	21.67 [a]	100 [a]	-
	100	16.67 [b]	18.33 [a]	13.33 [b]	48.33 [b]	51.67 [d]
	150	15.00 [b]	13.33 [a]	8.33 [bc]	36.67 [c]	63.33 [c]
	200	13.33 [b]	13.33 [a]	10.00 [b]	36.67 [c]	63.33 [c]
	250	10.00 [bc]	10.00 [a]	8.33 [bc]	28.33 [d]	71.67 [b]
	300	5.00 [c]	8.33 [a]	5.00 [c]	18.33 [e]	81.67 [a]
	F	21.31**	-	9.93**	287.87**	287.87**
	SED	3.86	3.87	2.09	1.93	1.93
	CD (0.05)	8.42	8.42	4.57	4.20	4.20
	CD (0.01)	11.79	11.81	6.41	5.89	5.89
Chloroform	Control	40.00 [a]	31.67 [a]	28.33 [a]	100 [a]	-
	100	6.67 [b]	5.00 [b]	-	11.67 [b]	88.33 [b]
	150	-	-	-	-	100[a]
	200	-	-	-	-	100[a]
	250	-	-	-	-	100[a]
	300	-	-	-	-	100[a]
	F	180.76**	31.77**	550.19**	485.43**	485.43**
	SED	1.82	3.33	0.71	2.30	2.30
	CD (0.05)	3.97	7.26	1.54	5.01	5.01
	CD (0.01)	5.57	10.17	2.16	7.03	7.03
Ethanol	Control	53.33 [a]	26.67 [a]	20.00 [a]	100 [a]	-
	100	13.33 [b]	11.67 [b]	11.67 [ab]	36.67 [b]	63.33 [c]
	150	8.33 [bc]	10.00 [b]	6.67 [bc]	25.00 [c]	75.00 [b]
	200	11.67 [b]	6.67 [b]	10.00 [abc]	28.33 [c]	71.67 [b]
	250	3.33 [c]	5.00 [b]	3.33 [c]	11.67 [d]	88.33 [a]
	300	3.33 [c]	1.67 [c]	3.33 [c]	8.33 [d]	91.67 [a]
	F	19.95**	19.95**	5.25**	291.40**	291.41**
	SED	4.13	3.01	4.47	2.21	2.21
	CD (0.05)	8.99	6.57	9.74	4.82	4.82
	CD (0.01)	12.61	9.22	13.66	6.76	6.76

** - Significant at p = 0.01 * - Significant at p = 0.05 SED = Standard Error Deviation CD = Critical Difference

Table 15

Ovicidal activity of petroleum ether, chloroform and ethanol extracts of *L. camara* flower against *C. quinquefasciatus*

Solvents used	Concentration in ppm	Egg hatchability %			Total egg hatchability	Total Egg mortality
		48 h	72 h	96 h		
Petroleum Ether	Control	50.00 [a]	28.33 [a]	21.67 [a]	100 [a]	-
	100	18.33 [b]	16.67 [b]	15.00 [b]	50.00 [b]	50.00 [c]
	150	15.00 [b]	11.67 [b]	13.33 [b]	40.00 [c]	60.00 [b]
	200	15.00 [b]	11.67 [b]	8.33 [b]	35.00 [cd]	65.00 [ab]
	250	11.67 [b]	10.00 [b]	8.33 [b]	30.00 [d]	70.00 [a]
	300	11.67 [b]	10.00 [b]	10.00 [b]	31.67 [cd]	68.33 [ab]
	F	17.39**	6.61**	5.94**	150.99**	150.99**
	SED	3.11	2.71	2.96	2.50	2.50
	CD (0.05)	6.78	5.90	6.44	5.45	5.45
	CD (0.01)	9.51	8.28	9.03	7.65	7.65
Chloroform	Control	40.00 [a]	31.67 [a]	28.33 [a]	100 [a]	-
	100	20.00 [b]	18.33 [b]	20.00 [ab]	58.33 [b]	41.67 [d]
	150	16.67 [bc]	13.33 [c]	15.00 [b]	45.00 [c]	55.00 [c]
	200	11.67 [cd]	10.00 [c]	13.33 [bc]	35.00 [d]	65.00 [b]
	250	11.67 [cd]	10.00 [c]	8.33 [c]	30.00 [de]	70.00 [ab]
	300	10.00 [d]	10.00 [c]	8.33 [c]	28.33 [e]	71.67 [a]
	F	35.69**	26.28**	9.67**	351.38**	351.40**
	SED	1.92	1.72	2.57	1.65	1.65
	CD (0.05)	4.19	3.76	5.61	3.59	3.59
	CD (0.01)	5.87	5.27	7.86	5.04	5.04
Ethanol	Control	53.33 [a]	26.67 [a]	20.00 [a]	100 [a]	-
	100	21.67 [b]	20.00 [b]	13.33 [d]	55.00 [b]	45.00 [c]
	150	20.00 [bc]	16.67 [bc]	10.00 [b]	46.67 [c]	53.33 [b]
	200	15.00 [cd]	15.00 [cd]	10.00 [b]	40.00 [cd]	60.00 [ab]
	250	13.33 [d]	11.67 [de]	8.33 [b]	33.33 [d]	66.67 [a]
	300	15.00 [cd]	8.33 [e]	10.00 [b]	33.33 [d]	66.67 [a]
	F	37.15**	27.30**	10.63**	227.81**	227.81**
	SED	1.70	1.64	2.13	1.95	1.95
	CD (0.05)	3.71	3.58	4.64	4.26	4.26
	CD (0.01)	5.19	5.02	6.51	5.97	5.97

** - Significant at p = 0.01 * - Significant at p = 0.05 SED = Standard Error Deviation CD = Critical Difference

Table 16

Ovicidal activity of petroleum ether, chloroform and ethanol extracts of *B. acuminata* flower against *C. quinquefasciatus*

Solvents used	Concentration in ppm	Egg hatchability %			Total egg hatchability	Total Egg mortality
		48 h	72 h	96 h		
Petroleum Ether	Control	50.00 [a]	28.33 [a]	21.67 [a]	100 [a]	-
	100	16.67 [b]	20.00 [b]	18.33 [b]	55.00 [b]	45.00 [c]
	150	16.67 [b]	11.67 [c]	15.00 [bc]	43.33 [c]	56.67 [b]
	200	13.33 [b]	10.00 [c]	13.33 [bc]	36.67 [cd]	63.33 [ab]
	250	11.67 [b]	10.00 [c]	13.33 [bc]	35.00 [d]	65.00 [a]
	300	11.67 [b]	8.33 [c]	11.67 [c]	31.67 [d]	68.33 [a]
	F	13.73**	16.17**	10.70**	227.02**	227.02**
	SED	2.53	2.52	2.11	1.99	1.99
	CD (0.05)	5.51	5.49	4.61	4.33	4.33
	CD (0.01)	7.72	7.69	6.46	6.07	6.07
Chloroform	Control	40.00 [a]	31.67 [a]	28.33 [a]	100 [a]	-
	100	25.00 [b]	16.67 [bc]	15.00 [b]	56.67 [b]	43.33 [b]
	150	20.00 [bc]	18.33 [b]	15.00 [b]	53.33 [b]	46.67 [b]
	200	15.00 [cd]	15.00 [bc]	11.67 [bc]	41.67 [c]	58.33 [a]
	250	15.00 [cd]	11.67 [c]	10.00 [c]	36.67 [c]	63.33 [a]
	300	14.67 [c]	11.67 [d]	10.00 [c]	36.34 [c]	63.66 [a]
	F	26.54**	11.12**	18.18**	154.10**	154.10**
	SED	2.02	2.09	1.71	2.32	2.32
	CD (0.05)	4.40	4.56	3.72	5.05	5.05
	CD (0.01)	6.17	6.39	5.22	7.08	7.08
Ethanol	Control	53.33 [a]	26.67 [a]	20.00 [a]	100 [a]	-
	100	21.67 [b]	18.33 [b]	15.00 [b]	55.00 [b]	45.00 [c]
	150	20.00 [b]	15.00 [b]	13.33 [bc]	35.00 [c]	51.67 [bc]
	200	13.33 [c]	15.00 [b]	13.33 [bc]	41.67 [bc]	58.33 [b]
	250	13.67 [c]	10.00 [c]	10.00 [c]	33.33 [c]	66.33 [a]
	300	13.33 [c]	10.00 [c]	10.00 [c]	33.33 [c]	66.67 [a]
	F	43.96**	25.37**	24.39**	271.31**	271.31**
	SED	1.69	1.54	1.34	1.79	1.79
	CD (0.05)	3.68	3.35	2.91	3.90	3.90
	CD (0.01)	5.16	4.70	4.08	5.47	5.47

** - Significant at p = 0.01 * - Significant at p = 0.05 SED = Standard Error Deviation CD = Critical Difference

Table 17

Lethal concentration of flower extracts of *C. gigantea* against eggs of *C. quinquefasciatus*

Solvents Used	Log LC_{50}	Log LC_{70}	Log LC_{90}	LC50 (ppm)	LC_{70} (ppm)	LC_{90} (ppm)	Regression Equation	95% Confidence Limits				χ^2	SE
								UCL (ppm)		LCL (ppm)			
								LC_{50}	LC_{90}	LC_{50}	LC_{90}		
Petroleum ether	2.12	2.38	2.75	133.33	242.44	574.76	Y=0.70+2.02X	157.61	864.00	112.79	382.35	1.11	0.67
Chloroform	2.07	2.40	2.88	117.52	253.37	2768.00	Y= 1.74+1.57X	151.10	1449.83	91.41	406.82	9.91	0.67
Ethanol	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 18

Lethal concentration of flower extracts of *T. peruviana* against eggs of *C. quinquefasciatus*

Solvents Used	Log LC_{50}	Log LC_{70}	Log LC_{90}	LC50 (ppm)	LC_{70} (ppm)	LC_{90} (ppm)	Regression Equation	95% Confidence Limits				χ^2	SE
								UCL (ppm)		LCL (ppm)			
								LC_{50}	LC_{90}	LC_{50}	LC_{90}		
Petroleum ether	1.92	2.09	2.33	84.95	124.53	216.30	Y=−1.09+3.15X	104.64	254.21	68.98	184.04	2.64	1.00
Chloroform	1.91	2.34	2.90	82.67	221.43	980.13	Y=2.77+1.17X	145.73	1231.90	46.42	361.82	1.19	0.67
Ethanol	2.05	2.37	2.84	112.86	237.03	691.85	Y=1.65+1.62X	192.14	2655.06	87.40	388.54	2.64	0.67

Table 19

Lethal concentration of flower extracts of *T. erecta* against eggs of *C. quinquefasciatus*

Solvents Used	Log LC_{50}	Log LC_{70}	Log LC_{90}	LC50 (ppm)	LC_{70} (ppm)	LC_{90} (ppm)	Regression Equation	95% Confidence Limits				χ^2	SE
								UCL (ppm)		LCL (ppm)			
								LC_{50}	LC_{90}	LC_{50}	LC_{90}		
Petroleum ether	1.99	2.32	2.80	97.97	211.87	645.16	Y=1.88+1.56X	134.39	1150.77	71.41	361.70	2.93	0.68
Chloroform	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethanol	1.86	2.12	2.49	73.85	132.87	310.16	Y=1.15+2.05X	103.58	402.24	52.66	239.15	7.66	0.75

LCL = Lower Confidence Limit UCL= Upper Confidence Limit χ^2 = Chi Square SE= Standard Error

Table 20

Lethal concentration of flower extracts of *L. camara* against eggs of *C. quinquefasciatus*

Solvents Used	Log LC_{50}	Log LC_{70}	Log LC_{90}	LC50 (ppm)	LC_{70} (ppm)	LC_{90} (ppm)	Regression Equation	95% Confidence Limits				χ^2	SE
								UCL (ppm)		LCL (ppm)			
								LC_{50}	LC_{90}	LC_{50}	LC_{90}		
Petroleum ether	2.10	2.41	2.85	127.18	257.83	715.14	Y=1.40+1.70X	156.77	1248.89	103.18	409.51	0.37	0.67
Chloroform	1.96	2.45	3.16	92.53	285.49	1452.04	Y=2.89+1.07X	151.06	5372.31	56.67	392.45	0.77	0.66
Ethanol	2.10	2.51	3.10	126.30	324.60	1268.05	Y=2.31+1.27X	167.29	3516.09	95.35	457.31	0.34	0.66

Table 21

Lethal concentration of flower extracts of *B. acuminata* against eggs of *C. quinquefasciatus*

Solvents Used	Log LC_{50}	Log LC_{70}	Log LC_{90}	LC50 (ppm)	LC_{70} (ppm)	LC_{90} (ppm)	Regression Equation	95% Confidence Limits				χ^2	SE
								UCL (ppm)		LCL (ppm)			
								LC_{50}	LC_{90}	LC_{50}	LC_{90}		
Petroleum ether	2.06	2.49	3.11	117.07	313.81	1302.64	Y=2.46+1.22	161.78	3840.17	84.72	441.88	0.45	0.66
Chloroform	2.16	2.54	3.10	146.25	353.88	1267.13	Y=2.04+1.36	182.21	3282.11	117.39	489.20	0.71	0.66
Ethanol	2.11	2.51	3.08	130.20	324.43	1212.04	Y=2.20+1.32	2.22	3179.70	100.23	462.00	0.48	0.66

LCL = Lower Confidence Limit UCL= Upper Confidence Limit χ^2 = Chi Square SE= Standard Error

Figure 2

Effect of petroleum ether extract of selected leaves on egg hatchability of *C. quinquefasciatus*

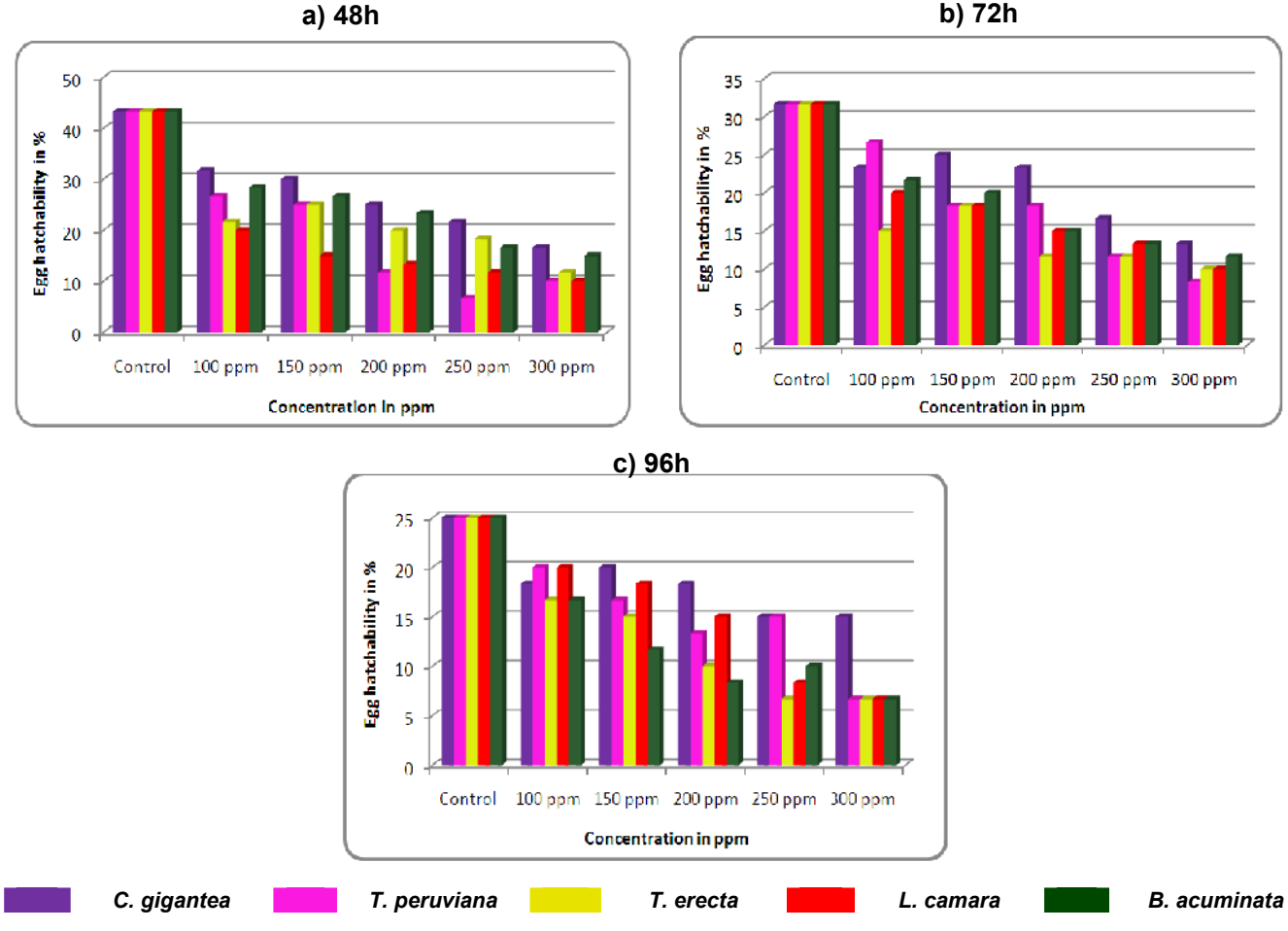


Figure 3

Effect of chloroform extract of selected leaves on egg hatchability of *C. quinquefasciatus*

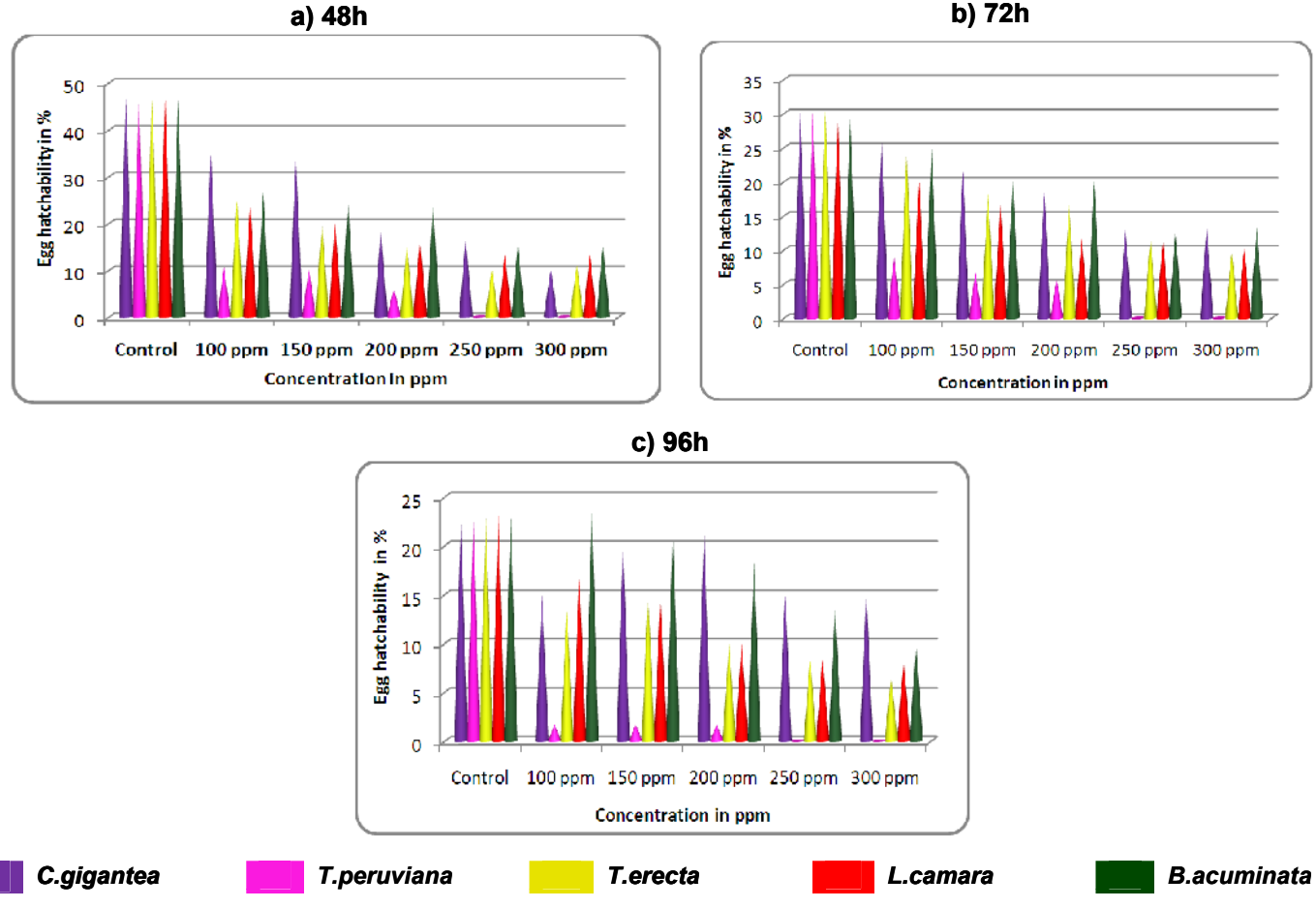


Figure 4

Effect of ethanol extract of selected leaves on egg hatchability of *C. quinquefasciatus*

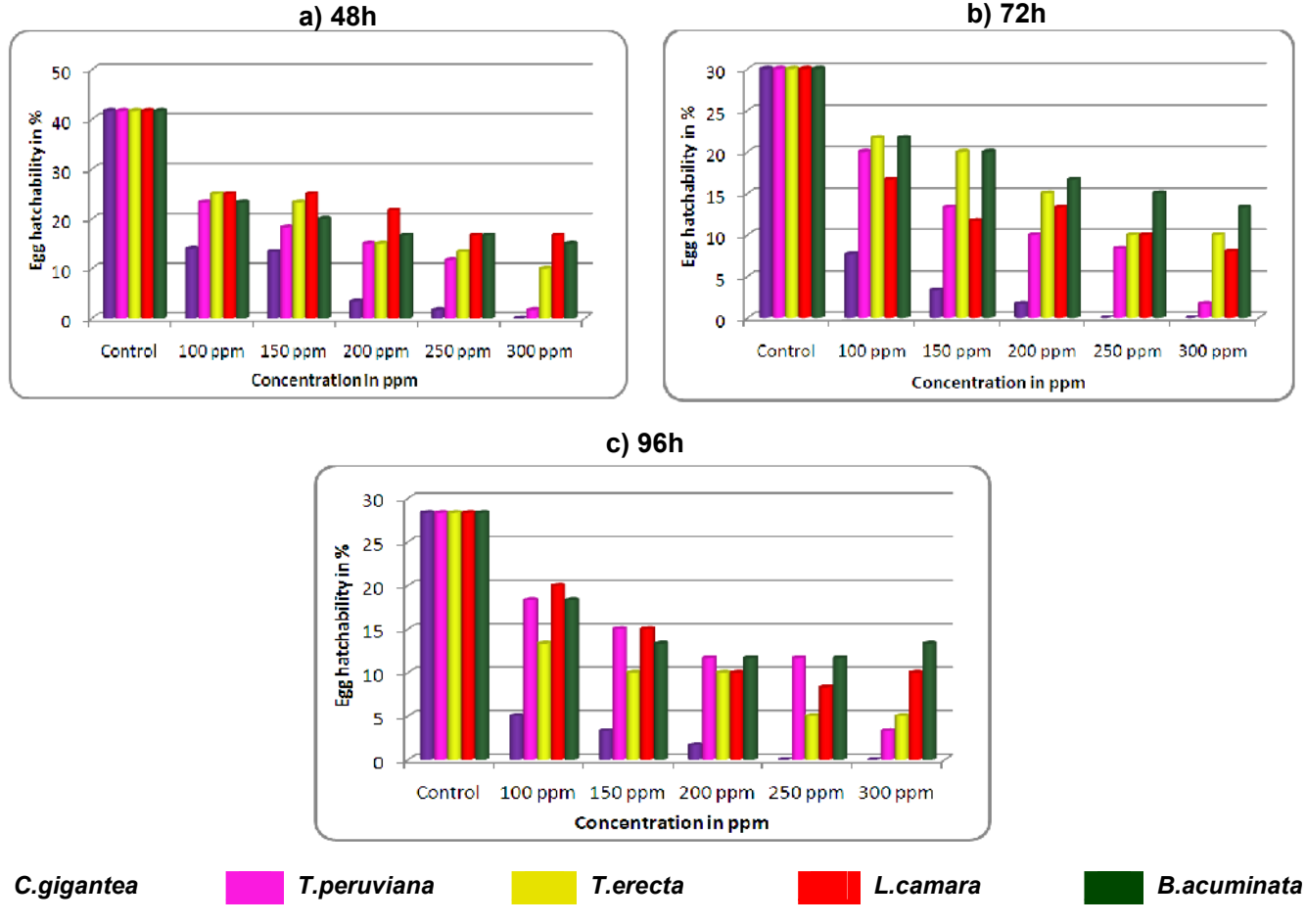
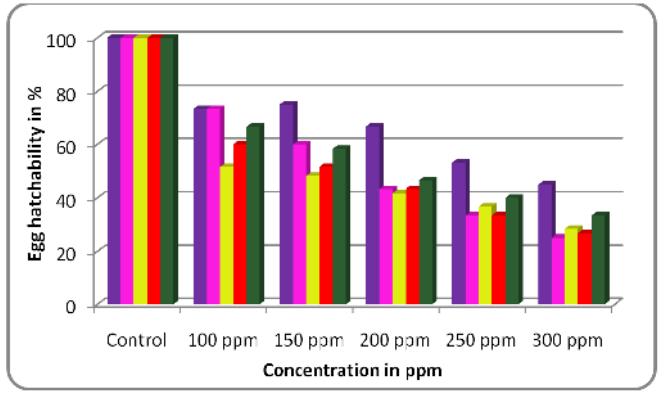


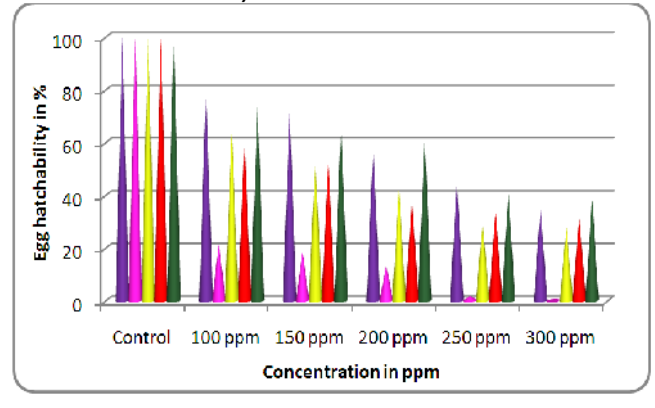
Figure 5

Effect of petroleum ether, chloroform and ethanol extract of selected leaves on total egg hatchability of *C. quinquefasciatus*

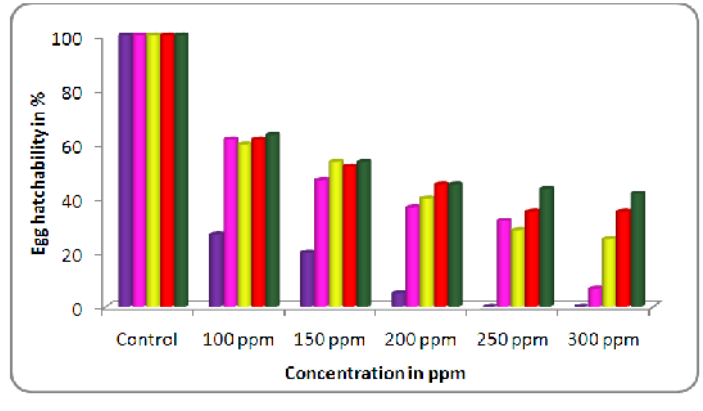
a) Petroleum ether extract



b) Chloroform extract



c) Ethanol extract

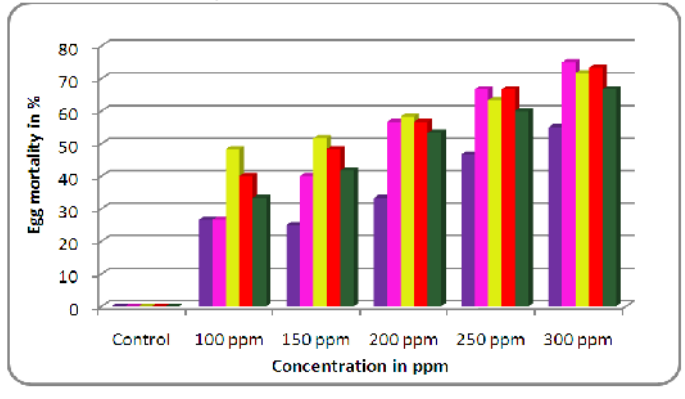


C.gigantea ***T.peruviana*** ***T.erecta*** ***L.camara*** ***B.acuminata***

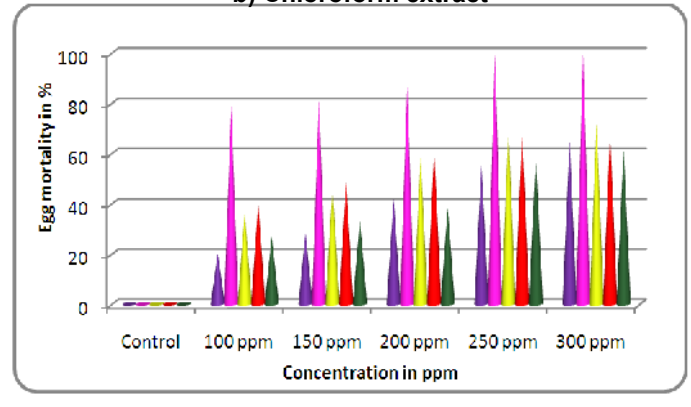
Figure 6

Effect of petroleum ether, chloroform and ethanol extract of selected leaves on total egg mortality of *C. quinquefasciatus*

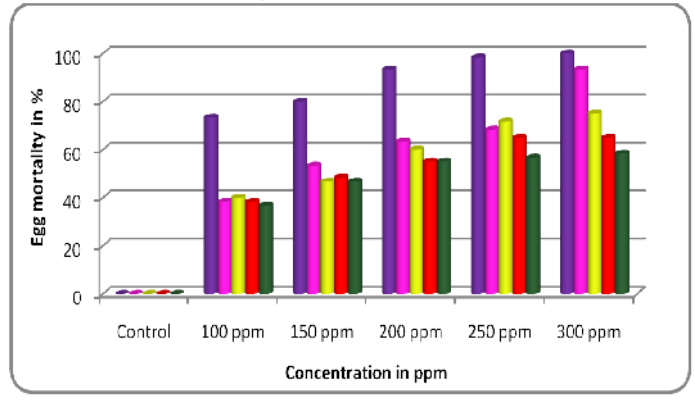
a) Petroleum ether extract



b) Chloroform extract



c) Ethanol extract



C.gigantea ***T.peruviana*** ***T.erecta*** ***L.camara*** ***B.acuminata***

Figure 7

Effect of petroleum ether extract of selected flowers on egg hatchability of *C. quinquefasciatus*

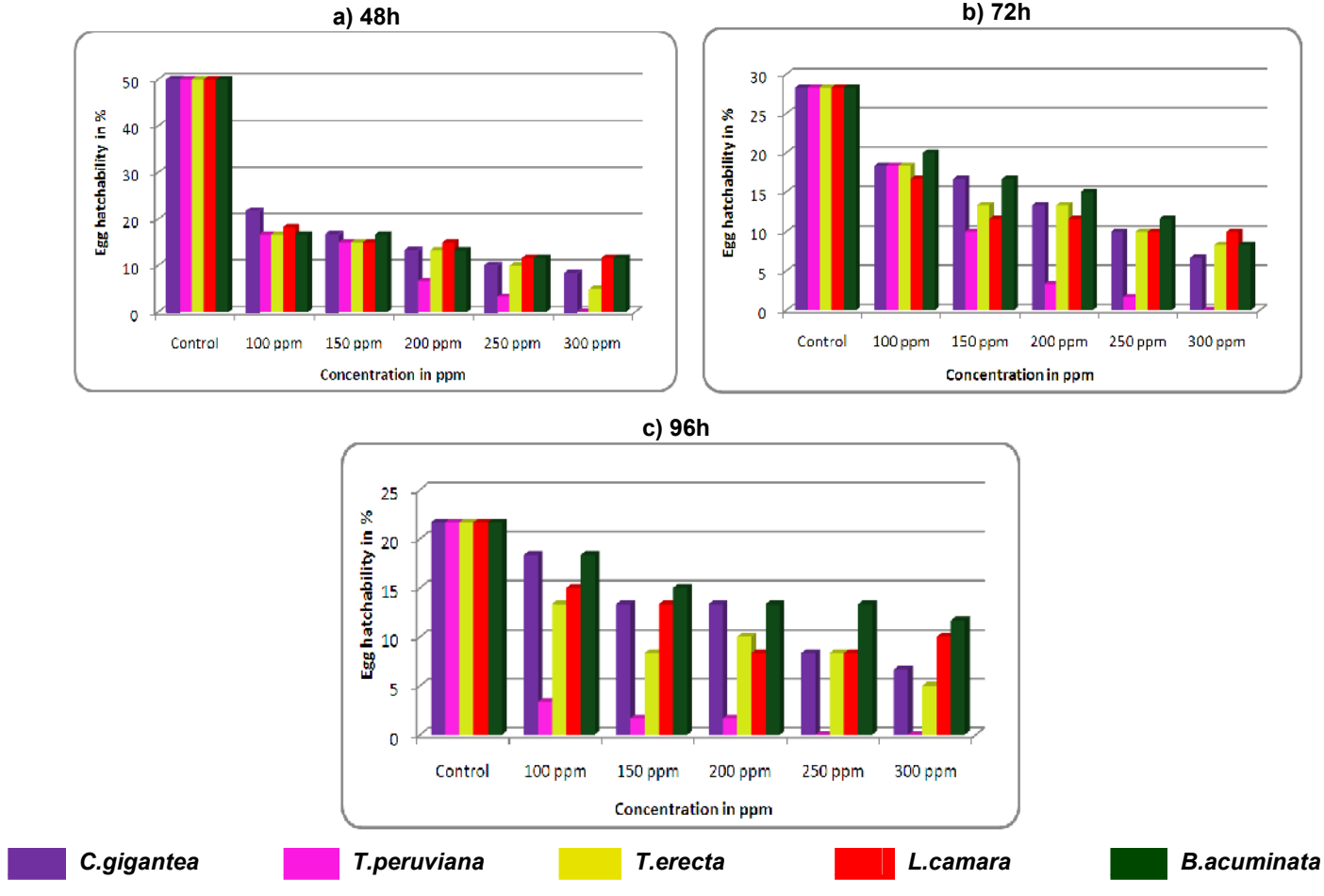


Figure 8

Effect of chloroform extract of selected flowers on egg hatchability of *C. quinquefasciatus*

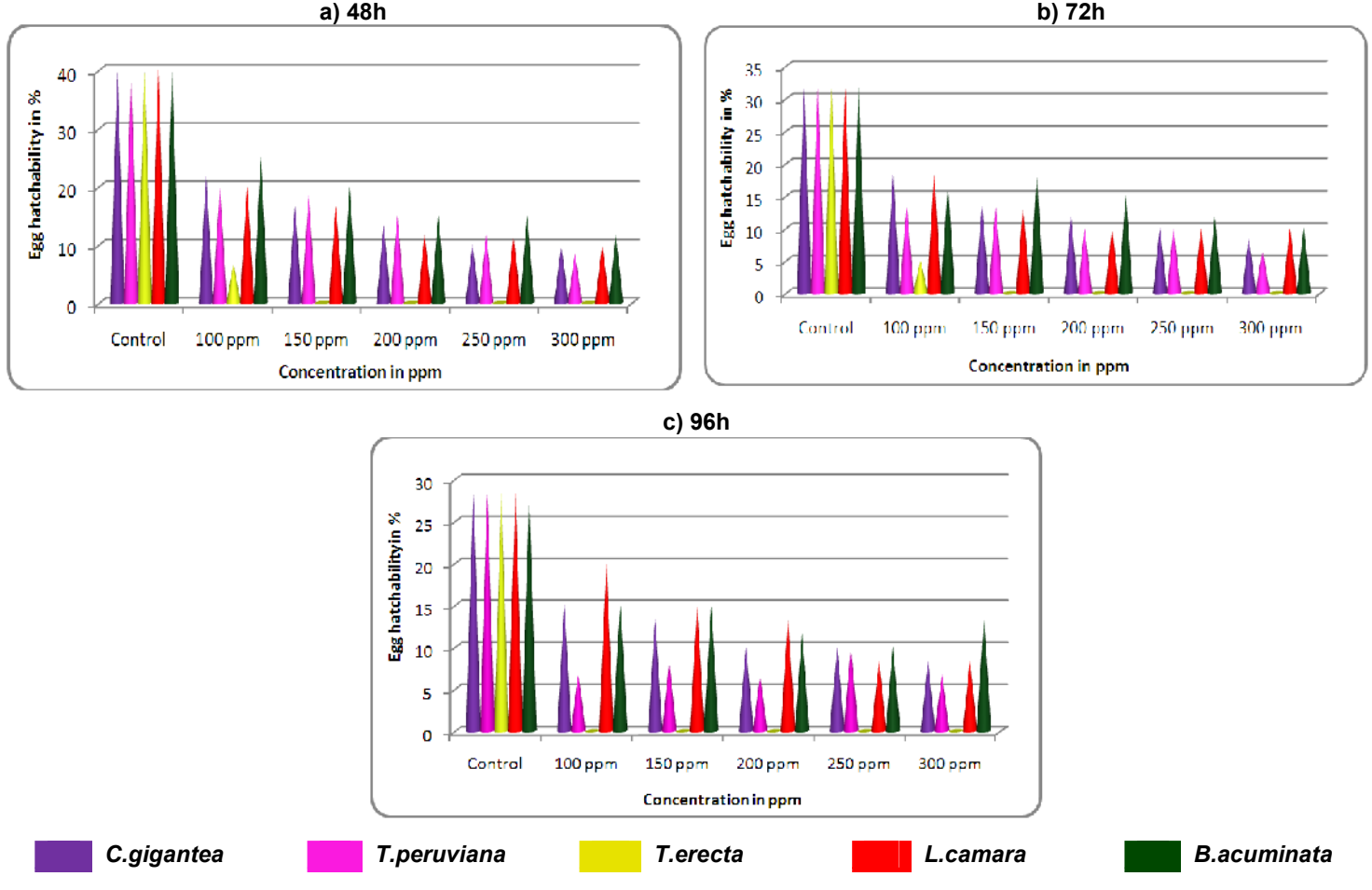


Figure 9

Effect of ethanol extract of selected flowers on egg hatchability of *C. quinquefasciatus*

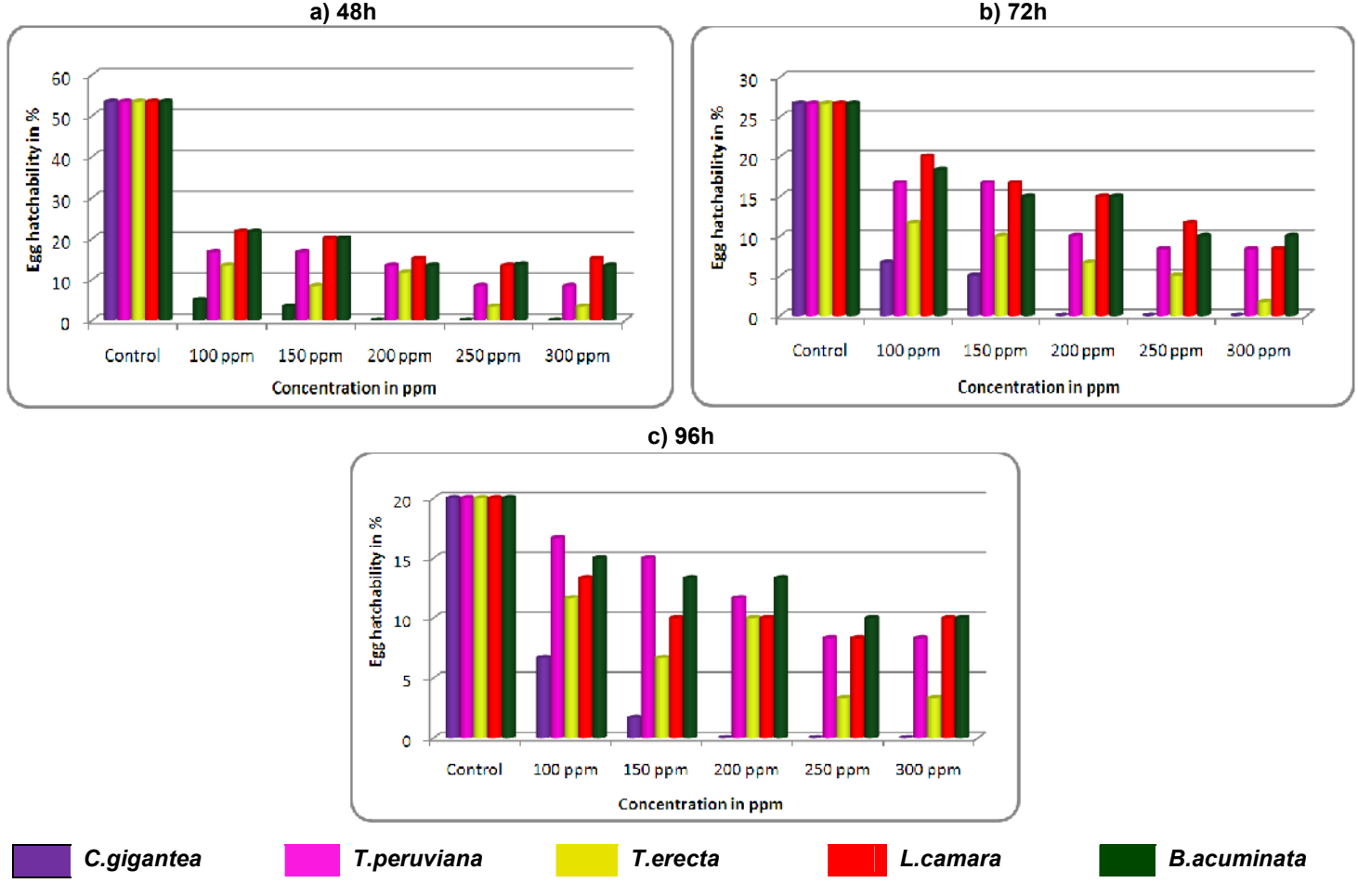
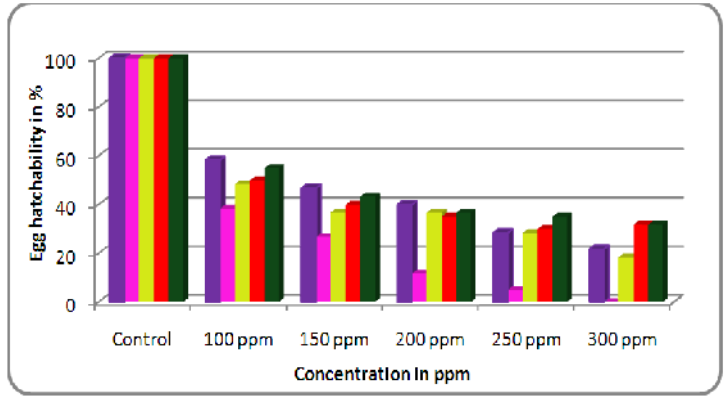


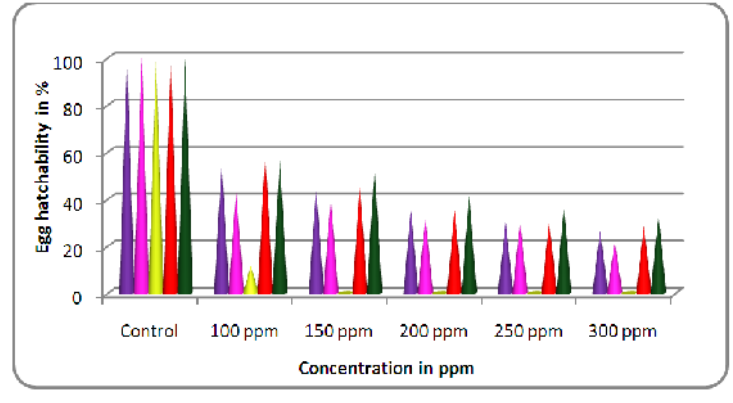
Figure 10

Effect of petroleum ether, chloroform and ethanol extract of selected flowers on total egg hatchability of *C. quinquefasciatus*

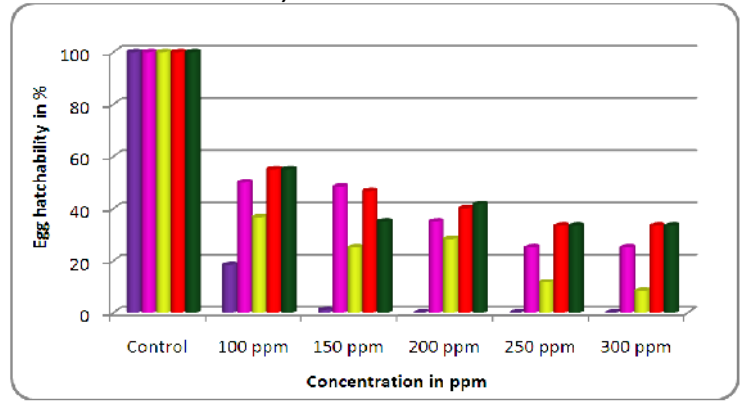
a) Petroleum ether extract



b) Chloroform extract



c) Ethanol extract

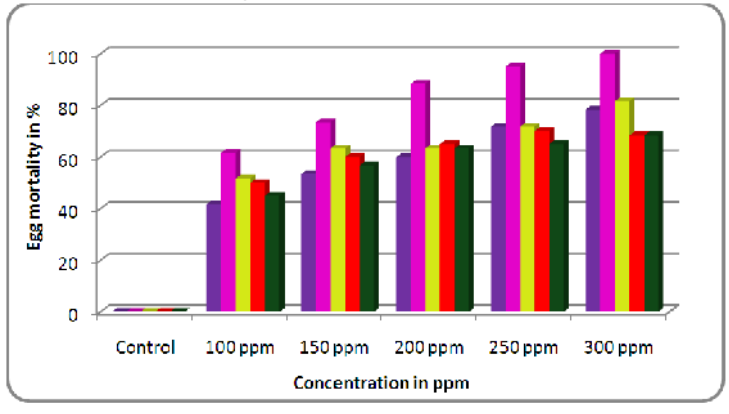


C.gigantea ***T.peruviana*** ***T.erecta*** ***L.camara*** ***B.acuminata***

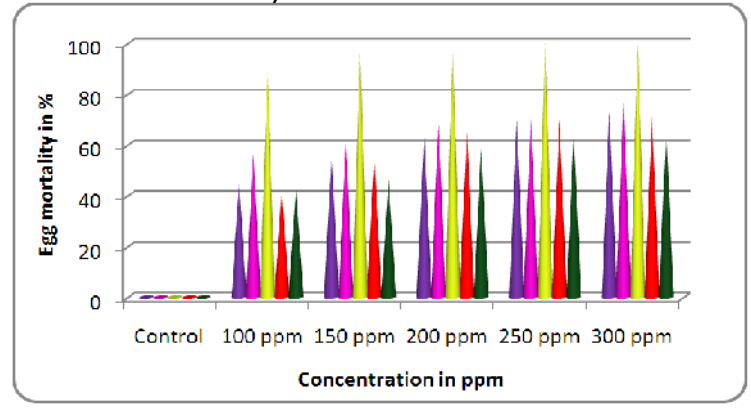
Figure 11

Effect of petroleum ether, chloroform and ethanol extract of selected flowers on total egg mortality of *C. quinquefasciatus*

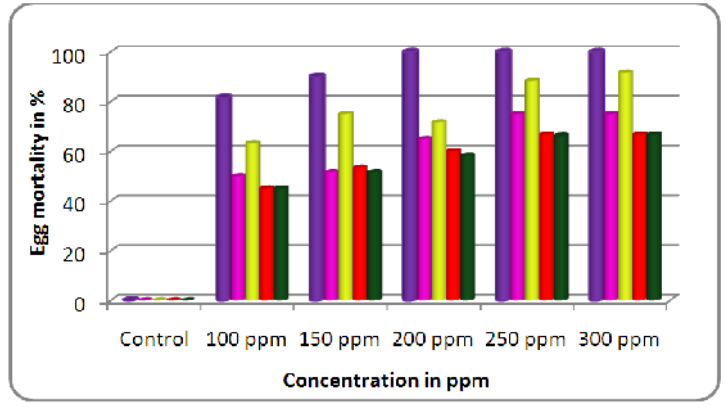
a) Petroleum ether extract



b) Chloroform extract



c) Ethanol extract



C.gigantea ***T.peruviana*** ***T.erecta*** ***L.camara*** ***B.acuminata***

Egg mortality

Among the three treatments petroleum ether extract of *T. peruviana* flower was found to be more effective producing 100% mortality at 300 ppm (Fig. 11a). Chloroform and ethanol extract induced 78.33% and 75% egg mortality respectively at the concentration of 300 ppm (Fig. 11b & c). The LC_{50} value of petroleum ether extract was 84.95 ppm with regression equation of Y=1.09+3.15X, UCL LC_{50} was 104.64 ppm and LCL LC_{50} value was 68.98 ppm (Table 15).

Flower extracts of *T. erecta*

Among the three extracts, chloroform extract possessed very high ovicidal activity imparting zero percentage egg hatchability in 150, 200, 250 and 300 ppm concentrations (Table 14 & Fig. 8) throughout the experiment period. Chloroform extract was followed by ethanol and petroleum ether extracts which displayed a total egg hatchability of 8.33% and 18.33% in 300 ppm concentration (Fig. 10c & a) respectively. In control treatments total egg hatchability was 100%.

Egg mortality

The chloroform extract showed the highest toxicity than the other two extracts producing a maximum of 100% egg mortality at the concentration ranging from 150 - 300 ppm (Fig. 11b). The effectiveness of chloroform extract was followed by ethanol extract. The total egg mortality was recorded as 91.67% in ethanol extract and 81.67% in petroleum ether extract in 300 ppm concentration (Fig. 11c & a). No mortality was observed in the control treatments. The percent egg mortality was found to be directly proportional to the concentration of extract. Statistical analysis of the experimental data was performed to find the values of LC_{50}, LC_{70} and LC_{90}, 95% confidence limit and regression equation (Table 19).

Flower extracts of *L. camara*

Maximum ovicidal activity was possessed in chloroform extract inducing total egg hatchability of 28.33% (Table 18 & Fig. 10b) at the higher concentration of 300 ppm. Petroleum ether and ethanol extracts exhibited 31.67 and 33.33 total egg hatchability percentage respectively (Fig. 10a & c). The control treatment showed 100% total egg hatchability.

Egg mortality

Among the three extracts, highest total egg mortality percentage of 71.67 was observed at the higher concentration of 300 ppm in the chloroform extract (Fig. 11b). The LC_{50} value of chloroform extract was displayed as 92.53 ppm with regression eqution of Y=2.89+1.07X. The 95% confidence limits, LC_{50} LC_{70} and LC_{90} values were also calculated. The UCL LC_{50} was 151.06 ppm and LCL LC_{50} value was 56.67 ppm (Table 20). Minimum egg mortality percentage (66.67%) was observed in ethanol extract of *L. camara* (Fig. 11c).

Flower extracts of *B. acuminata*

Petroleum ether extract showed maximum ovicidal activity by inducing total egg hatchability percentage of 31.67 at the concentration of 300 ppm (Table 16 & Fig. 10a). Ethanol extract of *B. acuminata* at the higher concentration of 300 ppm produce a total egg hatchability percentage of 33.33% (Fig. 10c). Minimum egg hatchability was recorded in chloroform extract inducing a total egg hatchability percentage of 36.34 (Fig. 10b). Total egg hatchability was found to be 100% in the case of control treatment.

Egg mortality

The highest egg mortality was found to be 68.33% and it was exhibited in petroleum ether extract at 300 ppm (Fig. 11a) and the LC_{50} value was 117.07 ppm with regression eqution of Y=2.46+1.22X. UCL LC_{50} value and LCL LC_{50} value were recorded as 161.78 ppm and 84.72 ppm respectively (Table 21). Minimum egg mortality was exhibited in chloroform extract imparting only 63.66% total egg mortality percentage (Fig. 11b).

The fresh eggs *of C. quinquefasciatus* treated with different concentrations of leaf and flower extracts caused ovicidal activity resulting in failure of egg hatching. Hundred percentage ovicidal activity was observed in the concentrations ranging from 150 - 300 ppm throughout the experimental period and was exhibited in chloroform extract of *T. erecta* flower. The ovicidal effects were generally dose dependent. The results of ovicidal activity in relation to dose of plant extracts were presented in the tables 2 to 21. Ethanol extract of *C. gigantea* flower induced zero

percentage egg hatchability in the concentrations ranging from 200-300 ppm during the study period.

Moderate ovicidal activity was observed in chloroform extract of *T. peruviana* leaf which recorded zero percentage egg hatchability at the concentration ranging from 250-300 ppm throughout the experimental period. The ovicidal activity of chloroform extract of *T. peruviana* leaf was followed by ethanol extract of *C. gigantea* leaf which interrupted the hatchability of eggs at 72 h and 96 h of the study period. The egg hatchability was found to be zero percentage throughout the study in 300 ppm concentration. The results were found to be statistically significant at $P< 0.01$ level. From the results, it can be concluded that the effect of the plant extracts were reported to be dose dependent as evident by an increase in percent mortality with increasing concentrations.

Among the flower extracts, zero percentage egg hatchability was noted in chloroform extract of *T. erecta* which possessed very high ovicidal activity at 150, 200, 250 and 300 ppm concentration followed by ethanolic extract of *C. gigantea* at concentrations ranging from 200-300 ppm.

Discussion

Extracts from plants may be an alternative source of mosquito control agent because they constitute a rich source of bioactive compounds that are bio degradable into nontoxic products and potentially suitable for controlling mosquitoes. Ovicidal compounds are able to interrupt embryo development, impair the survival of larvae inside the egg. In view of recently increased interest in developing plant based insecticides as an alternate to chemical insecticide, this study was undertaken to assess the ovicidal potential of the selected five plant extracts against the medically important mosquito vector *C. quinquefasciatus*.

The results of present study are comparable with earlier reports. In the present study, all extracts showed good ovicidal activity of which maximum ovicidal activity was exhibited by chloroform extract of *Tagetes erecta* flower which provided an excellent potential for controlling the vector mosquito, *C. quinquefasciatus*.

In the study, it was observed that percentage hatchability was inversely proportional to the concentration of extract and directly proportional to the eggs. Similar observations were documented by Govindarajan *et al* (2012) in which among the crude hexane, benzene, chloroform, ethyl acetate and methanol solvent extracts of *Delonix elata* tested against *Ae. aegypti* and *An. stephensi* mosquito vectors, a complete mortality was observed in 300 ppm for leaf methanol extract and 500 ppm for seed methanol extract of *D. elata* against *An. stephensi* and *Ae. aegypti* respectively.

In the present study, it was observed that the treatment of *C. quinquefasciatus* eggs with various plant extracts resulted in the decrease of percentage hatchability. Similar results have been obtained by Valarmathy *et al* (2011) against *C. quinquefasciatus* eggs as it caused embryonic death resulting in the failure to hatch when treated with different concentrations of the plant oil formulation.

Pannerselvam and Murugan (2013) studied the ovicidal potential of crude hexane, ethyl acetate, benzene, aqueous and methanol solvents extracts from medicinal plants *Andrographis paniculata, Cassia occidentalis* and *Euphorbia hirta* against the vector, *An. stephensi*. The percentage hatchability was inversely proportional to the concentration of the extract. Mortality of 100% with methanol extract of *A. paniculata* exerted in 150 ppm and aqueous, methanol extract of *C. occidentalis* and *E. hirta* were exerted in 300 ppm. In the present study, similar observation was recorded in which egg hatchability was inhibited in 150 ppm i.e., 100% egg mortality was observed in 150 ppm in the chloroform extract of *T. erecta* flower. As observed in the present study, similar results were reported by Elango *et al* (2011) in which the percent hatchability was inversely proportional to the concentration of extract and directly proportional to the eggs. A complete mortality with ethyl acetate and methanol extracts of *Andrographis paniculata, Eclipta prostrata* and *Tagetes erecta* were exerted at 998.85 mg/l. Similarly, the flower extracts of *T. erecta* showed high larvicidal activity against *Ae. aegypti*, against the larvae of *Meloidogyne incognita* and *Sitophilus oryzae* respectively (Natarajan *et al*., 2006; Pavela, 2002; Elango *et al*., 2009 and Broussalis *et al*., 1999).

The phytochemicals derived from plant sources possess a complex of chemicals with unique biological activity. It can act as larvcides, insect growth regulators, repellents and ovipositional attractants having deterrent activities as observed by different researches (Amer and Melhorn, 2006; Govindarajan *et al*., 2011b) The leaf extract of *Acalypha indica* with different solvents viz., benzene, chloroform, ethyl acetate and methanol were tested for larvicidal, ovicidal and ovipositional attractancy against *An. stephensi* (Govindarajan *et al*., 2008a). In addition, *Pelargonium citrosa* (Jeyabalan *et al*., 2003), *Cymbopogan citrates* (Pushpanathan *et al*., 2006) and *Mentha piperita* (Ansari *et al*., 2000) were shown to contain larvicidal and growth inhibitory activity against *An. stephensi*. Gokulakrishnan *et al* (2012) reported that the larvicidal and ovicidal efficacy of different solvent leaf extract of *Ariitolochia indica* against *An. stephensi*.

The bioactive compound *Azadirachtin* showed complete ovicidal activity against the eggs of *C. tarsalis* and *C. quinquefasciatus* exposed to 10 ppm concentration (Su and Mulla, 1998). The ovicidal activity of *Moschosma polystachyum* leaf extract against the egg rafts of *C. quinquefasciatus* showed 100% mortality at 0-3 h and 3-6 h with concentrations of 125, 150, 175 and 200 mg/l (Rajkumar and Jebanesan, 2004a). Similar observation was recorded by ethanol extract of *C. gigantea* leaf used in the present study in which egg hatchability was found to be totally inhibited in the higher concentrations of 300 ppm.

Prajapathi *et al* (2005) have revealed the oviposition deterrent, ovicidal and repellent activities of essential oils of *Cinnamomum zeylanicum, Zingiber officinale* and *Rosemarinus officinalis* against *An. stephensi, Ae. aegypti* and *C. quinquefasciatus.* Kovendan *et al* (2012) recorded the ovicidal activity of *Acalypha alnifolia* against *An. stephensi, Ae. aegypti* and *C. quinquefasciatus* in which the percentage hatchability was inversely proportional to the concentration of extract and directly proportional to the eggs. Mortality of 100% with the methanol extract of *A. alnifolia* was exerted at 125 and 300 ppm. These earlier reports were in agreement with the results obtained in the present study in which among the three soluble fractions tested chloroform extract of *T. peruviana* leaf was found to

be more effective than other two extracts inducing zero percentage egg hatchability throughout the experiment period at the concentration of 250 and 300 ppm.

Results of the present study reflected spectrum of activity of selected plants extracts against the eggs of medically important vector mosquito *C. quinquefasciatus.* Results pertaining to the experiment clearly revealed that the ovicidal activity was dose dependent. This was in accordance with the studies that were carried out by many researchers. Krishnappa *et al* (2013) observed that among two plant solvents tested, *Cissus quadrangularis* extracts were found exhibit most significant ovicidal activity i.e., 100% egg mortality (zero hatchability) was observed at 50 ppm and 350 ppm than *Combretum ovalifolium* and was found to be dose dependent. Krishnappa and Elumalai (2012) reported that the methanol extract of *Abutilon indicum* exerted 100% mortality (zero hatchability) at 120, 150 and 180 ppm for *Ae. aegypti*, *C. quinquefasciatus* and *An. stephensi,* respectively. Similarly, the methanol extract of *Diplocyclos palmatus* exerted 100% mortality (zero hatchability) at 200, 250 and 300 ppm for *Ae. aegypti*, *C. quinquefasciatus* and *An. stephensi* respectively.

Govindarajan *et al* (2008b) reported that methanolic extract of *Cassia fistula* leaf was tested for larvicidal and ovicidal activity against *C. quinquefasciatus* and *An. stephensi*. Similar observation was recorded in the present investigation in which ethanol extract of *C. gigantea* flower was found to be highly effective exhibiting zero percentage egg hatchability at concentrations ranging from 200 - 300 ppm throughout the experiment period inducing very high ovicidal activity. Govindarajan (2011a) studied the larvicidal and ovicidal activity of crude hexane, ethyl acetate, benzene, chloroform, and methanol extracts of the leaf of three plants, *Eclipta alba, Cardiospermum halicacabum* and *Andrographis paniculata*, against *An. stephensi*. Govindarajan (2011b) recorded the larvicidal and ovicidal efficacy of different extracts of *Cardiospermum halicacabum* against *C. quinquefasciatus* and *Ae. aegypti*. The crude extracts of acetone, benzene, ethyl acetate, hexane and methanol of *M. maderaspatana* exerted 100% egg mortality in 240, 200, 160, 160 and 120 ppm against *Ae. aegypti* (Baluselvakumar *et al.*, 2012).

In the present study, a remarkable decrease in the hatchability percentage of eggs laid by female *C. quinquefasciatus* were observed especially in the case of flower extracts. The hatchability of eggs decreased as the concentration of the extract increased. These results are in consistent with those obtained by many authors using different plant extracts against different mosquito species (El-Sheikh *et al*., 2012; Jeyabalan *et al.,* 2003; Nathan *et al*., 2006; Coria *et al.,* 2008; Pavela, 2009). The increase in mortality during the course of the exposure period could be due to several factors, which may be acting separately or jointly. For example the uptake of active moiety of the compound could be time dependent, leading to a progressive increase in the titer of the plant derived compounds tested and its effects on the egg, larvae or adults of mosquito.

Arivoli *et al* (1999) reported that the aqueous extract of *Leucas aspera* when tested against the *C. quinquefasciatus*, was found to be ovicidal against all three mosquito species and the ovicidal response was in the decreasing order, *Ae.aegypti* followed by *An. stephensi* and *C. quinquefasciatus*. Saxena *et al* (1993) also found a significant reduction of hatchability in *An. stephensi* treated with *Annona squamosa*. The larvicidal, ovicidal and repellent activities of *Pemphis acidula* Forst against filarial and dengue vector was reported by Samidurai *et al* (2009). The findings of the present investigation were comparable with the above ovicidal studies and revealed that the selected plant extracts possesses ovicidal activity against mosquitoes.

In the present investigation, ethanol extract of *T. erecta* flower recorded an egg mortality percentage of 91.67% in 300 ppm concentration. The percent of egg mortality was found to be directly proportional to the concentration. This is in accordance with the findings of Govindarajan (2011a) which exerted a mortality of 100% with methanol and ethyl acetate extract of *A. paniculata* and methanol extract of *E. alba* 200 ppm and methanol and benzene extract of *C. halicacabum* in 150 ppm. Here also the percent of egg mortality was found to be directly proportional to the concentration of extract. Remarkable ovicidal properties of *Ervatamia coronaria* and *Caesalpinia pulcherrima* plant extracts were studied by Govindarajan *et al* (2011a) against *C. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*.

Results pertaining to the present experiment clearly showed that chloroform extract of *T. erecta* flower exhibited significant ovicidal activity against the *C. quinquefasciatus* than any other extracts. This is comparable with the early reports in which Krishnappa *et al* (2012a) who investigated the potentiality of mosquitocidal activity of *Gliricidia sepium.*

In the present study, petroleum ether extract of *C.gigantea* and ethanol extract of *B. acuminata* displayed an ovicidal activity of 55% and 58.33% against the eggs of *C. quinquefasciatus* at 300 ppm concentration respectively. The ovicidal activity was comparable to early researches in alkaloids of *A. squamosa* (Saxena *et al*., 1993), ethyl acetate fractions (seeds) of *Calophyllum inophyllum* (Pushpalatha and Muthukrishnan, 1999), petroleum ether fractions of *Rhinacanthus nasutus* and ethyl acetate fractions of *Solanum suratense* (Muthukrishnan and Pushpalatha, 2001) which caused 32, 54, 40 and 55% ovicidal activity against *An. stephensi* Liston. Elumalai *et al* (2012) investigated the acetone and methanol extract of *Eranthemum roseum* leaves for its larvicidal, ovicidal and pupicidal activities against the important malarial vector, *Anopheles stephensi*.

Our results showed that plant extracts have significant ovicidal activity against *C. quinquefasciatus*. This result is also comparable to earlier reports of Vasudevan *et al* (1989) who noted that the ovicidal activity of the castor oil extracted from the castor seeds against mosquito *An. stephensi*, *C. fatigans* and *Ae. aegypti*. Prakash (1992) stated that ovicidal action of certain chitin synthesis inhibitors against mosquitoes, *C. quinquefasciatus*, *Ae. aegypti*, *An.stephensi*, *An. culicifacies*. Ovicidal effects of the seed extracts of *Atriplex canescens* was reported against *C. quinquefasciatus* (Ouda *et al*., 1998). Su and Mulla (1998) reported that the ovicidal activity of neem products *Azadirachtin* against mosquitoes *C. tarsalis* and *C. quinquefasciatus*. Rajkumar and Jebanesan (2004b) studied that ovicidal activity of *Solanum tribolatum* leaf extract against *C. quinquefasciatus* and *C. tritaeniorhynchus.*

It is evident from the present study that the exposure of mosquito eggs to the plant extracts elicits egg mortality. Miura *et al* (1976) showed that the age of

embryos at the time of treatment played a crucial role with regard to the effectiveness of the chitin synthesis inhibitor, dimilin to *C. quinquefasciatus*. Exposure time also has a vital role in causing toxicity. According to Smith and Salkeld (1966), differences in susceptibility to ovicides are due to differential rates of uptake, penetration through the chrocion, conversion to active inhibitor; detoxication and failure of the toxic to reach the target.

Plant extracts used in the present study may thus contribute greatly to the reduction of environmental contamination and to an overall reduction in the population density of *C. quinquefasciatus*. The flora of India has rich aromatic plant diversity with has a great potential to be developed as natural insecticides for the control of mosquito and other pests.

PHASE II

4.2 REPELLENT ACTIVITY

The solvent fractions of the leaf and flower extracts of the selected plants were evaluated for their repellent activity against the adults of *C. quinquefasciatus* mosquito. The petroleum ether, chloroform and ethanol extracts in different concentrations were tested against three day old blood starved adult mosquitoes to test their repellent activity. The repellent efficacy was determined in three concentrations viz., 1.0, 2.5, 5.0 mg/cm^2 under laboratory conditions (Table 22 to 31). The control treatment did not provide any protection even during the first trial.

Leaf extracts of *C. gigantea*

All the three extracts viz., petroleum ether, chloroform and ethanol showed dose dependent repellent activity. Maximum repellent activity was observed in ethanol extract (Table 22) in lower concentrations of 1.0 and 2.5 mg/cm^2 showed 100% protection from mosquito bites upto 60 min. In 1.0 and 2.5 mg/cm^2, protection of 99% and 99.66% respectively were recorded in 90 min. At the higher concentration of 5.0 mg/cm^2, the ethanol extract of *C. gigantea* leaf provided 100% protection upto 120 min of the experimental period against *C. quinquefasciatus*.

The ethanol extract of *C. gigantea* flower showed remarkable repellent activity against the adults of *C. quinquefasciatus*. In 1.0 mg/cm^2, it was observed that the efficacy of the extract decreased a little resulting in the non-repellency of 1.67% of mosquitoes making the percentage of repellency and protection to decrease from 99% to 98.33% after 90 min. The repellent activity was very high at the initial stage of exposure. Increase in the exposure period showed reduction in repellent activity and it depends upon the concentration of the extract and density of mosquitoes.

The results showed that repellent activity was dose dependent. The repellency of ethanol extract was followed by petroleum ether extract and chloroform extract that provided a protection of 98.33% and 94.66% respectively at the initial stage of 30 min in the higher concentration of 5.0 mg/cm^2, which was reduced with the increase in the exposure period.

Leaf extracts of *T. peruviana*

Among the three solvent fraction tested the chloroform extract of *T. peruviana* was found to have maximum repellency of 100% against the mosquitoes upto 30 and 60 min at the concentrations of 1.0 mg/cm^2 and 2.5 mg/cm^2 respectively. However, there after the repellency percentage was found to decrease in both the concentrations as they showed a non-repellency to 1% and 1.34% of mosquitoes respectively resulting in the variation from 100% to 99% and 98.66% after 30 and 60 min respectively (Table 23).

Moreover, at the highest concentration of 5.0 mg/cm^2 the maximum efficacy as a repellent was confirmed by chloroform extract which gave 100% protection against the mosquito species upto 90 min. Followed by chloroform extract, at the 30 min of the study the repellency was found to be high in petroleum ether extract which showed a repellency percentage of 95.66 and ethanol extract with 94% at the concentration of 5.0 mg/cm^2 respectively. The repellency of the leaf extract showed variation according to the variation of doses.

Leaf extracts of *T. erecta*

In all the concentration of 1.0, 2.5 and 5.0 mg/cm^2 remarkable efficacy as repellent was recorded by petroleum ether extract of *T. erecta* leaf inducing a repellency percentage of 94.66, 95.33 and 95.66 at the initial experimental period of 30 min. There after, the repellency percentage decreased in relation to the increase in the exposure time and concentration (Table 24). At 180 min of the study period in all the three concentrations (1.0, 2.5 and 5.0 mg/cm^2) a decreased repellency percentage of 92.33, 92.66 and 93 respectively were observed.

Chloroform solvent fraction was found to exhibit the minimum repellency providing a protection of 94% at the lower concentration of 1.0 mg/cm^2 upto 30 min of study period. At the higher concentration of 5.0 mg/cm^2 the extract was observed to exhibit a protection percentage which increased to 95.33. The protection percentage was found to decrease thereafter. As the dose is increased the repellency percentage was observed to get increased. Minimum repellency percentage was noted in ethanol extract.

Leaf extracts of *L. camara*

The study indicated that among the three solvents tested the ethanol soluble fraction showed the maximum effect than the other two samples. Ethanol soluble fraction provided 95% protection upto 30 min at the higher concentration of 5.0 mg/cm^2. When the concentration was decreased to 2.5 and 1.0 mg/cm^2 the repellency percentage was found to be decreased to 93 and 92.66% respectively (Table 25). At 60 min of the exposure period the repellency percentage of all the three concentrations viz., 1.0, 2.5 and 5.0 mg/cm^2 was found to be decreased to 91.66, 92.33 and 93% respectively. The increase in exposure period resulted in the decrease of repellency.

The repellency of ethanol extract was followed by petroleum ether and chloroform extract. Petroleum ether extract provided a protection of 94.66% at 30 min in the concentration of 5.0 mg/cm^2. At 180 min of exposure the protection percentage got down to 91.33%. The minimum repellency among the three solvents were observed in the case of chloroform extract of *L. camara* leaf which

showed a protection of 94% at 30 min and at 180 min the repellency percentage recorded was 92.66%. The results clearly showed that repellent activity depends upon the exposure period and also the dose.

Leaf extracts of *B. acuminata*

The efficacy of *B. acuminata* as the mosquito repellent was assessed and the observations revealed that among the three extracts ethanol extract was found to be very effective against *C. quinquefasciatus* mosquitoes. Ethanol extract at the higher concentration of 5.0 mg/cm^2 provided protection of 96% at initial stage of the study period (Table 26). Thereafter at 60, 90, 120, 150 and 180 min, the protection was decreased as 94%, 93.66%, 93.33%, 93.33% and 92.66% respectively.

Repellent efficacy of petroleum ether extract of *B. acuminata* leaf followed the ethanol extract. At 30 min of study period it provided a protection of 95.33% at the higher concentration of 5.0 mg/cm^2, which was decreased as 93.33% at 60 min because of the non-repellency of 2% of mosquitoes. As the exposure period was increased the repellency percentage was decreased as 93.33%, 93.33%, 92.66%, 91.33% and 91% at 60, 90, 120, 150 and 180 min respectively. Minimum repellent efficacy was exhibited by chloroform extract which at the lower and higher concentration induced only 92 and 94% repellency respectively at 30 min of exposure.

Flower extracts of *C. gigantea*

The study clearly demonstrated that the mosquito repellent efficacy of the petroleum ether, chloroform and ethanol flower extracts of *C. gigantea*. The results confirmed that among the three extracts, ethanol extract showed maximum repellency, even at the lower concentration of 1.0 mg/cm^2 provided 100% protection against *C. quinquefasciatus* mosquito upto 90 min. At the concentration of 2.5 mg/cm^2 also the 100% repellency was seen upto 90 min, which thereafter decreased as the exposure period increased. At 120 min the protection was decreased to 99% and 99.33% in the concentration of 1.0 and 2.5 mg/cm^2 respectively. At the higher concentration of 5.0 mg/cm^2 ethanol extract was found to provide 100% protection from bites upto 150 min, after which it was slightly decreased (Table 27).

Table 22

Repellent activity of *C. gigantea* leaf extracts against adults of *C. quinquefasciatus*

Solvent used	Concentration mg/cm^2	% of repellency					
		30 min	60 min	90 min	120 min	150 min	180 min
Control	-	0±0	0±0	0±0	0±0	0±0	0±0
Petroleum ether	1.0	94±0.0	93.66±0.47	93±0.0	92.33±0.47	90±0.81	91.33±0.47
	2.5	94.66 ±0.47	94±0.0	93.66±0.47	93.33±0.47	93±0.0	92±0.0
	5.0	98.33±1.24	96.66±0.47	95.33±0.47	94.33±0.47	94.66±0.47	94.33±0.47
Chloroform	1.0	94±0.0	93.33±0.47	93±0.0	92±0.0	92±0.0	91.66±0.47
	2.5	94.33±0.47	94±0.81	93±0.0	92.66±0.47	92±0.0	91±0.0
	5.0	94.66±0.47	95±0.81	94.66±1.69	93.66±0.47	92.66±0.47	92.33±0.47
Ethanol	1.0	100±0.0	100±0.0	99±0.81	98.33±1.24	97.33±0.47	96.33±0.47
	2.5	100±0.0	100±0.0	99.66±0.47	99.66±0.47	97.66±0.47	97±0.81
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	99.66±0.47	99±0.81

Each value ($\chi \pm SD$) represents average of three values

Table 23

Repellent activity of *T. peruviana* leaf extracts against adults of *C. quinquefasciatus*

Solvent used	Concentration mg/cm^2	% of repellency					
		30 min	60 min	90 min	120 min	150 min	180 min
Control	-	0±0	0±0	0±0	0±0	0±0	0±0
Petroleum ether	1.0	93.33±0.47	93.66±0.47	93±0.0	92±0.0	91±0.81	91.33±0.47
	2.5	93.66±0.47	94±0.81	93.66±0.47	92.66±0.47	92.33±0.47	92.33±0.47
	5.0	95.66±0.94	94.33±0.47	94±0.0	93.33±0.47	93±0.0	92.33±0.47
Chloroform	1.0	100±0.0	99±0.81	99±0.81	97.66±0.47	96.66±0.47	95.66±0.47
	2.5	100±0.0	100±0.0	98.66±0.94	97.66±0.47	98±0.0	97±0.81
	5.0	100±0.0	100±0.0	100±0.0	99±0.81	99.33±0.47	98±0.0
Ethanol	1.0	93.33±0.47	93.66±0.94	92.66±0.47	92.66±0.47	91.66±0.47	91.33±0.47
	2.5	94±0.0	93.33±0.47	93±0.81	93±0.0	92±0.0	92±0.0
	5.0	94±0.81	93.33±0.47	93.66±0.47	93±0.0	94±0.81	92.66±0.47

Each value ($\chi \pm SD$) represents average of three values

Table 24

Repellent activity of *T. erecta* leaf extracts against adults of *C. quinquefasciatus*

Solvent used	Concentration mg/cm^2	% of repellency					
		30 min	60 min	90 min	120 min	150 min	180 min
Control	-	0±0	0±0	0±0	0±0	0±0	0±0
Petroleum ether	1.0	94.66±0.47	94.33±0.47	94.33±0.47	93.33±0.47	92.33±0.47	92.33±0.47
	2.5	95.33±0.47	95±0.0	94.33±0.47	93.66±0.47	93±0.0	92.66±0.47
	5.0	95.66±0.47	95.33±0.47	94.33±0.47	94±0.81	93.33±0.47	93±0.0
Chloroform	1.0	94±0.81	93.66±0.47	92.33±0.94	92.33±0.47	91.66±1.24	91.33±0.47
	2.5	94±0.0	93.66±0.47	93.33±1.24	93±0.0	92±1.41	92±0.81
	5.0	95.33±0.47	94±0.0	94±0.81	94±0.81	92±1.41	93±0.0
Ethanol	1.0	93.66±0.47	93±0.0	92.33±0.47	93.33±0.47	92.33±1.24	92.33±0.47
	2.5	94±0.0	93.66±0.47	94±0.81	93.33±0.47	93±1.24	93.33±0.47
	5.0	94±0.81	95±0.0	94.33±0.47	94±0.81	93±0.0	93.33±0.47

Each value ($\chi \pm SD$) represents average of three values

Table 25

Repellent activity of *L. camara* leaf extracts against adults of *C. quinquefasciatus*

Solvent used	Concentration mg/cm^2	% of repellency					
		30 min	60 min	90 min	120 min	150 min	180 min
Control	-	0±0	0±0	0±0	0±0	0±0	0±0
Petroleum ether	1.0	92.66±0.47	92.33±0.47	91.66±0.47	91±0.0	90.66±1.24	90±0.81
	2.5	93.33±0.47	92.66±0.47	92.33±0.47	92±0.81	91±0.81	91±0.81
	5.0	94.66±0.81	93.33±0.47	93±0.0	93±0.81	92.33±0.47	91.33±0.47
Chloroform	1.0	93.33±0.47	92±0.81	92.66±0.47	91.33±0.47	91±0.81	90±0.81
	2.5	93.33±0.47	92.66±0.47	92.33±1.24	92.33±0.47	91.33±0.47	91±0.0
	5.0	94±0.0	93±0.0	92.66±0.47	93±0.0	92.66±1.24	92.66±1.24
Ethanol	1.0	92.66±0.47	91.66±0.94	91.66±0.94	91±0.81	91.33±1.69	90.33±0.94
	2.5	93±1.41	92.33±0.47	92.33±0.47	91.66±0.94	91.33±0.47	90.66±0.47
	5.0	95±0.81	93±0.94	93.33±1.41	93.33±0.94	91.66±0.47	92±0.0

Each value ($\chi \pm SD$) represents average of three values

Table 26

Repellent activity of *B. acuminata* leaf extracts against adults of *C. quinquefasciatus*

Solvent used	Concentration mg/cm^2	% of repellency					
		30 min	60 min	90 min	120 min	150 min	180 min
Control	-	0±0	0±0	0±0	0±0	0±0	0±0
Petroleum ether	1.0	92.33±0.94	92±0.0	91.66±0.47	90.66±0.47	90.66±0.47	89.33±0.47
	2.5	93.33±0.47	93±0.81	92.66±0.47	92.33±0.47	91.33±0.47	91±0.81
	5.0	95.33±0.47	93.33±0.47	93.33±1.24	92.66±0.47	91.33±1.24	91±0.81
Chloroform	1.0	92±0.81	91.33±0.47	90±0.47	90.33±0.47	89.33±0.47	89.33±0.47
	2.5	93.66±0.47	92.33±0.94	92±0.81	92±0.81	91±0.0	91±0.0
	5.0	94±0.81	93.66±0.47	93±0.81	92.66±0.94	91±0.81	90.33±0.47
Ethanol	1.0	93.66±0.47	93±0.0	93±0.81	92.66±1.24	92.33±0.47	92±0.81
	2.5	93.66±0.94	93±0.81	93.33±0.47	93±0.81	93±0.81	92.66±0.47
	5.0	96±0.81	94±0.81	93.66±0.47	93.33±0.94	93.33±1.24	92.66±0.47

Each value ($\chi \pm SD$) represents average of three values

Table 27

Repellent activity of *C. gigantea* flower extracts against adults of *C. quinquefasciatus*

Solvent used	Concentration mg/cm^2	% of repellency					
		30 min	60 min	90 min	120 min	150 min	180 min
Control	-	0±0	0±0	0±0	0±0	0±0	0±0
Petroleum ether	1.0	94.33±0.47	94±0	94.33±0.47	93±0.0	93±0.81	93±0.0
	2.5	99.66±0.47	96.33±0.47	95±0.81	94.3±0.47	94±0.81	94±1.63
	5.0	100±0	97.33±0.47	96±0.0	95.33±0.47	95±0.0	95±0.81
Chloroform	1.0	94.33±0.47	94.33±0.47	94±0.81	93.66±0.47	93±0.0	92.66±0.47
	2.5	95.33±0.47	95±0.81	94.33±0.47	93.66±0.47	94±0.0	93.33±0.47
	5.0	95.66±0.47	96±0.81	96±0.81	94.33±0.47	95±0.81	94.33±0.47
Ethanol	1.0	100±0.0	100±0.0	100±0.0	99±0.81	97.66±0.47	96.66±0.47
	2.5	100±0.0	100±0.0	100±0.0	99.33±0.47	97.66±0.47	97.66±0.47
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	98.33±0.47

Each value ($\chi \pm SD$) represents average of three values

Table 28

Repellent activity of *T. peruviana* flower extracts against adults of *C. quinquefasciatus*

Solvent used	Concentration mg/cm^2	% of repellency					
		30 min	60 min	90 min	120 min	150 min	180 min
Control	-	0±0	0±0	0±0	0±0	0±0	0±0
Petroleum ether	1.0	93.33±0.47	93.33±0.47	93.33±1.24	93.66±1.69	92.66±0.47	92.33±0.47
	2.5	94.66±0.47	94.33±0.47	94.33±0.47	94.66±0.47	93.33±0.94	92.33±0.47
	5.0	95.33±0.47	95.33±0.47	95±0.81	95±0.81	95±0.81	94.33±0.47
Chloroform	1.0	95.33±0.47	94.66±0.94	93.66±0.94	93±0.81	92.33±0.94	91.66±0.47
	2.5	96±0.0	94.66±0.94	94±0.81	94.33±0.47	93±.81	92±0.81
	5.0	97.66±0.47	96±0.0	95.33±0.47	95±0.0	93.66±0.47	92.33±0.94
Ethanol	1.0	94.66±0.47	94.66±0.47	93.33±0.47	92.33±0.47	92.66±0.47	92±0.0
	2.5	94±0.47	94±0.00	93.66±0.47	93±0.81	92.66±0.47	92.33±0.47
	5.0	96±0.0	95.66±0.47	95±0.81	94.66±0.47	94±0.0	93.66±0.47

Each value (χ ± SD) represents average of three values

Table 29

Repellent activity of *T. erecta* flower extracts against adults of *C. quinquefasciatus*

Solvent used	Concentration mg/cm^2	% of repellency					
		30 min	60 min	90 min	120 min	150 min	180 min
Control	-	0±0	0±0	0±0	0±0	0±0	0±0
Petroleum ether	1.0	100±0.0	100±0.0	99±0.0	95.6±0.47	95±0.81	94.6±0.47
	2.5	100±0.0	100±0.0	98.3±0.47	97±0.81	96±1.41	95±0.81
	5.0	100±0.0	100±0.0	100±0.0	97.3±1.24	96.6±1.24	96±0.81
Chloroform	1.0	100±0.0	100±0.0	100±0.0	100±0.0	99.6±0.47	99.3±0.47
	2.5	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	99.6±0.47
	5.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0
Ethanol	1.0	100±0.0	98.3±0.47	96.3±0.47	94.6±0.47	94.6±1.24	93.3±0.47
	2.5	100±0.0	99.3±0.47	96.3±0.47	96±0.0	95±0.81	94.3±0.47
	5.0	100±0.0	100±0.0	97±0.0	96.6±0.47	95.6±0.47	94.3±0.47

Each value ($\chi \pm SD$) represents average of three values

Table 30

Repellent activity of *L. camara* flower extracts against adults of *C. quinquefasciatus*

Solvent used	Concentration mg/cm^2	% of repellency					
		30 min	60 min	90 min	120 min	150 min	180 min
Control	-	0±0	0±0	0±0	0±0	0±0	0±0
Petroleum ether	1.0	93.6±0.47	93.3±0.94	93±0.0	92±0.81	91.6±0.47	91.6±0.47
	2.5	94.6±0.47	94.3±0.47	94±0.0	93.6±0.94	93.3±0.47	92.3±0.47
	5.0	95±0.81	94.6±0.47	94.3±0.47	94±1.41	93±0.00	93±0.00
Chloroform	1.0	95.3±0.00	93.6±0.47	93.6±0.94	92.6±1.24	92.6±0.47	91.6±0.47
	2.5	96±0.47	95.3±0.47	94.3±0.47	94±0.0	93.3±0.47	93.3±0.47
	5.0	96.6±0.47	95.3±0.47	94.6±0.47	94.3±0.94	93.6±0.47	93.6±0.47
Ethanol	1.0	93±0.0	92.6±0.47	91.6±0.47	92±0.81	92±0.81	91±0.81
	2.5	94.3±0.47	93.6±0.47	93±0.81	92.6±0.47	92.3±0.94	92±0.81
	5.0	95±0.0	94.3±0.47	93.6±0.47	93.6±0.47	93±0.0	92.3±0.94

Each value ($\chi \pm SD$) represents average of three values

Table 31

Repellent activity of *B. acuminata* flower extracts against adults of *C. quinquefasciatus*

Solvent used	Concentration mg/cm^2	% of repellency					
		30 min	60 min	90 min	120 min	150 min	180 min
Control	-	0±0	0±0	0±0	0±0	0±0	0±0
Petroleum ether	1.0	93±0.0	93.3±0.47	93.6±0.94	92±0.0	91.6±0.94	90±0.47
	2.5	94.3±0.47	93.6±0.47	93.6±1.24	93.3±0.47	93±0.81	92.3±0.94
	5.0	94.6±0.94	94±0.81	94.3±0.94	93.3±0.47	93±0.81	93.3±0.47
Chloroform	1.0	93±0.81	92.6±0.47	93.3±1.69	92.3±0.47	92±0.81	91.6±1.24
	2.5	94±0.0	94±0.0	94.6±0.47	94.3±0.47	93.3±0.47	93±0.0
	5.0	95±0.0	94.6±0.47	94.6±0.47	94.3±0.47	93.6±0.47	93.6±0.47
Ethanol	1.0	93.6±0.94	93.6±1.24	93.3±0.47	93.3±0.47	93±0.81	92.6±0.47
	2.5	95±0.81	94±0.81	92.6±0.47	93.3±0.94	93.3±0.47	93±0.81
	5.0	95.6±0.47	94.6±0.47	94.6±0.47	94±0.0	93.3±0.47	93±0.0

Each value ($\chi \pm SD$) represents average of three values

Petroleum ether extract also provided 100% protection from mosquito bites for 30 min at the concentration of 5.0 mg/cm^2. With the extract applied to the arms of human volunteers, it was observed that the efficacy of the extract decreased a little thereafter exhibiting a repellency of 97.33%, 96%, 95.33%, 95% and 95% at 60, 90,120,150 and 180 min respectively. Comparing the three extracts minimum repellent activity was recorded in chloroform extract inducing a protection of 94.33% and 95.66% against the mosquito bites at the lower and higher concentrations of 1.0 and 5.0 mg/cm^2 at the initial stage of the study period.

Flower extracts of *T. peruviana*

The three soluble fraction of the flower of *T. peruviana* were tested for their repellent efficacy. The three extracts showed only slight variation in their repellent efficiency. Among the three extracts, chloroform extract revealed maximum repellent activity than the other two flower extracts. At the initial stage of the study period the extract was observed to give a protection of 95.33%, 96% and 97.66% at the concentration of 1.0, 2.5 and 5.0 mg/cm^2. At the later stage of the experimental period i.e., at 180 min the repellent percentage was decreased exhibiting a protection of 91.66%, 92% and 92.33% respectively (Table 28).

Next to chloroform extract, the ethanol extract induced a repellency of 94.66% at the lower concentration of 1.0 mg/cm^2 showed an increase with the increase in concentration. At the higher concentration of 5.0 mg/cm^2 the protection was increased from 94.66% to 96%. The repellent efficacies of all the extracts were dose dependent. Minimum repellent activity was displayed by petroleum ether extract which at the lower concentration of 1.0 mg/cm^2 provided only 93.33% protection and higher concentration provided only 95.33% protection upto 30 min.

Flower extracts of *T. erecta*

The results showed that all the extracts exhibited 100% repellent activity with slight variation in the duration of protection. Among the three extracts, the chloroform extract of *T. erecta* was found to be most effective as the mosquito repellent as it showed 100% protection from the bites of *C. quinquefasciatus*

mosquito species throughout the study period at the concentration of 5.0 mg/cm^2 (Table 29). Even at the concentration of 2.5 mg/cm^2 the extract was found to provide a protection of 100% upto 150 min. Moreover, at the lower concentration of 1.0 mg/cm^2 the extract was found to exhibit high repellency percentage giving a protection of 100% upto 120 min. It is clear that the percentage of repellency was directly proportional to the concentration of the extract.

With the slight variation in the duration of protection chloroform extract was followed by the petroleum ether extract providing 100% protection upto 90 min at the higher concentration of 5.0 mg/cm^2. At the concentrations of 1.0 and 2.5 mg/cm^2 the extract was found to exhibit 100% protection upto 60 min each. Thereafter, with the increase in the exposure period the protection percentage was recorded to get down slightly. That is at 90 min in both the concentrations (1.0 and 2.5 mg/cm^2) the non repellency of 1% and 1.7% of mosquitoes caused the repellency to decrease from 100% to 99% and 98.3% respectively.

Efficiency of petroleum ether extract was followed by ethanol extract which at the concentration of 1.0 mg/cm^2 provided 100% protection at starting stage of the study but at the higher concentration of 5.0 mg/cm^2 the duration of 100% protection extended upto 60 min. The results suggested that the petroleum ether extracts of *T. erecta* flower are promising as repellent at 1.0, 2.5 and 5.0 mg/cm^2 concentration against *C. quinquefasciatus* and could be useful in the search for new natural repellent compounds.

Flower extracts of *L. camara*

Among the three extracts investigated for their repellent efficacies, chloroform extract of *L. camara* was found to be more effective providing a maximum protection of 96.6% at the higher concentration of 5.0 mg/cm^2 (Fig. 16a). When the exposure period was increased to 60 min the protection was 95.3% because of the non repellency of 1.3% of mosquitoes. With the decrease in the concentration also the repellency was decreased. At the lower concentration of 1.0 mg/cm^2 chloroform extract showed a repellency of only 95.3% at 30 min (Table 30).

The repellency efficiency of chloroform extract was followed by petroleum ether extract. Minimum repellent efficacy was shown by ethanol extract of *L. camara* which at the lower concentration of 1.0 mg/cm^2 showed a protection of only 93% at 30 min and was increased to 94.3% and 95% when the concentration was increased to 2.5 and 5.0 mg/cm^2 respectively. At 60 min of exposure the protection provided by the extract at 1.0, 2.5 and 5.0 mg/cm^2 was 92.6%, 93.6% and 94.3%. At 180 min of the study period the protection showed by three concentrations (1.0, 2.5 and 5.0 mg/cm^2) were decreased to 91%, 92% and 92.3% respectively. The results showed that there were significant differences in repellency provided by the different extracts against the tested *C. quinquefasciatus*.

Flower extracts of *B. acuminata*

Among the extracts tested ethanol extract was found to exhibit more repellent efficacy than the other two extracts. At the concentration of 1.0 mg/cm^2 the ethanol extract was found to exhibit a protection of 93.6% upto 60 min. When the concentration was increased the protection percentage was increased. At the concentration of 5.0 mg/cm^2 the protection exhibited by the extract at 30 min and was 95.6% (Table 31). Thereafter, the protection was decreased to 94.6%, 94.6%, 94%, 93.3%, 93% due to the increase in the exposure period i.e., 60, 90, 120, 150 and 180 min respectively.

At 30 min chloroform extract provided 95% protection from mosquito bites. Petroleum ether extract exhibited minimum repellent efficacy among the three extracts giving a protection of 94.6% at the concentration of 5.0 mg/cm^2. At the lower concentration of 1.0 mg/cm^2 both the extracts provided only 93% protection at the initial stage of the study, whereas at 180 min of the experiment the protection came down from 93% to 91.6% an 90% respectively in both the extracts confirming that repellent efficacy decreases with increase in the exposure period of the extract.

The results revealed the repellent effect of five plants corresponding to different botanical families on *C. quinquefasciatus*. The data pertaining to the repellent activity of plant extracts, the mode of treatment and observations

concerning repellent activity are given in Table 22 to 31. It is evident from our results that the repellent activity of plant extracts against *C. quinquefasciatus* is directly proportional to the rise in concentration and density of mosquitoes and inversely proportional to the duration of the exposure period of the extracts. The varying results obtained in the study were probably due to the differences in the levels of toxicity among the insecticidal ingredients of each plant and the effect of plant extracts which can vary significantly depending on plant species, plant part, age of plant part, solvent of extraction and mosquito species.

The results revealed that highest repellent activity was exhibited by chloroform extract of *T. erecta* flower which was found to be the most promising repellent agent which provided 100% repellent activity at the higher concentration of 5.0 mg/cm^2 throughout the study period making it the most remarkable natural repellent, which was followed by ethanol extract of *C. gigantea* flower which provided 100% repellency against adult *C. quinquefasciatus* mosquito upto 150 min at the higher concentration of 5.0 mg/cm^2. Comparatively moderate repellent activity was provided by ethanol extract of *C. gigantea* leaf which provided 100% protection from upto 120 min followed by chloroform extract of *T. peruviana* leaf which provided 100% protection from the mosquito bites upto 90 min at concentration of 5.0 mg/cm^2.

The reason for the repellent efficacy of these plants may be due to the presence of active principles present in the plant extracts. The identification of these active components is a part of further research for an efficient, eco-friendly and biodegradable mosquito repellent of plant origin.

Discussion

Botanicals have been used traditionally by human communities in many parts of the world against pest species of insects. Many plants produce secondary metabolites that inhibit the growth of insects. Though several plants from different families have been reported for mosquitocidal activities, only very few botanicals have been moved from the laboratory to field use. Simple crude extracts from plants have been used as insecticides for centuries (Govindarajan, 2011b). Crude

plant extracts often consist of complex mixtures of active compounds. Advances of using complex mixtures may act synergistically. They may show greater overall bioactivity compared to the individual constituents (Sumroiphon *et al*., 2006) and insect resistance is much less likely to develop with mixtures. These reasons support the use of crude chemically unrefined plant extracts, containing mixtures of bioactive plant compounds.

Personal protection measures, including repellents, are widely used to prevent the transmission of arthropod borne diseases by minimizing the contact between humans and vectors. In contrast to vaccines and chemoprophylaxis as means of personal protection, repellents are convenient, inexpensive and afford advantages in protection against a wide range of vectors (WHO, 1996). They are also the primary means of mosquito-borne diseases prevention measures available in areas where vector control is not practical (Gupta and Rutledge, 1994; Copeland *et al.,* 1995; Govindarajan *et al.*, 2011a). The repellent potential of plants to mosquitoes and other pest insects has been well known both prior to (Granett, 1940) and after (Thorsell *et al.*, 1998) the advent of synthetic chemicals. Various botanicals, viz., *Cymbopogan spp*. (Rutledge *et al*., 1983; ,Ansari and Razdan, 1995). *Eucalyptus maculats citriodon* (Collins *et al*., 1993), *Azadirachta indica* (Sharma *et al.*, 1993), *Pelargonium citrosum* (Matsuda *et al*., 1996), *Lantana camara* (Dua *et al*., 1996), and *Mentha piperita* (Ansari *et al*., 2000) have been reported as being repellent against adult mosquitoes.

The data obtained in the present investigation was pertinent to note that all the plant extracts showed excellent repellent activity of which strong repellent activity was observed in chloroform extract of *T. erecta* flower. The control treatment did not provide any protection even during the first trial. *T. erecta* was found to be most promising as the mosquito repellent as it offered 100% protection from the bites of *C. quinquefasciatus* mosquito species throughout the study period at the higher concentration of 5.0 mg/cm^2. The petroleum ether extract and ethanol extract of the flower also offered 100% protection upto 90 min and 60 min respectively. Similar observations were reported by Kovendan *et al* (2013) in which hexane, benzene, ethyl acetate, acetone and methanol extract of *Acalypha alnifolia*

plant extracts at various concentrations tested for repellent activity produced 100% protection against mosquito bites.

In the present study, it was observed that ethanol extract of *C. gigantea* flower produced 100% protection against *C. quinquefasciatus* mosquito upto 90 min, even at the lower concentration of 1.0 mg/cm^2. The repellency percentage was found to fall down thereafter as the exposure period increased. It was also noted that when the concentration is increased to 5.0 mg/cm^2 the protection time also increased, i.e., at the concentration of 5.0 mg/cm^2 the protection provided by the extract was upto 150 min. These results can be compared to the findings of Venkatachalam and Jebanesan (2001) who reported that the mean protection time and total percentage protection in relation to dose of *Ferronia elephantum* leaf extract showed that the percentage of protection is in relation to dose and time.

Vijaya kumar *et al* (2011a) showed the repellent activity of ethanol extract of *Vitex negundo* leaf against *Ae. aegypti, An. stephensi* and *C. quinquefasciatus*. Among the different concentrations tested, the highest protection time of 126.8 min was observed at the highest concentration of 0.02 ppm against *C. quinquefasciatus* followed by *An. stephensi* and *Ae. aegypti* and at the lowest concentration protection period decreased. The findings of the present study were found to be similar to these findings. In the present study, ethanol extract of *C. gigantea* leaf showed 100% protection from *C. quinquefasciatus* mosquito bite for 120 min at the concentration of 5.0 mg/cm^2. And when the concentration is lowered to 1.0 mg/cm^2 the protection period was decreased to 60 min. This implies that period of protection is directly proportional to the concentration of the plant extract.

The findings of the present study corporate the earlier reports of Baluselvakumar *et al* (2012) who showed that, a higher concentration of 3.0 mg/cm^2 crude extracts of acetone, benzene, ethyl acetate, hexane and methanol of *Melothria maderaspatana* produced 100% protection upto 80, 100, 120, 120 and 140 min against *Ae. aegypti.* Mullai *et al* (2008) have reported that the leaf extracts of *Citrulus vulgaris* with different solvents viz., benzene, petroleum ether, ethyl

acetate and methanol was tested for larvicidal, ovicidal, repellent and insect growth regulatory activities against *An. stephensi*. Repellent properties of essential oils from various parts of four plant species *Cympopogon citratus, Cinnamomum zeylanicum, Rosmarinus officinalis* and *Zingiber officinale* against *C. tritaeniorhynchus* and *An. subpictus* was reported by Govindarajan *et al.,* 2011a.

Different parts of plants contain a complex of chemicals with unique biological activity which is thought to be due to toxins and secondary metabolites, which act as attractants or deterrents. Our results showed that different solvent extracts of selected plants have significant repellent activity against the filarial vector mosquito, *C. quinquefasciatus*. These results can be compared with the early reports of Elango *et al* (2011) who observed that maximum repellent activity was observed in ethyl acetate extracts of *A. paniculata, E. prostrata* and methanol extracts of *T. erecta,* and the complete protection time ranged from 120 to 150 min with the different extracts tested.

Venkatachalam and Jebanesan (2001) reported the repellent activity of methanol extract of *Ferronia elephantum* leaves against *Ae*. *aegypti* at 1.0 and 2.5 mg/cm^2 concentrations produced 100% protection upto 2:14±0:16 h and 4:00±0:24 h respectively, and the total percentage protection was 45.8% at 1.0 mg/cm^2 and 59.0% at 2.5 mg/cm^2 for 10h. The percentage of protection was found to be directly proportional to the concentration of the extract. These findings were in accordance with the results of the present study were the chloroform extract of *T. peruviana* leaf provided 100% protection from the *C. quinquefasciatus* mosquito bite for a time period of 30 min at 1.0 mg/cm^2, 60 min at 2.5 mg/cm^2 and 90 min at 5.0 mg/cm^2.

The chemicals derived from plants have been projected as weapons in future mosquito control programmes as they function as general toxicant, growth and reproductive inhibitors, repellents and oviposition deterrent (Sukumar *et al*., 1991). The ethyl acetate extracts of *Hyptis suaveolens*, *Rhododendon tomentosum*, and *Myrica gale* significantly reduced biting activity of *Ae*. *aegypti (*Jaenson *et al*., 2006). Prabhu *et al* (2011) reported that methanolic extract from *M. oleifera* seeds

(0.5, 1.0 and 2.0 mg/cm^2) repelled against *An. stephensi*. They observed a decrease in number of bites in arms of human volunteers treated with the extract. This is in accordance with the findings of the present study.

From the results of the present investigations, it can be confirmed that different plants extracts have different repellent action against the target mosquito. This may be due to the difference in the concentration of secondary metabolites present in various plant extracts. It has been proved that some phytochemicals act as toxicants against both adult as well as larval stages of mosquitoes. Whereas, others interfere with growth and reproduction or produce an olfactory stimuli thus acting as repellent or attractant (Markouk *et al*., 2000; Pushpanathan *et al*., 2008). If the protection time of a compound is long and the percentage of biting is low, the compound had good efficiency in repelling mosquitoes and deters biting.

As reported in the present study, the crucial role played by the exposure period in causing repellency was reported by many researchers. Murugan and Jeyabalan (1999) reported that *Leucas aspera*, *Acalypha indica*, *Allium sativum* and *Curcuma longa* had a strong larvicidal, anti-emergence, adult repellency and anti-reproductive activity against *An. stephensi*. Rajkumar and Jebanesan (2004a) evaluated the toxicity of the plant *Moschosma polystachyum* against mosquito, *C. quinquefasciatus*.

Palsson and Jaenson (1999) conducted research in several villages in Guinea Bissau (West Africa) on *Ocimum canum* that was being used traditionally as mosquito repellents by native people. Krishnappa *et al* (2012b) reported that the development of plant-based alternative compounds for mosquito control has gained importance now-a-days, in view of increasing resistance in mosquito vectors to existing insecticides. The larvicidal and repellent activities of benzene, chloroform, hexane and methanol leaf extracts of Indian medicinal plant, *Adansonia digitata* were investigated against malarial vector, *An. stephensi.*

Insect repellents derived from plant extracts is needed to find alternatives that are safer but still effective. Some plant extracts, such as neem (*Azadirachta indica*), sweet basil (*O. basilicum*), and lemon eucalyptus (*Corymbia citriodora*)

have been studied as possible mosquito repellents and have demonstrated good efficacy against some mosquito species (Kirton, 2005; Sharma *et al*, 1993). Tawatsin *et al* (2001) reported the volatile oils extracted from turmeric (*Curcuma longa*), kaffir lime (*Citrus hystrix*), citronella grass (*Cymbopogon winterianus*) and hairy basil (*O. americanum*) showed strong repellency against three mosquito vectors (*Ae. aegypti*, *An. dirus* and *C. quinquefasciatus*). Repellents properly used are inexpensive means of reducing and preventing a wide range of vectors (Gupta and Rutledge, 1989).

Nowadays many plant extracts of terrestrial origin are suggested to be advantageous for many mosquito control programmes. Phytochemicals obtained from huge diversity of plant species are an important source of safe and biodegradable chemicals which could be screened for mosquito repellent and insecticidal activities. Repellents of plant origin do not pose hazards or toxicity to human and domestic animal and are easily biodegradable. Natural products are safe for human when compared with synthetic ones. The findings of the present investigation have re-emphasised the need to explore the possibility of using herbal based repellents as supplementary and complimentary measures for mosquito control which will in turn reduce the chemical burden on the environment.

PHASE III

4.3 PHYTOCHEMICAL SCREENING

Higher plants play an important role in producing large number of organic compounds as secondary metabolites, which can be used for self defense and they act as bioactive compounds. Quite a lot of plants are used in folk medicine and other traditional medicine as aseptic agents throughout the world. The use of herbs and medicinal plants as the first medicines is a universal phenomenon. Every culture on earth, through written or oral tradition, has relied on the vast variety of natural chemistry found in healing plants for their therapeutic properties (Serrentino, 1991). All drugs of the past were substances with a particular therapeutic action extracted from plants. There are more than two thousand plant species acknowledged to own medicinal value in the traditional Asian system of medicine (Agnese *et al*., 2001).

The health giving properties of medicinal plants are due to the presence of various complex chemical substances of different composition which occur as secondary metabolites (Ongsakul *et al*., 2009 and Stuffness and Douros, 1982). They are grouped as alkaloids, glycosides, corticosteroids, coumarin, flavonoids and essential oils. Natural products play an important role in drug development programs in the pharmaceutical industry (Baker *et al*., 1995). Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness.

QUALITATIVE ANALYSIS

Phytochemical screening of ethanol extract of *C. gigantea* leaf and flower, chloroform extract of *T. peruviana* leaf and chloroform extract of *T. erecta* flower were carried out to test the presence of secondary metabolites such as alkaloids, tannins, phenols, flavonoids, sterols, terpenoids, saponins, anthroquinones, proteins and quinones (Table 32) by using standard procedures described by Raman, 2006.

Table 32

Phytochemical constituents present in plant extracts

Sl. No.	Constituents	*C. gigantea*		*T. peruviana*	*T. erecta*
		Ethanol extract of leaf	Ethanol extract of flower	Chloroform extract of flower	Chloroform extract of leaf
1	Alkaloids	+	+	+	+
2	Tannins	-	+	+	+
3	Phenols	+	+	+	+
4	Flavonoids	+	+	+	+
5	Sterols	+	+	+	+
6	Terpenoids	-	-	+	+
7	Saponins	+	-	-	-
8	Anthroquinones	-	+	+	-
9	Proteins	+	+	+	+
10	Quinones	+	+	-	+

GC-MS ANALYSIS

Ethanol extract of *C. gigantea* leaf

The GC-MS analysis of ethanol extract of *C. gigantea* leaf showed 16 major peaks at retention times of 10.34, 11.17, 14.27, 14.74, 18.79, 20.32, 22.93, 23.25, 27.37, 30.86, 31.36, 35.50, 38.26, 43.86, 45.80 (Fig. 12). The mass spectrum at retention time 10.34 (Fig. 13a) showed base peak at m/e 83.1. The fragmentation pattern showed two (M-28) peak at m/e 111.1 and m/e 83.1 and (M-13) peak at m/e 70.1 revealing the occurrence of carbonyl group and methyl group in the compound respectively.

The (M-15) and (M-13) peaks recorded at m/e 83.0 and m/e 70.1 in the mass spectrum at retention time 11.17 denoted the presence of methyl group and hydrocarbon in the compound respectively (Fig. 13b). The base peak was recorded at m/e 70.1. The mass spectrum of the peak at retention time 14.27 (Fig. 13c) exhibited base peak at m/e 83.1. The presence of five (M-14) peaks at m/e 125.2, m/e 111.1, m/e 97.1, m/e 83.1 and m/e 69.1 indicated the presence of hydrocarbon in the compound. The (M-27) peak at m/e 139.2 indicated the presence of nitrogen in the compound.

The base peak was recorded at m/e 83.1 at retention time 14.74 in the mass spectrum of the peak (Fig. 13d). Characteristic peaks other than these noted in the mass spectrum were in m/e 126.1, m/e 111.1, m/e 97.1 and m/e 71.1. The fragmentation pattern revealed the presence of two (M-14) peaks at m/e 97.1 and m/e 83.1 denoting the presence of hydrocarbon in the compound. The (M-15) peak at m/e 111.1 indicated the presence of methyl group in the compound. The mass spectrum of the peak at retention time 18.29, 22.93 and 27.37 (Fig. 13e, g & i) recorded base at m/e 83.1. The fragmentation pattern showed other characteristic peaks such as m/e 125.1, m/e 111.1, m/e 97.1 and m/e 69.1. The (M-14) peaks observed at m/e 111.1 m/e 83.1 and m/e 69.1 showed the occurrence of hydrocarbon in the compound and (M-28) peaks at m/e 97.1 and m/e 83.1 revealed the presence of carbonyl group in the compound.

The peaks recorded in the mass spectrum at retention time 20.32 were at m/e 142.2, m/e 127.1, m/e 100.0, m/e 82.1 and m/e 71.0. The fragmentation pattern showed the presence of (M-15) peak at m/e 127.1 that which denoted the presence of methyl group in the compound. The (M-27) peak and (M-18) peak noted at m/e 100.0 and m/e 82.1 denoted the presence of nitrogen and hydroxyl group in the compound respectively. The base peak was displayed at m/e 127.1 (Fig. 13f). The mass spectrum of the peak at retention time 23.25 (Fig. 13h) showed base peak at m/e 99.0. In the fragmentation pattern other characteristic peaks were observed at m/e 176.1, m/e 148.0, m/e 133.1, m/e 177.0 and m/e 67.1. The (M-28) peak at m/e 148.0 indicated the presence of carbonyl group in the compound. The presence of (M-15) peak and (M-18) peak at m/e 133.1 and m/e 99.0 denoted the occurrence of methyl group and hydroxyl group in the compound respectively.

At retention time 30.86 characteristic peaks were observed at m/e 399.4, m/e 357.1, m/e 34.9, m/e 285.2, m/e 264.3, m/e 222.4, m/e 197.2, m/e 180.2 and m/e 142.0 (Fig. 13j). In the mass spectrum, the base peak was shown at m/e 69.1 in the mass spectrum (Fig. 13k) at retention time 31.36. The fragmentation pattern showed other characteristic peaks such as m/e 153.2, m/e 111.1 and m/e 97.1. The presence of (M-14) and (M-28) peak at m/e 97.1 and m/e 69.1 indicated the presence of hydrocarbon and carbonyl group in the compound respectively.

Peaks displayed in the mass spectrum at retention time 35.50 included m/e 267.9, m/e 207.0, m/e 177.5, m/e 125.2, m/e 111.1 and m/e 83.1. The base peak was observed at m/e 97.1. The presence of three (M-14) peaks at m/e 111.1, m/e 97.1 and m/e 83.1 indicated the presence of hydrocarbon in the compound (Fig. 13l). The fragmentation pattern of the peak at retention time 38.26 (Fig. 13m) exhibited base peak at m/e 149.0. The mass spectrum includes other characteristic peaks at m/e 167.0, m/e 113.1 and m/e 70.1. The presence of (M-18) peak at m/e 149.0 indicated the presence of hydroxyl group in the compound.

The fragmentation pattern of the peak at retention time 43.86 included characteristic peaks at m/e 155.2, m/e 127.1 and m/e 97.1. Base peak was

displayed at m/e 71.1 and the (M-28) peak at m/e 127.1 revealed the presence of carbonyl group in the compound (Fig. 13n). At retention time 45.80 base peak was seen at m/e 253.0. The fragmentation pattern (Fig. 13o) showed other characteristic peaks at m/e 462.1, m/e 429.2, 415.1, m/e 366.2, m/e 346.1, m/e 316.1, m/e 280.2, m/e 203.2, m/e 180.0, m/e 131.0, m/e 94.1 and m/e 81.0. The (M-14) peak m/e 415.1 indicated the presence of hydrocarbon in the compound. The (M-27) peak and (M-13) peaks at m/e 253.0 and m/e 81.0 indicated the presence of nitrogen and hydrocarbon in the compound respectively.

Chloroform extract of *T. peruviana* leaf

The GC-MS analysis of chloroform extract of *T. peruviana* leaf displayed 17 major peaks at retention times such as 10.31, 14.73, 17.30, 18.79, 20.80, 22.92, 23.24, 25.93, 27.36, 29.29, 31.36, 35.07, 35.56, 38.26, 39.12, 43.46 and 43.93 (Fig. 14). The mass spectrum recorded base peak at m/e 70.1 for the retention time 10.31 (Fig. 15a). The two (M-14) peaks at m/e 97.1 and m/e 70.1 indicated hydrocarbon in the compound.

At retention time 14.73 and 27.36 characteristic peaks were observed at m/e 125.1, m/e 111.1 and m/e 69.1 in the fragmentation pattern (Fig. 15b & i). The (M-28) peak at m/e 83.1 indicated the presence of carbonyl group and (M-14) peak at m/e 111.1 and m/e 69.1 indicated the presence of hydrocarbon in the compound respectively. Base peak was recorded at m/e 69.1. The pattern of fragmentation in the mass spectrum at retention time 17.30 (Fig. 15c) showed base peak at m/e 19.11. The presence of (M-15) peak at m/e 191.1 revealed the occurrence of methyl group in the compound.

The base peak was observed at m/e 83, m/e 97.1 and m/e 69.1 in the mass spectrum at retention times 18.79, 35.56 and 43.93 respectively (Fig. 15d, m & q). The presence of six (M-14) peaks at m/e 125.1, m/e 111.1, m/e 97.1, m/e 81.1, m/e 83.1 and m/e 69.1 indicated the presence of hydrocarbon in the compound. The (M-18) peak at m/e 149.0 and m/e 81.1 respectively noted at retention time 20.80 and 38.26 in the mass spectrum revealed the presence of hydroxyl group in the

compound. In the mass spectrum, the fragmentation pattern displayed the base peak at m/e 149.0 and m/e 99.1 respectively (Fig. 15e & n).

The base peak was noted at m/e 97.1 in the fragmentation pattern of the mass spectrum at retention times 22.92, 31.36 and 43.46 (Fig. 15f, k & p). Other peaks included in the mass spectrum were in m/e 125.1 and m/e 69.1. The two (M-28) peaks at m/e 97.1 and m/e 69.1 denoted the presence of carbonyl group in the compound. At the retention time 23.24 base peak was observed at m/e 99.0. The (M-28) peak at m/e 148.1 revealed the presence of carbonyl group in the compound and (M-18) peak at m/e 99.0 indicated the presence of hydroxyl group in the compound. Other than these peaks at m/e 176.1, m/e 148.1, m/e 117.1, m/e 79.0 and m/e 67.1 were also observed in the spectrum (Fig. 15g).

Figure 12

Gas Chromatogram of ethanol extract of *C. gigantea* leaf

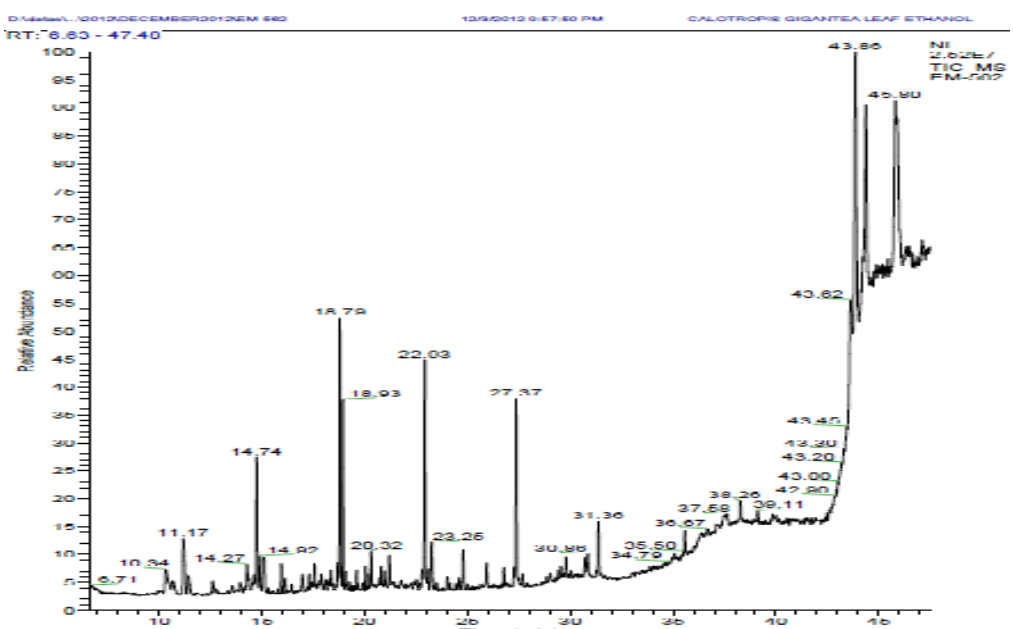
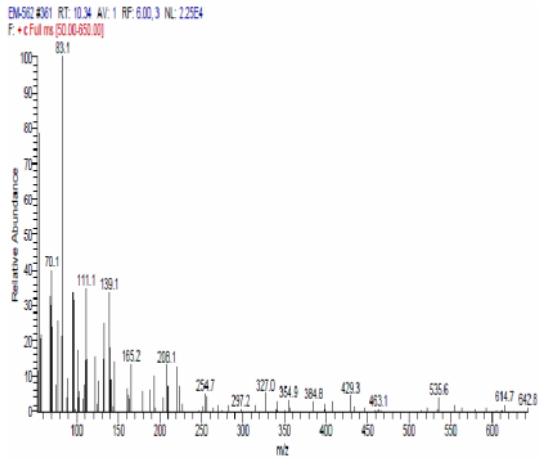
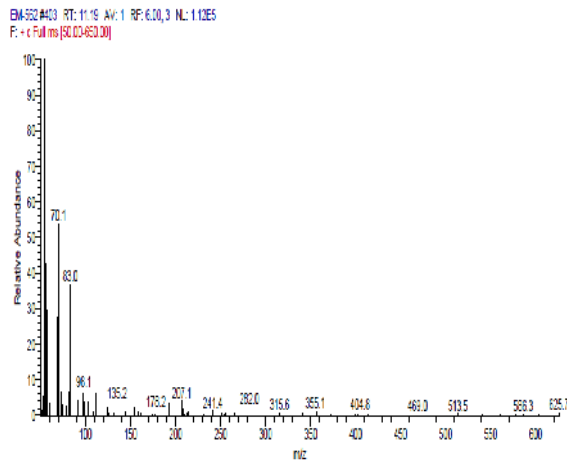


Figure 13

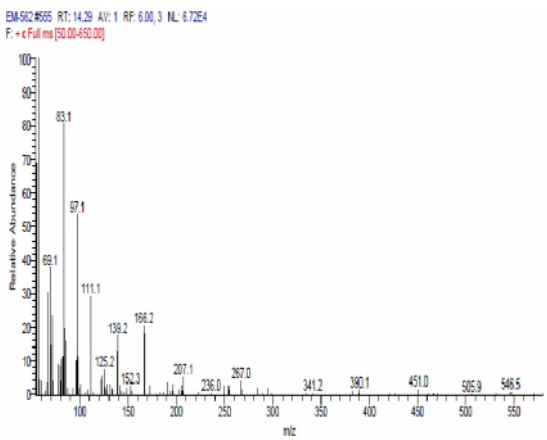
Mass spectrum of ethanol extract of *C. gigantea* leaf



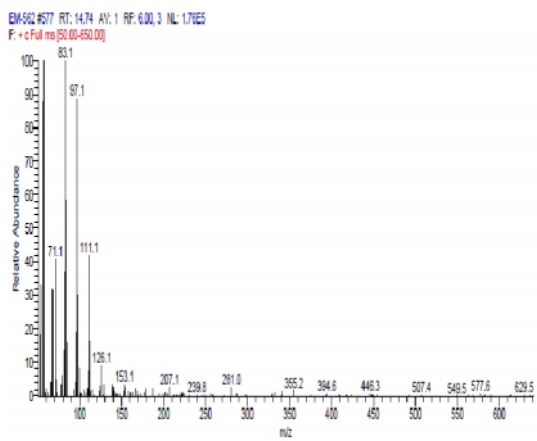
a) RT: 10.34



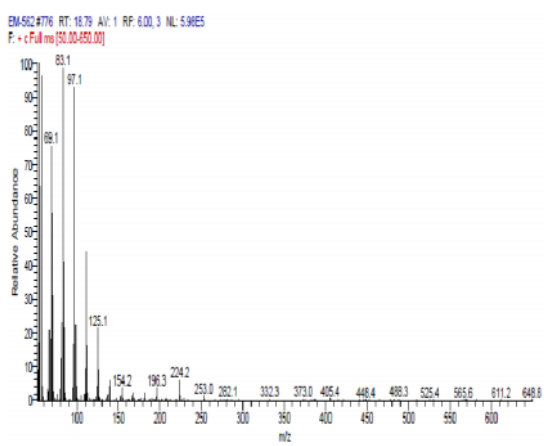
b) RT:11.17



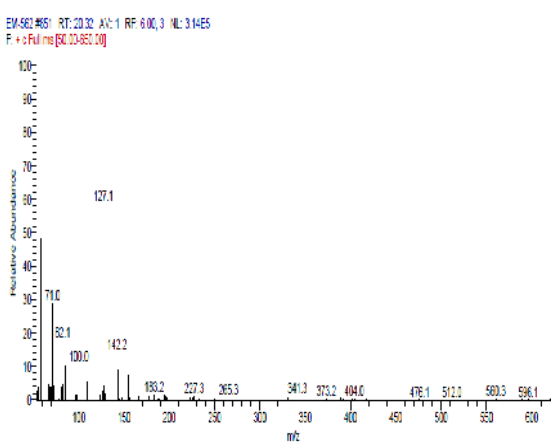
c) RT: 14.27



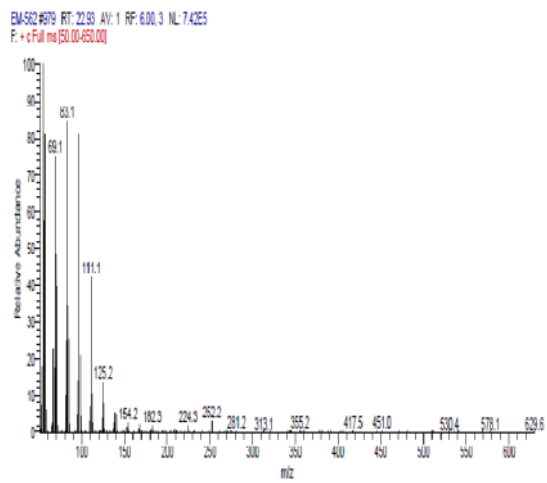
d) RT: 14.74



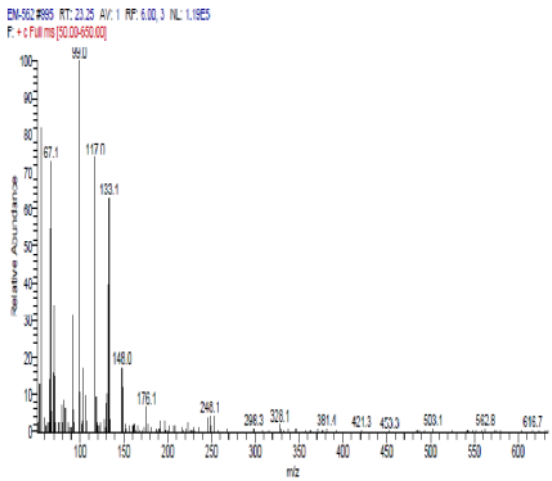
e) RT: 18.79



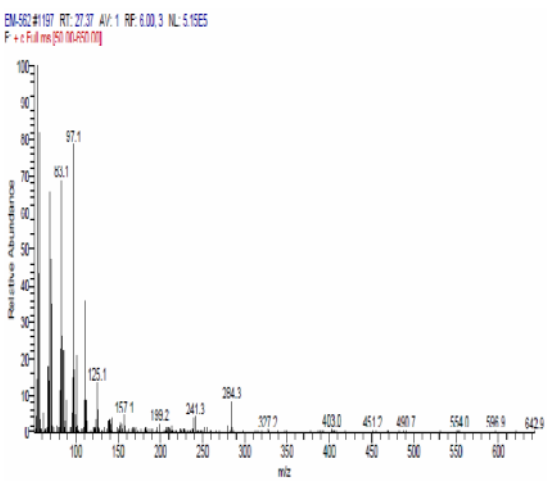
f) RT: 20.32



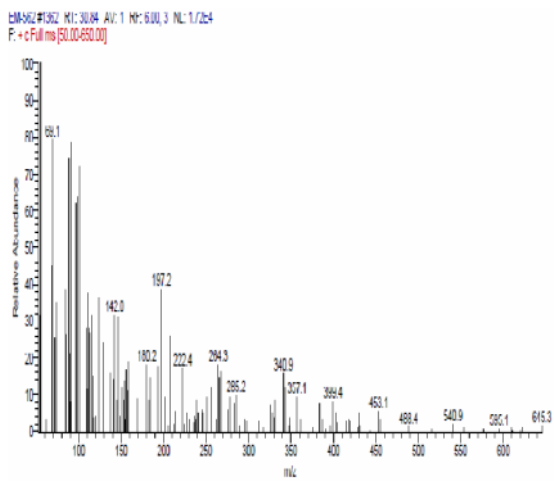
g) RT: 22.93



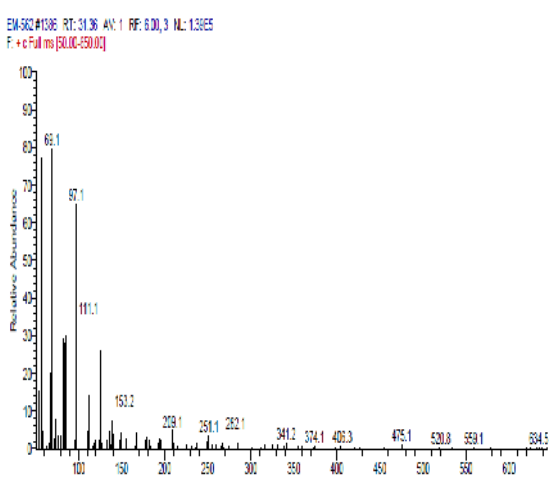
h) RT: 23.25



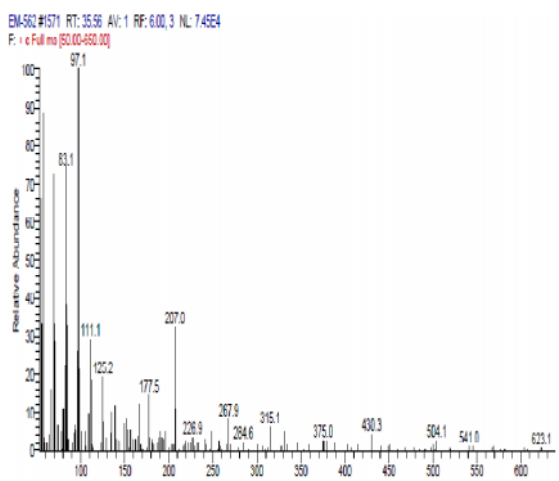
i) RT: 27.37



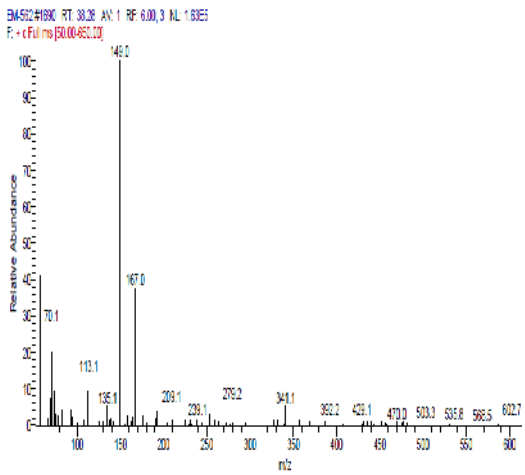
j) RT: 30.86



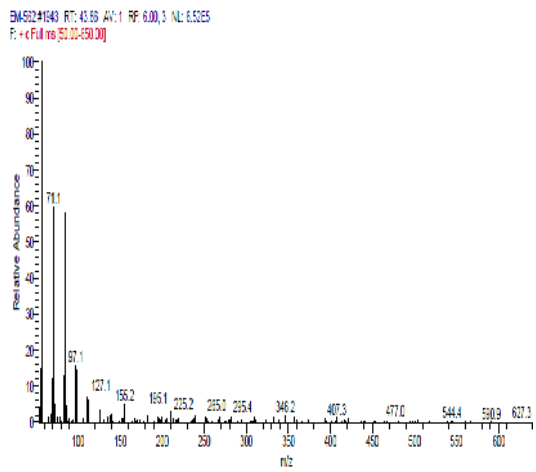
k) RT: 31.36



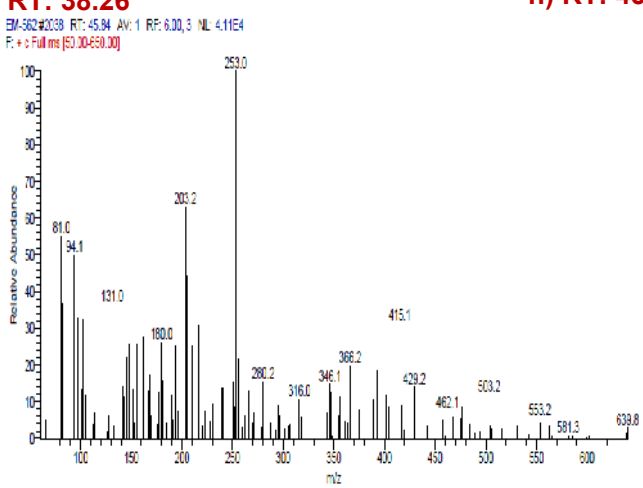
l) RT: 35.50



m) RT: 38.26



n) RT: 43.86



o) RT: 45.80

Figure 14

Gas Chromatogram of chloroform extract of *T. peruviana* leaf

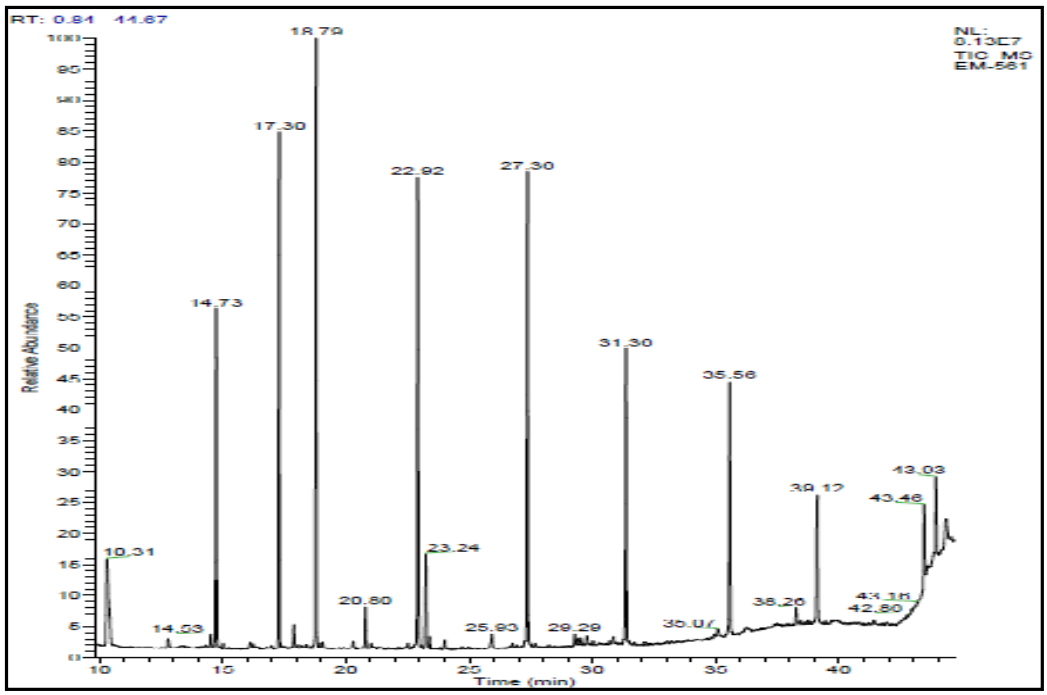
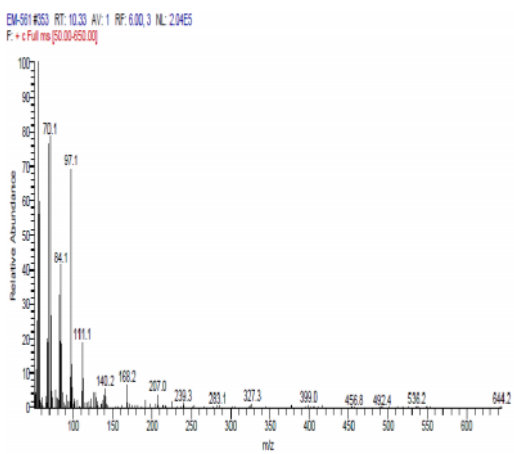
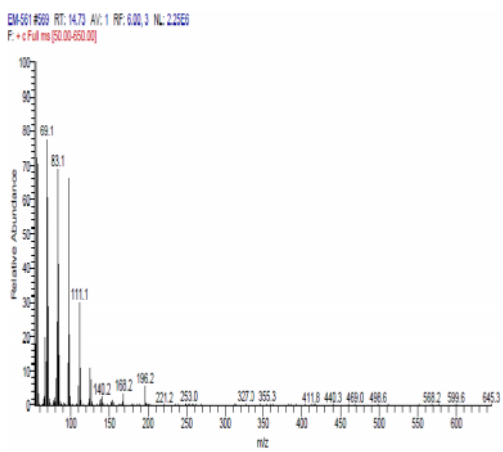


Figure 15

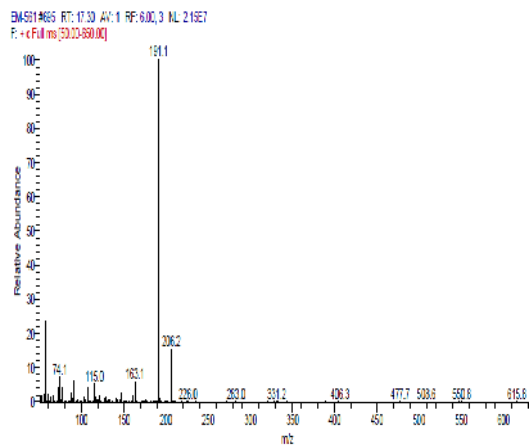
Mass spectrum of chloroform extract of *T. peruviana* leaf



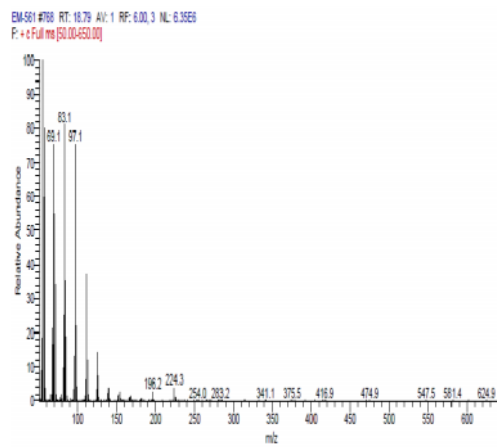
a) RT:10.31



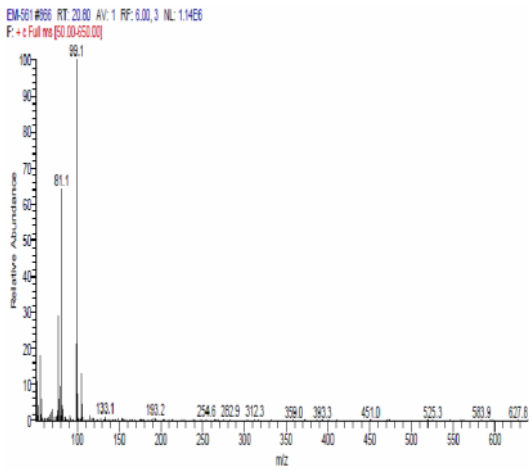
b) RT: 14.73



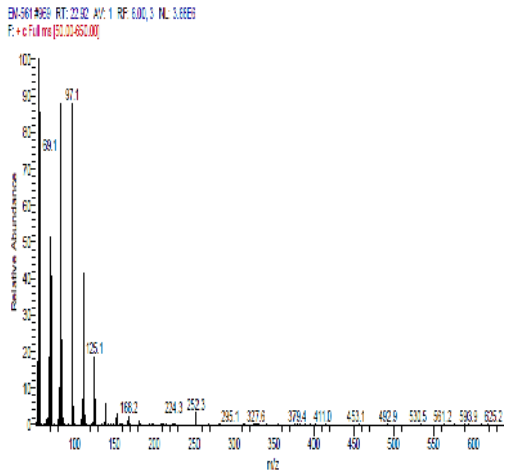
c) RT: 17.30



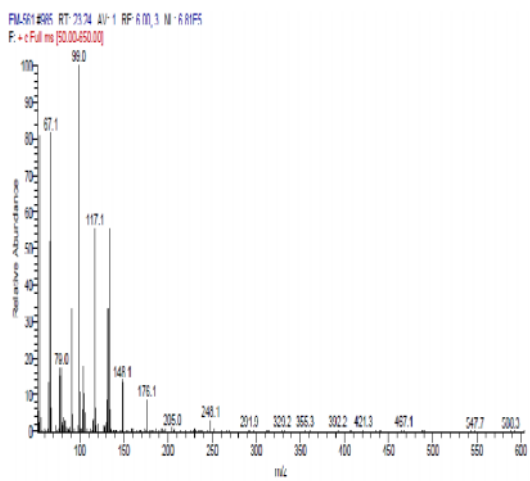
d) RT: 18.79



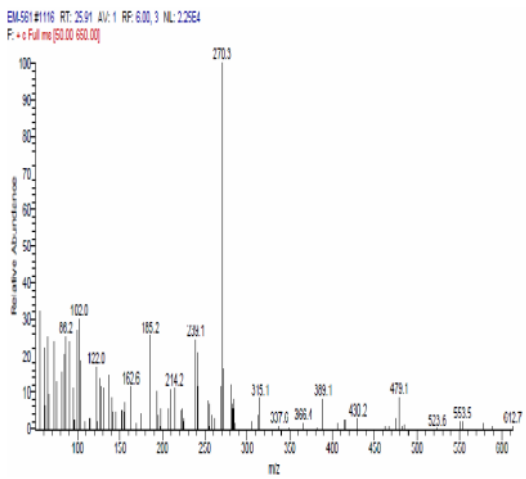
e) RT: 20.80



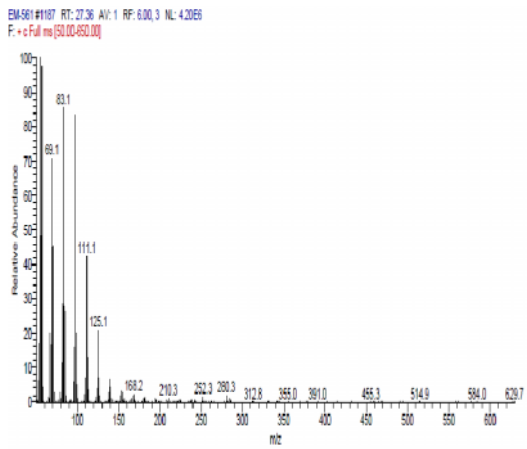
f) RT: 22.92



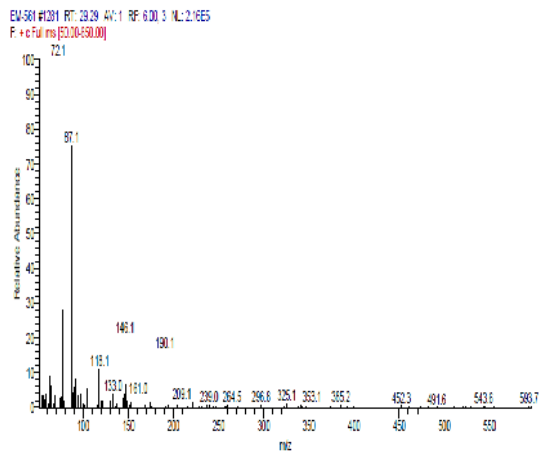
g) RT: 23.24



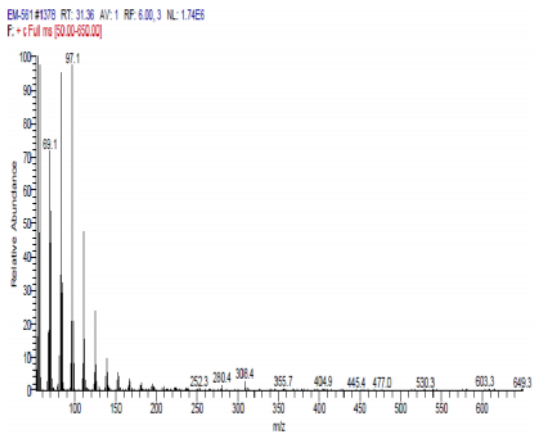
h) RT: 25.93



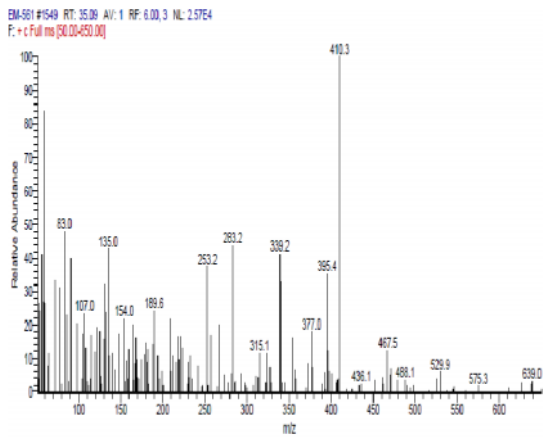
i) RT: 27.36



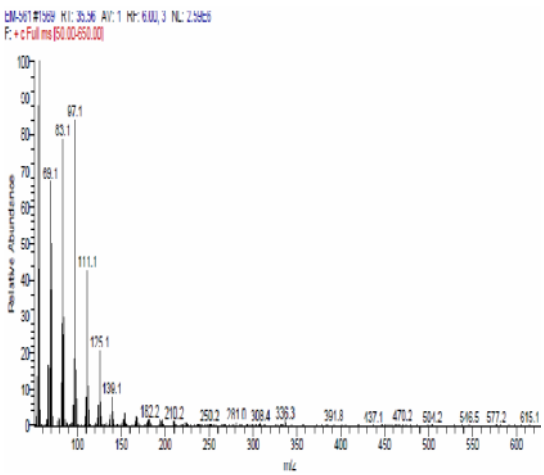
j) RT: 29.29



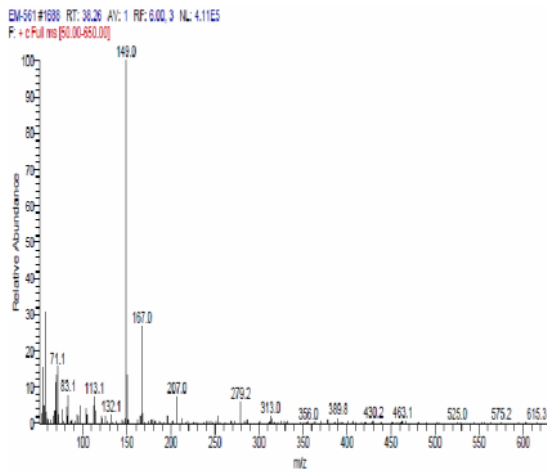
k) RT: 31.36



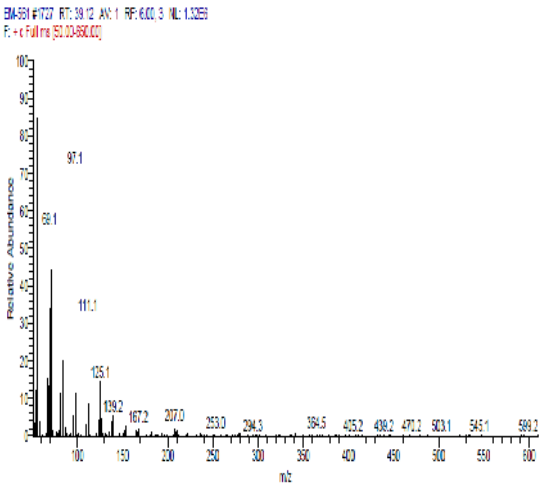
l) RT: 35.07



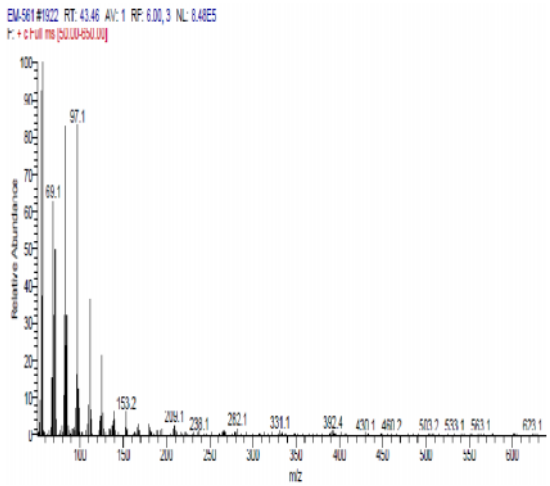
m) RT:35.56



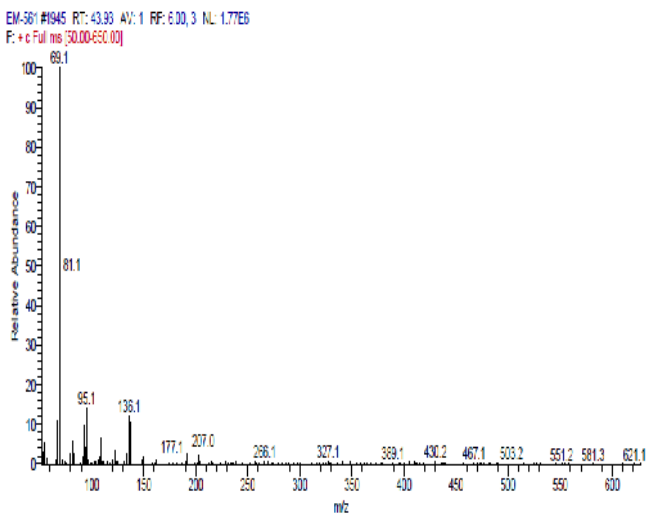
n) RT: 38.26



o) RT: 39.12



p) RT: 43.46



q) RT: 43.93

Figure 16

Gas Chromatogram of ethanol extract of *C. gigantea* flower

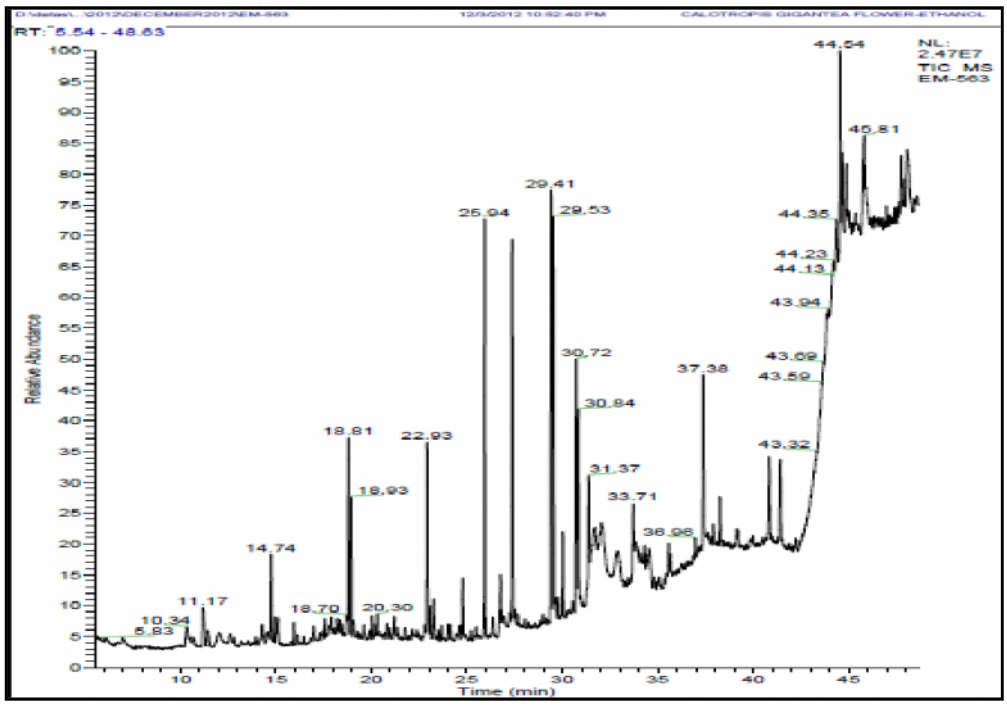
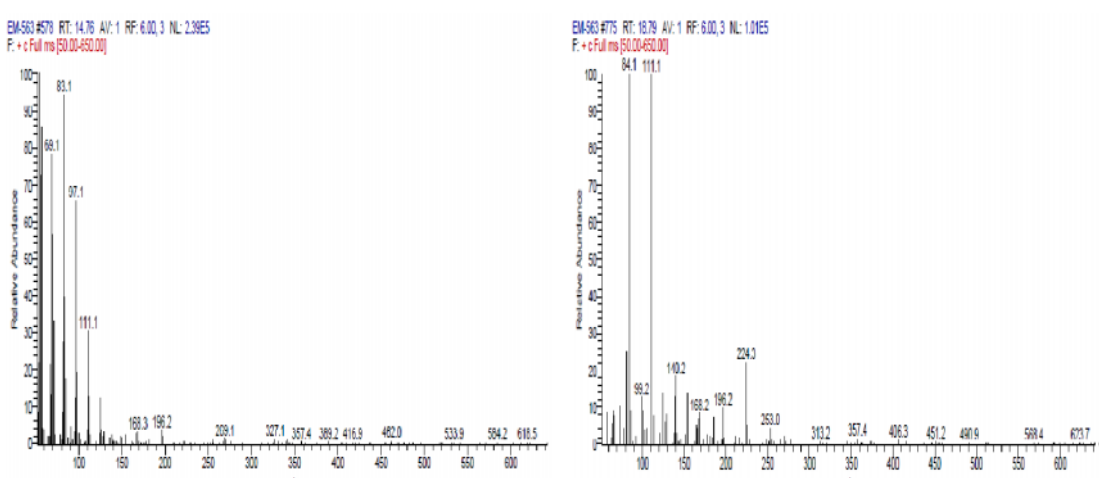
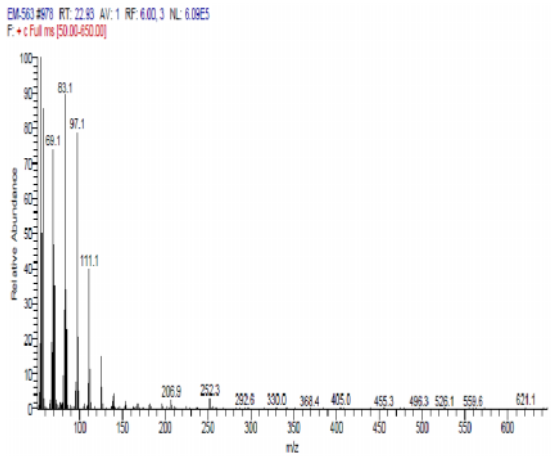


Figure 17

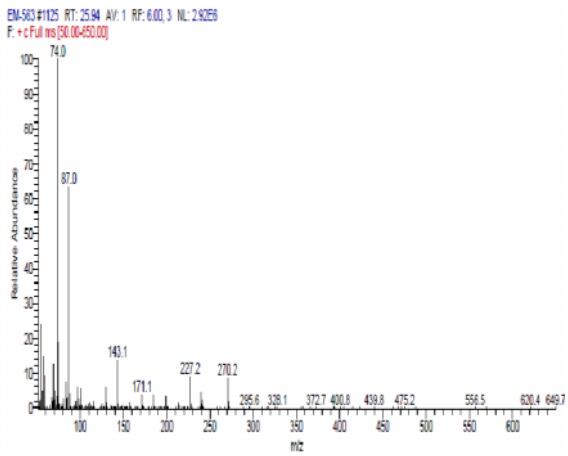
Mass spectrum of ethanol extract of *C. gigantea* flower



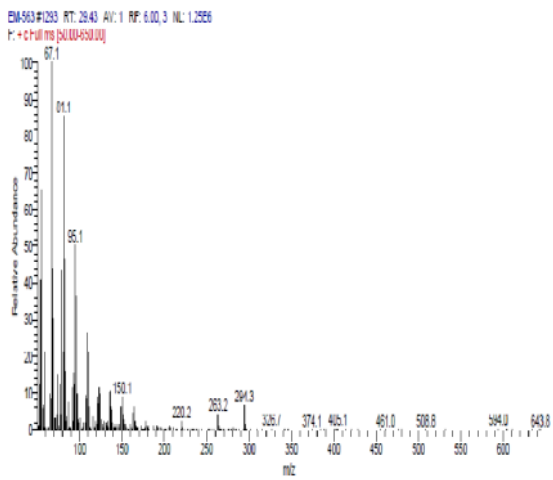
a) RT: 14.74 **b) RT: 18.81**



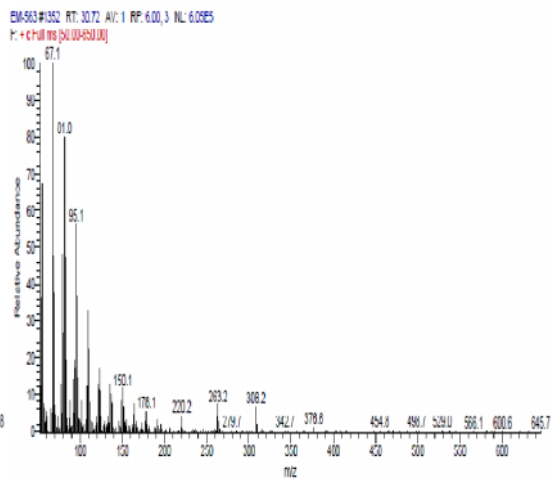
c) RT: 22.93



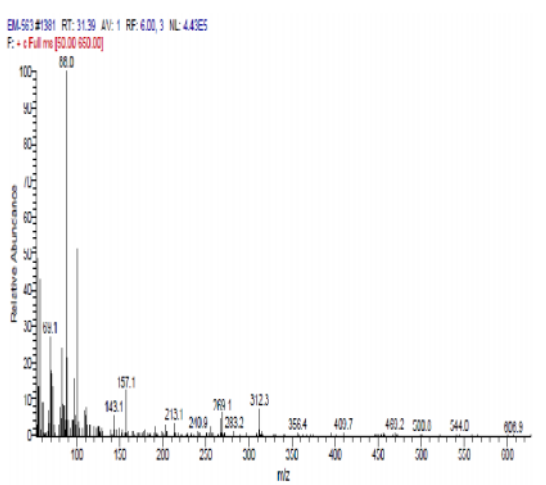
d) RT: 25.94



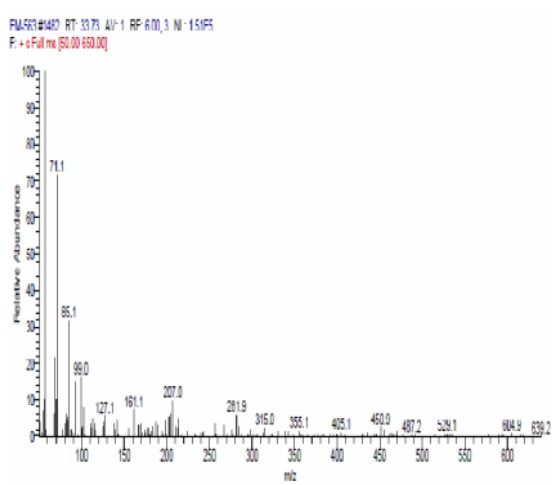
e) RT: 29.41



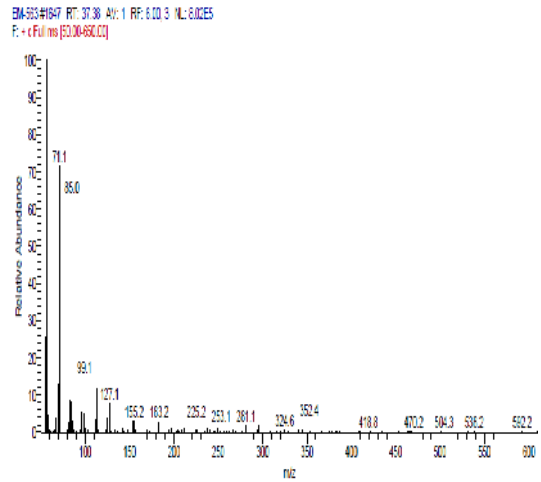
f) RT: 30.72



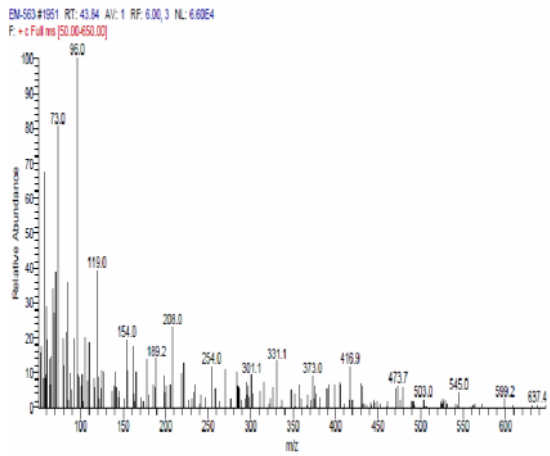
g) RT: 31.37



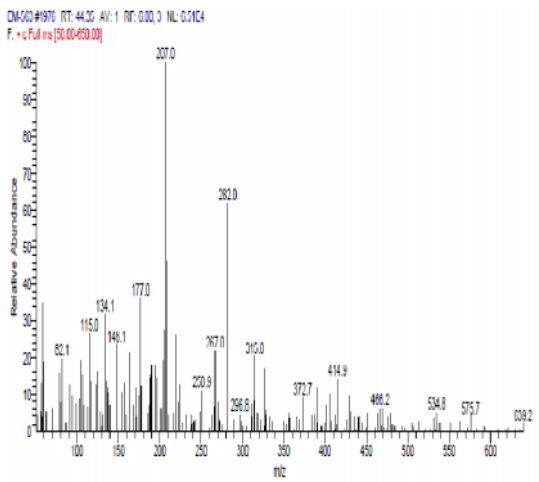
h) RT: 33.71



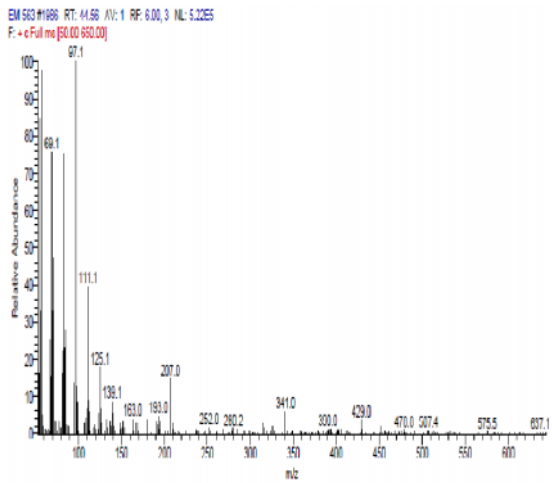
i) RT: 37.38



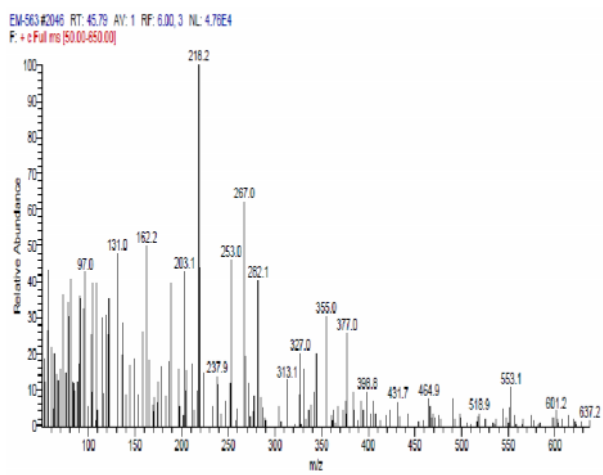
j) RT: 43.94



k) RT: 44.35



l) RT: 44.54



m) RT: 45.81

Figure 18

Gas Chromatogram of chloroform extract of *T. erecta* flower

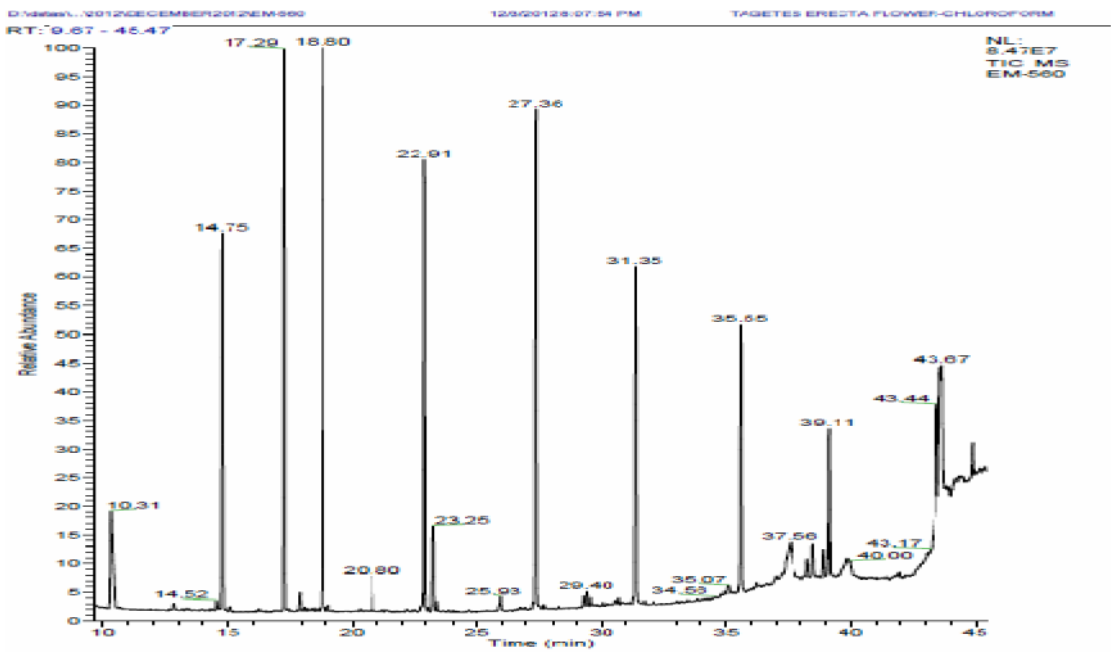
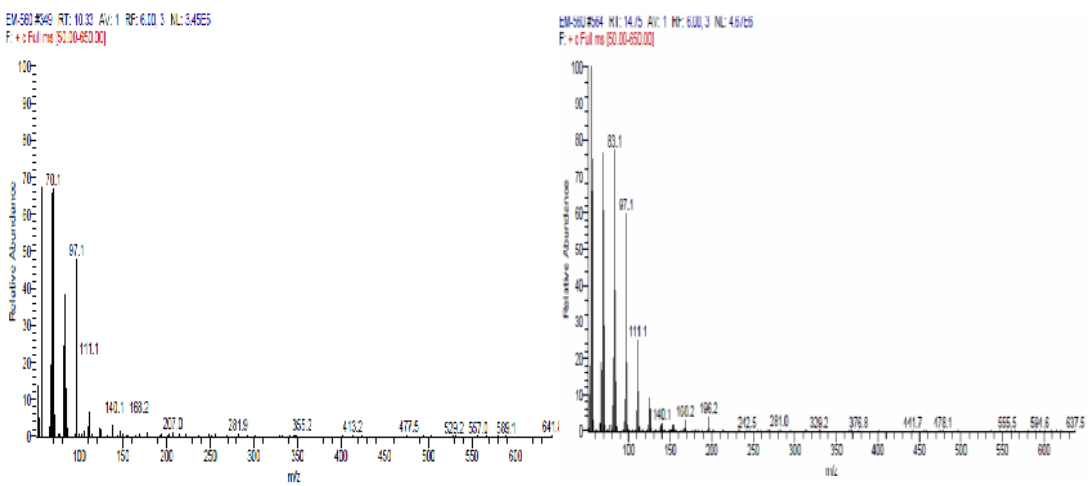
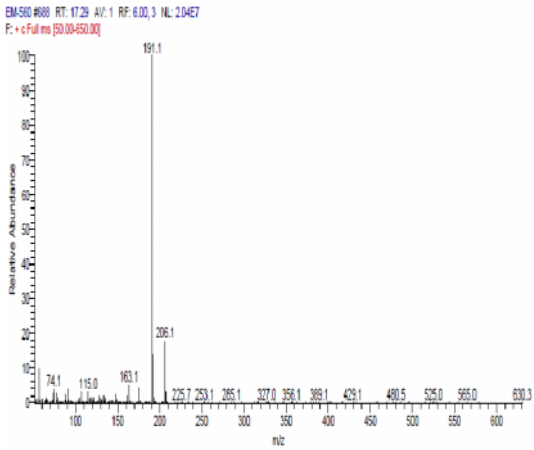


Figure 19

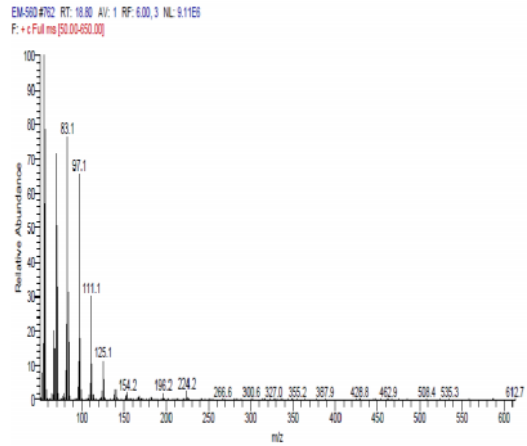
Mass spectrum of chloroform extract of *T. erecta* flower



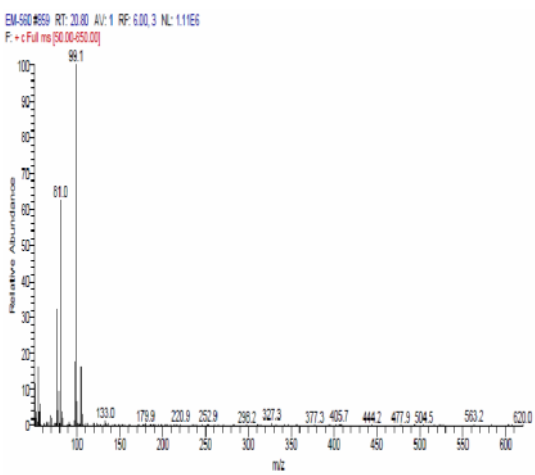
a) RT: 10.31 **b) RT: 14.75**



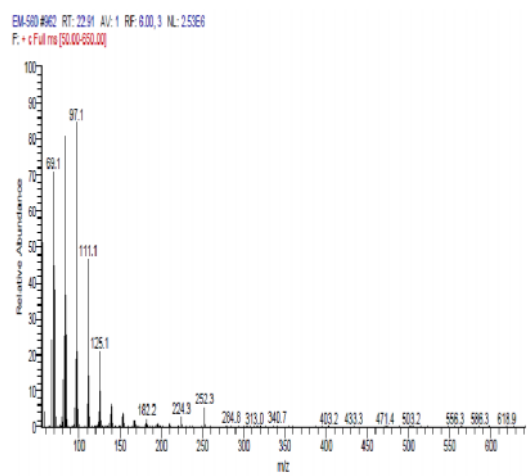
c) RT: 17.29



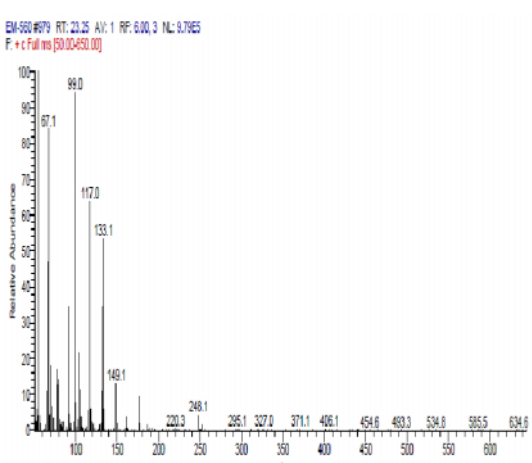
d) RT: 18.80



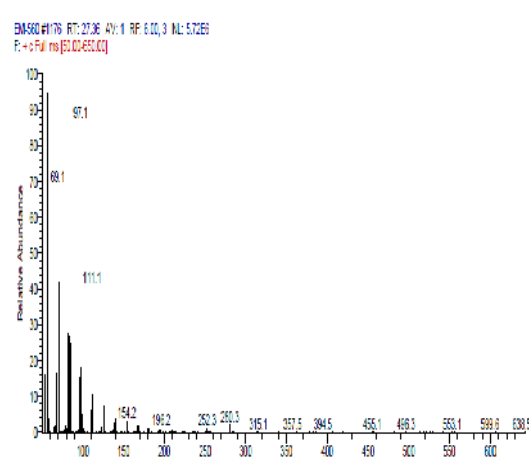
e) RT: 20.80



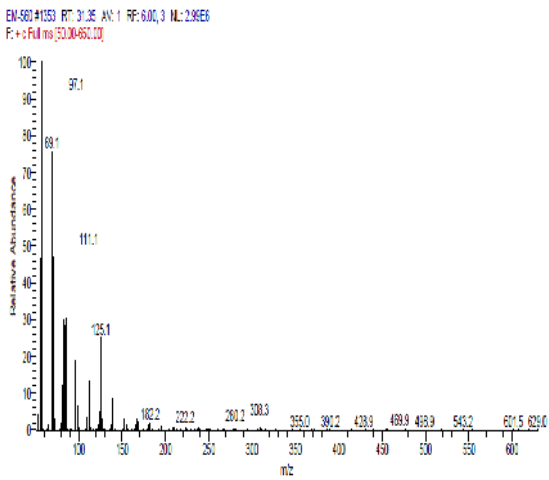
f) RT: 22.91



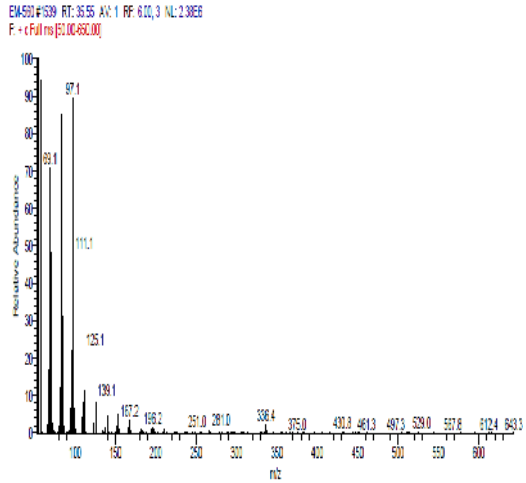
g) RT: 23.25



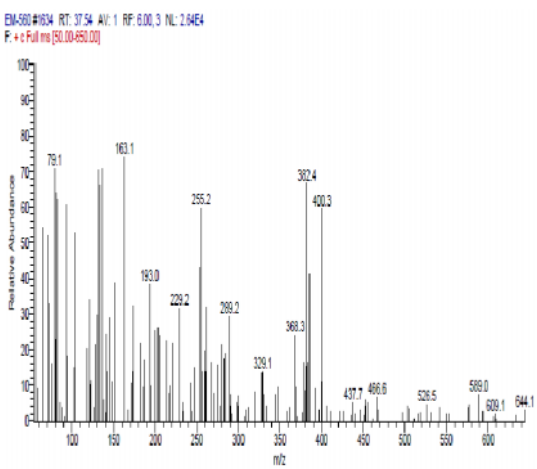
h) RT: 27.36



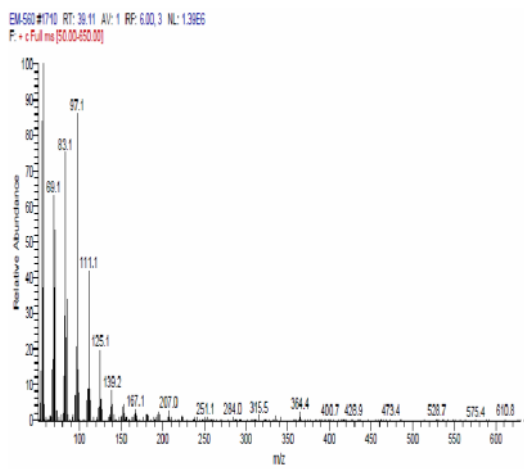
i) RT: 31.35



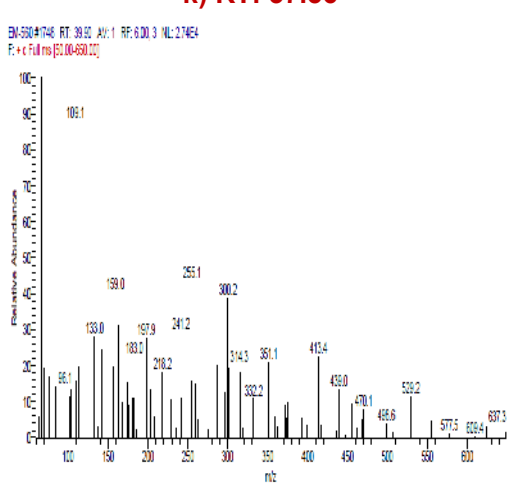
j) RT: 35.55



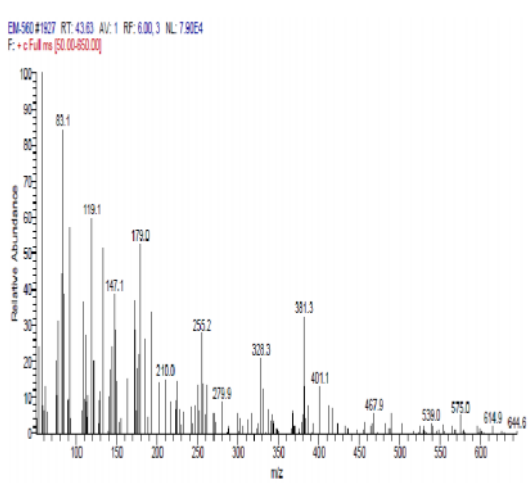
k) RT: 37.56



l) RT: 39.11



m) RT: 40.00



n) RT: 43.67

The fragmentation pattern of the peak at retention time 29.29 (Fig. 15j) recorded base peak at 72.1. Other peaks were observed in the spectrum at m/e 190.1, m/e 146.1, m/e 118.1 and m/e 87.1. The (M-44) peak at m/e 146.1 indicated the presence of carboxylic acid group in the compound. The (M-28) at m/e 118.1 indicated the presence of carbonyl group and (M-15) peak at m/e 72.1 revealed the occurrence of methyl group in the compound.

Retention time of 35.07 in the mass spectrum displayed base peak at m/e 410.3 (Fig. 15l). Other peaks observed were at m/e 395.4, m/e 377.0, m/e 339.2, m/e 315.1, m/e 283.2, m/e 253.2, m/e 189.6, m/e 154.0, m/e 135.0, m/e 107.0 and m/e 83.0. The (M-15) peak at m/e 395.4 denoted the presence of methyl group in the compound. The (M-18) and (M-28) peaks at m/e 377.0 and m/e 107.0 showed the presence of hydroxyl group and carbonyl group in the compound respectively.

Base peak at m/e 97.1 was recorded in the mass spectrum of the peak at retention time 39.12. The two (M-14) peaks at m/e 111.1 and m/e 97.1 indicated the presence of hydrocarbon in the compound and (M-28) peak at m/e 69.1 indicated the presence of carbonyl group in the compound (Fig. 15o). Other peaks observed in the spectrum were at m/e 125.1 and m/e 111.1.

At retention time 25.93 (Fig. 15h) base peaks were seen at m/e 270.3 in the mass spectrum. Other characteristic peaks observed were at m/e 369.1, m/e 315.1, m/e 239.1, m/e 214.2, m/e 185.2, m/e 162.6, m/e 122.0, m/e 102.0 and m/e 86.2. The (M-14) peak at m/e 270.3 showed the presence of hydrocarbon.

Ethanol extract of *C. gigantea* flower

The GC-MS analysis of the ethanol extract of *C. gigantea* flower showed 13 major peaks (Fig. 16) at retention time 14.74, 18.81, 22.93, 25.94, 29.41, 30.72, 31.37, 33.71, 37.38, 43.94, 44.35, 44.54, 45.81. The mass spectrum of the peak at retention time 14.74 and 22.93 is shown in figure 17a and 17c. The fragmentation pattern showed (M-14) peaks only at m/e 97.1, m/e 83.1 and m/e 69.1 denoting the presence of hydrocarbon in the compound.

The mass spectrum of the peak at retention time 18.81 noted base peak at m/e 84.1. Other characteristic peaks in the spectrum were noted in m/e 224.3, m/e

196.2, m/e 168.2, m/e 140.2, m/e 111.1, m/e 99.2 and m/e 84.1. Three (M-28) peaks were seen at m/e 196.2, m/e 168.2 and 140.2 respectively which indicated the presence of carbonyl group. The (M-15) peak was seen at m/e 84.1 which is the characteristic for methyl group (Fig. 17b).

At retention time 25.94 base peak was observed at m/e 74.0 in the mass spectrum. Other peaks included in the spectrum were observed in m/e 227.2, m/e 171.1, m/e 143.1 and m/e 87.0. The presence of (M-28) peak at m/e 143.1 and (M-13) peak at m/e 74.0 showed the presence of carbonyl group and methyl group in the compound. Two (M-14) peaks at m/e 81.1 and m/e 76.1 and two (M-14) peaks at m/e 85.1 and m/e 71.1 were noted in the mass spectrum of the peak at retention time 29.41 and 33.71 (Fig. 17e & h) respectively, which indicated the presence of hydrocarbon in the compound.

Base peak was recorded at m/e 67.1 in the mass spectrum (Fig. 17f) of the peak at retention time 30.72. The presence of (M-45) peak at m/e 263.2 revealed the presence of carboxylic acid functional group in the compound. The presence of (M-28) peak at m/e 150.1 showed the presence of carbonyl group and the presence of two (M-14) peak at m/e 81.0 and m/e respectively indicated the presence of hydrocarbon in the compound.

At retention time 31.37 the presence of (M-19) peak at m/e 69.1 denoted the presence of hydroxyl group in the compound. The mass spectrum displayed the presence of base peak at m/e 88.0 (Fig. 17g). Base peak was recorded at m/e 71.1 and m/e 97.1 in the mass spectrum at retention time 37.38 (Fig. 17i). The presence of (M-28) peak at m/e 127.1, m/e 99.1 and m/e 69.1 indicated the presence of carbonyl group. The presence of (M-14) peak at m/e 125.1, m/e 111.1, m/e 97.1, m/e 85.0 and m/e 71.1, indicated the presence of hydrocarbon in the compound.

The fragmentation pattern of the peak at retention time 43.94 (Fig. 17j) exhibited base peak at m/e 96.0. The (M-44) peak at m/e 373.0 indicated the presence of carboxylic acid group in the compound. Other peaks were recorded in m/e 119.0, m/e 96.0 and m/e 73.0. The base peak was observed at m/e 207.0 in

the fragmentation pattern of the peak at retention time 44.35 (Fig. 17k). Other peaks observed in the spectrum include m/e 372.7, m/e 315.0, m/e 282.0, m/e 267.0, m/e 250.9, m/e 207.0, m/e 177.0, m/e 148.1, m/e, 134.1, m/e 115.0 and m/e 82.1. The (M-15) peak at m/e 267.0 denoted the presence of methyl group in the compound. The presence of (M-44) peak at m/e 207.0 indicated the presence of carboxylic acid functional group in the compound. The (M-14) peak at m/e 134.1 showed the occurrence of hydrocarbon in the compound.

The mass spectrum of the peak at retention time 44.54 (Fig. 17l) displayed base peak at m/e 97.1. Three (M-14) peaks at m/e 125.1, m/e 111.1 and m/e 97.1 showed hydrocarbon in the compound. The (M-28) peak denoted the occurrence of carbonyl group in the compound. At retention time 45.81, the mass spectrum indicated the presence of peaks at m/e 464.9, m/e, 431.7, m/e 398.8, m/e 377.0, m/e 355.0, m/e 327.0, m/e 313.0, m/e 282.1, m/e 267.0, m/e 253.0, m/e 237.9, m/e 218.2, m/e 203.1, m/e 162.2, m/e 131.0 and m/e 97.0. The base peak was noted at m/e 218.2. The (M-28) peak at m/e 327.0 indicated the presence of carbonyl group in the compound. Presence of two (M-14) peaks at m/e 313.1 and m/e 253.0 and three (M-15) peaks at m/e 267.0, m/e 237.9 and m/e 203.1 denoted the presence of hydrocarbon in the compound (Fig. 17m).

Chloroform extract of *T. erecta* flower

The GC-MS analysis of chloroform extract of *T. erecta* flower showed 14 major peaks at retention time 10.31, 14.75, 17.29, 18.80, 20.80, 22.91, 23.25, 27.36, 31.35, 35.55, 37.56, 39.11, 40.00 and 43.67 (Fig. 18). The mass spectrum of the peak at retention time 10.31 (Fig. 19a) exhibited base peak at m/e 70.1. The fragmentation pattern showed (M-14) peak indicating the presence of hydrocarbon and (M-27) peak indicating the presence of nitrogen in the compound.

The mass spectrum displayed base peak at m/e 83.1 and m/e 97.1 at retention time 14.75 and 39.11 (Fig. 19a & l) respectively. Other peaks in the mass spectrum were displayed at m/e 125.1, m/e 111.1, m/e 97.1, m/e 83.1 and m/e 69.1. The fragmentation pattern showed only (M-14) peaks. Hence, the compound may be hydrocarbon. At retention time 17.29 the base peak was noted at m/e 191.1

in the mass spectrum. The presence of (M-15) peak denoted methyl group in the compound (Fig. 19c).

At retention time 18.80 (Fig. 19d), base peak was recorded at m/e 83.1 in the mass spectrum. The other characteristic peaks in the spectrum were observed in m/e 125.1, m/e 111.1, m/e 97.1 and m/e 83.1. The fragmentation pattern showed the presence of (M-14) peak indicating the presence of hydrocarbon in the compound. The mass spectrum recorded base peak at m/e 99.1 in the retention time 20.80 and 23.25 (Fig. 19e & g). Other peaks were seen at m/e 149.1, m/e 133.1, m/e 117.0, m/e 88.0 and m/e 67.1. The characteristic (M-18) peak denoted the presence of hydroxyl group in the compound.

The (M-14) peak and (M-28) peak in the mass spectrum at retention time 22.91 (Fig. 19f) clearly indicated hydrocarbon and carbonyl group in the compound. The base peak was seen at m/e 97.1. The fragmentation pattern in the mass spectrum of the peak at retention time 27.36, 31.35 and 35.55 (Fig. 19h, i & j) exhibited (M-14) peak at m/e 97.1, m/e 111.1 and m/e 125.1 denoting the presence of hydrocarbon fragment in the compound. The (M-28) peak at m/e 69.1 and m/e 97.1 in the mass spectrum indicated the presence of carbonyl group respectively. Other characteristic peaks were seen at m/e 139.1, m/e 125.1, m/e 111.1, m/e 97.1 and m/e 69.1.

The mass spectrum of the peak at retention time 37.56 showed in figure 19k exhibited base peak at m/e 163.1. The fragmentation pattern showed (M-14) peak at m/e 368.3 reporting the occurrence of hydrocarbon fragment in the compound. (M-28) peak at m/e 437.7 showed the presence of carbonyl group in the compound.

Two (M-14) peaks were observed in the mass spectrum of the peak at retention time 40.00 at m/e 300.2 and m/e 183.0. The peaks denoted the presence of hydrocarbon in the compound. The presence of (M-18) peak may be due to the presence of hydroxyl group in the compound (Fig. 19m). The base peak was exhibited at m/e 109.1. The mass spectrum of the peak (Fig. 19n) at retention time

43.67 exhibited base peak at m/e 83.1. The (M-28) peak at m/e 119.1 indicated the presence of carbonyl group in the compound.

Discussion

The identification of organic compounds from the extracts of plants is of great importance, mainly because they can be used as an excellent source of pharmaceutical products. Since plant extracts are complex mixtures of organic compounds, they need appropriate fractionation techniques to allow a better analysis of their individual constituents (Jacques *et al*., 2007). The functional properties such as antibacterial, antifungal and insecticidal activities of essential oils and plant extracts are widely used in processed food preservation, pharmaceuticals, cosmetics, alternative medicine and natural therapies (Bakkali *et al*., 2008). The pharmacological properties, such as anti-inflammatory, antioxidant, antispasmodic, anti-hemorrhoidal, antimalarial, anticoagulant, hair tonic, insect repellent, anti-diarrhea and anti-dysentery activities of different species of plants have been reported by many researchers (Candan *et al*., 2003; Thakong, 1999; Saraf *et al*., 1991; Abubakar *et al*., 2011; Kasturi *et al*., 1973).

The GC-MS is an ideal technique for qualitative and quantitative analysis of volatile and semi volatile compounds (Iordache *et al*., 2009). Therefore, the present investigation is an attempt to screen the phytochemicals and also to isolate the functional groups present in the selected plants, to evaluate the bioactive potential and to characterize them by GC-MS analysis.

Some studies had established that long chain aliphatic methyl ketones showed repellence to arthropods, including blood-sucking insects (Ndungu *et al*., 1995; Blum *et al*., 1966; Torr *et al*., 1996; Barton, 2003; Roe, 2004; Gikonyo *et al*., 2002). The efficacy of aliphatic methyl ketones against *An. gambiae*, a malaria vector, in a study found that odd-carbon compounds including 2-pentadecanone were more effective than even-carbon compounds especially at high concentrations. The C15 compound was compared favourably with DEET a repellent to various insects including mosquitoes (Innocent *et al*., 2008).

A compound 6,10,14-trimethyl-2-pentadecanone, which is a C15 aliphatic methyl ketone was revealed by Ogunlesi *et al* (2009) by carrying out GC-MS analysis in the dried leaves of *Euphorbia hirta.* The plant was found to be effective repellent against *Anopheles* species because of the presence of 6,10,14 -trimethyl-2-pentadecanone. This result can be compared to the present study in which the presence of various secondary metabolites with repelling efficacy may have helped in repelling the *C. quinquefasciatus* mosquito.

A study carried out by Jemaa *et al* (2011) reported the chemical composition and the repellent activity of *Laurus nobilis* essential oil against adults of the cigarette beetle *Lasioderma serricorne*. Essential oil chemical composition was assessed via, GC-MS analysis. 1,8-cineole, linalool, eugenylmethylether, isovaleraldehyde and camphene were the major compounds. Similar observations were recorded in the present investigation in which the phytochemical constituents present in the ethanol extract of *C. gigantea* leaf and flower, chloroform extract of *T. peruviana* leaf and chloroform extract of *T. erecta* flower exerted 100% repellent efficacy against the target mosquito.

Results of our study can be compared favorably with other investigations in which the selected flower and leaf extracts produced significant activity against pest insects. In this context, Erler *et al* (2006) reported repellent activity of *Laurus nobilis* essential oil against the adult females of *C. pipiens*. Besides, Cosimi *et al* (2009) demonstrated the repellency of some essential oils from Mediterranean plants against three stored beetles *Sitophilus zeamais*, *Cryptolestes ferrugineus* and *Tenebrio molitor*. Additionally, Papachristos and Stampoulos (2002) showed that essential oil from *L. nobilis* presented a repellent activity against *Acanthoscelides obtectus*.

In the present investigation the selected plant and leaf extracts which were subjected to GC-MS analysis possessed excellent ovicidal and repellent efficacy against the eggs and adults of *C. quinquefasciatus* respectively, which may be due to the efficient phytochemical constituents present in them. These results were in concordance with the findings of Vijaya Kumar *et al* (2011b) who subjected petroleum ether extract of *Annona squamosa* leaf to GC-MS and reported that it

possessed fifteen active compounds and concluded that it may be responsible for the active anti-larvicidal, oviposition deterrent and repellent activity of *A. squamosa* leaves.

An investigation conducted by Zibbu and Batra (2011) reported that the highest peak area of 17.05 was obtained by 2-Nonanol at retention tine 18.87 in the methanol extract of *Nerium oleander*. Whereas, methanol extract of another plant *T. peruviana* provided the highest peak area of 48.83% for dipropyl ether at RT 12.62. In the present study, the GC-MS analysis of the chloroform extract of *T. peruviana* produced 17 major peaks in the mass spectrum at the retention time ranging from 10.31 - 43.93.

The bioactive compounds of *Indigofera aspalathoides* have been evaluated using GC-MS by Abirami and Rajendran (2011a). GC-MS analysis of the methanol extract of *I. aspalathoides* showed the presence of ten major peaks and revealed the existence of the two major compounds. Similar to our studies Kalaivani *et al* (2012) aimed to examine the phytochemical constituents present in *Andrographis paniculata* by GC-MS analysis. The results revealed the presence of thirteen major peaks thereby revealing the presence of thirteen major compounds. Ghannadi and Dezfuly (2011) also identified seventeen compounds from *Myrtus communis*. Chibani *et al* (2011) characterized eighteen compounds from *Ferula communis* using GC-MS analysis.

Sampathkumar and Ramakrishnan (2011) revealed the presence of medicinally active constituents such as carbohydrates, protein, lipids, phenols, flavonoids, saponin, alkaloid and quinone in the ethanol extract of *Naringi crenulata*. Hashemi *et al* (2008) reported the presence of phytochemicals in *Euphorbia hirta* namely volatile oil, alkaloids, tannins, saponins and steroids. These findings were similar to the observations made in the present study that also revealed the presence of various secondary metabolites such as alkaloids, tannins, phenols, flavonoids, sterols, proteins etc in the tested plant extracts.

Velanganni *et al* (2011) reported fourteen major compounds from the ethanol root extract of *Mallotus philippensis*. Diethyl phthalate was present as the major

constituent eluted as a major peak at retention time 11.25. Patil and Rajput (2012) identified twenty compounds in RRT 31.87 and sixteen compounds in RRT 32.86 in the GC-MS analysis of chloroform extract of leaves of *Butea monosperma.* Similarly in the present study, the ethanol extract of *C. gigantea* leaf produced fourteen major peaks and corresponding retention times were recorded. These results were in agreement with the earlier studies done with other plant species of *Asteraceae* family (Boukamcha *et al*., 2004; Oueslati *et al*., 2004).

Tasdemir *et al* (2003) detected and identified a total of 200 compounds from organic extracts, while the water extracts contained only traces of few volatiles. The ethanol extract of the *Rhododendron luteum* flowers was found to exhibit the most diverse composition of thirty four compounds. Ethanol was highly volatile and capable of dissolving a wide class of flavours and volatiles as reported by Ceva-Antunes *et al* (2003). The stronger extraction capacity of ethanol could have produced number of active constituents responsible for many biological activities. In the present study also, the ethanol extract of *C. gigantea* leaf and flower extract showed sixteen and thirteen major peaks respectively in the mass spectrum. Ethanol is a polar solvent that has an ability to extract most of the bio-active compounds such as flavonoids and tannins (Nino *et al*., 2006).

Phytochemical screening studies have been carried out in different parts using GC-MS (Wu *et al*., 2010; Vohra and Kaur, 2011; Sangeetha and Vijayalakshmi, 2011; Janakiraman *et al*., 2012). In the present study, the gas chromatogram showed the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicated the relative concentrations of the components present in the plant. The mass spectrometer analyzes showed the compounds eluted at different times to identify the nature and structure of the compounds. The large compound, fragments into small compounds giving rise to appearance of peaks at different m/z ratios. In addition to this, the results of the GC-MS profile can be used as pharmacognostical tool for the identification of the plant. The result of the present study supported and supplemented the previous observations (Wu *et al*., 2010; Vohra and Kaur, 2011; Sangeetha and Vijayalakshmi, 2011; Roy *et al*., 2010).

The GC-MS result revealed the presence of the functional groups namely hydrocarbon, methyl, hydroxyl, nitrogen, carbonyl and carboxylic acid in the ethanol leaf and flower extract of *C. gigantea*, chloroform extract of *T. peruviana* leaf and chloroform extract of *T. erecta* flower. Based on these findings, compounds were selected from PubChem databases for further *in silico* studies.

PHASE IV

4.4 *IN SILICO* ANALYSIS OF PHYTOCHEMICAL COMPOUNDS

Molecular docking is a widely used computational tool for the study of molecular recognition, which aims to predict the binding mode and binding affinity of a complex formed by two or more constituent molecules with known structures. An important type of molecular docking is protein-ligand docking because of its therapeutic applications in modern structure based drug design (Huang and Zou, 2010). Computational biology and bioinformatics have the potential not only in speeding up the drug discovery process thus reducing the costs, but also in changing the way drugs are designed. The site of drug action, which is ultimately responsible for the pharmaceutical effect, is a receptor (Richon, 1994). Docking is frequently used to predict the binding orientation of small molecule, drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule (Prakash *et al*., 2010). Hence, docking plays an important role in the rational design of drugs. Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking (Pratheepa, 2012).

BIOLOGICAL ACTIVITY PREDICTION

Totally fourteen compounds has been selected for docking studies. Out of fourteen compounds, ten were processed. The structure and properties of the ten secondary metabolites were shown in table 33. The structural formulas of the processed ten secondary metabolites were given as an input for PASS server. The PASS server provides all the possible activity of the given secondary metabolites and the activities of the compounds were shown in tables 34 to 43.

Table 33: Structure and properties of secondary metabolites

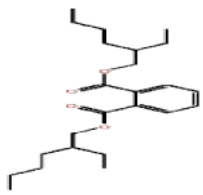
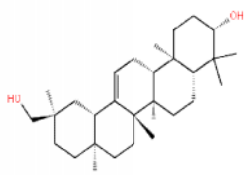
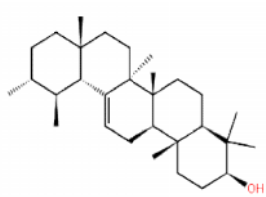
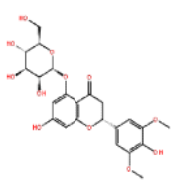
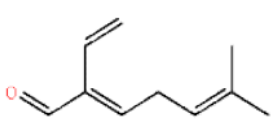
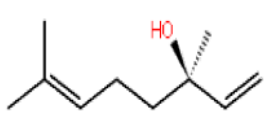
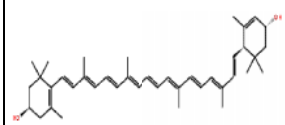
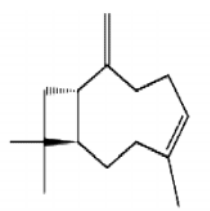
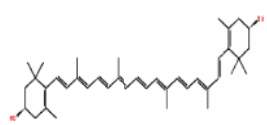
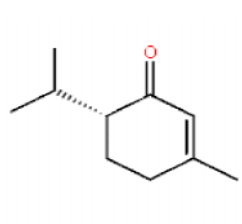
S. No.	Plant Source	Compound Structure	Compound Name	Molecular Formula	Molecular Weight [g/mol]
1	*C. gigantea*		Di (2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.55612
2	*C. gigantea*		Beta amyrin	$C_{30}H_{50}O_2$	442.7168
3	*C. gigantea*		Alpha amyrin	$C_{30}H_{50}O$	426.7174
4	*T. peruviana*		Peruvianoside I	$C_{23}H_{26}O_{12}$	494.44534
5	*T. erecta*		Cis-ocimene	$C_{10}H_{14}O$	150.21756
6	*T. erecta*		Linalool	$C_{10}H_{18}O$	154.24932

Table 33 Contd….

S. No.	*Plant Source*	Compound Structure	Compound Name	Molecular Formula	Molecular Weight [g/mol]
7	*T. erecta*		Lutein	$C_{40}H_{56}O_2$	568.87144
8	*T. erecta*		Beta carophyllene	$C_{15}H_{24}$	204.35106
9	*T. erecta*		Zeaxanthin	$C_{40}H_{56}O_2$	568.87144
10	*T. erecta*		Pipertone	$C_{10}H_{16}O$	152.23344

Each biologically active compound possesses a number of biological activities. Its specificity of action is always relative and is defined by the peculiarities of object, dose, route, etc. On the contrary, the biological potential of compound includes all activities, which can be discovered under some specific experimental conditions. The biological potential is called the biological activity spectrum.

Table 34

Activity of Di (2-ethylhexyl) phthalate

Pa	Pi	Activity	Pa	Pi	Activity
0,973	0,002	Eye irritation, inactive	0,773	0,004	Phenol O-methyltransferase inhibitor
0,949	0,003	Skin irritation, inactive	0,777	0,010	5-O-(4-coumaroyl)-D-quinate 3'-monooxygenase inhibitor
0,927	0,002	Cutinase inhibitor	0,771	0,005	Anesthetic general
0,888	0,005	Sugar-phosphatase inhibitor	0,771	0,010	Carboxypeptidase Taq inhibitor
0,876	0,008	Alkenylglycerophosphocholine hydrolase inhibitor	0,772	0,012	Arginine 2-monooxygenase inhibitor
0,847	0,004	Lipid metabolism regulator	0,793	0,036	Aspulvinone dimethylallyltransferase inhibitor
0,854	0,016	Ubiquinol-cytochrome-c reductase inhibitor	0,764	0,009	IgA-specific serine endopeptidase inhibitor
0,836	0,004	Acetylesterase inhibitor	0,768	0,017	Sphinganine kinase inhibitor
0,848	0,017	Phobic disorders treatment	0,749	0,008	Lipoprotein lipase inhibitor
0,824	0,004	Gluconate 5-dehydrogenase inhibitor	0,769	0,029	CYP2J substrate
0,831	0,014	Acrocylindropepsin inhibitor	0,750	0,012	Exoribonuclease II inhibitor
0,831	0,014	Chymosin inhibitor	0,768	0,044	CYP2C12 substrate
0,831	0,014	Saccharopepsin inhibitor	0,744	0,023	CYP2J2 substrate
0,820	0,004	All-trans-retinyl-palmitate hydrolase inhibitor	0,723	0,002	Sclerosant
0,829	0,015	Polyporopepsin inhibitor	0,727	0,010	Macrophage colony stimulating factor agonist
0,813	0,007	Pullulanase inhibitor	0,730	0,016	Alkylacetylglycerophosphatase inhibitor
0,819	0,015	Antiseborrheic	0,721	0,013	Lysine 2,3-aminomutase inhibitor
0,803	0,014	Pro-opiomelanocortin converting enzyme inhibitor	0,711	0,005	Anthranilate-CoA ligase inhibitor
0,785	0,004	Spasmolytic, Papaverin-like	0,705	0,008	Cholesterol antagonist
0,784	0,012	Membrane permeability inhibitor	0,702	0,006	Poly(beta-D-mannuronate) lyase inhibitor
0,787	0,016	5-Hydroxytryptamine release stimulant	0,704	0,008	Poly(alpha-L-guluronate) lyase inhibitor
0,797	0,026	Testosterone 17beta-dehydrogenase (NADP+) inhibitor	0,703	0,021	Fibrinolytic

Pa – probability of active Pi – probability of inactive

Table 35

Activity of Beta amyrin

Pa	Pi	Activity	Pa	Pi	Activity
0,974	0,002	Caspase 3 stimulant	0,816	0,001	ICAM1 expression inhibitor
0,957	0,002	Insulin promoter	0,823	0,015	Alkenylglycerophosphocholine hydrolase inhibitor
0,938	0,002	Hepatoprotectant	0,808	0,002	Antinociceptive
0,934	0,001	Transcription factor NF kappa B stimulant	0,806	0,004	Antineoplastic (lung cancer)
0,934	0,001	Transcription factor stimulant	0,790	0,004	Antiulcerative
0,912	0,005	Antineoplastic	0,794	0,027	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
0,906	0,002	Antiviral (Influenza)	0,766	0,003	Chitinase inhibitor
0,903	0,002	Oxidoreductase inhibitor	0,763	0,004	Lipid peroxidase inhibitor
0,899	0,004	Apoptosis agonist	0,754	0,005	Phosphatase inhibitor
0,894	0,004	Lipid metabolism regulator	0,751	0,003	Nitric oxide antagonist
0,879	0,001	Caspase 8 stimulant	0,769	0,029	CYP2J substrate
0,880	0,003	Membrane integrity antagonist	0,736	0,007	Antisecretoric
0,875	0,006	Mucomembranous protector	0,723	0,001	DNA ligase (ATP) inhibitor
0,860	0,003	Hepatic disorders treatment	0,716	0,004	Gastrin inhibitor
0,855	0,003	Chemopreventive	0,713	0,004	Wound healing agent
0,850	0,005	Antiinflammatory	0,714	0,005	Antineoplastic (breast cancer)

Pa – probability of active Pi – probability of inactive

Table 36

Activity of Alpha amyrin

Pa	Pi	Activity	Pa	Pi	Activity
0,934	0,002	Insulin promoter	0,835	0,002	Nitric oxide antagonist
0,926	0,002	Hepatoprotectant	0,826	0,019	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
0,911	0,004	Apoptosis agonist	0,808	0,003	Lipid peroxidase inhibitor
0,901	0,005	Antineoplastic	0,808	0,017	Alkenylglycerophosphocholine hydrolase inhibitor
0,897	0,002	Transcription factor NF kappa B stimulant	0,793	0,003	Antiviral (Influenza)
0,897	0,002	Transcription factor stimulant	0,789	0,002	Caspase 8 stimulant
0,890	0,003	Chemopreventive	0,788	0,002	Antinociceptive
0,889	0,004	Antiinflammatory	0,781	0,011	Alkylacetylglycerophosphatase inhibitor
0,885	0,003	Oxidoreductase inhibitor	0,773	0,004	Phosphatase inhibitor
0,878	0,003	Antiprotozoal (Leishmania)	0,772	0,003	Wound healing agent
0,876	0,003	Hepatic disorders treatment	0,782	0,015	Acylcarnitine hydrolase inhibitor
0,864	0,004	Caspase 3 stimulant	0,753	0,001	DNA ligase (ATP) inhibitor
0,865	0,007	Mucomembranous protector	0,755	0,006	Antisecretoric
0,851	0,005	Hypolipemic	0,731	0,004	Gastrin inhibitor
0,840	0,003	Antiulcerative	0,756	0,033	CYP2J substrate
0,839	0,005	Membrane integrity antagonist	0,716	0,005	Antineoplastic (lung cancer)

Pa – probability of active Pi – probability of inactive

Table 37

Activity of Peruvianoside I

Pa	Pi	Activity	Pa	Pi	Activity
0,975	0,001	Monophenol monooxygenase inhibitor	0,845	0,007	Antineoplastic
0,975	0,002	Lipid peroxidase inhibitor	0,840	0,004	UGT1A9 substrate
0,975	0,001	Free radical scavenger	0,836	0,003	Lactase inhibitor
0,971	0,001	Hepatoprotectant	0,837	0,012	Benzoate-CoA ligase inhibitor
0,961	0,000	4-Coumarate-CoA ligase inhibitor	0,828	0,005	Caspase 3 stimulant
0,962	0,001	Anticarcinogenic	0,808	0,003	Antiviral (Influenza)
0,962	0,001	Chemopreventive	0,804	0,003	Antitussive
0,960	0,002	UDP-glucuronosyltransferase substrate	0,790	0,001	Capillary fragility treatment
0,957	0,002	UGT1A substrate	0,780	0,004	UGT1A1 substrate
0,957	0,003	Membrane integrity agonist	0,770	0,005	3-Phytase inhibitor
0,926	0,003	Anaphylatoxin receptor antagonist	0,767	0,006	Antiprotozoal (Leishmania)
0,924	0,003	Membrane permeability inhibitor	0,776	0,020	Sugar-phosphatase inhibitor
0,920	0,007	CDP-glycerol glycerophosphotransferase inhibitor	0,759	0,004	HMOX1 expression enhancer
0,910	0,003	Antihypercholesterolemic	0,753	0,001	Iodide peroxidase inhibitor
0,900	0,001	Laxative	0,758	0,008	Cytostatic
0,899	0,003	Cardioprotectant	0,743	0,003	Hemostatic
0,898	0,002	Proliferative diseases treatment	0,739	0,004	Mycothiol-S-conjugate amidase inhibitor
0,889	0,005	TP53 expression enhancer	0,741	0,008	Antifungal
0,885	0,001	UGT1A7 substrate	0,740	0,008	Analeptic
0,877	0,004	CYP3A4 inducer	0,746	0,016	HIF1A expression inhibitor
0,861	0,004	Vasoprotector	0,726	0,002	Sweetener
0,857	0,001	Skin whitener	0,748	0,026	CYP2H substrate
0,852	0,002	Expectorant	0,727	0,007	Vasodilator
0,849	0,001	Alpha glucosidase inhibitor	0,716	0,004	CYP2C9 inducer
0,852	0,004	Membrane integrity antagonist	0,720	0,013	Apoptosis agonist
0,851	0,004	CYP3A inducer	0,705	0,003	CYP2E1 inducer
0,842	0,002	Histamine release stimulant	0,705	0,014	Respiratory analeptic
0,842	0,003	Antioxidant			

Pa – probability of active Pi – probability of inactive

Table 38

Activity of Cis-ocimene

Pa	Pi	Activity	Pa	Pi	Activity
0,906	0,002	Fatty-acyl-CoA synthase inhibitor	0,780	0,039	Ubiquinol-cytochrome-c reductase inhibitor
0,897	0,005	Antieczematic	0,744	0,005	Undecaprenyl-phosphate mannosyltransferase inhibitor
0,896	0,005	CYP2J substrate	0,731	0,002	Deoxyribose-phosphate aldolase inhibitor
0,889	0,003	CYP2E1 inhibitor	0,766	0,038	CDP-glycerol glycerophosphotransferase inhibitor
0,882	0,012	Aspulvinone dimethylallyltransferase inhibitor	0,748	0,021	Pro-opiomelanocortin converting enzyme inhibitor
0,833	0,003	Allyl-alcohol dehydrogenase inhibitor	0,720	0,001	2,3-Oxidosqualene-lanosterol cyclase inhibitor
0,835	0,011	Mucomembranous protector	0,726	0,009	3-Hydroxybenzoate 6-monooxygenase inhibitor
0,823	0,002	BRAF expression inhibitor	0,728	0,011	Bisphosphoglycerate phosphatase inhibitor
0,810	0,003	Prenyl-diphosphatase inhibitor	0,725	0,010	Limulus clotting factor B inhibitor
0,811	0,008	Protein-disulfide reductase (glutathione) inhibitor	0,742	0,030	Polyporopepsin inhibitor
0,791	0,005	CYP2E1 substrate	0,747	0,040	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
0,787	0,002	TRPA1 agonist	0,715	0,013	Phosphatidylcholine-retinol O-acyltransferase inhibitor
0,786	0,005	CYP2E substrate	0,703	0,005	Glutarate-semialdehyde dehydrogenase inhibitor
0,782	0,012	GST A substrate	0,706	0,009	All-trans-retinyl-palmitate hydrolase inhibitor
0,769	0,002	Dolichyl-phosphatase inhibitor	0,716	0,030	Sugar-phosphatase inhibitor
0,779	0,014	Feruloyl esterase inhibitor	0,704	0,022	Beta-adrenergic receptor kinase inhibitor
0,756	0,005	Amine dehydrogenase inhibitor	0,704	0,022	G-protein-coupled receptor kinase inhibitor
0,760	0,010	CYP2B6 substrate	0,711	0,030	CYP2J2 substrate
0,752	0,002	Alpha-pinene-oxide decyclase inhibitor			

Pa – probability of active Pi – probability of inactive

Table 39

Activity of Linalool

Pa	Pi	Activity	Pa	Pi	Activity
0,978	0,002	Mucomembranous protector	0,781	0,004	Undecaprenyl-phosphate mannosyltransferase inhibitor
0,910	0,003	Cell adhesion molecule inhibitor	0,761	0,009	Phosphatidylcholine-retinol O-acyltransferase inhibitor
0,896	0,009	Aspulvinone dimethylallyltransferase inhibitor	0,725	0,004	Gastrin inhibitor
0,868	0,003	Fatty-acyl-CoA synthase inhibitor	0,743	0,023	Membrane permeability inhibitor
0,860	0,007	Beta-adrenergic receptor kinase inhibitor	0,741	0,026	Sugar-phosphatase inhibitor
0,860	0,007	G-protein-coupled receptor kinase inhibitor	0,751	0,039	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
0,852	0,005	GST A substrate	0,711	0,002	Antiviral (Rhinovirus)
0,844	0,003	Prenyl-diphosphatase inhibitor	0,747	0,042	CDP-glycerol glycerophosphotransferase inhibitor
0,836	0,002	Ecdysone 20-monooxygenase inhibitor	0,716	0,013	UDP-glucuronosyltransferase substrate
0,832	0,002	BRAF expression inhibitor	0,728	0,026	CYP2J2 substrate
0,803	0,005	Lipid metabolism regulator	0,719	0,022	TP53 expression enhancer
0,798	0,004	Antisecretoric	0,727	0,058	Ubiquinol-cytochrome-c reductase inhibitor
0,811	0,018	CYP2J substrate	0,715	0,048	Gluconate 2-dehydrogenase (acceptor) inhibitor
0,808	0,017	Antieczematic	0,703	0,039	Chlordecone reductase inhibitor
0,786	0,008	Antiinflammatory			

Pa – probability of active Pi – probability of inactive

Table 40

Activity of Lutein

Pa	Pi	Activity	Pa	Pi	Activity
0,952	0,002	CYP2J substrate	0,773	0,004	Phenol O-methyltransferase inhibitor
0,942	0,002	Reductant	0,777	0,010	5-O-(4-coumaroyl)-D-quinate 3'-monooxygenase inhibitor
0,934	0,002	Chemopreventive	0,771	0,005	Anesthetic general
0,910	0,005	Antineoplastic	0,771	0,010	Carboxypeptidase Taq inhibitor
0,895	0,002	Radioprotector	0,772	0,012	Arginine 2-monooxygenase inhibitor
0,891	0,004	Dermatologic	0,793	0,036	Aspulvinone dimethylallyltransferase inhibitor
0,888	0,005	Apoptosis agonist	0,764	0,009	IgA-specific serine endopeptidase inhibitor
0,876	0,001	Keratolytic	0,768	0,017	Sphinganine kinase inhibitor
0,877	0,003	Phosphatidylcholine-retinol O-acyltransferase inhibitor	0,749	0,008	Lipoprotein lipase inhibitor
0,868	0,007	Beta-adrenergic receptor kinase inhibitor	0,769	0,029	CYP2J substrate
0,868	0,007	G-protein-coupled receptor kinase inhibitor	0,750	0,012	Exoribonuclease II inhibitor
0,863	0,003	Antipsoriatic	0,768	0,044	CYP2C12 substrate
0,854	0,001	Retinol dehydrogenase inhibitor	0,744	0,023	CYP2J2 substrate
0,853	0,003	All-trans-retinyl-palmitate hydrolase inhibitor	0,723	0,002	Sclerosant
0,845	0,002	CYP4A11 substrate	0,727	0,010	Macrophage colony stimulating factor agonist
0,842	0,005	CYP2B6 substrate	0,730	0,016	Alkylacetylglycerophosphatase inhibitor
0,835	0,008	CYP2C substrate	0,721	0,013	Lysine 2,3-aminomutase inhibitor
0,831	0,005	CYP2B substrate	0,711	0,005	Anthranilate-CoA ligase inhibitor
0,828	0,004	Prostate cancer treatment	0,705	0,008	Cholesterol antagonist
0,815	0,004	Antiulcerative	0,702	0,006	Poly(beta-D-mannuronate) lyase inhibitor
0,817	0,013	CYP3A4 substrate	0,704	0,008	Poly(alpha-L-guluronate) lyase inhibitor
0,804	0,005	Lipid metabolism regulator			

Pa – probability of active Pi – probability of inactive

Table 41

Activity of Beta caryophyllene

Pa	Pi	Activity	Pa	Pi	Activity
0,915	0,005	Antineoplastic	0,780	0,031	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
0,897	0,005	Antieczematic	0,745	0,011	Antiinflammatory
0,847	0,005	Apoptosis agonist	0,734	0,005	Antipsoriatic
0,792	0,003	Transcription factor NF kappa B stimulant	0,734	0,006	Dermatologic
0,792	0,003	Transcription factor stimulant	0,722	0,002	NF-E2-related factor 2 stimulant
0,799	0,021	CYP2J substrate	0,709	0,011	Phosphatase inhibitor
0,763	0,005	Antineoplastic (lung cancer)	0,746	0,048	CYP2C12 substrate
0,760	0,004	MMP9 expression inhibitor			

Pa – probability of active Pi – probability of inactive

Table 42

Activity of Zeaxanthin

Pa	Pi	Activity	Pa	Pi	Activity
0,980	0,001	CYP2J substrate	0,855	0,009	CYP3A4 substrate
0,954	0,001	Phosphatidylcholine-retinol O-acyltransferase inhibitor	0,846	0,002	CYP2C8 inhibitor
0,952	0,002	Beta-adrenergic receptor kinase inhibitor	0,835	0,003	TNF expression inhibitor
0,952	0,002	G-protein-coupled receptor kinase inhibitor	0,834	0,005	CYP2C9 substrate
0,945	0,001	All-trans-retinyl-palmitate hydrolase inhibitor	0,832	0,011	CYP3A substrate
0,947	0,004	CYP2C substrate	0,823	0,005	UDP-glucuronosyltransferase substrate
0,941	0,003	Dermatologic	0,820	0,002	CYP4A substrate
0,936	0,003	Lipid metabolism regulator	0,819	0,002	Growth stimulant
0,928	0,002	HMOX1 expression enhancer	0,816	0,003	Antioxidant
0,925	0,004	Apoptosis agonist	0,813	0,004	Chemopreventive
0,924	0,003	CYP2C19 substrate	0,812	0,005	Anticarcinogenic
0,919	0,001	Keratolytic	0,803	0,001	Retinoic acid beta receptor agonist

Table 42 Contd…

Pa	Pi	Activity	Pa	Pi	Activity
0,920	0,004	TP53 expression enhancer	0,802	0,002	CYP2E1 inducer
0,917	0,003	Reductant	0,795	0,005	UGT1A substrate
0,915	0,001	CYP4A11 substrate	0,789	0,002	Oxidizing agent
0,915	0,001	BRAF expression inhibitor	0,805	0,017	Antieczematic
0,918	0,005	Antineoplastic	0,790	0,005	CYP2D substrate
0,917	0,004	CYP2A6 substrate	0,782	0,003	CYP1B substrate
0,914	0,004	CYP2A substrate	0,785	0,007	CYP3A5 substrate
0,908	0,001	Retinoic acid receptor agonist	0,778	0,003	Antiacne
0,909	0,002	Antipsoriatic	0,770	0,004	Antipruritic
0,907	0,001	Retinol dehydrogenase inhibitor	0,768	0,004	Proliferative diseases treatment
0,907	0,004	CYP1A1 substrate	0,763	0,002	Chemoprotective
0,902	0,004	CYP2B6 substrate	0,758	0,001	Retinal dehydrogenase inhibitor
0,901	0,004	CYP2B substrate	0,761	0,004	UGT1A1 substrate
0,900	0,004	CYP2E1 substrate	0,755	0,005	CYP2D6 substrate
0,896	0,004	CYP2E substrate	0,748	0,004	CYP1A inducer
0,891	0,002	MMP9 expression inhibitor	0,744	0,004	Prostate cancer treatment
0,887	0,003	CYP2C8 substrate	0,767	0,034	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
0,888	0,007	Ubiquinol-cytochrome-c reductase inhibitor	0,744	0,012	Immunosuppressant
0,877	0,002	Cytochrome P450 stimulant	0,722	0,004	UGT2B substrate
0,866	0,002	Beta-carotene 15,15'-monooxygenase inhibitor	0,719	0,001	EIF4E expression inhibitor
0,867	0,005	CYP1A substrate	0,716	0,002	ABCA1 expression enhancer
0,864	0,003	Radioprotector	0,717	0,005	Antiulcerative
0,862	0,001	Dolichyl-phosphatase inhibitor	0,718	0,006	Carminative
0,858	0,000	Factor XIIIa stimulant	0,712	0,002	Protein-S-isoprenylcysteine O-methyltransferase inhibitor
0,859	0,004	Oxidoreductase inhibitor	0,706	0,002	CYP1B1 substrate
0,854	0,005	CYP1A2 substrate			

Pa – probability of active Pi – probability of inactive

Table 43

Activity of Piperitone

Pa	Pi	Activity	Pa	Pi	Activity
0,907	0,002	Carminative	0,775	0,016	Acylcarnitine hydrolase inhibitor
0,890	0,005	Antieczematic	0,760	0,031	Mucomembranous protector
0,873	0,011	Ubiquinol-cytochrome-c reductase inhibitor	0,734	0,008	Vasoprotector
0,878	0,017	CYP2C12 substrate	0,717	0,024	Glutamyl endopeptidase II inhibitor
0,826	0,019	Testosterone 17beta-dehydrogenase (NADP+) inhibitor	0,705	0,012	Phosphatase inhibitor
0,812	0,018	CYP2J substrate	0,704	0,016	Alkane 1-monooxygenase inhibitor
0,810	0,016	Alkenylglycerophosphocholine hydrolase inhibitor	0,718	0,032	Membrane permeability inhibitor
0,783	0,011	Alkylacetylglycerophosphatase inhibitor			

Pa – probability of active Pi – probability of inactive

PASS algorithm was selected by theoretical and empirical comparison of many different mathematical methods to provide high accuracy of prediction and robustness of calculated estimates (Filimonov, 1995). It was shown that the mean accuracy of prediction with PASS is about 86% in LOO cross-validation (PASS, 2013). PASS uses CSV, SD or MOL-files (MDLI, 2012) as input and the results of prediction (output) can be obtained as TXT or SD-files. Since the prediction of biological activity spectra for 1,000 compounds in usual PC takes about 1 minute, PASS can be effectively applied to predict biological potential of separate compounds and to analyze large chemical databases.

Biological activity spectrum of compound can be predicted on the basis of structure-activity relationships found by the analysis of the known data from the training set. Based on the analysis of large training set consisting of tens of thousands of the known biologically active compounds, computer program PASS provides the means to evaluate any new compound in huge chemical-pharmacological space. Pa and Pi are the estimates of probability to be active and inactive respectively. Their values vary from zero to one.

MOLECULAR DOCKING STUDIES

Three dimensional structure of the odorant binding protein of *C. quinquefasciatus* (PDB id 2L2C) was showed in figure 1. Protein was prepared according to the parameters (Fig 1a & b). Grid generated for all the amino acids from the workspace was selected with the parameter of 20A^{o} distance. Ligand docking panel was rewarded with no constraints, no rotatable groups and no extended volumes chosen in the grid generating panel. The reason for selecting all amino acid for docking was to find out post docking active site prediction mechanism. In specific, whichever site the ligand interacts will be the active site for this study.

Resulting grid file were further generated with complex of all conformation to dock with ligands. This aims to dock with Polar and SASA (Surface Accessible Solvent Area) surface based profile of the ligand. Ligands were retrieved from PubChem database. Retrieved ligands were carried out for preparation. All fourteen compounds were prepared of which, only ten compounds were binding with mosquito OBP and were used for further docking studies.

The glide score, number of H-bonds, distance of H-bonds, interacted residues and ligand atom of docked compounds were showed in table 44. Compound id 42608013 (Peruvianoside I) exhibited good glide score (-7.55 A^{0}**)** and formed 2 H-bonds with target odorant binding protein (Fig 20a), followed by compound id 201783 (Beta amyrin) which showed a glide score (-6.73A^{0}**)** and formed 1 H-bond with target odorant binding protein (Fig 20b).

Table 44

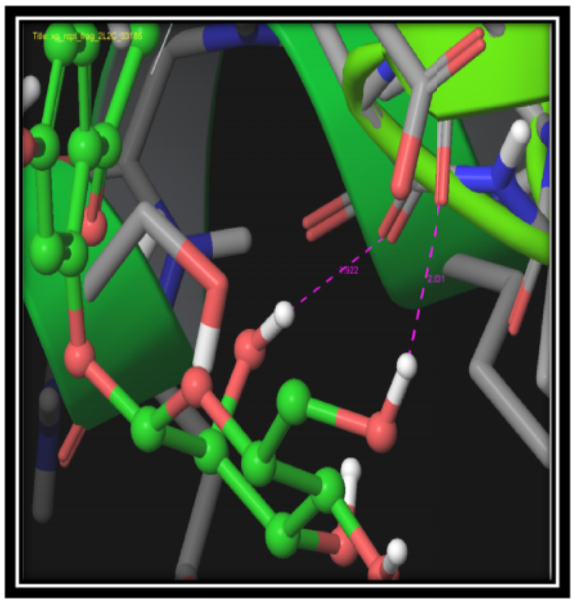
Docking score and H-bond interaction of ligands against mosquito odorant binding protein (PDB id 2L2C)

Sl. No	Name of compound	Compound id	G score	No. of H bonds	Distance	Protein residues	Ligand atom
1	Di(2-ethylhexyl) phthalate	8343	-8.66	-	-	-	-
2	Beta amyrin	201783	-6.73	1	2.308	HIS111:(N) NE2	H
3	Alpha amyrin	73170	-5.7	-	-	-	-
4	Peruvianoside I	42608013	-7.55	2	2.031 1.922	GLU 61:(O) O ALA 62:(O) O	H H
5	Cis-ocimene	5369951	-3.7	1	1.986	TRP 114: (H)HE1	O
6	Linalool	6549	-3.0	1	2.256	VAL 125: (O)OXT	H
7	Lutein	5281243	-6.12	-	-	-	-
8	Beta caryophyllene	5281515	-5.61	-	-	-	-
9	Zeaxanthin	5280899	-5.42	-	-	-	-
10	Piperitone	6987	-4.19	-	-	-	-

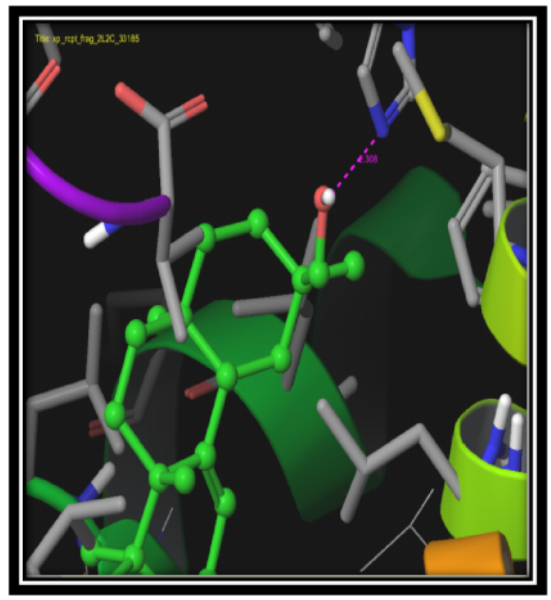
Activity of compound id 201783 (Beta amyrin) was followed by compound id 5369951 (Cis-ocimene) having glide score (-3.7 A^0) which formed 1 H- bond with target odorant binding protein (Fig 20c). The compound id 6549 (Linalool) displayed glide score (-3.0 A^0) and formed 1 H- bond with target odorant binding protein (Fig 20d). Remaining compounds (8343, 5281243, 73170, 5281515, 5280899 and 6987) were highly binding with *C. quinquefasciatus* odorant binding protein but there was no H-bond interaction. So the compound id 42608013 (Peruvianoside I), id 201783 (Beta amyrin), id 536995 (Cis-ocimene) and id 6549 (Linalool) were chosen for further analysis as they exhibited maximum glide scores. The complex structures of ligands 42608013 (Peruvianoside I), 201783 (Beta amyrin), 536995 (Cis-ocimene) and 6549 (Linalool) and the structure of target mosquito odorant binding protein were taken for further molecular dynamics approach.

Figure 20

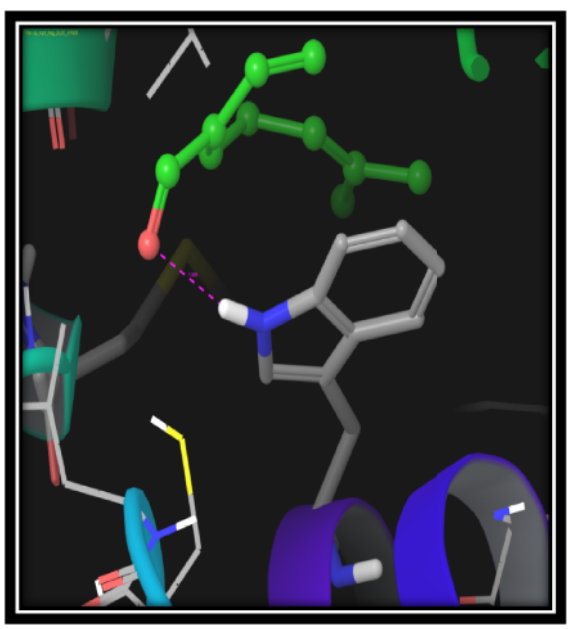
Compounds docked against odorant binding protein (PDB id 2L2C) of *C. quinquefasciatus*



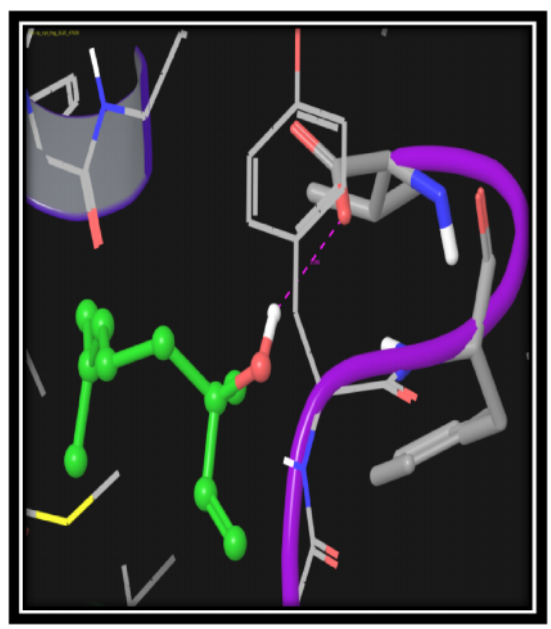
a) Compound 42608013 (Peruvianoside I)



b) Compound 201783 (Beta amyrin)



c) Compound 536995 (Cis-ocimene)



d) Compound 6549 (Linalool)

MOLECULAR DYNAMICS STUDIES

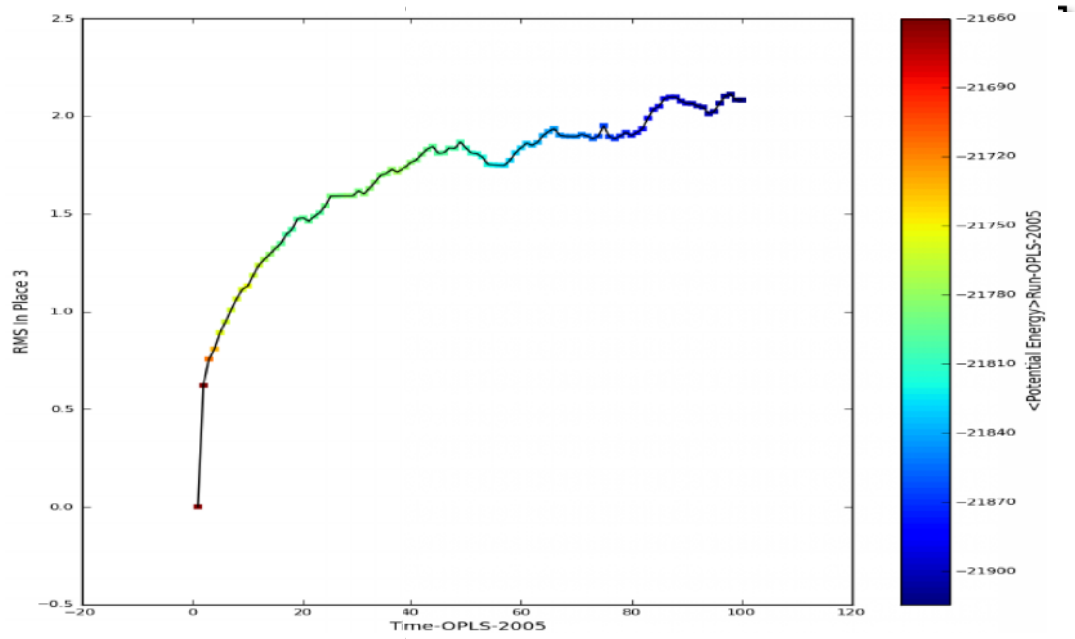
The molecular dynamic simulation was carried out for the complex structures of compounds 42608013, 201783 and 536995 with mosquito odorant binding protein (PDB id 2L2C) to evaluate the structural stability. The final trajectory files were taken for calculating the RMSD of the complex structures. While running MD simulation for 42608013 (Peruvianoside I) - 2L2C complex for 100 ps, the RMSD plot shows the stability of the complex structures at 80ps (Fig 21a). Graphical representation of Time vs. Potential energy map for 42608013 (Peruvianoside I) - 2L2C complex structure during molecular dynamics simulation for 100ps is displayed in figure 21b.

For the complex of compound 201783, MD simulation was carried out with mosquito odorant binding protein (PDB id 2L2C). The final trajectory files were taken for calculating the RMSD of the complex structures. While running MD simulation for 201783 (Beta amyrin) - 2L2C complex for 100 ps, the RMSD plot shows the stability of the complex structures at 90ps (Fig 21c). Graphical representation of Time vs. Potential energy map for 201783 (Beta amyrin) - 2L2C complex structure during molecular dynamics simulation for 100ps is shown in figure 21d.

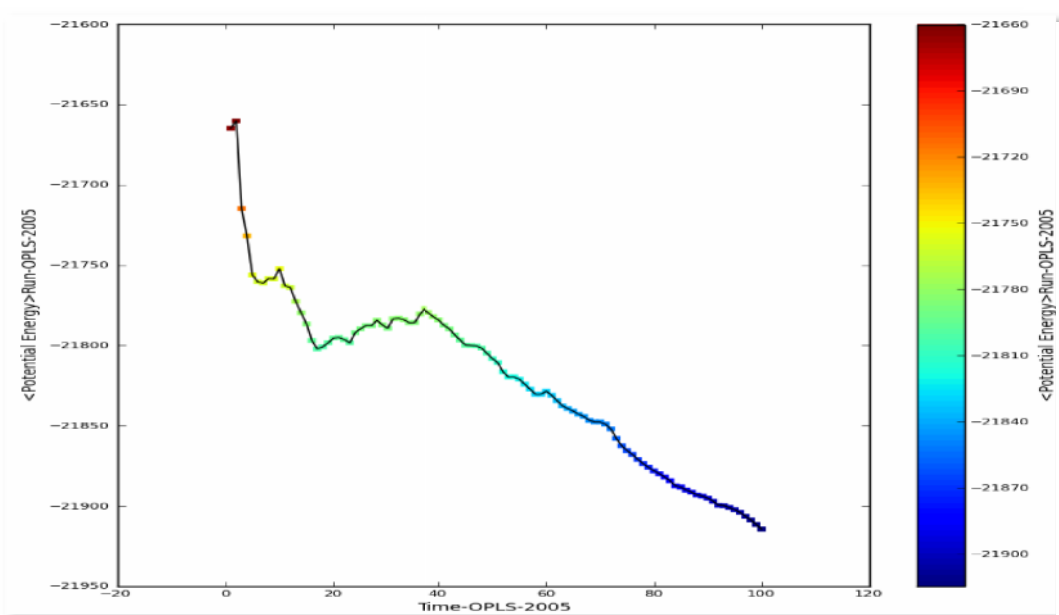
The MD simulation was carried out for the complex of compound 5369951 with mosquito odorant binding protein (PDB id 2L2C) to evaluate the structural stability. The final trajectory files were taken for calculating the RMSD of the complex structures. In the MD simulation for 5369951 (Cis-ocimene) - 2L2C complex for 100 ps, the RMSD plot shows the stability of the complex structures at 90ps (Fig 21e). Graphical representation of Time vs. Potential energy map for 5369951 (Cis-ocimene) - 2L2C complex structure during molecular dynamics simulation for 100ps is exhibited in figure 21f.

Figure 21

Graphical representation of molecular dynamics simulation studies for docked complex

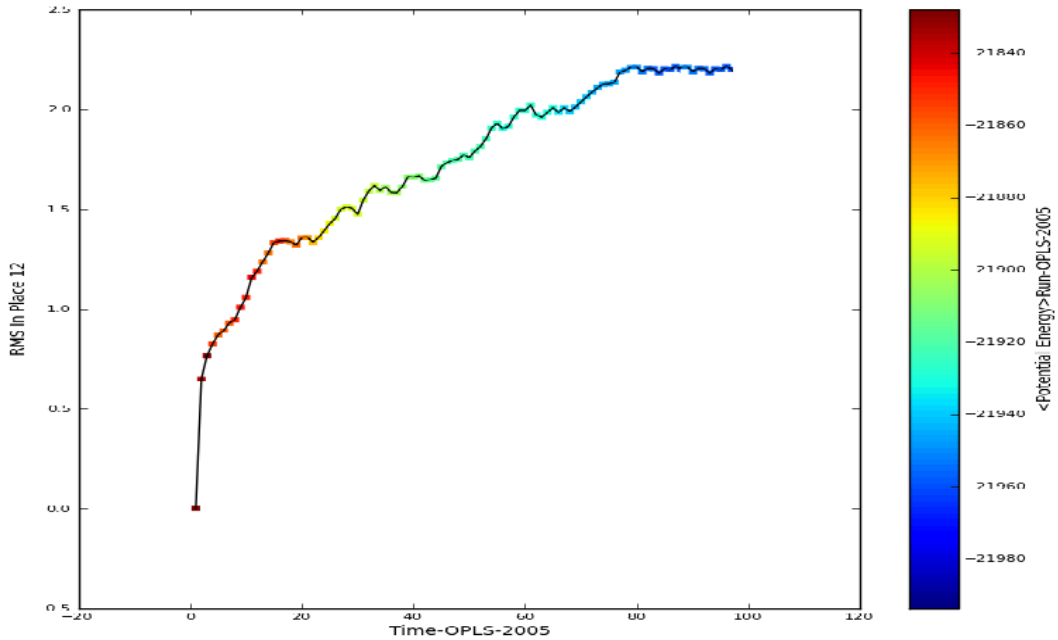


a) Time vs. RMS map for compound 42608013 (Peruvianoside I) - 2L2C complex structure for 100ps

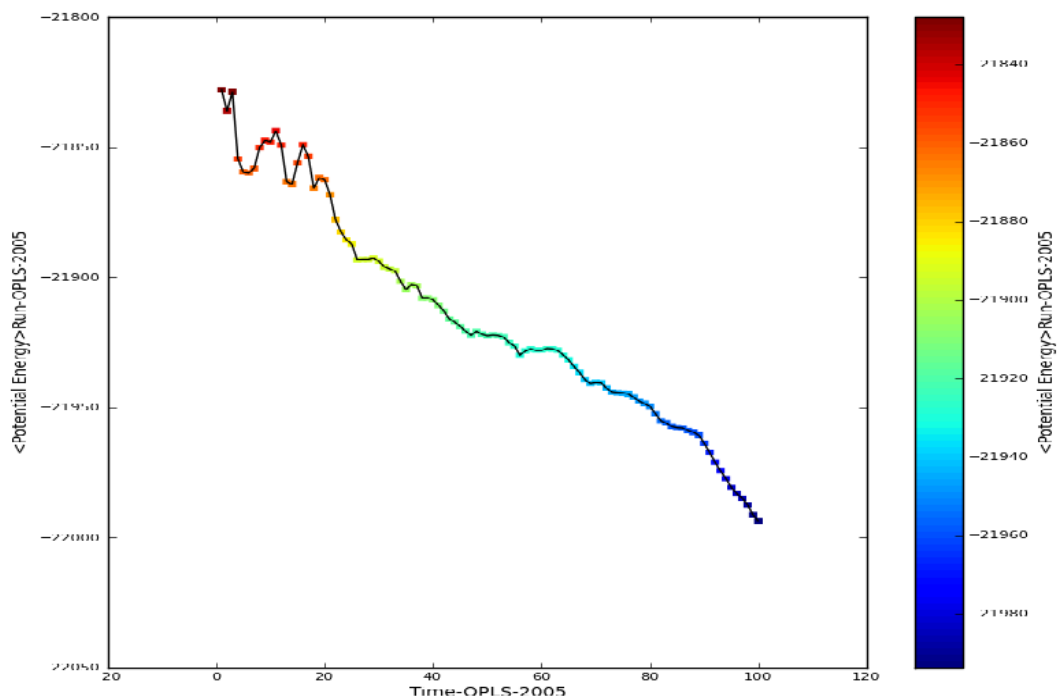


b) Time vs. Potential energy map for compound 42608013 (Peruvianoside I) - 2L2C complex structure for 100ps

Figure 21 Contd…

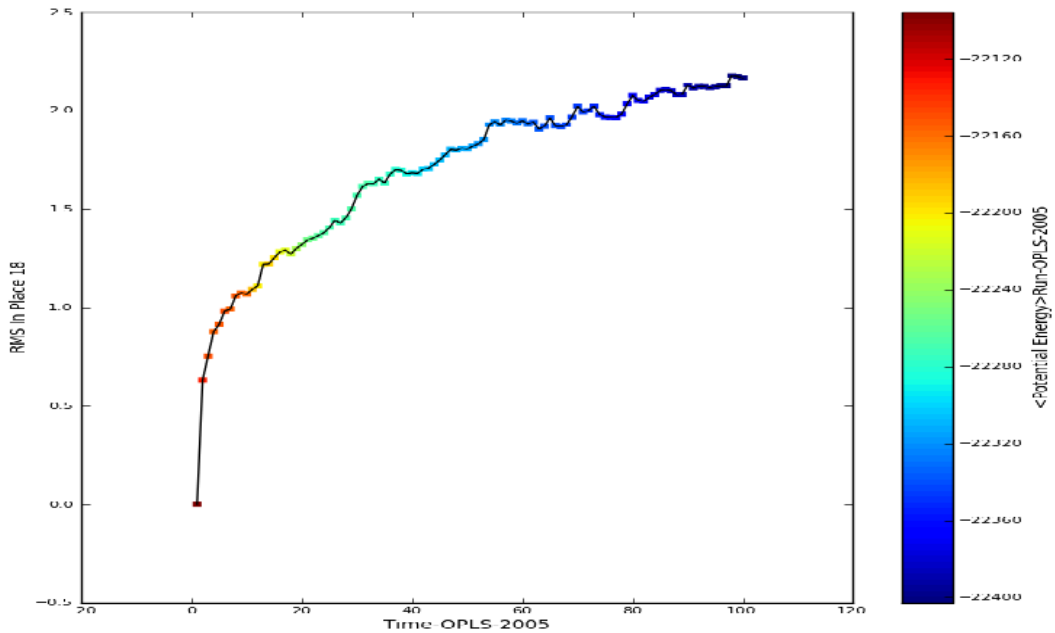


c) Time vs. RMS map for compound 201783 (Beta amyrin) - 2L2C complex structure for 100ps

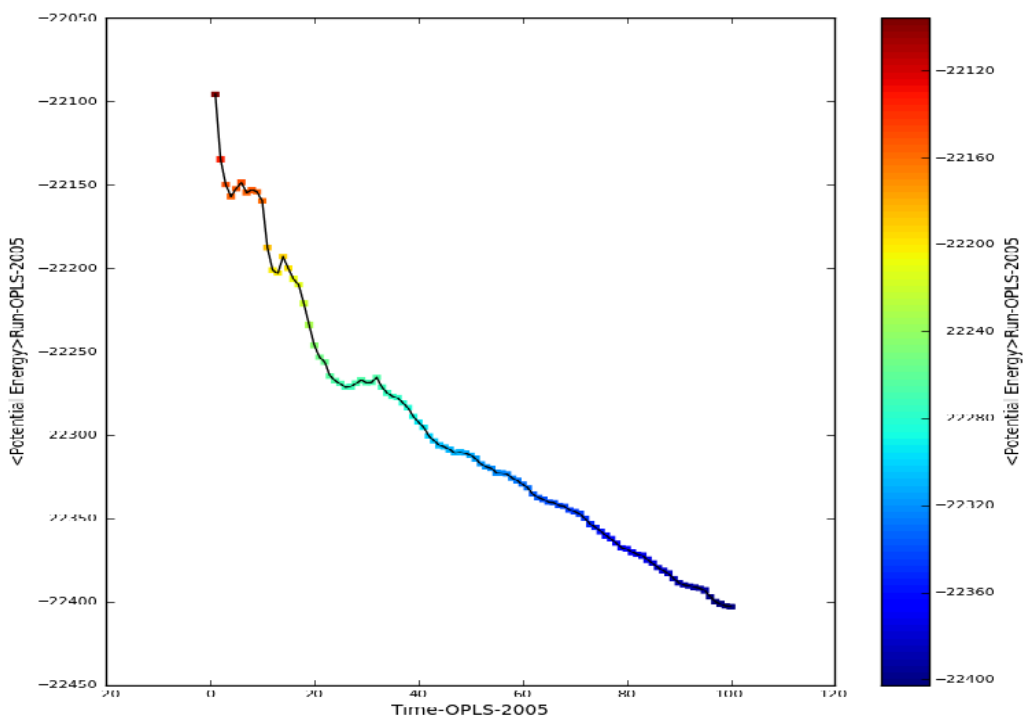


d) Time vs. Potential energy map for compound 201783 (Beta amyrin) - 2L2C complex structure for 100ps

Figure 21 Contd…



e) Time vs. RMS map for compound 5369951 (Cis-ocimene) - 2L2C complex structure for 100ps



f) Time vs. Potential energy map for compound 5369951 (Cis-ocimene) - 2L2C complex structure for 100p

As the molecular dynamics simulations studies were performed, in order to check whether the compounds would be anchored and estabilized in the pockets of the target protein, via the interactions predicted by the docking studies, the above results corroborated the docking results, showing the stability of complex structures.

Discussion

In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules. Computational prediction of receptor-odour interactions has the potential to enable systems level analysis of olfactory receptor repertoires in organisms (Boyle *et al*., 2013). Many chemical components of herbs are tested *in silico* for drug designing, and may give new effective drug against many killer mosquito borne diseases.

Precise detection and coding of odours by the peripheral olfactory neurons are subsequently processed, transformed and integrated in the central nervous system to generate specific behavioral responses that are critical for survival such as finding food, finding mates, avoiding predators etc (Naters and Carlson, 2006). More than 2000 odours in the environment have been catalogued from a small fraction of plant sources alone (Knudsen et al., 2006). The present study is a computational approach to study the interactions between the odorant binding protein (PDB id 2L2C) of *C. quinquefasciatus* mosquito and the ligand structures of selected plants.

In the present study, the structural formula of the processed ten secondary metabolites namely di(2-ethylhexyl) phthalate, beta amyrin, lutein, alpha amyrin, beta caryophyllene, zeaxanthin, peruvianoside I, piperitone, cis-ocimene and linalool of the selected plants viz., *T. erecta*, *C. gigantea* and *T. peruviana* were given as an input for PASS server. The PASS server provides all the possible activities of the given secondary metabolites. PASS can be effectively applied to predict biological potential of compounds and to analyze large chemical databases.

PASS predicted search results show all the available information on the pharmacological and toxicological activity of all the three compounds analysed. Similar observation in accordance with the present study using PASS server was already reported by many researchers. De Britto *et al* (2001) used PASS to predict the biological activity profile of seven secondary metabolites and successfully compared the PASS predictions with the available information on the pharmacological and toxicological activity of these compounds. The antiviral activities of selected seven compounds were confirmed in another work by Narayanan and Velmurugan (2013).

In the present investigation, the chemical structures of the ligands from the selected plants were retrieved from PubChem databases and were used for docking studies by Maestro in Schrodinger's computational programs. Similar study was done by Hetal *et al* (2013) in which the chemical structures of Tulsi and Mamejavo retrieved from databases like linalool, catechin, carvacrol, caryophyllene, rosmarinic acid, ajmalicine, luteolin, eugenol, apigenin, swertiamarin etc. was used for docking studies by Argus lab and Swissdock.

Oliferenko *et al* (2013) found that the insect olfactory system is believed to be the prime target for many natural repellents. Normally an odorant penetrates through the pores in the sensillum cuticle to the hemolymph, which bathes dendrites of the olfactory receptor neurons (ORN). Odorant binding proteins (OBPs) present in the hemolymph recognize and encapsulate hydrophobic odorants for further transportation through the hemolymph to specialized odorant receptors (ORs) residing in the ORN membrane (Naters and Carlson, 2006*;* Blomquist and Vogt, 2003; Ha and Smith, 2009; Sato and Touhara, 2009). Stimulation of an odorant receptor by an odorant initiates a sequence of biochemical events amplifying the action potential (Jacquin Joly and Merlin, 2004). One odorant can elicit responses of different intensities from different ORs, whereas ORs can be broadly or narrowly tuned for a wide or restricted panel of odours (Hallem *et al*., 2004; Wang *et al*., 2010). The odour code reflects not only the odorant chemical structure but also depends on its concentration (Hallem and

Carlson, 2006; Malnic *et al*., 1999) and presence of other volatiles (Martin *et al*., 2011).

GC-MS analysis and *in silico* molecular docking studies of mosquito repellent compounds from *Hyptis suaveolens* using Schrodinger Maestro software was conducted by Gaddaguti *et al* (2012). Among all methanolic compounds of *H. suaveolens*, gamma sitosterol exhibit maximum insect repellent activity when compared with known DEET against 3N7H crystal structure of odorant binding protein 1 from *An. gambiae*. Glide score of gamma-sitosterol was -5.99. These results can be compared with the present study which showed that among the ligands tested peruvianoside I exhibited maximum mosquito repellent activity against odorant binding protein (PDB ID 2L2C) of *C. quinquefasciatus* with a good glide score of -7.55.

Highly abundant in mosquitoes, odorant binding protein is the most probable candidate for the host-seeking and oviposition behavior, as it is overly expressed in the female antennae, not in the male ones (Biessmann *et al*., 2010 and Xu *et al*., 2010). Boyle *et al* (2013) reported that in addition to provide new insights into the nature of the interactions between odorants and their receptors, the computational screening could also aid the development of novel insect repellents, or compounds that mask the odours used by disease causing insects to identify their hosts. It could also be used in the future to develop novel flavours and fragrances.

Oliferenko *et al* (2013) conducted an experiment to evaluate the repellent efficacy of thirty four compounds against the female *Ae. aegypti* mosquitoes and results revealed that the compound treated clothes were repelling the mosquitoes. Observation lead to the conclusion that 2-phenylcyclohexanol is a viable scaffold for developing more diverse active repellent compounds. Molecular docking with Glide against the *Aaeg*OBP1 3D structure helped to identify highly promising scaffolds and individual compounds possessing mosquito repellent activity. The computational findings were confirmed by behavioral bioassay with *Ae. aegypti* mosquito species. Similar observations were found in the present study in which

the ligands of the selected plants which showed repellent activity against *C. quinquefasciatus* mosquito viz., beta amyrin, peruvianoside I, cis-ocimene and linalool exhibited good glide scores and were highly interacting with the odorant binding protein of *C. quinquefasciatus* 2L2C.

In the present study the compound peruvianoside I produced the highest glide score with two hydrogen bonds and was found to be highly interacting with the target odorant binding protein protein 2L2C. This is in accordance with the study of Seniya and Vyas (2013) in which Balanitin 6 binded in hydrophilic domain of the malarial protein PvDHFR with minimum binding energy thereby inhibiting its activity and could be a promising potential drug candidate against malaria.

Liu *et al* (2011) in his study reported α Pinene and 1, 8-cineole to be the two main components of *E. robusta* essential oil by GC-MS analysis. α Pinene has been shown to possess repellent activity against many insects, such as American cockroaches, *Periplaneta americana* (Sun *et al*., 1985), mosquitoes (Jaenson *et al*., 2006), stored product insects (Ngoh *et al*., 1998) and common tick (Traboulsi *et al*., 2005). 1, 8-Cineole has showed strong repellent activity against several insects, e.g. stored product insects (Yoon *et al*., 2007; Obeng-Ofori *et al*., 1997). 1, 8-cineole was effective against mosquitoes *C. pipiens molestus* bites offering complete protection for 2h (Traboulsi *et al*., 2005). The above findings suggested that α Pinene and 1, 8-cineole may have potential to be developed as new natural repellents. In accordance with this in the present study the main compounds beta amyrin of *C. gigantea* (Suresh *et al*., 2012) peruvianoside I of *T. peruviana* (Tewtrakul *et al*., 2002) and cis-ocimene (Brijesh *et al*., 2012) and linalool (Martinez *et al*., 2009) of *T. erecta* were found to be binding with the OBP of *C. quinquefasciatus* (PDB id 2L2C) by producing hydrogen bonds and good glide scores and may be responsible for the repellent activity against the target mosquitoes. The mosquito repellent activity of linalool is already reported by Ryan and Byrne, 1988 and Kaufman *et al.,* 2010.

In the present study, the best docked complexes were taken for molecular dynamic simulation studies to check the stabilization of the bonds and to confirm

the docking results. The Root Mean Square Deviation (RSMD) results confirmed that the MD simulations corroborated the docking results, showing that these compounds bind independently in monomers, remaining anchored and estabilized during the simulation. Similar observation was reported by Affonso *et al* (2013) in the docking and molecular dynamics study performed on potential ligands on the odorant binding protein of the mosquito *An. gambiae* (*Ag*OBP1) which suggested eugenyl acetate as a better repellent than DEET. In order to check the docking results, molecular dynamics simulations was run. The temporal RMSD results suggested that the compounds accommodate well inside the binding sites during the MD simulations, showing stabilization of the systems and confirming the results obtained by the total energy calculations.

Computer aided simulation of molecular interaction and prediction of possible active site moieties can be done using bioinformatics tools which help drug target prediction. Newer drugs are required to satisfy the numerous unmet clinical needs in many disease indications. *In silico* virtual screening and computer-aided drug design have become increasingly important to design new active novel pharmacophores, and enzyme inhibitors that bind to the active sites of parasite protein and bocks their action.

In the search for more stable and potent ovicidals and repellents against mosquitoes an integrated computational approach would be highly relevant. It can shorten discovery time and lower cost by reduction of the vast resources required for classical trial-and-error methods based on screening of large compound libraries. In the present study, a fundamental effort integrating computational and experimental approach to design novel mosquito ovicidal and repellent agents is reported. Molecular docking along with molecular dynamic simulation are integrated with experimental bioassay to guide rational design of mosquito ovicidals and repellents in a way similar to that widely used in drug discovery. An integrated approach to test the potential of the selected plant extracts to control the *C. quinquefasciatus* mosquito in the present investigation can also be applied in the future for the development of broader class of vector control systems.