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Original Research Article

Phytochemical Profiling and Mosquitocidal Properties of Grape Fruit Pedicel Extract Against Malarial, Dengue and Filarial Vectors

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ABSTRACT:
The dengue, malaria and filariasis are serious global disease which caused by the mosquitoes, *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus*. These species cause high morbidity and mortality to the human population and the development of resistance to chemical insecticides resulting in rebounding vectorial capacity. Plants may be alternative sources of mosquito control agents. The GC-MS analysis of grape fruit pedicel was done and five major compounds were identified in the methanolic grape pedicel extract namely, N-Hexadecanoic Acid, 1,E-11,Z-13-Octadecatriene, 9,12-Octadecadienoic Acid, 9-Octadecynoic Acid and 6,8-Dodecadien-1-OL (6Z,8E). The mosquitocidals activity of methanol extracts from grape fruit pedicel against immature and adult of *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* (L.) were studied. After 24 hrs the mortality was noted and Lethal Concentration (LC_{50}) was calculated against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. The LC_{50} of *An. stephensi* were 133.263ppm, 178.275ppm, 235.619ppm, 284.472ppm and 380.630ppm for I, II, III, IV Instar and pupae, respectively. Similarly, LC_{50} for *Ae. aegypti* were 89.093ppm (I Instar), 196.560ppm (II Instar), 241.043ppm (III Instar), 323.565ppm (IV Instar) and 363.515ppm (pupae) and for *Cx. quinquefasciatus* were 190.073ppm, 261.693ppm, 295.404ppm, 289.067ppm and 348.430ppm for I Instar, II Instar, III Instar, IV instar and Pupae, respectively. After the treatment of grape pedicel extract the percentage of egg hatchability (Ovicidal activity) was observed. No eggs were hatched out after 400ppm of three mosquito species. In ovipositional deterrent study the number of eggs laid in control and treatment water was observed and based on this the Effective Repellency (ER) was calculated. The ER ranging from 69.83% to 88.43 % for *An. stephensi;* 72.18% to 89.14% for *Ae. aegypti*; 69.66% to 88.81% for *Cx. quinquefasciatus*, was investigated. It is thus concluded that the grape fruit pedicel extract has an effective toxicity against *An. stephensi*, *Ae. Aegypti* and *Cx. quinquefasciatus*. Hence, this pedicel extract can be used as an insecticide.

Keywords: Grape pedicel, Phyto-Chemical, Mortality, Hatchability.

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INTRODUCTION

Arthropods are the diverse group of animals includes insects, arachnids, crustaceans, and more. Arthropods are acting as the vector of a number of life threatening pathogens, parasites etc., across the world. Among them, mosquitoes are playing a leading role in transmitting parasites, viruses, bacterial diseases, fungal infection from individual to individual within a short period and pull them to dead (Murugan *et al*., 2015a,b,c; Beneli *et al*., 2015a,b).

Mosquitoes can transmit more diseases than any other group of arthropods and affect millions of people throughout the world. WHO has declared the mosquitoes as "public enemy number one" (World Health Organization, 1996). Mosquito borne diseases are prevalent in more than 100 countries across the world, infecting over 700 million people every year globally and 40 million of the Indian population. They act as a vector for most of the life threatening diseases like malaria, yellow fever, dengue fever, chikungunya ferver, filariasis, encephalitis, West Nile virus infection, *etc.*, in almost all tropical and subtropical countries and many other parts of the world. Member States in three WHO regions regularly report the annual number of cases. The number of cases reported increased from 284 million in 2015 to 528 million dengue infections in recent time (WHO, 2018).

Control measure against this vector in the short-term is the use of conventional insecticides. But the extensive use of synthetic organic insecticides during the last five decades have resulted in environmental pollution and also in the development of physiological resistance in major vector species in addition to the increased costs of insecticides. In this context, several researchers have conducted experimental studies on application of alternative insecticides resources which have minimal or absent undesirable effect on environment and human health. The extracts and/or essential plant oils have been tested against insects and mosquito vectors. The biological agents are easily degradable into less or nontoxic compounds and proven to be safely used for mosquito control programs. In the literature, several experiments were carried out to examine the effect of plant extracts or essential oils against mosquito larvae and showed positive results (see Rasheed *et al*., 2005; Amer and Mehlhorn 2006a, b; Rahuman *et al*. 2008a, b).

Secondary metabolites such as phenolics, flavonoids, terpenoids, coumarins and alkaloids are active against some of the target insects, and potentially suitable for Integrated Pest Management (IPM) programs, they could lead to the development of new classes of safer insect control agents (Park *et al.,* 2002; Mansour *et al.,* 2004; Panneerselvam *et al.,* 2016). Plant-borne chemicals can act as an ovicides, larvicides, pupicides, adult repellents and oviposition deterrents against a wide number mosquito species of economic importance (Panneerselvam *et al.,* 2013; Govindarajan *et al.,* 2016; Aziz *et al.,* 2018). Previously, mosquito control using phytochemical products arising as a valuable alternative, due to their reduced toxicity towards non-targets and high biodegradability (Ravikumar *et al.,* 2011; Benelli *et al.,* 2015a). Flavonoids protects the plants from insects feeding as well as other herbivores animals by altering the palatability of the plants and reducing their nutritive value, or even act as toxins (Harborne and Williams, 2000). Flavonoids are also known as a vitamin pelicit since it possess a variety of medicinal activities such as antidiuretic, antioxidant, antimicrobial and anticancer properties (Usha and, Bopaiah, 2011; Shakya, 2016). Phyto-compounds, including phenolics, terpenoids, and alkaloid (Wink, 1993; Kim *et al.,* 2001) had good insecticidal activity against both crop and human pest (Assabugi *et al.,* 1997).

Grapes are so useful in that every part of it can be used for culinary purposes. They can be very nutritious and beneficial to human beings at the same time. The skins can be used for food coloring and the pulp and juice can be used for jams, raisins and wines. The seeds are used as oil and the leaves for dolmas. Compounds in grapes help fight cancer, heart disease, degenerative nerve disease and others. Grape skins contain resveratrol, which is a powerful antioxidant that may prevent

cancer and cardiovascular disease. The antioxidant helps to lower the levels of cholesterol circulating in the body and hence reduces cholesterol deposition in the arteries. Several studies have shown that the antioxidant also has protective effect against prostate cancer. Grape seed extract is used as a natural antihistamine and anti-inflammatory agent. These medicinal uses of the plant to enhance of interest to find out the activity of remaining part of the plant. In this study, we highlighted the phytochemical analysis and insecticidal activity of the Grape pedicel methonolic extract. Further, we investigated that ovicidal and ovideterrent toxicity of Grape pedicel extract.

MATERIALS AND METHODS

Collection of Grape pedicel

The Grape pedicels were collected from in and around fruit stall of Komarapalayam, Namakkal District, Tamilnadu, India. The specimen has been deposited in the Department of Zoology, J. K. K. Nataraja College of Arts and Science, Komarapalayam-638183, Tamilnadu, India.

Preparation of pedicel extract

The collected pedicels were washed with tap water, shade tried at room temperature. The dried pedicels were powdered with electrical blender. The powdered grape pedicel (1.0 kg) was then subjected to extraction in methanol (5.0 L) using soxhexlet extraction apparatus for 8 hours individually. The extract was filter through a Buchner funnel with Whatman number 1 paper. The filtrate was evaporated to dryness under reduced pressure using rotary vacuum evaporator. One gram of the pedicel residue was dissolved in 100-mL of methanol (stock solution) and considered as 1% stock solution. From this stock solution, different concentrations were prepared ranging from 100 to 500 ppm, respectively.

GC-MS analysis

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250μm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1μL of extract sample injected into the instrument the oven temperature was as follows: 60°C (2 min); followed by 300°C at the rate of 10 °C min−1; and 300°C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 230°C; ion source temperature 230°C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS National Institute of Standards and Technology (2008) library.

Laboratory colonization of mosquitoes

For the different bioassays enormous amount of different stages of mosquito colony needed. The egg rafts/eggs of three mosquito species such as *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* were obtained from PG and Research Department of Zoology, J. K. K. Nataraja College of Arts and Science, Komarapalayam-638183, Tamilnadu, India. The laboratory colony was maintained at 70-85% RH, 28±2ºC temperature and 12; 12 light and dark photoperiod cycle. The larvae were fed on powdered mixture of dog biscuits and yeast powder in 3:1 ratio. The adults were provided with 5% glucose solution and honey was given to male and female with one week old chick blood meal. Eggs, larvae and adult females were continuously available for the bioassays from these laboratory colonized mosquitoes**.**

Bioassay

Larvicidal and pupicidal activity

The larvicidal and pupicidal activity of Grape pedicel extract was evaluated as per the method recommended by World Health Organization (2005). The extract was tested for larvicidal and pupicidal activity against *An. stephensi*, *Ae. aegypti* and *Cx. qinquefasciatus*. Batches of 25 first instar, second instar, third instar, fourth instar larvae and pupae of *An. stephensi*, *Ae. aegypti* and. *Cx. qinquefasciatus* were separately transferred to a small disposable test cups, each containing 200ml of water. The appropriate volume of dilution was

added to 200ml of water in the cups to obtain the desire target dosage, starting with the lower concentration. The larval mortality was observed and recorded after 24 h. each test was replicated five times and equal number of controls was set up simultaneously using tap water. To this 1 ml of methanol was added. The percentage of mortality was calculated by using Abbott's formula (Abbott, 1925). The LC_{50}, $LC_{90,}$ 95 percent confidence limit of lower confident limit (LCL) and upper confidence limit (UCL) and chi-square values were calculated by using profit analysis (Finney, 1947).

Oviposition deterrent Activity

The oviposition deterrent test was performed using the method of Xue *et al*. (2001). Fifteen gravid female *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* were (10 days old, 4 days after blood feeding) transferred to each mosquito cage (45 x 38x 38 cm) covered with a plastic screen, with a glass top, and a muslin sleeve for access. A 10% sucrose solution was available at all times. Serial dilutions of pedicel extract were made in methanol. Enamel bowls containing 100 ml of rainwater or stagnant water were treated with pedicel extract to obtain test solutions of 100, 200, 300, 400 and 500ppm. Two enamel bowls holding 100 ml of rainwater were placed in opposite corner that contained 1% methanol. The positions of the bowls were alternated between the different replicates so as to nullify any effect of position on oviposition. Three replicates for each concentration were run, with cages placed side by side for each bioassay. All experiments were run at ambient temperature (27 ± 2ºC) with relative humidity of 70-80%. After 24h, the number of eggs laid in treated and control bowls was recorded.

The percent effective repellency for each leaf extract concentration was calculated using the following formula

$$ER\ (\%) = \frac{\text{Number of eggs in control} - \text{Number of eggs in treatment}}{\text{Number of eggs in control}} \times 100$$

Where
ER= Percent effective repellency
NC= Number of eggs in control
NT= Number of eggs in treatment

Determination of oviposition activity index (OAI)

The results of the oviposition experiment were expressed as mean number of eggs, and oviposition activity index (OAI), which was calculated using the formula.

$$OAI = \frac{NT - NS}{NT + NS}$$

Where NT is the total number of eggs in the test solution and NS is the total number of eggs in the control solution. Index values lie within the range +1 to -1. Positive values indicate that more eggs were deposited in the test cups than in the control cups and that the test solutions were attractive. Conversely, negative values indicated that more eggs were deposited in the control cups than in the test cups and that the test solutions were a deterrent.

Ovicidal activity

The method of Su and Mulla (1998) was slightly modified and used to test the ovicidal activity. The eggs of *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* were collected from in and around Komarapalayam, Namakkal. The Grape pedicel extract was diluted in the methanol to achieve various concentrations (100, 200, 300, 400 and 500ppm). Before treatment the egg rafts of *Cx. quinquefasciatus* and eggs of *Ae. aegypti* and *An. stephensi* were counted under microscope individually. Eggs of these mosquito species (100 numbers of 12-18h old eggs) were exposed to each concentration of crude extracts (100 numbers of 0-6, 6-12 and 12-18h old eggs) until they hatched or died. After treatment the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under microscope. Each experiment was replicated five times along with appropriate control. The hatch rate was assessed 48 h post treatment by following formula.

$$\textbf{\% of egg hatchability} = \frac{\textbf{Number of hatched lavae}}{\textbf{Total number of eggs}} \times \mathbf{100}$$

Statistical analysis

All data were subjected to Analysis of Variance (ANOVA). LC_{50} and LC_{90} values and their 95% confidence limits were estimated by getting a probit regression

model to the observed relationship between percentage mortality of larvae and logarithmic concentration of the substance. The goodness of fitness of the model was tested using Chi-Square test AP value of less than 0.05 was considered as a significant departure of the model from the observations. In case of significant departure a heterogeneity factor was used to calculate the 90% confidence limit for LC_{50} and LC_{90}. All analysis was carried out using SPSS Software version 16.0.

RESULTS

The bioactive compounds present in methanol extract obtained from Grape Pedicel are shown in Table 1. Their identification and characterization were based on their elution order in a GC-MS column. Based on abundance, there were five major compounds present in the methanolic pedicel extract were N-Hexadecanoic Acid (21.973%), 1,E-11,Z-13-Octadecatriene (16.670%), 9,12-Octadecadienoic Acid (Z,Z), 9-Octadecynoic Acid and 6,8-Dodecadien-1-OL (6Z,8E) (14.754%). The GC chromatograms of the grape pedicel extract presented in Figure 1 show the retention time in the column and the detected peaks which correspond to the bioactive compounds present in the extract. Spectra and chemical structure of phytocompounds were represented in Figure 1A.

Figure 1: Phytoconstituents detected in the methanol extract of grape pedicel using gas chromatography-mass spectrometry.

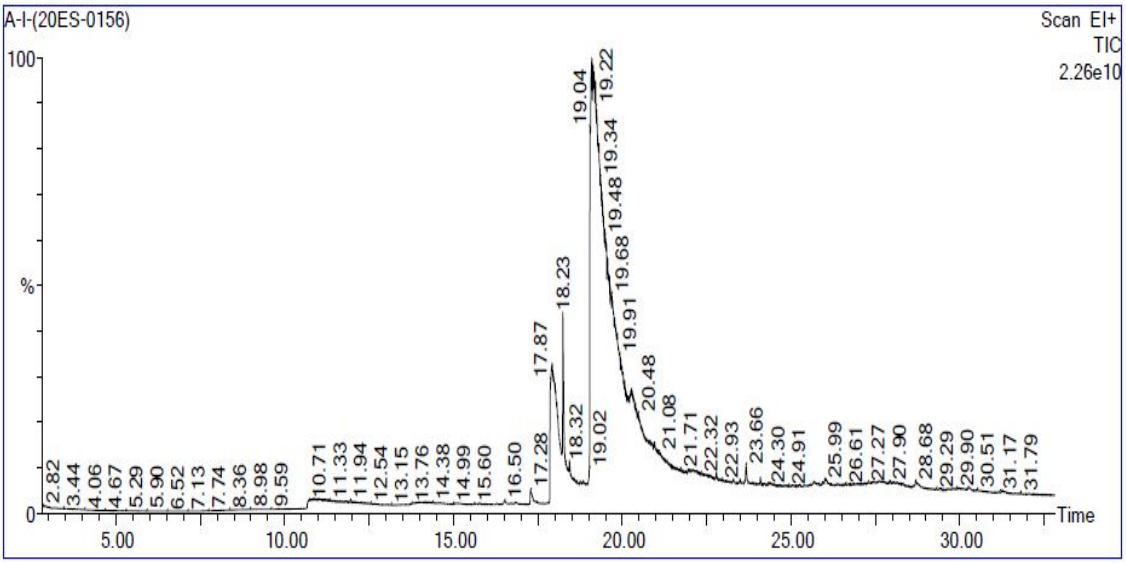
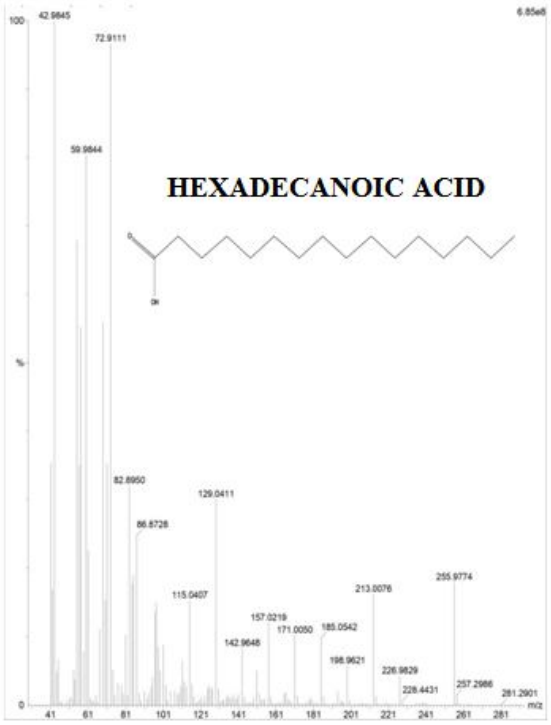
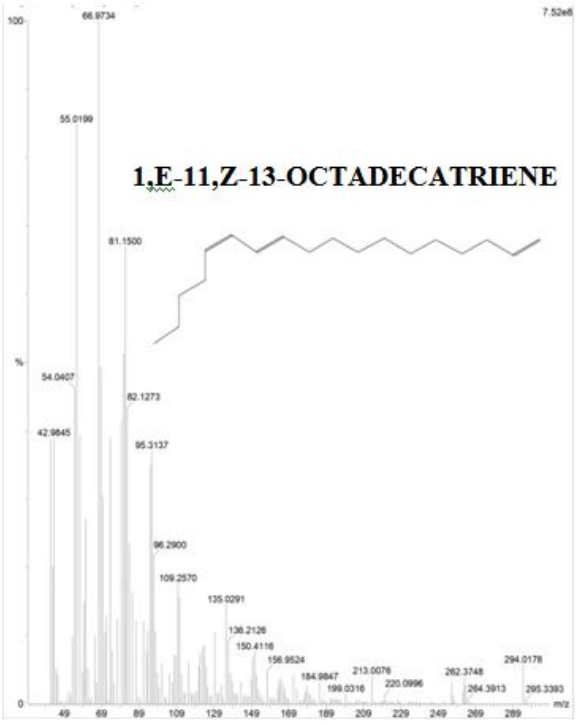
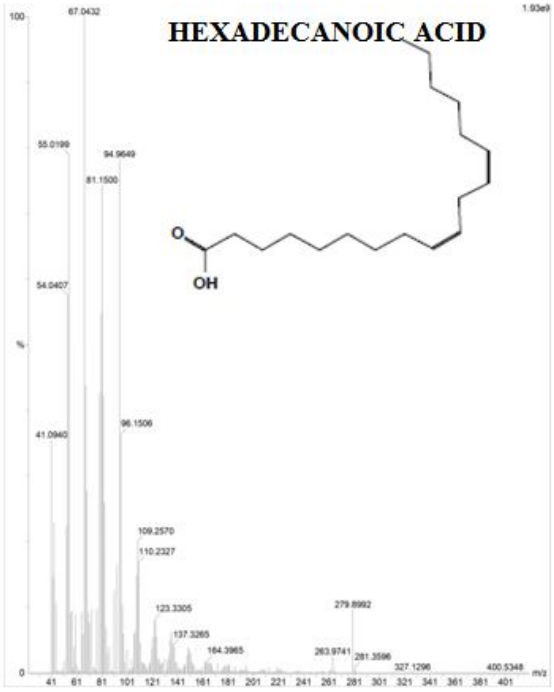


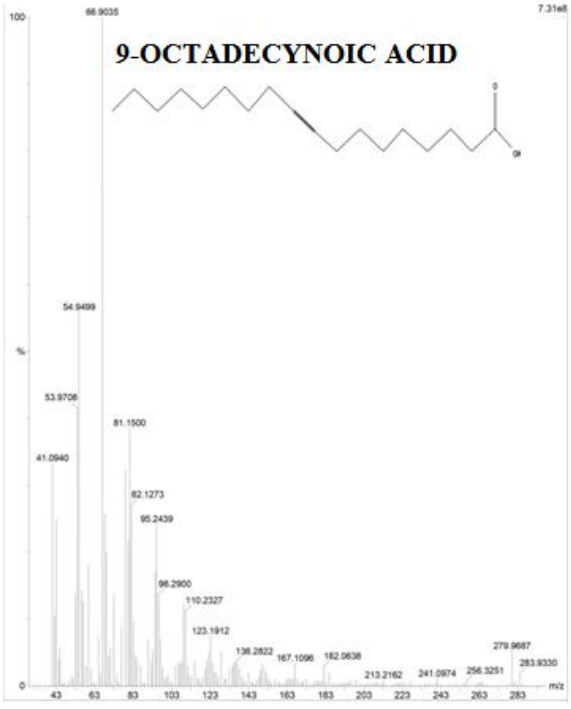
Table 1: Gas-Chromotography mass spectrometry analysis of Methanolic grape fruit pedicel extract

Peak No.	Compound Name	Molecular Weight	Formula	Nist	Retention time (min)	Scan	Height	Area	Area%	Norm%
1.	N-Hexadecanoic Acid	256	$C_{16}H_{32}O$	198159	17.909	3021	6,712,750,592	1,528,519,808.0	8.825	11.80
2.	1,E-11,Z-13-Octadecatriene	248	$C_{18}H_{32}$	28512	18.234	3086	8,290,862,080	331,930,176.0	1.916	2.56
3.	9,12-Octadecadienoic Acid (Z,Z)-	280	$C_{18}H_{32}O_2$	198648	19.095	3258	21,187,698,688	12,953,250,816.0	74.789	100.00
4.	9-Octadecynoic Acid	280	$C_{18}H_{32}O_2$	2949	20.275	3494	4,521,505,792	1,908,788,608.0	11.021	14.74
5.	6,8-Dodecadien-1-OL (6Z,8E)	182	$C_{12}H_{22}O$	28894	20.951	3629	1,734,596,480	597,269,056.0	3.448	4.61


HEXADECANOIC ACID


1,E-11,Z-13-OCTADECATRIENE


HEXADECANOIC ACID
OH


9-OCTADECYNOIC ACID

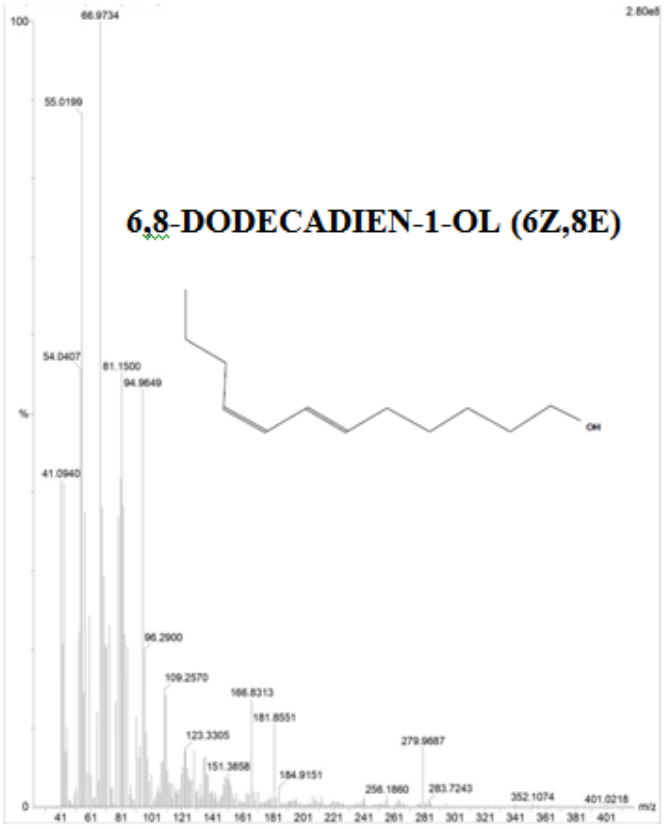


Figure 1 (A): Spectra and Chemical structure of five phytocompounds identified.

Larvicidal and pupicidal activity of Grape Fruit Pedicel Extract (GFPE) at various concentrations against the malarial vector, *An. stephensi* is given in the Figure 2A. Considerable mortality was evident after the treatment of GFPE for all larval instars and pupae. Mortality was increased as concentration increased, for example, 46.6 % mortality was noted at I instar larvae by the treatment of GFPE at 100ppm whereas; it has been increased to 96.0 % at 500ppm of GFPE treatment. Similarly, the same trend has been noted for all larval stages and pupae of *An. stephensi* at different concentrations of GFPE treatment. The LC_{50} and LC_{90} values represented as follows: LC_{50} value of I, II, III, IV instars and pupa were 133.263ppm, 178.275ppm, 235.619ppm, 284.472ppm and 380.630ppm, respectively. LC_{90} value of I, II, III, IV instars and pupa were 424.174ppm, 468.131ppm, 567.052ppm, 746.610ppm and 663.636ppm, respectively (given in table-2). The obtained chi-square values states that there is no much difference between the expected and observed mortality. The chi-square values were significant at $p<0.05$ level. The similar trends has been noted all treatments.

Table 2: Larvicidal and Pupicidal activity grape fruit pedicel extract *against Anopheles stephensi*

Larval instars	LC $_{50}$ (LC_{90})	Regression equation	95% Confidence limit		Chi-Square value (x^2)
			LCL LC_{50}(LC_{90})	UCL LC_{50}(LC_{90})	
I	133.263 (424.174)	*y*=0.587+0.004 *x*	85.560 (383.625)	168.105 (482.387)	1.147*
II	178.275 (468.131)	*y*=0.788+.004 *x*	139.596 (424.764)	208.626 (530.340)	1.783*
III	235.619 (567.052)	*y*=0.911+0.004 *x*	121.552 (453.436)	308.721 (898.684)	6.496*
IV	284.472 (746.610)	*y*=1.360+0.004*x*	301.874 (577.686)	360.959 (736.649)	2.343*
Pupae	380.630 (663.636)	*y*=1.724+0.005*x*	321.461 (546.661)	469.751 (950.709)	5.643*

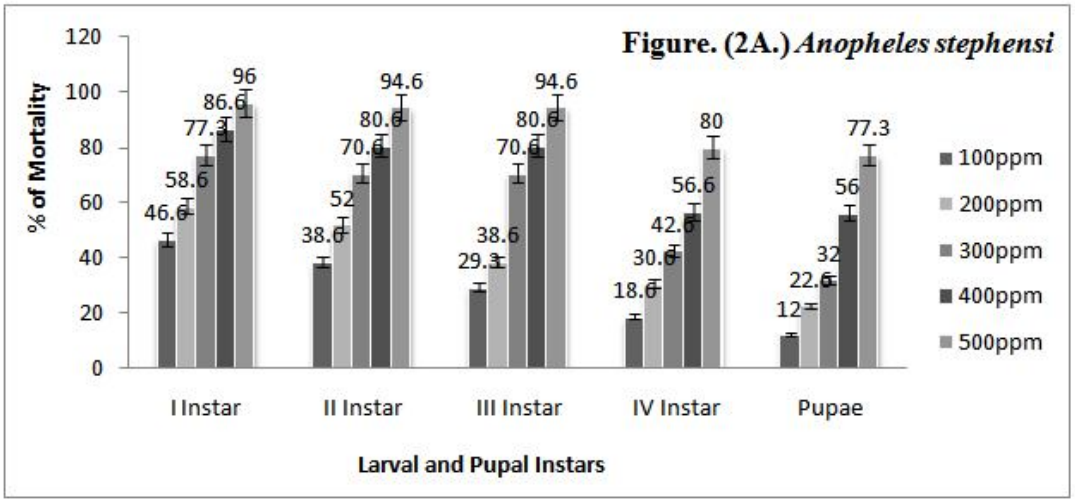
The larval mortality is expressed as mean ± SD of five replicates. Nil mortality was observed in the control. Within a column means followed by the same letter(s) are not significantly different at 5% level by Duncan's multiple range tests. *LFL* – Lower Fiducidal Limit; *UFL* - Upper Fiducidal Limit. x^2, Chi-Square value. *Significant at $p < 0.05$ level.

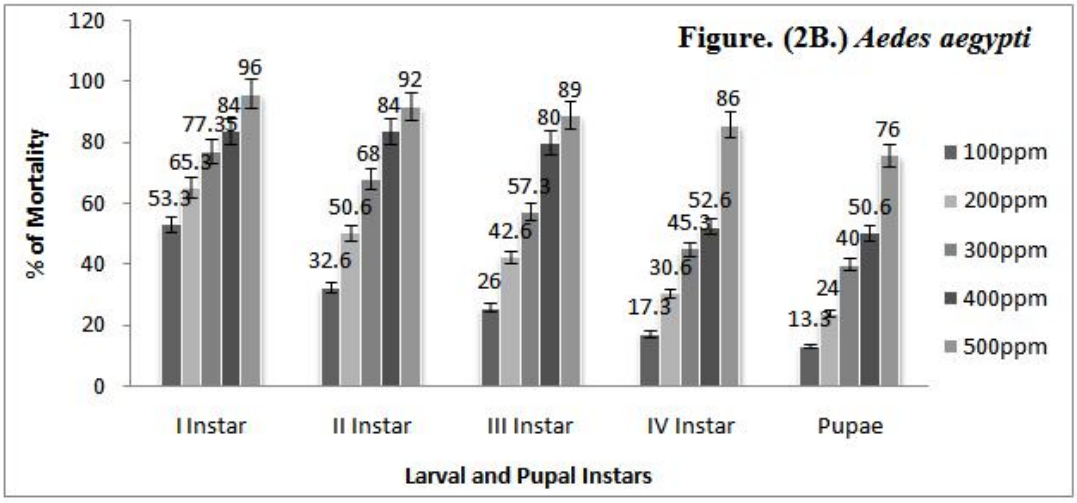
Figure 2B demonstrate the effect of GFPE at different concentrations on the mortality and toxicity of larval instars and pupae of dengue vector, *Ae. aegypti*. Significant mortality rate was observed after the treatment of grape pedicel extract. Maximum mortality was noted in all larval and pupal instars of *Ae. aegypti*. Highest mortality rate (96%) was observed in I Instar larva and lowest mortality rate (13.3%) was observed in pupae at 100ppm concentration of GFPE. LC_{50} and LC_{90} values were calculated using the observed mortality and LC_{50} values for GFPE are 89.093 ppm, 196.560ppm, 241.043ppm, 323.565ppm, 363.515ppm and LC_{90} values are 438.650ppm, 468.954ppm, 510.031ppm, 609.192ppm and 658.027 ppm for I, II, III, IV larval instars and pupae, respectively (given in table 3).

Table 3: Larvicidal and Pupicidal activity grape fruit pedicel extract *against Aedes aegypti*

Larval instars	LC $_{50}$ (LC_{90})	Regression equation	95% Confidence limit		Chi-Square value (x^2)
			LCL LC_{50}(LC_{90})	UCL LC_{50}(LC_{90})	
I	89.093 (438.650)	*y*=0.327+0.004 *x*	16.660 (390.173)	136.368 (513.435)	2.219*
II	196.560 (468.954)	*y*=0.925+0.005 *x*	468.954 (428.055)	224.135 (526.273)	0.098*
III	241.043 (510.031)	*y*=1.148+0.005 *x*	212.578 (466.665)	266.436 (570.636)	0.990*
IV	323.565 (609.192)	*y*=1.452+0.004 *x*	243.948 (486.475)	419.217 (988.338)	8.633*
Pupae	363.515 (658.027)	*y*=1.582+0.004 *x*	335.917 (594.806)	395.294 (751.046)	2.065*

The larval mortality is expressed as mean ± SD of five replicates. Nil mortality was observed in the control. Within a column means followed by the same letter(s) are not significantly different at 5% level by Duncan's multiple range tests. *LFL* – Lower Fiducidal Limit; *UFL* - Upper Fiducidal Limit. x^2, Chi-Square value. *Significant at p <0.05 level.





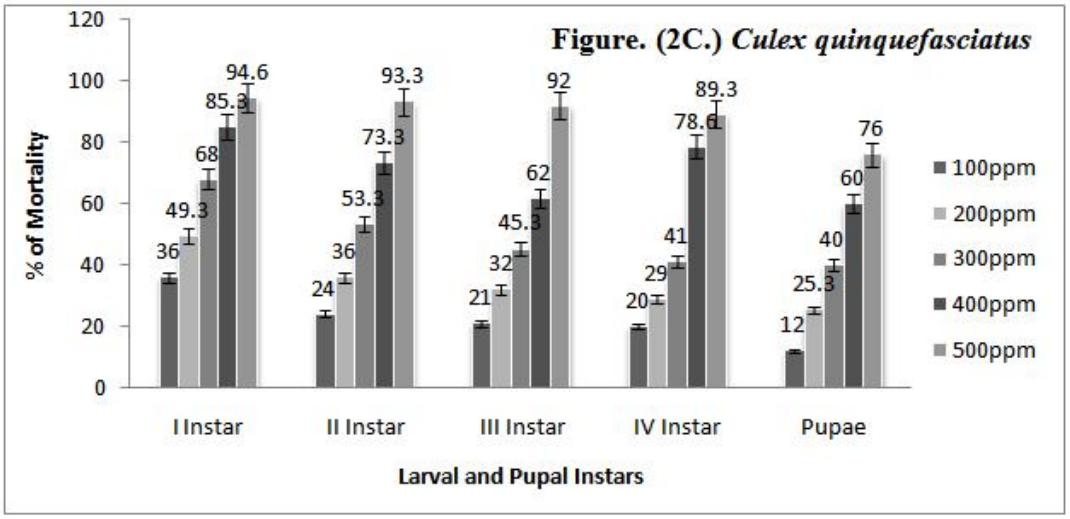


Figure 2: Mortality percentage of grape fruit pedicel extract against immature stage of three mosquitoes

The larvicidal and pupicidal activity of the GFPE at various concentrations against *filarial vector, Cx. quinquefasciatus* is shown in figure 2C. Considerable mortality was evident after GFPE treatment for all larval instars and pupae. Mortality increased with the concentration. For example, the mortality at the first instar stage at a 100ppm concentration was 36%; however, mortality increased to 94.6% when the concentration was increased to 500ppm. The mortality in the pupal stage was 12% at a 100ppm concentration; it increased to 76% at 500ppm concentration. The result of the study proved that mortality was increased as concentration increased. The LC_{50} and LC_{90} values were shown as follows: the LC_{50} values of I instar, II instar, III instar, and IV instar were 190.073ppm, 261.693ppm, 295.404ppm and 289.067ppm, respectively; and the LC_{90} values of I instar, II instar, III instar, and IV instar were 452.455ppm, 509.515ppm, 555.663ppm and 521.474ppm, respectively. The LC_{50} and LC_{90} values for pupae were 348.430ppm and 622.557ppm, respectively (given in table 4). The chi-square values were significant at $p<0.05$ level.

Table 4: Larvicidal and Pupicidal activity grape fruit pedicel extract *against Culex quinquefasciatus*

Larval instars	LC_{50} (LC_{90})	Regression equation	95% Confidence limit		Chi-Square value (x^2)
			LCL LC_{50}(LC_{90})	UCL LC_{50}(LC_{90})	
I	190.073 (452.455)	y=0.928+0.005 x	157.087 (413.663)	217.099 (506.348)	1.194*
II	261.693 (509.515)	y=1.353+0.005 x	236.779 (468.930)	285.100 (565.110)	4.163*
III	295.404 (555.663)	y=1.455+0.005 x	210.905 (447.136)	379.919 (878.741)	9.773*
IV	289.067 (521.474)	y=1.594+0.006x	215.227 (429.612)	360.513 (754.710)	9.354*
Pupae	348.430 (622.557)	y=1.629+0.005 x	322.834 (567.534)	376.660 (701.273)	0.107*

The larval mortality is expressed as mean ± SD of five replicates. Nil mortality was observed in the control. Within a column means followed by the same letter(s) are not significantly different at 5% level by Duncan's multiple range tests. *LFL* – Lower Fiducidal Limit; *UFL* - Upper Fiducidal Limit. x^2, Chi-Square value. *Significant at p <0.05 level

Table 5 illustrates that ovicidal activity of GFPE at different concentrations against eggs of *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus*. The percentage of egg hatchability was noted. The hatchability rate was significantly reduced after the treatment of GFPE. In control 100, 99.2 and 100% of eggs were hatched out from *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus*, respectively. Whereas, the percentage of egg hatchability was considerably reduced at 100 and 200ppm it's ranging from 37.3 to 2.4 %, respectively. After the 400ppm the eggs were completely stopped their hatchability.

Table 5: Ovicidal activity of grape fruit pedicel extract

Mosquito species	Percentage of egg hatchability Concentrations (ppm)					
	Control	100	200	300	400	500
Anopheles stephensi	100±0.0	37.3±0.8	15.6±0.9	NH	NH	NH
Aedes aegypti	99.2±0.6	43.7±0.6	21.5±0.1	5.2±0.3	NH	NH
Culex quinquefasciatus	100±0.0	39.4±0.4	19.2±0.2	2.4±0.7	NH	NH

NH- No Hatchability

Table 6 shows that the ovipositional deterrent activities of GFPE against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* . The percentage of egg lying was very high in control when compared with the treated sample. The different concentration of GFPE (100, 200, 300, 400 and 500ppm) exhibits significant Oviposition Active Index (OAI) and the percentage of Effective Repellency (ER) were calculated. The percentages of Effective Repellency of *An. stephensi* were 69.83%, 76.94%, 83.52%, 86.16% and 88.43%. The percentage of Effective Repellency of *Ae. aegypti* were 72.18%, 74.24%, 82.64%, 86.68% and 89.14% and the percentages of Effective Repellency (ER) of *Cx. quinquefasciatus* were 69.66%, 76.89%, 83.24%, 86.38% and 88.81%, respectively. The results indicated that the oviposition activity was dose dependent. At the highest concentration (500ppm) of the GFPE deterred the egg laying of three mosquito vectors with high percentage and at lower concentration (100ppm) exhibited the deterrent activity of was reduced. The oviposition Active Index (OAI) was also calculated and the negative values of OAI indicate the GFPE as high deterrent agent.

Table 6: Ovipositional deterrent activity of grape fruit pedicel extract *against Anopheles stephensi*

Concentrations (ppm)	Mean number of eggs in bowl (±SD)		ER (%)	OAI
	Treated	Control		
Anopheles stephensi				
	83.5	276.7	69.83	-0.53
200	72.7	315.5	76.94	-0.62
300	60.8	369.6	83.52	-0.71
400	54.2	388.9	86.16	-0.75
500	48.8	423.4	88.43	-0.79
Aedes aegypti				
100	80.2	287.8	72.18	-0.56
200	78.5	304.8	74.24	-0.59
300	65.8	379.2	82.64	-0.70
400	52.8	399.8	86.68	-0.76
500	46.7	430.3	89.14	-0.80
Culex quinquefasciatus				
100	84.5	269.7	69.66	-0.52
200	74.8	323.8	76.89	-0.62
300	60.2	359.2	83.24	-0.71
400	53.5	385.6	86.38	-0.76
500	45.9	410.3	88.81	-0.79

ER- Effective Repellency; OAI- Oviposition Active Index

DISCUSSION

Phytochemicals are botanicals which are naturally occurring insecticides obtained from floral resources. Applications of phytochemicals in mosquito control were in use since the 1920s (Shahi *et al*., 2010), but the discovery of synthetic insecticides such as DDT in 1939 side tracked the application of phytochemicals in mosquito control programme. After facing several problems due to injudicious and over application of synthetic insecticides in nature, re-focus on phytochemicals that are easily biodegradable and have no ill-effects on non-target organisms was appreciated. Since then, the search for new bioactive compounds from the plant kingdom and an effort to determine its structure and commercial production has been initiated. At present phytochemicals make upto 1 per cent of world's pesticide market (Isman, 1997).

The main chemicals in pedicel of grapes include: N-Hexadecanoic Acid, 1,E-11,Z-13-Octadecatriene (16.670%), 9,12-Octadecadienoic Acid (Z,Z), 9-Octadecynoic Acid and 6,8-Dodecadien-1-

OL (6Z,8E) (14.754%) were estimated. Palmitic Acid is a saturated long-chain fatty acid with a 16-carbon backbone. Palmitic acid is found naturally in palm oil and palm kernel oil, as well as in butter, cheese, milk and meat. Palmitic acid ($CH_3(CH_2)_{14}COOH$), *n*-hexadecanoic acid, is a saturated fatty acid. Many fatty acids are known to have antibacterial and antifungal properties (Agoramoorthy *et al*., 2007). Fatty acids can modulate immune responses by acting directly on T cells (Lawrence *et al*., 1993).

Stearic Acid (fatty acid) is also denoted as n-octadecanoic acid or as 18:0. Major sources of this long-chain saturated fatty acid include milk fat, which contains 5% to 15% stearic acid, as well as lard, cocoa butter fat, and shea butter, which can contain from 10% to 35% stearic acid. It is generally found in small quantities in seeds and marine oils. However, high stearic acid and high oleic acid/high stearic acid seed oils have been obtained by breeding soybean (Bubeck et al., 1989) and sunflower (Osorio, 1995). The fatty acid used for Cancer prevention, flavor, hyper cholesterolemic 5-alpha reductase inhibitor, antiandrogenic perfumery, insectifuge, anti- inflammatory, anemiagenic, dermatitigenic, choleretic (Boham and Kocipai, 1994).

The botanical extracts from the plant leaves, roots, seeds, flowers, and bark in their crude form have been used as conventional insecticides for centuries. In fact, many researchers have reported the effectiveness of plant extracts against mosquito larvae (Rasheed *et al*., 2005; Amer and Mehlhorn, 2006a). Plant bioactive secondary metabolites, such as alkaloids, phenols and terpenoids, either alone or in combination, contribute to mosquito larvicidal activity. Selective plant preparations can be developed into mosquito larvicidal products that are suitable for integrated mosquito management and are of great interest because they are target-specific, biodegrade to nontoxic products, have few harmful effects on nontarget organisms, and are environmentally non-persistent (Sukumar *et al*., 1991; Shaalan *et al*., 2005; Isman, 2006; Pavela, 2015).

These potential larvicidal products can be applied to habitats of mosquito larvae in the same manner as conventional larvicides. Certain plant preparations are toxic to different mosquito species larvae. Mosquito larvicidal compounds may act as toxicants, insect growth regulators, anti-microbials against endosymbionts of the larvae, or serve as juvenile hormone blockers in physiological changes such as metamorphosis (Isman, 2006; Pasquale *et al*., 2012).

The results of this study show that the grape fruit pedicel extract can disrupt the life cycle of *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* by preventing their eggs from hatching and by preventing the development of L1 larvae to L4 and pupal stages. As far as we know, this is the first demonstration that the grape fruit pedicel extract and/or their ingredients can disrupt the larval and pupal stage of the *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus*. It is evident from our results that a rise in the concentration of pedicel extract was the main cause of mortality in *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* larvae and pupae. Similar study was conducted by Govindarajan *et al*. (2014) revealed that the root extract of *Asparagus racemosus* possess remarkable ovicidal, larvicidal and adulticidal activity against medically important vector mosquitoes. Venkatachalam and Jebanesan (2001) they have been reported that methanolic extracts of few plants exhibited larvicidal activity against *Cx. quinquefasciatus*. Rajkumar and Jebanesan (2004) reported that increase in the concentration of leaf extract of *Solanum aerianthum* induced the oviposition attractant activity in *Cx. quinquefasciatus*. Earlier, Mathivanan *et al*. (2010) reported that the methanol extract of *Ervatamia coronaria* showed promising larvicidal and ovicidal activity against *An. stephensi*.

In the present study methanolic grape fruit pedicel extract showed 96% larvicidal activity against the first instar larvae of *An. stephensi* and *Ae. aegypti* when compared to *Cx. quinquefasciatus*. The LC_{50} and LC_{90} estimates for the pedicel extract were ranging from 89.093 to 380.630ppm and 424.174 to 663.636ppm, respectively against the three mosquito larvae and pupae. The lowest LC_{50} were calculated in I Instar larvae of *Ae. aegypti* ($LC_{50=}$ 89.093ppm)

and the highest LC_{50} were calculated in pupae of *An. stephensi* ($LC_{50=}$ 380.630ppm). Other extracts of several plants have also been proved to have larvicidal activity against *An. Stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. The methanol extract of *Ervatamia coronaria* (Family: Apocynaceae) leaves showed good larvicidal activity against larvae of *Cx. quinquefasciatus* (LC_{50}=72.41 mg/L; LC_{90}=65.67 mg/L at 24 h), *Ae. aegypti* (LC_{50}=62.08 mg/L; LC_{90}=136.55 mg/L at 24 h) and *An. Stephensi* (LC_{50}=127.24 mg/L; LC_{90}=120.86 mg/L at 24 h) (Mathivanan *et al*., 2010). Likewise, LC_{90} value of first to fourth instars larvae and pupae 687.14, 913.10, 1011.89, 1058.85 and 1141.65 ppm, respectively. Ramar *et al*. (2014) reported the bioefficacy of pupicidal potential with the LC_{50} values (in ppm) of some essential oils against *Cx. quinquefasciatus* and *An. stephensi* viz., clove (106.3 and 110.5), tulsi (133.6 and 144.2) and cinnamon (141.0 and 150.1) after 24 hours respectively.

In the present study grape fruit pedicel extract showed prominent ovicidal activity, this might be due to the volatile compounds present in the extract. The pedicel extract treated eggs exhibited an allayed hatchability and this may be due to the action of phytochemicals present in the extract. The extract may inhibit the hatchability of the eggs by interfering with their chorion. It is evident from the present study that exposure of *An. Stephensi* eggs to the pedicel extract of methonolic solvent not only elicited egg mortality but also delayed hatchability to larval stages. Similar kind of observation was also noted earlier by several workers (Rajkumar *et al*., 2011; Aarthi and Murugan, 2011). The ovicidal activity indicated an important finding that the larvae which hatched out of the treated eggs were succumbed to death within an hour or two. Similarly, ovicidal and gravid mortality effects of ethanolic extract of *Andrographis paniculata* was assessed by Kuppusamy *et al.* (2008) against *An. stephensi*. Larvicidal and oviposition activity of *Cassia obtusifolia* leaf extract against *An. stephensi* was also evaluated by Rajkumar and Jebanesan (2009). Likewise, Govindarajan *et al*. (2008a) reported that the younger age groups of egg rafts or eggs showed poor hatchability rate when exposed to higher concentrations of extract and that older age groups of egg rafts or eggs showed high hatchability rate when exposed to lower concentrations of extract. The methanol containing water that served as a control showed 94% hatchability in 0–3-h-old egg rafts/eggs, but the 100% hatchability was noted in egg rafts/eggs beyond the age of 0–3 h old in leaf methanol (90%) extract of *Cassia fistula* against egg raft of *Cx. quinquefasciatus* (Govindarajan *et al*., 2008b).

In the present study, the methonol extract of grape pedicel recorded the highest ovicidal activity of 100% nil hatchability was observed at 300, 400 and 500 ppm concentration against the eggs of *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus*, respectively. Previously, some investigators studied the ovicidal activity of plant extracts against mosquito eggs. Elango *et al.* (2009) reported that *Cocculus hirsutus* methanol extract caused 86% and 100% ovicidal activity at 500 ppm and 1000 ppm, respectively against *An. subpictus*. In another study, 100% ovicidal activity was recorded by a methanol extract of *Andrographis paniculata* at 150 ppm concentration in *An. stephensi* eggs (Panneerselvam and Murugan, 2013). Furthermore, the same methonol extract of grape pedicel showed highest oviposition deterrent activity (88.43%, 89.14% and 88.81%) at 500 against *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* adult females, respectively. Previously, some investigators reported the oviposition deterrent effect of plant extracts against vector mosquitoes. Coria *et al*. (2008) reported 100% oviposition deterrent effect obtained with *Melia azedarach* L. leaf extract at 1 g/L concentration against *Ae. aegypti*. Autran *et al.* (2009) recorded the oviposition deterrent effect of essential oil obtained from leaves, inflorescence, and stem of *Piper marginatum Jacq*. Their results showed that essential oil of leaves and stems of *P. marginatum* exhibited oviposition deterrent effect on *Ae. aegypti* females at 50 ppm and 100 ppm concentration and that the number of eggs laid was significantly lower (<50%) compared to control. Similarly, Prajapati *et al.* (2005) reported that the bark oil of *Cinnamomum zeylanicum* reduced the oviposition of *Ae. aegypti* to 50% at 33.5 ppm concentration.

Generally the active toxic ingredients of plant extracts are secondary metabolites that are evolved to protect them from herbivores. The insects feed on these secondary metabolites potentially encountering toxic substances with relatively non-specific effects on a wide range of molecular targets. These targets range from proteins (enzymes, receptors, signaling molecules, ion-channels and structural proteins), nucleic acids, biomembranes, and other cellular components (Rattan, 2010). This in turn, affects insect physiology in many different ways and at various receptor sites, the principal of which is abnormality in the nervous system (such as, in neurotransmitter synthesis, storage, release, binding, and re-uptake, receptor activation and function, enzymes involved in signal transduction pathway) (Rattan, 2010). A recent study by Ramkumar et al. (2019) showed that buprofezin and azadirachtin affected embryonic development and egg hatchability through hormonal alterations. The current study showed that egg un-hatchability and pupal mortality were grape pedicels concentration dependent. The same trend was observed in the response of freshly laid eggs of *C. pipiens* when treated with different insect growth regulators (Suman et al., 2013).

CONCLUSION

Majority of the mosquito control programmes are targeting the immature stages of the mosquitoes as the principal breeding habitats are man-made and can be easily identified. Chemical insecticides have been used arbitrarily during the past few decades for vector borne disease control which has led to the development of resistance in many insect species including mosquito vectors. The finding of the current investigation revealed that the pedicel extracts of grape possess potential mosquito larvicidal, pupicidal, ovicidal and ovipositional deterrent activity against *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus*. Since there is no availability of previous larvicidal, pupicidal, ovicidal and ovipositional deterrent activity of the pedicel extract, this investigation serves as first-hand information. The grape pedicel could be an excellent alternative source for mosquito larvicides because they constitute a potential source of bioactive chemical and generally free from harmful effects to the environment and non-target organisms. Use of this pedicel extract as larvicides in mosquito control instead of synthetic insecticides could reduce the cost, adverse environmental effects and pollution. This study reveals the excellent mosquitocidal potentiality of the locally available grape pedicel. Further studies on identified active compounds are needed to recommend the active fraction of the pedicel extract for development of eco-friendly larvicides for control of insect vectors.

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Conflicts Of Interest
The Authors declare no conflicts of interest.

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