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Research article

Antidengue potential of leaf extracts of *Pavetta tomentosa* and *Tarenna asiatica* (Rubiaceae) against dengue virus and its vector *Aedes aegypti* (Diptera: Culicidae)

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ABSTRACT

The aim of the present study was to screen the anti-dengue potential of crude leaf extracts of two plants from *Pavetta tomentosa* and *Tarenna asiatica*. For larvicidal assay, the acetone extract of both plants showed maximum effects, with the least LC_{50} and LC_{90} values (*P. tomentosa* (5.968 and 7.493 μg/ml) and *T. asiatica* (1.288 and 1.992 μg/ml)) and the same extract of both plants exhibited better pupicidal potency. The adulticidal activity of both plants (0–60 min interval periods) recorded best results in acetone extracts and the LC_{50} and LC_{90} values were recorded as *P. tomentosa* (32.105 and 41.001 μg/ml) and *T. asiatica* (09.012 and 11.854 μg/ml). Among the two plants *P. tomentosa* acetone leaf extract have good antiviral property against Dengue viral cell line. In addition, the phytochemical nature of the plant reveals the presence of saponins, flavonoids and alkaloids in all the tested extracts of both plants. GC-MS analysis revealed Hexanedioic acid, Bis(2-Ethylhexyl) Ester (22.54) and 2,6,10,14,18,22- Tetracosahexane, 2,6,10, 15, 19,15,19,23- Hexamethyl-(ALL-E)- (25.33) identified as two major phytoconstitutents in *P. tomentosa* and Tetracontane (23.580) is a major compound identified from *T. asiatica* acetone extracts. The functional groups of chemical compounds (aromatis, alkanes, alkyls and carboxylic acids) from *P. tomentosa* and *T. asiatica* were analyzed by FT-IR spectrum.

1. Introduction

Dengue virus is mainly transmitted by female mosquitoes of *Aedes aegypti* and *Ae. albopictus*, it comprises four distinct serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) which belong to the genus Flavivirus (family Flaviviridae). Distinct genotypes have been identified within each serotype, highlighted the extensive genetic variability of the dengue serotypes. Among them, World health Organization (2012) reported the "Asian" genotypes of DEN-2 and DEN-3 are frequently associated with severe diseases and closed with secondary dengue infections. The World Health Organization estimates that around 50 to 400 million peoples are annually at risk of dengue (WHO, 2015). The dengue and other infections have been dramatically risen in recent decades, due to increased urbanization, trade and travel etc. No effective drug or vaccine is available so far for control of dengue (Panneerselvam et al., 2012). Mosquitoes are an important vector that is capable of transmitting potential pathogens to human beings and responsible for several infectious diseases like dengue, zika, filariasis, malaria, yellow fever and chickungunya etc (Nauen, 2007). They become a challenging issue to public health worldwide, and it has a serious social and economic impact especially in tropical and sub-tropical countries (Bossche and Coetzer, 2008). In India, for the past two years more than one lakh cases are confirmed with dengue infection. During the year 2017, National vector borne disease control programme have been reported 11,552 cases dengue affected and 245 people leading to death (NVBDC, 2017).

The chemicals from plant origin, are now recognized as potent alternative insecticides to replace the synthetic agents for mosquito control programs (Regnault-Roger et al., 2012). Medicinal plant extracts and their secondary metabolites could not produce any toxic to humans, biodegradable, and potentially suitable for use in the control of mosquito larvae (Govindarajan et al., 2005; Govindarajan and Sivakumar, 2012). A small group of plants (69 nos) around the world have been reported as the potential antiviral agents especially against the dengue (Kadir et al., 2013). The natural molecule potential of medicinal plants to fight against Dengue (Suroowan et al., 2016).

The chosen plants (*Pavetta tomentosa* and *Tarenna asiatica*) are

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shrubby in nature, belongs to family Rubiaceae and distributed in India. In tamil, *P. tomentosa* called as Kattukkaranai and Karanai, *T. asiatica* known as Thaerani. *P. tomentosa* found in terrain and hilly regions with greyish, brown bark, elliptic or oblong-lanceolate, coriaceous leaves, white, fragrant flowers in cymes and black, multi-seeded berries. The plant is used for suppuration in boils and skin diseases (Amutha et al., 2012). The *P. tomentosa* bark in decoction or administered pulverized form especially to children for correct visceral obstructions (Kirtikar and Basu, 2003). The decoctions of leaves are used to alleviate the pains caused by haemorrhoids. The pulverized root of *P. tomentosa* was mixed with the ginger and rice-water, used as remedy for dropsy. A local fermentation with the leaves are used in relieving the pain of piles. *T. asiatica* fruit juice was used to arrest eye infection and leaves are served as medicine for gastric troubles (Kamsuk et al., 2007). The solvent extracts of leaves (or) powders of *Tarenna* species found to be better antimicrobial (Jayasinghe et al., 2002), antioxidant (Yang et al., 2007) and anti-inflammatory (Amutha et al., 2012) properties.

With the brief introduction, we performed the phytochemical profile and test the mosquitocidial potency of *P. tomentosa* and *T. asiatica* plant crude extracts against dengue vector and viral cell lines under laboratory condition.

2. Materials and methods

2.1. Collection and extraction of plant materials

The fresh and healthy plant leaves of *P. tomentosa* and *T. asiatica* were collected from Yercaud hills, Eastern Ghats of Tamil Nadu, India and plants name was authenticated by Botanical Survey of India, Coimbatore, Tamilnadu, India (BSI/SRC/5/23/2016/Tech./1069 (*P. tomentosa*) and BSI/SRC/5/23/2016/Tech./584 (*T. asiatica*). The plant leaves were washed with running tap-water for removal of the soils, dust particles and dried under room temperature for three weeks. Then the materials were powdered using electric blender. The known amount of *P. tomentosa* and *T. asiatica* leaf powders (25g) were extracted with five different solvents (hexane, ethyl acetate, chloroform, acetone and methanol) separately in 250ml of Soxhlet apparatus and the boiling point was differed at 50–80 °C. The extracts were concentrated under reduced pressure (22–26 mm Hg) at 45 °C. Then, the obtained crude extracts were collected in sterilized container and stored at room temperature for further analysis.

2.2. Mosquito rearing

The mosquito (*Ae. aegypti*) larvae were obtained from the National Centre for Disease Control (NCDC), Mettupalayam, Tamil Nadu, India and kept in plastic trays containing tap water and maintained under laboratory condition. All the experiments were done an ideal environmental condition (i.e. 27 ± 2 °C and 75–85% relative humidity under 14:10 light and dark photoperiods). Dog biscuits along with yeast powder is used as feed supplements to the larvae (in the ratio of 3:1) and maintained in the laboratory.

2.3. Larvicidal and pupicidal assay

Larvicidal and pupicidal activity of different concentrations (100–900 μg/ml) leaf extracts of both plants were carried out as per the modified protocol of World Health Organization (2005). The extracts were dissolved in 1 ml of 0.5% DMSO, and then it was diluted with 249 ml of filtered tapwater to obtain the desired concentration of the each extract. The control was prepared using 1ml of 0.5% DMSO in 249 ml of water. The 4^{th} instar larvae and pupae (25Nos) were introduced into each solution. For each concentration, three replicates were maintained and the larval and pupal mortality was recorded, after 24 and 48 h exposure. During this period, no food was administered to the larvae and pupae of test mosquito (Govindarajan et al., 2016).

2.4. Adulticidal bioassay

Adulticidal bioassay of plants leaf extracts were performed as per the protocol of WHO (2009). Using clean glass test tube and different concentrations of solvent extracts (ranging from 100-900 μg/ml) were placed in test tube and allowed to dry. Three replicates were maintained per concentration and twenty unfed female mosquitoes were released in each replicate. Tubes were tightly covered with mosquito net and coated with solvent alone served as control and the mortality was observed, after 24 h.

2.5. Maintenance and sub-culturing of cell line

Monolayer culture of C6/C36 mosquito cell line was brought from the local veterinary college, Tripathi, Andrapradesh and the cell line was maintained in the Prof. D.V.R. Sai Gopal Laboratory, Department of Viorology, Sri Vengateswara University, Tripathi. The cell culture plates were covered with spirit soaked cotton for removal of the adhering dust particles. The growth medium was discarded and then add 4–5 ml of MEM (without FCS) and gently rinsed. The dead cells and excess FCS were washed out and discarded the medium. TPVG was added over the cells and the plates were incubated at 37 °C for 5 min for disaggregation of cells. The cells became individual and it's present as suspension. Add, 5ml of 10% MEM with FCS using serological pipette. Then, the passaging cells were split into 1:2 and 1:3 ratio for cytotoxicity assessment (Mossman, 1983).

2.6. MTT assay

The MTT (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide) assay of sample was done as per the modified protocol of Mossman (1983). The each well was washed with MEM (w/o) FCS for 2–3 times, then add 200μl of MTT concentration (5 mg/ml) and incubated for 6–7 h in 5% CO_2 incubator, for studying the cytotoxicity assay. After incubation, 1ml of DMSO was added in each well and mixed by pipette and then left aside for 45sec. If any viable cells are seen after adding solubilizing reagent (DMSO) it shows the purple color formation. The suspension was transferred into the cuvette from spectrophotometer and taken Optical Density values at 595nm including the blank (DMSO). Cell viability (%) = Mean OD/Control OD x 100.

2.7. Preparation of crude extracts

The acetone extracts of two plants (10mg) was dissolved in 10 mL of serum free MEM yielded a concentration of 1mg/1mL. The freshly prepared stock was filtered through 0.45 filter, before to perform the experiment. The working concentration of plant crude extracts was maintained in the range of 1 mg/ml to 125 mg/ml.

2.8. Invitro antiviral tests by MTT assay

The mosquito cells (C6/C36) were seeded at a concentration of 5000 cells/well in 96 well culture plates along with different concentrations of acetone extract of both plants and dengue virus (2.6×10^5 $TCID_{50}$). Cells without plant extract and dengue virus cell used as controls. All the assays were tested in triplicates and the plates were incubated at 37 °C at 5% CO_2 atmosphere and observed upto 72 h. A specific cytopathic change induced by dengue virus was noticed at different time intervals of 24, 48 and 72 h of post inoculation periods. After 72 h of post inoculation, the cells were stained with trypan blue and the viable cells were counted (Chiow et al., 2016) and marked.

2.9. Phytochemical analysis

The preliminary phytochemical analysis of both plants extracts were carried out by the modified protocol of Harbone (1973).

2.10. GC–MS analysis

Gas Chromatography Mass Spectrometry analysis of both plants crude extract was performed in the Agilent 6890 GC equipped with 5,973 N mass selective detectors and an HP-5 capillary column. The carrier gas was helium at a flow rate of 1.0 ml/min^{-1} (constant flow). The sample (0.2 µl) was injected with a split of 20:1 and the temperatures were maintained at 230 °C and 150 °C, respectively (Govindarajan et al., 2011).

2.11. Fourier transmission-infra red analysis

The FT-IR spectrum of samples was measured using Arid Zone FT-IR spectrometer equipped with a DTGS detector. About 5mg of plants sample was mixed with 100 mg of dry potassium bromide (KBr) and the mixture was compressed to yield the small pellet. The pellet was analysed under FTIR spectrophotometer in the range of 4,000 - 500cm-1 at room temperature. An absorbance spectrum was acquired with 4cm^{-1} resolution and signal-averaged over 32 scans. Interferograms were Fourier transformed using cosine apodization for an optimum linear response. Spectra were baseline corrected, scaled for mass differences and normalized to the methylene peak at 2927 cm-1 (Cecilia et al., 2014).

2.12. Statistical analysis

The obtained data were analyzed using SPSS software version 16.0 (Statistical Package of Social Sciences). The average mortality data of all stages of mosquito were subjected to probit analysis for calculate LC_{50},

Table 1
Mosquitocidal activity of *P. tomentosa* and *T. asiatica* plant extracts against *Ae. aegypti*.

S.No	Mosqui to stages	Observ ations	Name of the Extract	*P. tomentosa*			*T. asiatica*		
				LC_{50} (LCL-UCL) (95% confidence limit) µg/ml	LC_{90} (LCL-UCL) (95% confidence limit) µg/ml	$\chi 2$	LC_{50} (LCL-UCL) (95% confidence limit) µg/ml	LC_{90} (LCL-UCL) (95% confidence limit) µg/ml	$\chi 2$
1	Larvae	24 h	Hexane	1.432 (99.454 ± 182.971)	1.967 (147.818 ± 239.279)	1.47	1.702 (52.595 ± 67.059)	2.518 (17.995 ± 35.819)	1.80
			Ethyl acetate	1.030 (68.757 ± 135.833)	1.442 (104.185 ± 180.778)	7.43	1.741 (54.046 ± 71.381)	2.561 (17.753 ± 38.581)	2.82
			Chloroform	1.582 (111.571 ± 199.949)	2.161 (164.761 ± 260.217)	3.75	0.952 (68.893 ± 11.625)	1.690 (41.695 ± 24.445)	7.62
			Acetone	9.325 (66.278 ± 119.326)	1.223 (91.693 ± 150±972)	26.7	1.288 (14.610 ± 22.518)	1.992 (89.510 ± 15.988)	10.0
			Methanol	9.325 (66.278 ± 119.326)	1.223 (91.693 ± 150.972)	22.4	1.284 (33.052 ± 44.059)	1.994 (16.738 ± 38.029)	3.29
		48 h	Hexane	8.625 (59.854 ± 112.167)	1.170 (86.276 ± 146.052)	8.6	1.200 (31.878 ± 43.251)	1.898 (17.633 ± 43.330)	5.01
			Ethyl acetate	6.914 (49.607 ± 88.653)	9.060 (68.057 ± 112.408)	6.21	1.140 (26.678 ± 37.355)	1.825 (16.428 ± 40.696)	13.3
			Chloroform	5.968 (67.808 ± 113.982)	1.182 (91.822 ± 143.217)	9.1	1.295 (36.244 ± 48.035)	2.007 (17.834 ± 41.461)	7.17
			Acetone	9.122 (44.270 ± 74.958)	7.493 (5.760 ± 91.677)	18.1	1.388 (62.845 ± 12.165)	2.193 (40.888 ± 60.166)	10.9
			Methanol	8.540 (66.005 ± 104.061)	1.054 (84.205 ± 125.513)	25.1	1.661 (23.285 ± 30.673)	2.386 (95.438 ± 15.224)	13.5
2	Pupae	24 h	Hexane	2.044 (1441.700 ± 4100.200)	8.519 (4208.333 ± 3627.885)	3.366	1.990 (1416.453 ± 4156.741)	6.429 (3341.125 ± 2755.465)	3.02
			Ethyl acetate	3.001 (1788.332 ± 9116.596)	2.771 (9119.005 ± 3217.527)	2.58	2.351 (1564.964 ± 5414.272)	1.245 (5725.772 ± 8132.208)	1.96
			Chloroform	2.512 (1632.583 ± 6615.054)	1.060 (4629.115 ± 7121.918)	4.52	2.429 (1568.960 ± 7654.611)	7.925 (3559.356 ± 6774.002)	3.29
			Acetone	1.361 (989.185 ± 2427.717)	1.682 (6815.540 ± 9859.402)	2.78	1.682 (1222.217 ± 2975.700)	1.169 (5499.152 ± 4800.661)	0.54
			Methanol	3.115 (1739.066 ± 1840.852)	5.348 (1338.774 ± 1420.311)	1.95	1.761 (1308.797 ± 3049.634)	7.153 (3844.482 ± 2353.708)	2.35
		48 h	Hexane	1.682 (1222.217 ± 2975.700)	1.169 (5499.152 ± 4800.661)	0.54	3.008 (1680.562 ± 1158.286)	5.723 (1382.214 ± 1728.322)	0.97
			Ethyl acetate	2.823 (1558.046 ± 1184.202)	7.612 (1597.633 ± 3845.940)	3.8	1.915 (1191.397 ± 5493.939)	5.036 (1260.239 ± 1332.072)	6.9
			Chloroform	2.298 (1539.874 ± 5038.395)	1.419 (6079.018 ± 7887.307)	4.6	3.975 (2113.849 ± 1759.681)	4.219 (1139.459 ± 9881.153)	3.24
			Acetone	3.273 (288.684 ± 366.054)	1.306 (1069.195 ± 1712.828)	28.01	4.555 (312.841 ± 399.326)	1.276 (1039.616 ± 1698.116)	28.9
			Methanol	5.888 (513.858 ± 692.573)	3.281 (2256.514 ± 5815.468)	11.1	4.186 (537.441 ± 734.930)	3.540 (2391.093 ± 6481.277)	14.6
3	Adult	0–60 mins	Hexane	11.810 (09.1245 ± 19.078)	44.215 (39.021 ± 48.197)	9.27	12.142 (09.11.700 ± 17.200)	28.019 (24.333 ± 36.185)	2.82
			Ethyl acetate	31.581 (28.146 ± 33.0980)	71.533 (44.910 ± 88.001)	11.3	30.901 (16.132 ± 41.596)	42.071 (19.124 ± 44.982)	7.21
			Chloroform	30.845 (29.501 ± 35.816)	66.201 (64.219 ± 78.235)	3.11	22.354 (21.0014 ± 25.964)	31.568 (28.467 ± 32.991)	2.91
			Acetone	32.105 (28.463 ± 36.589)	41.001 (35.350 ± 44.301)	1.31	09.012 (07.551 ± 11.328)	11.854 (9.254 ± 12.007)	7.31
			Methanol	55.510 (51.891 ± 71.526)	63.598 (59.887 ± 67.258)	0.76	30.210 (19.924 ± 33.215)	48.373 (44.215 ± 51.2543)	0.789

Significant at $p < 0.05$.
LC_{50} lethal concentration (50 % mortality), LC_{90} lethal concentration (90 % mortality), LCL lower confidence limits, UCL upper confidence limits, x^2 – Chi Square, df degrees of freedom– 3.

LC $_{90}$, 95 % fiducial limits of upper and lower confidence limits, chi-square and CC_{50} values. Results with $p < 0.05$ were considered as statistically significant (Finney, 1971).

3. Results

The crude extracts of both plants exhibits better larvicidal, pupicidal and adulticidal activity against *Ae. aegypti* (Table 1). The acetone and methanol extracts of *P. tomentosa* shown good mortality rate in *Ae. aegypti* with the LC_{50} and LC_{90} values of 9.325 and 1.223 μg/ml followed by *T. asiatica* having LC_{50} and LC_{90} values of 1.288 and 1.992, 1.284 and 1. 994 μg/ml (after 24 h) respectively. Whereas, the ethyl acetate and hexane extracts of *P. tomentosa* found to be moderate effect and *T. asiatica* noticed significant effect against *Ae. aegypti*. The least activity was noted in chloroform extract of both plants, after 24 h exposure period.

After 48 h treatment of both plants crude extract against the *Ae. aegypti* mosquito showed better mortality rate with least LC_{50} and LC_{90} values in tested extracts. i.e. acetone (9.122 and 7.493 μg/ml), ethyl acetate (6.914 and 9.060 μg/ml), hexane (8.625 and 1.170 μg/ml) and chloroform (5.968 and 7.493 μg/ml) respectively.

Table 2
Antiviral property of mosquito cell line (C6/C36) using *P. tomentosa* and *T. asiatica* acetone extracts against Dengue virus.

S. No	Cell culture status	Concentration (μg/ml) of the crude extracts	Cell viability (in %)	
			P. tomentosa	*T. asiatica*
1.	Normal Cell line as Control	Nil	100	100
2.	Virus infected cell line	10^{-6} virus dilution	10	10
3.	Virus infected cell line	1000	9.12	22.18
4.	Virus infected cell line	500	11.45	34.35
5.	Virus infected cell line	250	15.29	49.27
6.	Virus infected cell line	125	18.38	68.17

Cytotoxicity concentration, denotes significant difference at $P < 0.05$, compared to the negative control using ANOVA followed by post hoc Duncan Multiple Range Test (DMRT), performed by SPSS software.

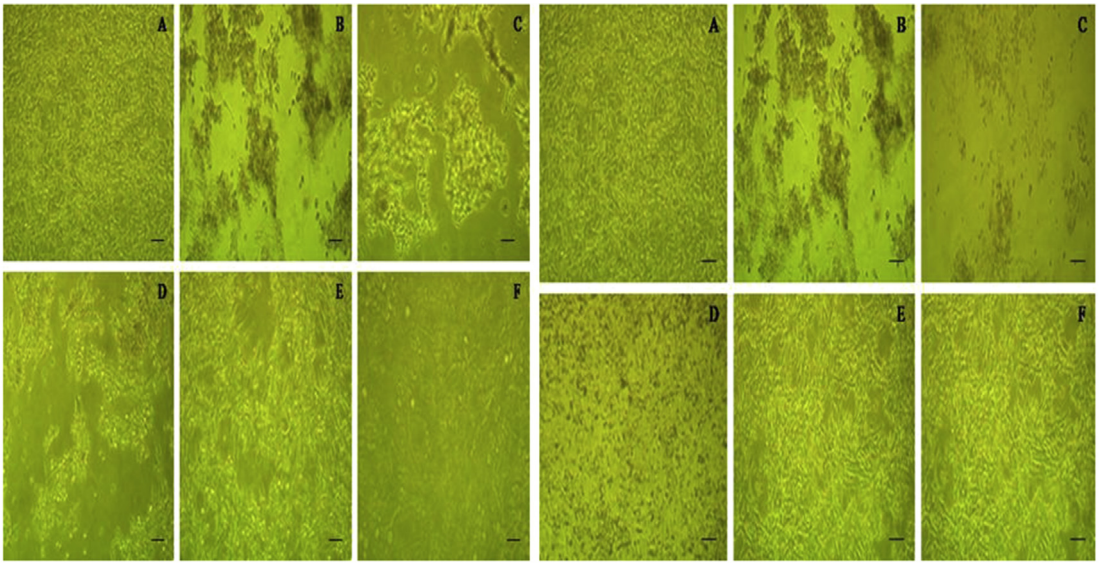
The pupicidal assay of plants extracts showed an excellent effect against test mosquito, after 24&48 h. Among them, acetone extract reflects highest pupicidal effect with the least LC_{50} and LC_{90} values i.e. 3.273 and 1.306 μg/ml, after 48 h exposure. Whereas, other tested crude extracts found to be more or less similar mortality rate, after 48 h of treatment.

The adulticidal activity results of both plants were observed at 0–60 min time intervals. The crude extracts of plants shown notable adulticidal activity against *Ae. aegypti*. Among them, acetone and hexane extracts expressed good mortality and the best LC_{50} and LC_{90} values of *P. tomentosa* (32.105and 41.001 μg/ml, 11.810 and 44.215) and *T. asiatica* (09.012 and 11.852 μg/ml, 12.142 and 28.019 μg/ml) was noticed after 60 min.

The antiviral property of plant extracts shown no significant cytopathic changes in cells treated with acetone extracts of *T. asiatica* and the best CC_{50} value was recorded in the concentration of 500 μg/ml, but *P. tomentosa* acetone extracts failed to restrict the virus cell lines at concentration of 125 μg/ml (Table 2 and Fig. 1) it means that *P. tomentosa* expressed good antiviral potential.

The preliminary phytochemical analysis of both plants revealed the presence of saponins, flavonoids and alkaloids in all the tested extracts. Amino acids and tannins were occurred in the acetone and methanol extracts. Proteins were present only in the chloroform extract and carbohydrates were found in chloroform and acetone extracts of both plants. Whereas, phenols are present only in the ethyl acetate extract of *P. tomentosa* (Table 3).

The GC-MS chromatogram results reflected forty nine compounds present in the acetone extract of *P. tomentosa* and seven major compounds (Table 4 & Figs. 2 and 3) were identified as follows: Hexanedioic acid, Bis(2-Ethylhexyl) Ester, 2,6,10,14,18,22-Tetracosahexane, 229 2,6,10,15,19,23- Hexamethyl-(ALL-E)-, 3,7,11,15- Tetramethyl-2-Hexadecen-1 OC, N-Hexadecanoic acid, Octadecanoic acid, Vitamin E and 1-Naphthalenepropanol, Alpha-Ethydecahydro-5-(Hydroxymethyl)-. But incase of *T. asiatica* have three major compounds like Tetracontane, 2- methyltetracosane and Eicosane (Table 5 & Figs. 4 and 5). The functional groups of compounds were identified based on the peak values i.e. aromatic compounds (1569.45cm^{-1}), alkanes, alkyls (1689.25^{-1}) and carboxylic acids (2926.48^{-1}) are majorly present in *P. tomentosa* and *T. asiatica* have aromatic compounds (1450.66^{-1}), ethers (1231.33^{-1}), alkyl halides (1344.35^{-1}) and alcohols (3345.49^{-1}). The bonding



Normal Cell Control, B- Virus Control, C-F - Extract concentrations (C- 1000 μg/ml, D- 500μg/ml, E- 250 μg/ml & F- 125 μg/ml), Cells - (40X) and Scale bar (50 μm)

Fig. 1. Antidengue potential of different concentrations of *P. tomentosa* and *T. asiatica* plant crude extracts against Dengue virus.

Table 3
Preliminary Phyto-chemical analysis of *P. tomentosa* crude extracts.

S. No	Phytochemical Name	Name of the Test	*T. asiatica*					*P. tomentosa*				
			Hx	EA	C	A	M	Hx	EA	C	A	M
1	Phenols	$FeCl_2$	–	–	–	+	+	–	+	–	–	–
2	Flavonoids	NaOH	+	+	+	+	+	+	+	+	+	+
3	Alkaloids	Wanger's	+	+	+	+	+	+	+	+	+	+
4	Saponins	Foam	+	+	+	+	+	+	+	+	+	+
5	Tannins	Braymer's	–	–	–	+	+	–	+	–	+	+
6	Glycosides	Keller Killiani	–	+	–	–	–	–	–	+	+	–
7	Proteins	Biuret	+	+	+	–	–	–	–	+	–	–
8	Amino Acid	Ninhydrin	–	+	+	+	+	–	–	–	+	+
9	Quinones	Quinone test	+	+	–	–	–	+	–	+	+	+
10	Carbohydrates	Fehlings	–	–	–	+	–	–	–	+	+	–

+ = Present, – = Absent.
Hx- Hexane, EA- Ethyl Acetate, C- Chloroform, A- Acetone, M- Methanol.

Table 4
GC-MS analysis from *P. tomentosa* acetone extract.

S. No	Peak area	Compound name	Nature of the compound	Molecular weight	Molecular formula	Biological activities	References
1	18.12	3,7,11,15- Tetramethyl- 2-Hexadecen-1-OC	Terpene alcohol	296	$C_{20}H_{40}O$	Cancer preventive, Antimicrobial and Fragrance compound	Sharmila et al. (2016), Venkatesh et al. (2014)
2	20.04	N-Hexadecanoic acid	Palmitic acid	256	$C_{16}H3_2O_2$	Potent mosquito larvicide, Antioxidant, Antiinflamatory, pesticide, flavour and Nematicide,	Vanitha et al. (2018), Sharmila et al. (2016), Venkatesh et al. (2014)
3	21.50	Octadecanoic acid	Stearic acid	284	$C_{20}H_{36}O_2$	Antimicrobial, Cancer preventive and Insectifuge	Ananthi and Ranjitha Kumari (2013), Abubakar and Majinda (2016)
4	22.54	Hexanedioic acid, Bis(2-Ethylhexyl) Ester	Diisooctyl adipate (DEHA)	370	$C_{22}H_{42}O_4$	-	-
5	25.33	2,6,10,14,18,22-Tetracosahexane, 2,6,10,15,19,23-Hexamethyl-,(ALL-E)-	Squalene, Organic compound, Triterpene	410	$C_{30}H_{50}$	Antibacterial, Antioxidant, Antitumor, Antiinflammatory, Hypocholesterolemic and Immunostimulant	Sharmila et al. (2016)
6	27.07	Vitamin E	Vitamin compound	430	$C_{29}H_{36}O_2$	Antidermatitic, Antileukemic, Antispasmodic	Santhosh Kumar et al. (2014)
7	30.08	1-Naphthalenepropanol, Alpha-Ethydecahydro- 5-(Hydroxymethyl)-	-	308	$C_{20}H_{36}O_2$	-	-

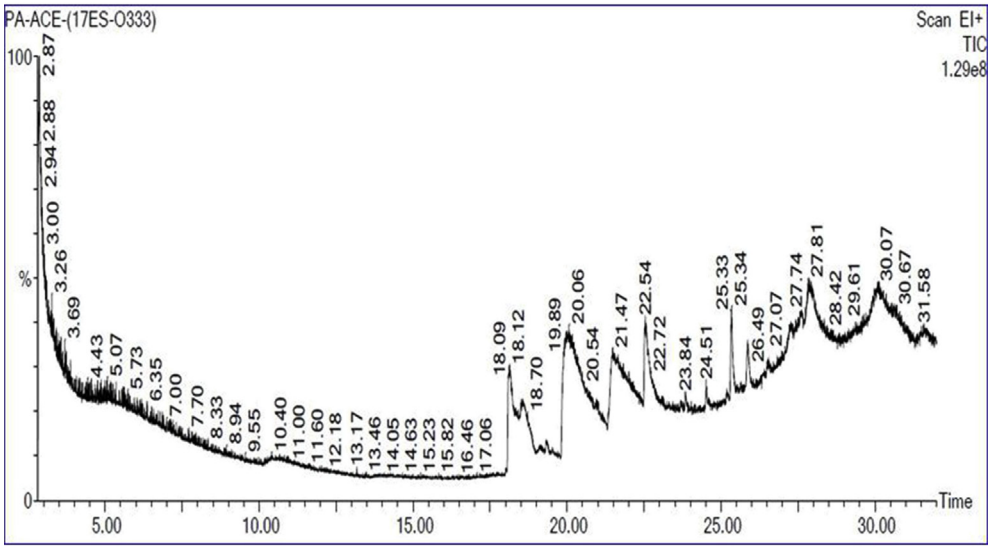


Fig. 2. GC-MS chromatogram of *P. tomentosa* acetone extract.

patterns of bioactive compounds are mainly C=C, O–H and C–H stretch vibrations of *P. tomentosa* and Ring C =C, =C–O–C, C–F and O–H stretch vibrations of *T. asiatica* (Table 6 & Figs. 6 and 7). The highest sharp peak was noticed in aromatic compounds, so it may be a chance for presence of phenolic or flavonoids compounds in the plants.

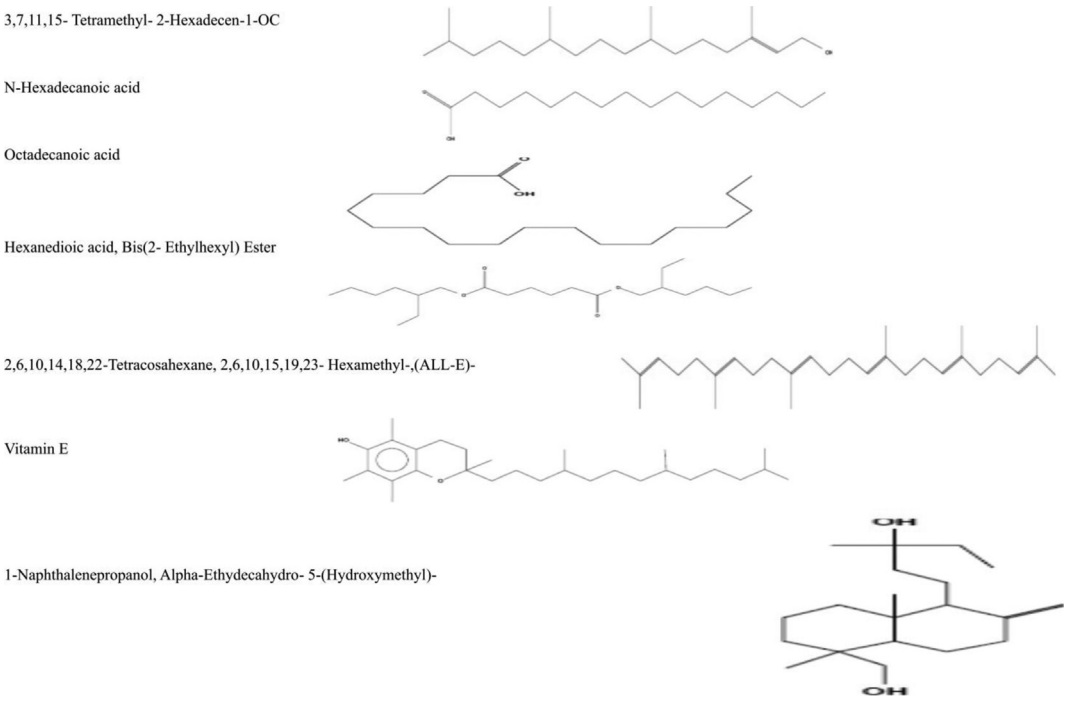


Fig. 3. GC-MS identification of major chemical compounds and their structures of acetone extract of *P. tomentosa.*

Table 5
GC-MS spectrum of *T. asiatica* acetone extract.

S. No	Peak area	Compound name	Nature of the compound	Molecular weight	Molecular formula	Biological activities	References
1	23.580 & 23.775	Tetracontane	Alkane	618	$C_{44}H_{90}$	Hypoglycaemic and Antioxidant activity	Arora and Saini (2017)
2	25.547	2-Methyltetracosane		352	$C_{25}H_{52}$	Free radical scavenging	Arora and Kumar (2017)
3	25.722	Eicosane	Alkane	282	$C_{20}H_{42}$	Antifungal, Antibacterial, Antitumor and Cytotoxic	Arora and Saini (2017)

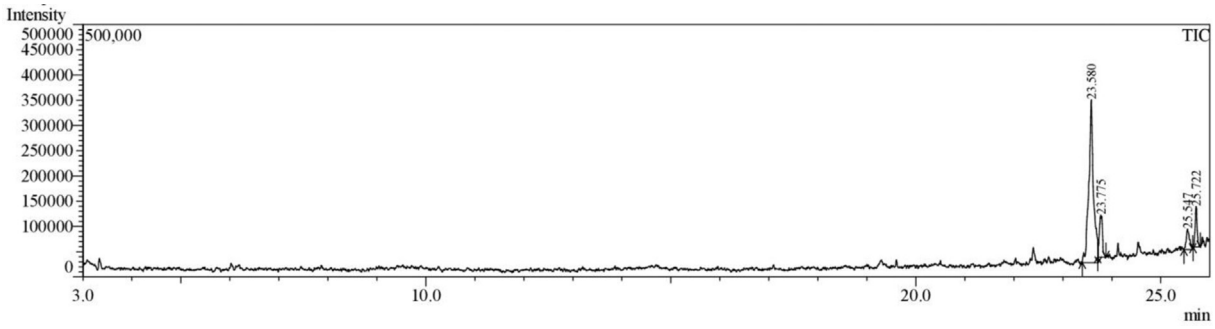


Fig. 4. GC-MS analysis of acetone extract of *T. asiatica*.

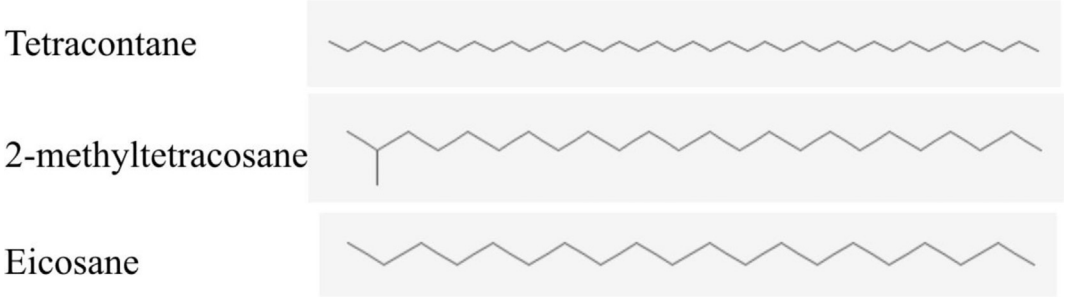


Fig. 5. GC-MS identification of major chemical compounds and their structures of acetone extract of *T. asiatica.*

4. Discussion

The present study was aimed to investigate into the antiviral-cum-mosquitocidal property of two plants against dengue virus vis-a-vis its vector. Different solvents (acetone, methanol, chloroform, ethyl acetate and hexane) of leaf extracts of *P. tomentosa* and *T. asiatica* were found to be more effective against an important vector mosquito, *Ae. aegypti*. So far, there is no report pertaining to the mosquitocidal activity against *P. tomentosa* and *T. asiatica* plant. Nazar et al. (2009) and Dhanasekaran et al. (2013) investigated the larvicidal and repellent potential of *Spermacoce hispida* crude extracts against *Ae. aegypti*. The outcome of present study was comparable with the previous work done by Jayaraman et al., (2015) who reported that the larvicidal potential of various solvent extracts from seven aromatic plants against three mosquito vectors including *Ae. aegypti* and better larval mortality was recorded after 12 and 24 h of exposure. Previously, many medicinal plants reported as having potent larvicidal agents against dengue vector i.e. leaf extract of *Solanum xanthocarpum* (Mahesh kumar et al., 2012) *Asparagus racemosus* root extract (Govindarajan and Sivakumar, 2014) Orange peel extract of *Citrus sinensis* (Murugan et al., 2012), leaf of *Cassia fistula* (Govindarajan, 2009), *Calotropis procera* (Singh et al., 2005) *Ocimum gratissimum* and *O. cannun* (Pratheeba et al., 2015) respectively.

We clearly noticed that acetone extract of both plants have good antiviral activity against dengue virus through MTT assays. Similarly,

Table 6
FT- IR analysis of acetone extracts from *P. tomentosa* and *T. asiatica*.

S. No	*P. tomentosa*			*T. asiatica*		
	Peak value	Functional group	Bonding pattern	Peak value	Functional group	Bonding pattern
1	3402.11	Carboxylic acids	O–H str	3918.52	Amines (RR'N–H)	N–H str
2	2926.48	Alkanes and Alkyls	C–H Str	3406.93	Amines (R–NH2)	N–H Symmetric &Asym. (twobands)
3	1736.84	Esters	C=O Str	3284.99	Amides (R–C(O)–NH2)	N–H Symmetric &Asym. Str
4	1689.25	Carboxylic acids	O–H str	3345.49	Alcohols (C=C–CH2–OH)	O–H str
5	1569.45	Aromatic compounds	C=C str	3095.75	Alkenes	=C–H str
6	1463.16	Alkanes and Alkyls	C–H b	2981.55	Alkanes and Alkyls	C–H str
7	1377.34	Alkanes and Alkyls	CH_3 C–H b	2717.27	Aldehydes (R–CH = O)	H–C=O str
8	1342.12	Alkyl halides	C–F str	2612.30	Carboxylic Acids (R–C(O)–OH)	O–H str
9	1297.25	Esters (Aromatic)	O=C–O–C str	1806.01	Acylchlorides (R–C(O)–Cl)	C=O str
10	1160.26	Esters (Aliphatic)	O=C–O–C str	1768.45	Esters (R–C(O)–O-Ar)	C=O str
11	1080.51	Alcohols	C–O str	1450.66	Aromatic compounds (two or Four bands)	Ring C=C str
12	1027.72	Ethers	=C–O–C sym & Asym. Str	1344.35	Alkyl halides	C–F str
13	926.26	Alkenes	=C–H b	1231.33	Ethers	=C–O–C sym & Asym. str
14	777.64	Alkyl halides	C–Cl str	1153.65	Alkyl halides	C–F str
15	720.09	Monosubstitutes	C–H b	1078.18	Alcohols	C–O str
16	621.23	Alkynes	=C–H b	803.14	Alkenes (RCH = CR'R")	=C–H b
17	571.51	Alkyl halides	C–Br str	743.49	Aromatic Compounds	C–H b
18	486.15	Alkyl halides	C–I str	686.46	Alkenes Cis-RCH = CHR′	=C–H b
				632.46	Alkyl halides (R–Br)	C–Br str

str- Stretch, b- Bend.

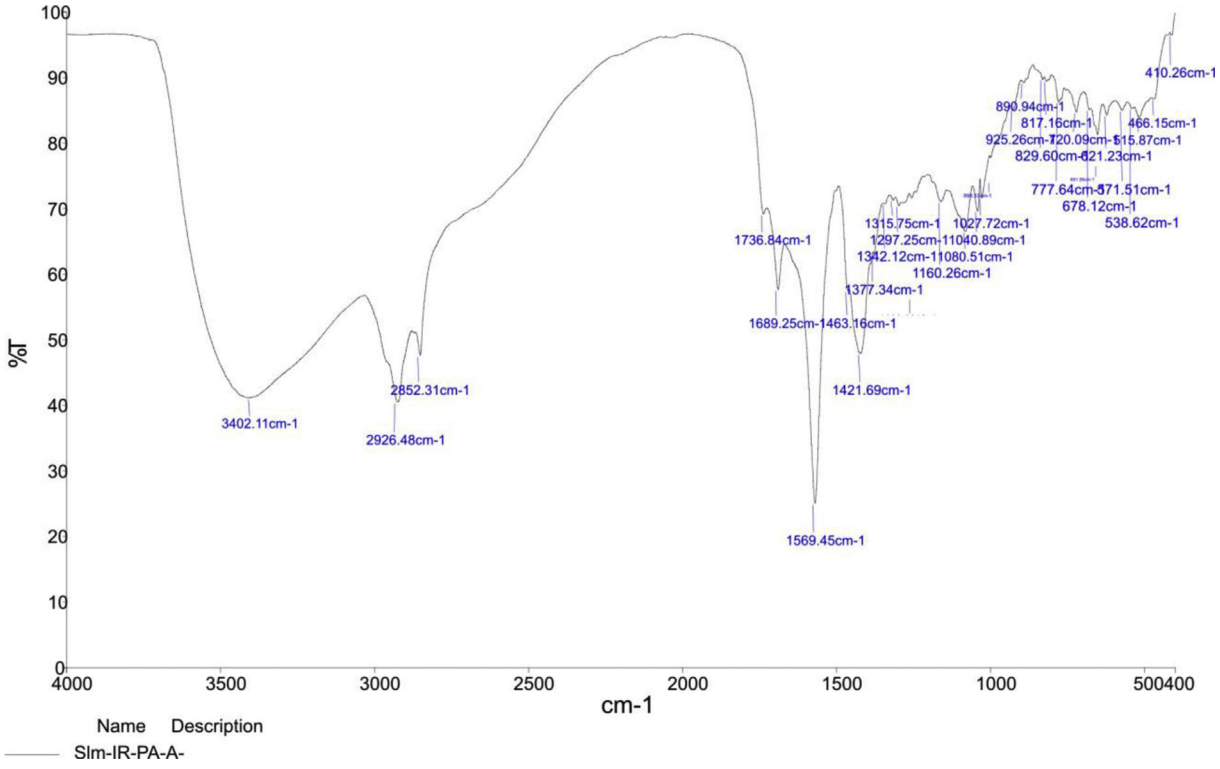


Fig. 6. FT-IR Spectrum of *P. tomentosa* acetone extracts.

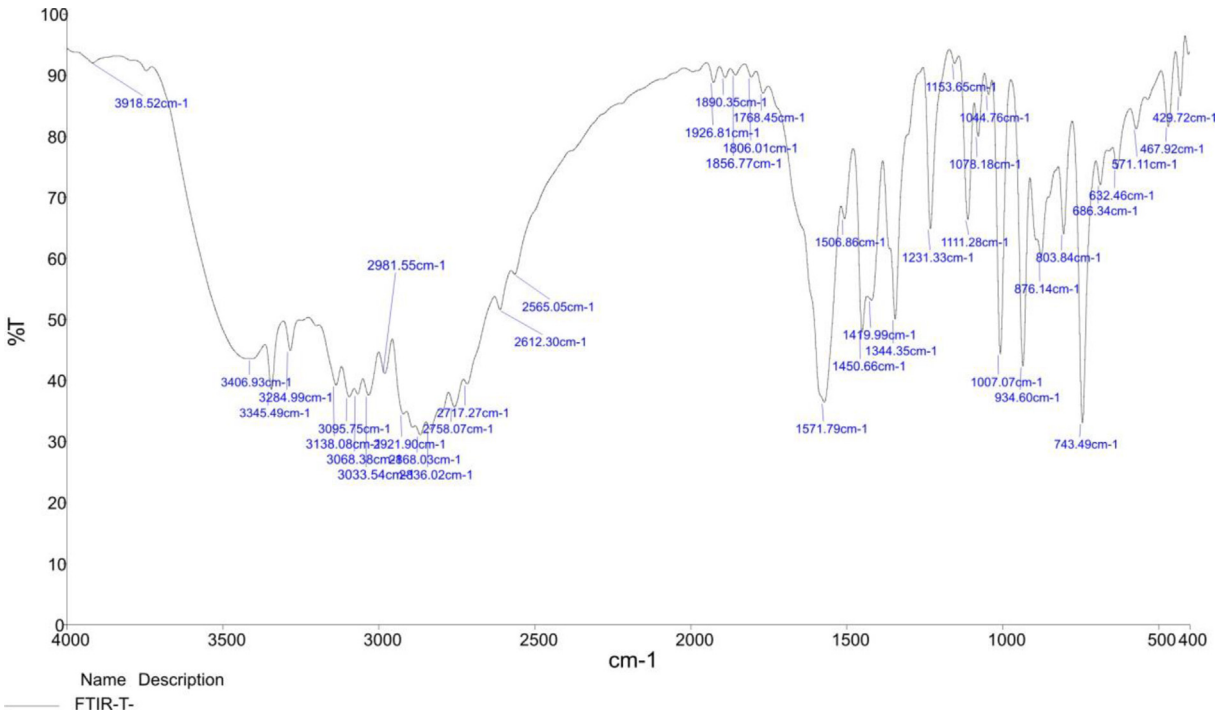


Fig. 7. FT-IR Spectrum of acetone extracts from *T. asiatica*.

Rothan et al. (2014) reported the ethanol extract of *Tridax procumbens* and methanol extract of *Vernonia cinerea* have a high inhibitory activity against dengue virus. Tang et al. (2012) proved the methanol extracts of *Andrographis paniculata* and *Momordica charantia* have the ability to inhibit dengue virus. On the other hand, Sood et al. (2015) studied the use of *Cissampelos pariera* and *Ficus septica* (Huang et al., 2017) have a potent antiviral activity against dengue virus. Similarly, Castillo Maldonado et al. (2017) investigated methanol extracts of *Sambucus nigra* leaves and flowers exhibited DENV-2 virus at 400 μg/ml.

Whereas, the outcome of present study noticed better results against dengue virus cell using *P. tomentosa* crude extracts at 125 μg/ml Klawikkan et al. (2011) reported that Thai medicinal plant extracts expressed better antiviral property against DENV-2 by MTT assays. Similarly, Ling et al. (2014) reported that *O. sanctum* extracts showed maximum inhibitory effects toward DENV-1.

The preliminary phytochemical analyses of extracts from both plants observed the kind of results was observed by Borokini and Omotayo (2012) the presence of alkaloids in *M. tomentosa*. Praveena and Suriyavathana (2013) and Krishnakumar et al. (2015) analyzed the phytochemical nature of *Toddalia asiatica* for the presence of alkaloids, flavonoids and saponins. GC-MS from *P. tomentosa* plant leaf extracts shown major compounds may be responsible for potent larvicidal effect against *Ae. aegypti*. The bioactive compounds like alkaloids (Young et al., 1996), Flavonoids (Lopes et al., 2004; Cardoso et al., 2005; Pinto et al., 2008) have been identified from may Rubiaceae plants. Similarly, Moharana et al. (2015) identified the essential oil from *P. tomentosa* leaves by GC-MS. GC-MS and FT-IR results of plant samples reflect the several bioactive compounds and its functional groups. N-Hexadecanoic acid, Palmitic acid, Octadecanoic acid, Squalene, Vitamin and Carboxylic acids, Esters, alkenes, alkynes, alcohol were present in *P. tomentosa* and *T. asiatica* crude extracts. Similarly, Fadipe (2014) reported the palmitic acid and ester were identified by GC-MS and FT-IR. The results of present investigation was comparable with the report of Owolabi et al. (2018) who found, the functional groups of bioactive compounds from *Feretia apodanthera* plant crude extracts. Similarly, Vanitha et al. (2018) studied the GCMS & FT-IR analysis of *Benkara malabarica* leaf extracts show the compounds along with functional groups (like N-Hexadecanoic acid, Octadecanoic acid, Vitamin E and Squalene and Aromatic rings and alkynes). The bioactive compounds and its functional groups were reported from *Benkara brieyi* (Odo et al., 2017), *Neolamarckia cadamba* (Zayed et al., 2014) and *Mentha spicata* (Jain et al., 2016) by various researchers using GCMS and FTIR analysis.

5. Conclusion

In conclusion, we report the leaf extracts of *P. tomentosa* and *T. asiatica* had significant mosquitocidal property against *Ae. aegypti* vector mosquito. The remarkable antiviral activity of dengue virus was found in *P. tomentosa* than *T. asiatica*. The preliminary phytochemicals analysis results showed the presence of flavonoids, alkaloids and saponins in all extracts of *P. tomentosa*. GC-MS result reflects the presence of organic compounds, squalene or triterpene nature of compounds and FT-IR result indicate the functional groups of aromatic compounds, carboxylic acids, alkanes and alkyls. This is the first hand scientific informations on *P. tomentosa* and *T. asiatica* plant leaf extracts play a dual role for prevention of the dengue virus and its vector. Further study, in related to isolation of pure/active mosquitocidal and antiviral compounds from plants are under progress.

Declarations

Author contribution statement

D. Natarajan: conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

T. Pratheeba: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

V. Taranath: Performed the experiments; Contributed reagents, materials, analysis tools or data.

DVR Sai Gopal: Analyzed and interpreted the data; Contributed

reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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