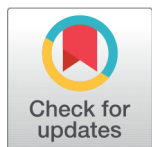


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The phyto-compounds Fumaric acid, di(1-adamantylmethyl) ester and 2-Pentamethylidisilanyloxypentane Borne from *Physalis longifolia* Against Various Medical and Agronomic Pests

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Abstract

Objectives: To identify the major phytochemicals of *Physalis longifolia* leaf methanol extract and assess the ovicidal and larvicidal toxicity of target medical and agronomic pests of *Aedes aegypti*, *Culex quinquefasciatus*, *Spodoptera litura* and *Helicoverpa armigera* eggs and larvae. **Methods:** In this study, the phyto-compounds identification was made by GC-MS analysis. The selected phyto-compounds were applied towards eggs were tested various concentrations 4-16 µg/ml and larvae were tested various concentrations 2-200 µg/ml of medical and agronomic pests. Overall mortality was examined using probit analysis to calculate LC₅₀/LC₉₀. **Findings:** The most common an Indian medicinal flora, *Physalis longifolia* leaf methanol extract and its derived major phyto-bioactive constituents Fumaric acid, di(1-adamantylmethyl) ester and 2-Pentamethylidisilanyloxypentane were authentically isolated by GC-MS analysis. By phyto-chemical screening, more numbers of bio-active phytochemicals were occupied in high polar leaf extract. *P. longifolia* leaf methanol extract and its derived major phyto-bioactive constituents Fumaric acid, di(1-adamantylmethyl) ester and 2-Pentamethylidisilanyloxypentane were showed toppest toxicity above 90% which reached nearly 100% and the larval toxicity of LC₅₀/ LC₉₀ values were 11.01/18.73, 10.56/ 17.90, 14.19/ 26.59, 14.88/ 27.80 µg/ml and 10.91/ 18.75, 10.70/ 18.26, 15.77/ 29.29 and 14.86/26.55 µg/ml against the medical and agronomic pests of *Ae. aegypti*, *Cx. quinquefasciatus*, *S. litura* and *H. armigera*, respectively. **Novelty:** The bio-toxicity of selected *P. longifolia* leaf methanol extract and its derived major phyto-bioactive constituents were well effective, environmental friendlier and produced least toxicity on non-target organisms. The medicinal flora, *P. longifolia* originated phyto-products could be a valuable farmer and eco-friendly bio-toxic weapon on medical and agronomic pests which promote

lesser susceptible on non-target organisms.

Keywords: Phytoproducts; Ecotoxicity; *Physalis longifolia*; Medical pest; Agronomic pest

1 Introduction

Around the world, vector control and its related medical field have been frequently facing diverse of challenges protecting public and animals against from pathogenic mosquitoes⁽¹⁾. Mosquitoes are key vector for spreading many diseases and causing immense trouble to all types of blood yielding faunas⁽²⁾. Globally, the mosquitoes are topmost blood sucking ecto-parasitic medicinal pests which causing millions of uncountable defects to human and other vertebrates in every year⁽³⁾. *Spodoptera litura* is a prime polyphagous insect pest in agronomic field they severely consume above 200 floras (All are revenue crops) which covered around 40 families in worldwide. Recently, they lead progressively developed into significant position in among the farmer⁽⁴⁾. *Helicoverpa armigera* is a significant polyphagous pest of many revenues agronomic crops and it causing significant revenue losses around 10 billion US\$ every year. This pest voraciously consuming every part of flora as the results valueless yield and consumption⁽⁵⁾. Globally, few decades the farmers turned towards the use of unadvisable synthetic chemical pesticides for pest controlling prepossess which are highly toxic and gives speedy results and its extensive usage making high level environmental hazards, faunal and floral health defects, generate pest resistance, toxicity in food remains⁽⁶⁾. In addition, most of the medical and agronomic pests are sufficiently capable of endurance against all types of commercial pesticides⁽⁷⁾. This incident is an urgent sign for searching of newer as well as traditional method and it definitely required alternate remedy for use of commercial pesticides. Moreover, they should be inexpensive, reliable, low price, easy preparative, selective toxicity, eco-friendly, etc.,⁽⁸⁾. But all the problems can be ratified through exploration phyto-products. India is a vast floral diversity country around 2000 floras has insecticidal properties. They have divers of phyto-compounds which are excellent toxoid to varieties of pests⁽⁹⁾. *P. longifolia* is ever greenish herb which has many small branches. It can be used as medicine for cure different types of urinary infections as well as many different diseases. Therefore, the present works focused for preliminary phytochemical screening and selected major phyto-bioactive constituents of *P. longifolia* against common medical and agronomic pests.

2 Methodology

2.1 Floral collection, processing and extraction

The floral selection was based on their abundance and its viability of medicinal values /various traditional usages. Therefore, the fresh green and cleaned *P. longifolia* floral leaves (Figure 1 A-C) were collected from local agronomic field, Cuddalore and Mayiladuthurai District, Tamil Nadu, India which were immediately carried to laboratory for identification and used for extract preparation. The required quantity of fresh green leaves was washed with tap water and allowed to dry at optimal temperature ($28 \pm 2^{\circ}\text{C}$). Around 1Kg of fresh dried leaf (15-20 Days) was ground finely which extracted with high polar solvent methanol by using Soxhlet apparatus. The methanol leaf extract was keenly filtered and condensed through rotary vacuum evaporator. The condensed extract 50 mg and were preserved at deep refrigerator (0 to- 4°C).

2.2 Target pests rearing

Medical pest culture: Eggs/larvae/ pupae of *Ae. aegypti* and *Cx. quinquefasciatus* were collected from locally available aquatic areas (Pond/pool/sewage treatment plant/house hold container) of Cuddalore and Mayiladuthurai District, Tamil Nadu, India. The aquatic juvenal stage of medical pests was maintained at glass/plastic container with required volume of H₂O. Medical pest larvae fed with the composite diet of 1% yeast: biscuits 1%: natural *Apis florea* honey 1% and it was followed by the prescribed protocol⁽¹⁰⁾. Agronomic pest culture: Eggs/larvae/ pupae of *S. litura* and *H. armigera* were collected from locally available agronomic areas (Cotton field/ groundnut field/ bhendi field) of same District. The host plant of respective pests was allowed to rear and it was followed by the prescribed protocol⁽¹¹⁾. They were properly monitored at 28±2°C temperature and 75–80 RH under 12 hours Light:12 hours Dark. The selected (medical and agronomic) pests 0-5 hrs. old eggs and 3rd instar larvae were used for further bio-assay activities.



Fig 1. A green flora of *P. longifolia* (A), Grinded leaf powder (B) and Condensed leaf methanol extract (C)

2.3 Toxicity of target pests eggs and larvae

The medical and agronomic pests *Ae. Aegypti*, *Cx. quinquefasciatus* and *S. litura* and *H. armigera* eggs (0-5 hrs age old) were 25 Nos. counted which tested with various concentrations (4-16µg/ml) of *P. longifolia* leaf methanol extract and its derived phyto-bioactive constituents of Fumaric acid, di(1-adamantylmethyl) ester and 2-Pentamethylidisilanyloxypentane⁽¹²⁾. Each and every concentration of ovicidal toxicity replicated five times. Control was maintained without floral properties and toxicity keenly surveillance by using microscope. The percent egg mortality was observed at 24 hours exposure period⁽¹³⁾. The medical pest larval toxicity: 3rd instar larvae (25 Nos.) of selected medical pests were collected from mother culture which tested under laboratory condition at various concentrations (2-200µg/ml). The concentration was made by based on the preliminary screening and the control was maintained without floral properties, the larval death counted and percent mortality was observed after 24 hrs. of exposure period⁽¹⁴⁾. The agronomic pest larval toxicity: the fresh tender host leaves of castor for *S. litura* and tomato for *H. armigera* were separately allowed with required concentrations of leaf methanol extract and phyto-bioactive constituents⁽¹⁴⁾. Agronomic pests 25 larvae introduced in separate containers and five replicates maintained at each test. The larval toxicity noticed and the percent mortality corrected by standard protocol⁽¹³⁾.

2.4 Isolation of phyto-bioactive constituent

The leaf methanol extracts of *P. longifolia* were assessed to phyto-chemicals analysis and GC-MS was examined by a mass detector Turbo mass gold-Perkin Elmer with Elite-5MS slender segment. It was more useful tool for identify and isolate the all different phyto-bioactive constituents through retention time, peak, area and others⁽¹⁵⁾.

2.5 Statistical analysis

The collected raw data were corrected into mean ± standard deviation as well as other related statistical observation also done (LC₅₀/ LC₉₀, Chi-square, Regression, ANOVA, Tukey's multiple range tests) by Probit Analysis and IBM-SPSS- 26.0 V Software.

3 Results and Discussion

3.1 Phytochemical screening

The availability of different bio-active phytochemicals was shown in various leaf extracts of *P. longifolia* which are clearly displayed in Table 1. Particularly, more numbers of bio-active phytochemicals were occupied in high polar leaf extract (leaf methanol extract). '+' represented as availability of bio-active phytochemicals and '-' represented as zero/nil of bio-active phytochemicals. The plants are having massive quantities of highly valuable phytochemicals which can be used as various bio-activities including insecticidal properties. The similar kind of observation were noticed from various medicinal flora^(16,17).

Table 1. The various qualitative phyto-chemicals detected from leaf extracts of *P. longifolia*

Sl. No.	Phytochemical screening	<i>P. longifolia</i> different leaf extracts				
		Hex	DEE	DCM	ETA	MET
1.	Carbohydrates	-	+	-	-	+
2.	Alkaloids	-	-	+	-	+
3.	Flavonoids	+	-	-	+	+
4.	Saponins	-	-	-	-	+
5.	Tannins	-	-	-	+	+
6.	Triterpenes	+	-	+	-	+
7.	Resins	+	-	-	-	-
8.	Coumarins	-	-	-	-	+
9.	Anthraquinones	+	+	+	+	-
10.	Phenolics	-	-	+	-	+

HNE: Hexane; DEE: Diethyl ether; DCM: Dichloromethane; ETA: Ethyl acetate; MET: Methanol

+: phytochemical group Zero

-: phytochemical group Appeared

3.2 Identification of bio-active compounds by GC-MS analysis

P. longifolia leaf methanol extract was evaluated by GC-MS spectral analysis for identify the phyto-bioactive constituents their availability which shown in Figure 2 as well as other related analytic parameters were authentically displayed in Table 2. A sum of 20 phyto-bioactive constituents obtaining 100% and the major phyto-bioactive constituents were Fumaric acid, di(1-adamantylmethyl) ester (Peak- 8, Retention time- 37.295, Area- 87701, Area %- 18.74, Height- 40003 and Height %- 17.75) and 2-Pentamethyldisilanyloxy pentane (Peak- 19, Retention time- 39.476, Area- 80149, Area %- 17.13, Height- 35467 and Height %- 15.74). The identified major phyto-bioactive constituents were evidently confirmed by MS analysis which displayed in Figures 3 and 4. The present study *P. longifolia* GC-MS analysis output has been strongly agreed with the findings of earlier works, *Loranthes pentandrus* against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*⁽¹⁸⁾, *Clitoria ternatea* against *Aedes* species⁽¹⁹⁾.

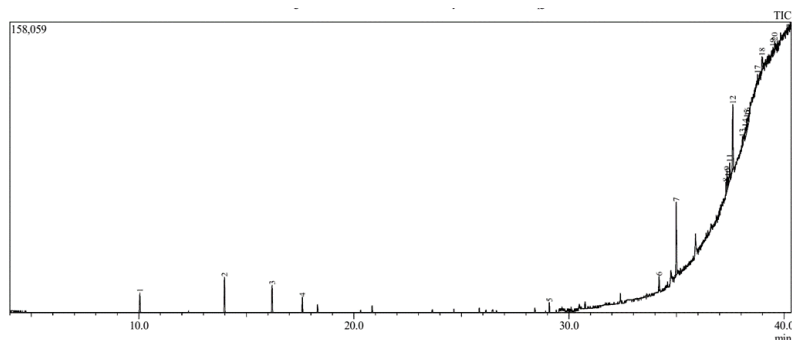
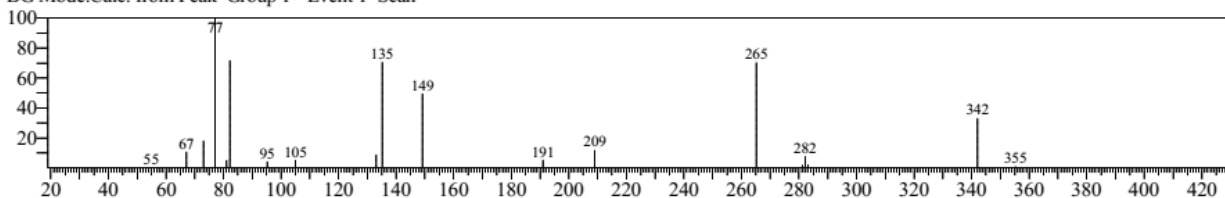


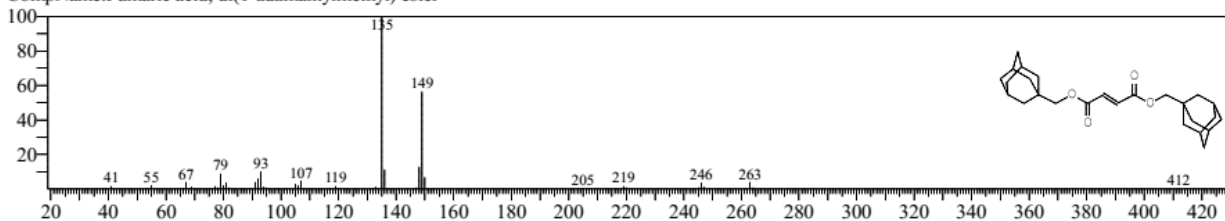
Fig 2. GC-MS chromatogram of *P. longifolia* leaf methanol extract

<< Target >>

Line#:8 R.Time:37.295(Scan#:6660) MassPeaks:20
 RawMode:Averaged 37.290-37.300(6659-6661) BasePeak:77.10(674)
 BG Mode:Calc. from Peak Group 1 - Event 1 Scan



Hit#:1 Entry:244067 Library:NIST14.lib
 SI:56 Formula:C26H36O4 CAS:0-00-0 MolWeight:412 RetIndex:2750
 CompName:Fumaric acid, di(1-adamantylmethyl) ester



Hit#:2 Entry:246534 Library:NIST14.lib
 SI:53 Formula:C26H42O4 CAS:0-00-0 MolWeight:418 RetIndex:2788
 CompName:Fumaric acid, 1-adamantylmethyl undecyl ester

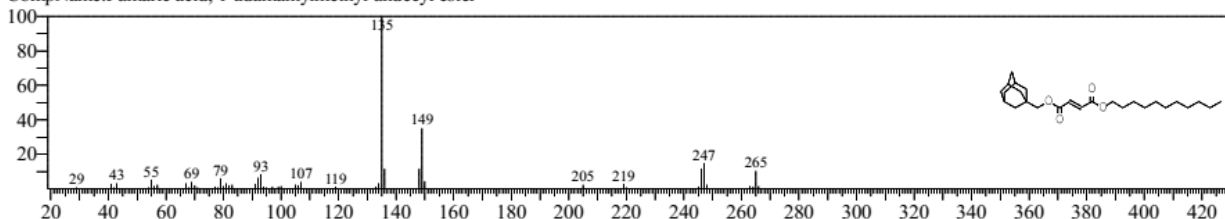
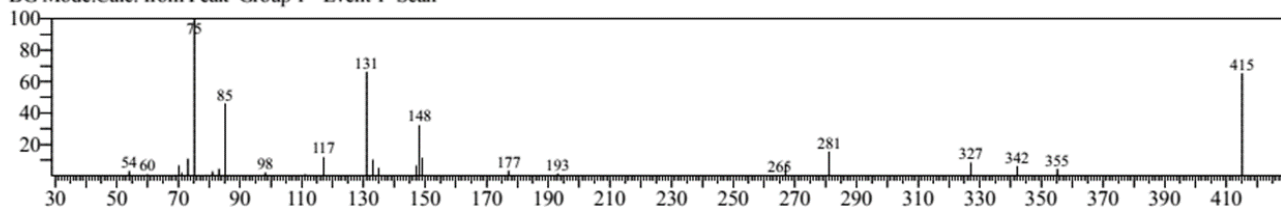


Fig 3. Mass spectrum and structure of Fumaric acid, di(1-adamantylmethyl) ester phyto-compound

Line#:19 R.Time:39.475(Scan#:7096) MassPeaks:30
 RawMode:Averaged 39.470-39.480(7095-7097) BasePeak:75.10(1050)
 BG Mode:Calc. from Peak Group 1 - Event 1 Scan



Hit#:1 Entry:81275 Library:NIST14.lib
 SI:59 Formula:C10H26OSi2 CAS:0-00-0 MolWeight:218 RetIndex:847
 CompName:2-Pentamethyldisilyloxypentane

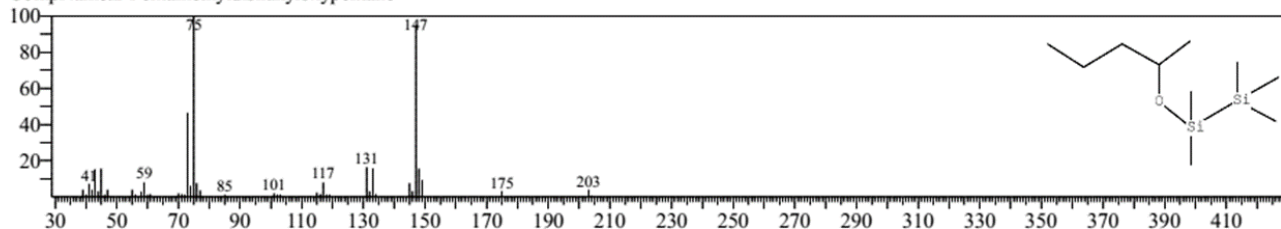


Fig 4. Mass spectrum and structure of 2-Pentamethyldisilyloxypentane phyto-compound

Table 2. GC-MS analysis of *P. longifolia* leaf methanol extract

PE	RT	ST	ET	AR	AR %	HE	HE %	A/H	CN
1	10.046	10.01	10.08	20802	4.44	10880	4.83	1.91	BENZOIC ACID, 2,5-BIS(TRIMETHYLSILOXY)-, TRIMETHYLSILYL ESTER
2	13.982	13.95	14.015	9870	2.11	5104	2.27	1.76	Cyclohexasiloxane, dodecamethyl-
3	16.195	16.165	16.23	27577	5.89	15053	6.68	1.83	PENTADECANE
4	17.602	17.58	17.63	14781	3.16	8414	3.73	1.76	1,3-DIPHENYL-1-((TRIMETHYLSILYL)OXY)-1(Z)-HEPTENE
5	29.084	29.065	29.105	9137	1.95	5558	2.47	1.64	(3.ALPHA.,5.ALPHA.)-3,4,4-TT METHYLCHOLEST-7-EN-3-OL
6	34.186	34.145	34.225	16479	3.52	7847	3.48	2.1	2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20-ICOSAMETHYLCYCLODECASILOXANE #
7	34.986	34.94	35.035	7742	1.65	7048	3.13	2.19	1,2-BENZENEDICARBOXYLIC ACID
8	37.295	37.29	37.315	87701	18.74	40003	17.75	1.1	Fumaric acid, di(1-adamantylmethyl) ester
9	37.349	37.315	37.37	26280	5.62	10934	4.85	2.4	3-METHYL-2-PENTENE-1,5-DIOL
10	37.395	37.37	37.405	10370	2.22	5405	2.4	1.92	1H-1,2,3-TRIAZOLE, BENZAMIDE DERIV.
11	37.473	37.405	37.5	27810	5.94	9879	4.38	2.82	Heptasiloxane, hexadecamethyl-
12	37.615	37.5	37.65	16951	3.62	6219	2.76	2.26	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
13	38.055	38.035	38.09	10658	2.28	5319	2.36	2	Adipic acid, 3-heptyl tetradecyl ester
14	38.195	38.175	38.25	34117	7.29	19356	8.59	2.73	1-(4-Amino-furazan-3-yl)-5-piperidin-1-ylmethyl-1H- (1,2,3)triazole-4-carboxylic acid ethyl ester
15	38.273	38.25	38.29	12372	2.64	6960	3.09	1.78	SILICONE GREASE, SILICONFETT
16	38.305	38.29	38.37	19076	4.08	6347	2.82	3.01	9-Bromononanoic acid
17	38.765	38.75	38.78	4252	0.91	5178	2.3	0.82	Tris(tert-butyl dimethylsilyloxy)arsane
18	38.974	38.945	39.025	22551	4.82	8147	3.62	2.77	CYCLONONASILOXANE, OCTADECAMETHYL-
19	39.476	39.465	39.52	80149	17.13	35467	15.74	1.93	2-Pentamethyldisilanyloxypentane
20	39.558	39.52	39.57	9331	1.99	6213	2.76	1.5	Glutaric acid, 2-methylpent-3-yl 2-isopropoxyphenyl ester

PE: Peak; RT: Retention Time; ST: Start time; ET: End Time; CA: Composition Area; CA%: Composition Area%; CAH: Composition Hight; CAH%: Composition Hight%; CA/H: Composition Area/ Hight; CN: Compounds Name

3.3 Ovicidal activity of major bio-active compounds

Egg toxicity of *P. longifolia* floral derived phyto-bioactive constituents of Fumaric acid, di(1-adamantylmethyl) ester and 2-Pentamethyldisilanyloxypentane against freshly laid eggs of medical pests *Ae. aegypti* and *Cx. quinquefasciatus* are apparently shown in Table 3. On perusal of the data indicates that statistically significant of egg toxicity was observed against *Ae. aegypti* and *Cx. quinquefasciatus* with their values were 26.6%, 49.6%, 74.2%, 95.8%, 28.8%, 46.7%, 77.8%, 95.5% and 27.4%, 44.6%, 73.4%, 98.2%, 22.4%, 48.4%, 78.5% and 98.6% observed towards 4, 8, 12, and 16 $\mu\text{g/ml}$ concentrations respectively. The same major phyto-bioactive constituents Fumaric acid, di(1-adamantylmethyl) ester and 2-Pentamethyldisilanyloxypentane tested on eggs of agronomic pests *S. litura* and *H. armigera* with their values were 22.2%, 37.6%, 73.9%, 94.8%, 21.4%, 42.2%, 75.2%, 96.5% and 19.8%, 45.5%, 74.6%, 95.4%, 18.3%, 44.0%, 76.8% and 94.8% noticed towards 4, 8, 12, and 16 $\mu\text{g/ml}$ concentrations respectively. Among the three different phyto-products tested, the most predominant egg toxicity was showed by major phyto-bioactive constituents at all concentration from low to high on the eggs of medical and agronomic pests. The phyto-bioactive constituents from various flora were provided an excellent tool for controlling many pests, the floral extracts of *Cyathocline purpurea* and *Blumea lacera* showed highest eggs toxicity 70–90% on *Ae. aegypti*⁽²⁰⁾. Nonadecanoic acid borne from *Solanum pseudocapsicum* against the eggs of *H. armigera* and *S. litura* with their toxic values were 81.70 and 80.10%, respectively⁽²¹⁾.

Table 3. Ovicidal activity of *P. longifolia* phyto-products against the eggs of medical and agronomic pests

% Ovicidal activity of medical and agronomic pests, freshly laid (0-6 hours age old) eggs				
Concentration	<i>Ae. aegypti</i>	<i>Cx. quinquefasciatus</i>	<i>Ae. aegypti</i>	<i>Cx. quinquefasciatus</i>
Fumaric acid, di(1-adamantylmethyl) ester			2-Pentamethyldisilanyloxypentane	
4 µg/ml	26.6±1.2	28.8±1.2	27.4±1.7	22.4±1.8
8 µg/ml	49.6±1.4	46.7±2.3	44.6±2.4	48.4±1.4
12 µg/ml	74.2±2.5	77.8±3.9	73.4±2.8	78.5±3.4
16 µg/ml	95.8±4.6	95.5±2.8	98.2±5.6	98.6±3.6
Concentration	<i>S. litura</i>	<i>H. armigera</i>	<i>S. litura</i>	<i>H. armigera</i>
4 µg/ml	22.2±1.2	21.4±1.6	19.8±1.2	18.3±1.5
8 µg/ml	37.6±1.4	42.2±1.7	45.5±1.5	44.0±1.8
12 µg/ml	73.9±2.5	75.2±1.2	74.6±2.3	76.8±1.8
16 µg/ml	94.8±4.6	96.5±1.7	95.4±1.6	94.8±1.6

Value represents mean ± S.D. of five replications. Values in a column with a different superscript alphabet are significantly different at P <0.05 level DMRT Test.

3.3.1 Values of major bio-active compounds

P. longifolia leaf methanol extract derived major phyto-bioactive constituent Fumaric acid, di(1-adamantylmethyl) ester was tested on selected medical and agronomic pests larvae which showed predominant toxicity and their LC₅₀/ LC₉₀ value of 11.01/18.73, 10.56/ 17.90, 14.19/ 26.59 and 14.88/ 27.80 µg/ml were noticed against the medical and agronomic pests of *Ae. aegypti*, *Cx. quinquefasciatus* and *S. litura* and *H. armigera*, respectively (Table 4). Larval toxicity of major phyto-bioactive constituent 2-Pentamethyldisilanyloxypentane derived from medicinal flora of leaf methanol extract which tested against the 3rd instar larva of selected medical and agronomic pests *Ae. aegypti*, *Cx. quinquefasciatus* and *S. litura* and *H. armigera*. Lethal toxicity of major phyto-bioactive constituents 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester and their LC₅₀/ LC₉₀ value of 10.91/ 18.75, 10.70/ 18.26, 15.77/ 29.29 and 14.86/26.55 µg/ml were noticed against the medical and agronomic pests of *Ae. aegypti*, *Cx. quinquefasciatus*, *S. litura* and *H. armigera*, respectively. The statistical analysis of the data clearly indicates that a major phyto-bioactive constituents induced outstanding larval lethality were observed on selected pests. The larval toxicity of phyto-products were an immense workout towards selected pests as well as it has been compared with previously reported many works, the Indian medicinal flora *Jussiaea repens* originated major bio-active floral-compound 4-piperidineacetic acid, 1-acetyl-5-ethyl-2-[3-(2-hydroxyethyl)-1H-indol-2-yl]-á-methyl-, methyl ester was showed well effective larval death (LC₅₀ 118.3 µg/ml, 116.1 µg/ml and 114.4 µg/ml) against larvae of dengue, malarial, and filarial vectors, respectively⁽²²⁾. *Citrus limetta* derived Corynan-17-0l,18,19-didehydro-10-methoxy-, acelate (ester) was showed best larval toxicity on *Ae. albopictus*, *An. maculatus* and *Cx mimulus* LC₅₀ values 5.56, 13.72 and 11.45 µg/ml, respectively⁽²³⁾.

Table 4. LC values of *P. longifolia* phyto-products against 3rd instar larvae of medical and agronomic pests

Target pests	LC50 (µg/ml)	LCL-UCL (µg/ml)	LC90 (µg/ml)	LCL-UCL (µg/ml)	R-value	χ ²
Fumaric acid, di(1-adamantylmethyl) ester						
<i>Ae. aegypti</i>	11.01	10.21-11.78	18.73	17.52-20.30	1.88+0.17	4.426
<i>Cx. quinquefasciatus</i>	10.56	9.79-11.30	17.90	16.76-19.37	1.98+0.19	6.920
<i>S. litura</i>	14.19	12.85-15.43	26.59	24.71-29.06	1.55+0.11	2.766
<i>H. armigera</i>	14.88	13.52-16.14	27.80	25.81-30.43	1.49+0.1	1.757
2-Pentamethyldisilanyloxypentane						
<i>Ae. aegypti</i>	10.91	10.10-11.69	18.75	17.52-20.35	1.84+0.17	5.316
<i>Cx. quinquefasciatus</i>	10.70	8.15-12.97	18.26	15.44-24.44	1.94+0.18	9.135
<i>S. litura</i>	15.77	14.40-17.05	29.29	27.14-32.15	1.5+0.1	2.180
<i>H. armigera</i>	14.86	13.62-16.04	26.55	24.77-28.84	1.62+0.11	1.703

LC₅₀: Lethal Concentration showed 50% mortality; LC₉₀: Lethal Concentration showed 90% mortality; LCL: Lower Confidence Limit; UCL: Upper Confidence Limit; R- value: Regression value; χ² value: Chi-square value

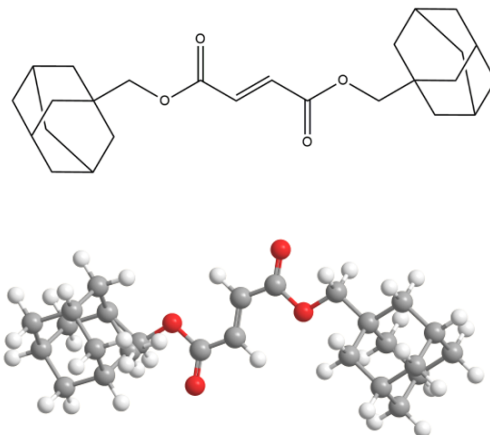


Fig 5. The 2D and 3D structure of phyto-compound Fumaric acid, di(1-adamantylmethyl) ester

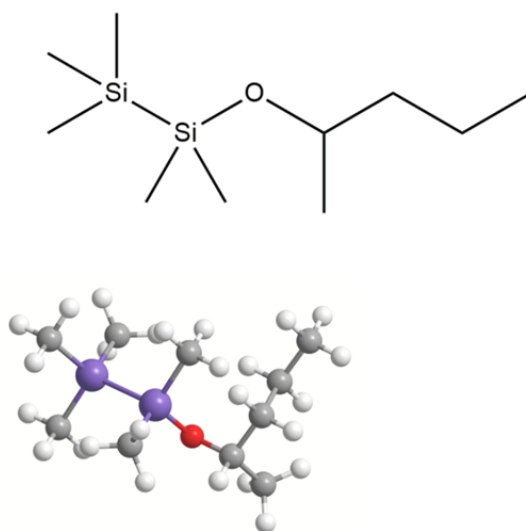


Fig 6. The 2D and 3D structure of phyto-compound 2-Pentamethyldisilanyloxypentane

4 Conclusion

The phyto-bioactive constituents of *P. longifolia* provided an excellent target specificity against medical and agronomic pests as well as comparatively poor/ less effects on non-target organisms. It could be a friendlier approach to various biotic species and most reliable as well as harmless tool for farmer handling.

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