Biocatalysis and Agricultural Biotechnology 19 (2019) 101137

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Chemical constituents and larvicidal efficacy of *Naringi crenulata* (Rutaceae) plant extracts and bioassay guided fractions against *Culex quinquefasciatus* mosquito (Diptera: Culicidae)

T. Pratheeba[a], P. Vivekanandhan[b], A.K. Nur Faeza[c], D. Natarajan[a,*]

[a] Natural Drug Research Laboratory, Department of Biotechnology, School of Biosciences, Periyar University, Salem, 636 011, Tamil Nadu, India
[b] Molecular Entomology Laboratory, Department of Biotechnology, School of Biosciences, Periyar University, Salem, 636011, Tamil Nadu, India
[c] Medical Entomology Laboratory, School of Biological Sciences, University of Sains Malaysia, Penang, 11800, Minden, Malaysia

ARTICLE INFO

Keywords:
Culex quinquefasciatus
Larvicidal activity
Naringi crenulata
HPLC analysis
GC-MS analysis
Mosquitoes

ABSTRACT

Mosquitoes transmitted several vector borne diseases like malaria, dengue, lymphatic filariasis and now Zika virus. Synthetic chemicals cause much illness to humans, non-target organisms and natural ecosystem. In the present study, larvicidal efficacy of *Naringi crenulata* (Rutaceae) different leaf extracts and bioassay guided fractions against *Culex quinquefasciatus*, phytochemical screening and secondary metabolites were characterized by TLC, HPLC, GC-MS and FT-IR analysis. Our results show highest larval mortality was found in acetone extract. For 24h LC_{50} and LC_{90} values were, 1.020, 1.931 mg/l. And 48h LC_{50} and LC_{90} values were, 3.728, 4.771 mg/l respectively. Fraction 9th showed remarkable larvicidal activity on larvae of *Cx. quinquefasciatus*. For 24h LC_{50} and LC_{90} values were, 5.312, 9.130 mg/l), for 48h LC_{50} and LC_{90} values were, 5.000, 9.538 mg/l. Phytochemical screening of *N. crenulata* crude extract contains phenols, alkaloids, tannins, saponins in acetone extracts. GC-MS analysis results show, 9,12-Octadecadienoic Acid (Z,Z)- (26.846%) is a major chemical constituent may be involved in larvicidal activity. Thin layer chromatography analysis shows nine molecules were separated using chloroform: methanol in the ratio 9.7: 0.3 solvent system. A sharp single peak was obtained in the 9th fraction with retention time of 1.376min with a purity of 99%. FT-IR analysis results confirmed presence of alkynes, alcohols, phenols, carboxylic acids, stretching, wagging and bending of oxygenated bonding. The present study shows that chemical constituents obtained from *N. crenulata* extracts and 9th fraction have strong larvicidal properties these botanical derived molecules offered alternative to synthetic chemical insecticides.

1. Introduction

Mosquitoes transmitted several vector-borne diseases namely, malaria, dengue, yellow fever, lymphatic filariasis and recently zika virus etc. in tropical and subtropical climatic conditions (Benelli and Duggan, 2018). *Culex quinquefasciatus* is vector of lymphatic filariasis in several countries (Benelli, 2015a). Lymphatic filariasis distributed in tropical zones with around 120 million peoples was infected in worldwide and 44 million peoples having the common chronic manifestation (Bernhard et al., 2003; Govindarajan and Benelli, 2016). Chemical insecticides a major concern for the control of mosquito vectors. Repeated usage of same or different types of chemical insecticides cause several illness to environment, humans and other non-target organisms, major problems is mosquitoes get insecticide resistance capacity in very quickly (Ramkumar and Shivakumar, 2015).

Currently several researchers to find the biological derived mosquito larvicides from plant, bacteria and fungus and their metabolites against larvae, pupae and adult of major mosquito species (Benelli, 2015b; Vivekanandhan et al., 2018a,b,c,d). Especially fungus and medicinal plant extracts very effective in mosquito control program, the plant and microbes play an important role in reduce the mosquito populations and low effects to environments and non-target organisms (Benelli, 2016). Plant and fungal secondary metabolites provide an alternative source in the vector control program (Zeinab and Abou-Elanga, 2014; Vivekanandhan et al., 2018a, e). Medicinal plants, entomopathogenic fungus and fungal derived secondary metabolites are right choice for control of mosquito population because these metabolites has several advantages like, ecofriendly in nature, biodegradable, efficacy, target specific, cheaper and low toxicity to environment and non-target organisms (Vivekanandhan et al., 2018a,b; Benelli, 2015b; Vivekanandhan et al., 2019).

Naringi crenulata (wild) is a medicinal tree, belonging to Rutaceae

* Corresponding author.
E-mail address: natarajpu@gmail.com (D. Natarajan).

https://doi.org/10.1016/j.bcab.2019.101137
Received 5 November 2018; Received in revised form 9 April 2019; Accepted 14 April 2019
Available online 16 April 2019
1878-8181/

family found in India and other part of the world. It is commonly called as Narinarakam, Kattunarakam and Tamil name it is called Megavilvam or Magavilvam. *Naringi* genus it's growing as understory trees in evergreen forests up to 1200m. Plant contain several medicinal properties namely, the plant root is used to curing vomiting, dysentery and colic disorders in India (Rajith and Ramachandran, 2010). Fruit decoction is used as an insect repellent agent and also isolates Pectic polysaccharides for several biological activities (Senthilkumar et al., 2006; Saroj et al., 2003). Bark juice is applied externally for getting speedy relief in sprain (Udayan et al., 2008). *N. crenulata* plant extracts show good Anthelmintic activity (Ramani et al., 2010; Sarada et al., 2011). The *Naringi crenulata* (wild) plant contain several active phytochemical constituents namely, alkaloids, flavonoids, quinines, steroids, terpenoids and glycosides phytochemicals may be involved in the mosquitocidal activity. In the present study, larvicidal efficacy of *Naringi crenulata* (Rutaceae) leaf extracts and bioassay guided fractions against *Culex quinquefasciatus* mosquito (Diptera: Culicidae), phytochemical screening and secondary metabolites characterized by TLC, HPLC, GC-MS and FT-IR analysis.

2. Materials and methods

2.1. Plant materials

N. crenulata leaves (Fig. 1) were collected from Vellimalai hills, Eastern Ghats of Tamil Nadu, India. Botanical nomenclature of the plant was identified by Dr. D. Natarajan, Assistant Professor, Department of Biotechnology, Periyar University, Tamil Nadu, India.

2.2. Preparation of plant extracts

Plant leaves were washed with running tap-water for remove the dust particles and leaves were shade-dried at room temperature (28 ± 2 °C) for three weeks in Natural Drug Research Laboratory, Department of Biotechnology, Periyar University. Dried leaves were grind for making a powder using electrical steel blender. Leaf powder (300g) was extracted with different solvent system like, hexane, ethyl acetate, acetone and methanol (each 400 ml separately) with help of Soxhlet apparatus (boiling point range at 50–80 °C) for 8 h. Extract was concentrated under reduced pressure (22–26 mmHg) at 50 ± 2 °C, and the obtained residue was stored at 4 °C.

2.3. Mosquito culture

Cx. quinquefasciatus larva were procured from National Centre for Disease Control (NCDC), Mettupalayam, Tamil Nadu, India. Mosquito larvae were reared and maintained in the laboratory condition. Larvae were fed with dog biscuits and millet powder and yeast powder in 3:3:1 ratio (Vivekanandhan et al., 2018c).

Fig. 1. *Naringi crenulata* plant.

2.4. Larvicidal bioassay

Larvicidal bioassay of *N. crenulata* plant extract and isolated fractions were evaluated as per method of World Health Organization (WHO, 2005; Pratheeba et al., 2015). About 25 4th instar larva were transfer into bioassay trays containing 249 ml of tap water and 1.0 ml of the desired plant extract in various concentrations (100, 200, 300, 400, 500, 600, 700, 800 and 900 mg/l) dissolved in DMSO and controls setup were treated with DMSO. Larval mortality was calculated after 24h post treatment. Each concentration has three replicates and each replicate has 25 larvae.

2.5. Phytochemical screening

Preliminary phytochemical analysis was done using different solvent crude extracts of *N. crenulata* with slightly modified method of Satheesh et al. (2012) and Thamaraiselvi et al. (2012).

2.6. GC–MS analysis

Gas chromatography mass spectrometry analysis of *N. crenulata* plant extract was performed on an Agilent 6890. GC equipped with 5,973 N mass selective detectors with HP-5 capillary column. Carrier gas was helium at a flow rate of 1.0 ml/min (constant flow). Sample (0.2 µl) was injected with a split of 20:1 and the temperatures were maintained at 230 and 150 °C.

2.7. Thin-layer chromatography

Thin-layer chromatography was done with help of acetone crude extract of *N. crenulata* using different mobile phase for separation of different chemical constituents present in the acetone crude extract. Glass chamber was selected chloroform: methanol solvent system in the ratio of 9.7:0.3 covered and left untouched for few min. Next, the sample was loaded on the Silica TLC plates (Merck; size, 15 × 2 cm) and the spots were allowed to dry. Then, the plate was taken from the chamber and the solvent front was marked immediately with using a pencil. TLC plate was allowed to dry completely, for solvent may evaporate. To visualize the different zones/spots of the crude extract passed on the TLC plate. TLC plates were exposed to UV chamber. Finally, the R_f value was calculated using the following formula (Torres et al., 2015).

$$R_f = \frac{\text{Distance traveled by compound}}{\text{Distance traveled by solvent}}$$

2.8. Column chromatography

Acetone crude extract of *N. crenulata* (10 g) was subjected to column chromatography over silica gel (60–120 mesh) and it was eluted with chloroform followed by the combination of chloroform/methanol in the ratio of 9.7:0.3. Fractions were eluted with solvents gradually increasing and collected in a 50-ml test tube. Eluted fraction was combined based on the TLC results were obtained. A single fraction (50 ml) was collected and then concentrated to dryness in a rotary vacuum evaporator. Concentration fraction was collected in glass vials and stored at 4 °C further use.

2.9. Purity checking

9th fractions were confirmed by TLC, following the above-

Table 1
Larvicidal potential of *N. crenulata* leaf extracts against *Cx. quinquefasciatus* after 24h and 48h treatments.

Observation	Extracts	Total no of larvae	LC_{50} (LCL-UCL) mg/l	LC_{90} (LCL-UCL) mg/l	χ^2	Df
24hrs	Acetone	375	1.02 (0.56–15.58)	1.93 (0.81–22.83)	98.43	16
	Hexane	375	9.74 (3.40–25.90)	2.34 (1.96–26.54)	15.04	16
	Ethyl acetate	375	1.81 (0.51–17.62)	4.14 (2.67–29.32)	73.87	16
	Methanol	375	1.13 (0.68–18.14)	2.24 (3.65–32.86)	42.25	16
48hrs	Acetone	375	3.72 (0.00–36.00)	4.77 (1.10–35.90)	15.00	16
	Hexane	375	6.17 (3.74–33.51)	1.14 (0.95–29.15)	16.95	16
	Ethyl acetate	375	1.05 (1.0–24.97)	2.38 (1.15–32.15)	25.30	16
	Methanol	375	9.04 (3.56–42.51)	1.75 (1.0–26.44)	40.33	16

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, χ^2 Chi square, df degrees of freedom.

Table 2
Phytochemical screening of *N. crenulata* leaf extracts.

S. No	Phytochemical test	Name of the test	Hexane	Ethyl Acetate	Acetone	Methanol
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.

mentioned protocol to use chloroform and methanol mobile phase in all cases. For visualization of the resolute compounds of the TLC plates were exposed to UV chamber and the visible spots were marked with a pencil. Respective R_f values of different components of each fraction were calculated. Fractions having compounds with similar R_f values were mixed together. Finally, the residue of the single fraction was labeled and stored at 4 °C for further use.

3. High performance liquid chromatography analysis

9th fraction from *N. crenulata* was done HPLC analysis using a Waters Nova-Pack C18 column (4 Km, 3.9 × 150 mm), adapted to Waters Nova-Pack C18 60 Å, guard column (3.9 × 20 mm) and flow rate of 1 mL/min. Acetonitrile (CH_3CN) was HPLC grade from Merck. The analyses were carried out at room temperature (25 °C) with a volume of 20 µL of three injections. Peaks were identified by comparison of their retention times (*t*R). A fraction was quantified by a calibration curve with at least five data points covering the concentration range of 5–1400 mg/ml.

3.1. Fourier transmission-infra red analysis

FT-IR spectrum of the samples was measured on Arid Zone FT-IR

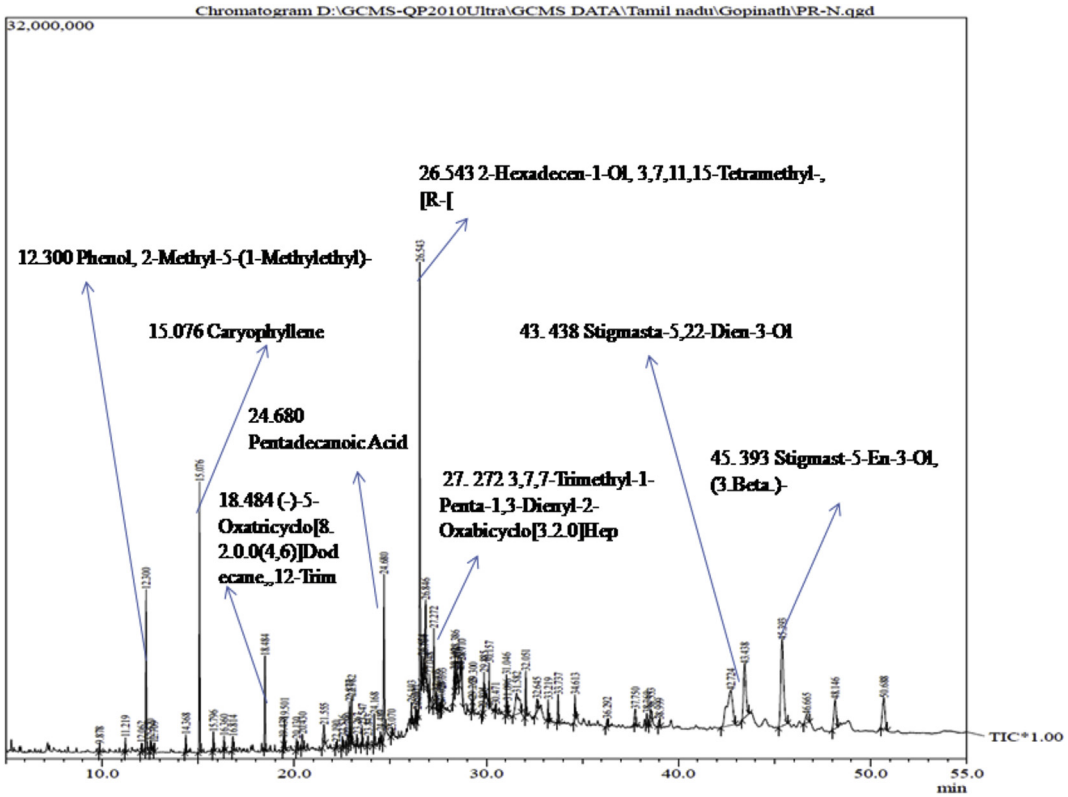

Fig. 2. Major chemical constituents were identified from *N. crenulata* acetone extract by GC-MS analysis.

Table 3
Major chemical constituents screening from *N. crenulata* acetone extracts using GC- MS analysis.

S.no	R T	Molecular Formula	Molecular Weight	Compound Name	Biological Activity	Reference
1	26.543	$C_{20}H_{40}O$	296	2-Hexadecen-1-Ol, 3,7,11,15-Tetramethyl-, [R-[	Antimicrobial activity	Sarada et al. (2011)
2	15.076	$C_{15}H_{24}$	204	Caryophyllene	Larvicide, Mosquito, Antimicrobial and Anticancer activity	Sargunam et al. (2011)
3	12.300	$C_{10}H_{14}O$	150	Phenol, 2-Methyl-5-(1-Methylethyl)-	Antifungal, Antimicrobial and Antioxidant activity	Sarada et al. (2011)
4	24.680	$C_{15}H_{30}O_2$	242	Pentadecanoic Acid	Antifungal and Antimicrobial activity	Bashir et al. (2012)
5	18.484	$C_{15}H_{24}O$	220	(−)-5-Oxatricyclo[8.2.0.0(4,6)]Dodecane,12-Trim	Nematicide, insectifuge and Antiacne activity	Sudha et al. (2013)
6	26.846	$C_{18}H_{32}O_2$	280	9,12-Octadecadienoic Acid (Z,Z)-	Insecticidal activity	Susitra Manjari et al. (2014); Vivekanandhan et al. (2018c)
7	27.272	$C_{14}H_{20}O$	204	3,7,7-Trimethyl-1-Penta-1,3-Dienyl-2-Oxabicyclo[3.2.0]Hep	Antimicrobial activity	Sudha et al. (2013)
8	45.393	$C_{29}H_{50}O$	414	Stigmast-5-En-3-Ol, (3.Beta.)-	Antihepatotoxic, Antiviral, Antioxidant, Cancer preventive and Hypocholesterolemic activity	Lakshmi and Viji Stella Bai (2015)
9	43.438	$C_{29}H_{48}O$	412	Stigmasta-5,22-Dien-3-Ol	Antioxidant activity	Lakshmi and Viji Stella Bai (2015)

spectrometer equipped with a DTGS detector. 5 mg of 9th fraction sample was mixed with 100 mg of dry potassium bromide (KBr) and the mixture was compressed to prepare the small pellet. Pellet was analysed under FT-IR spectrophotometer in the range of 4,000 - 500cm-1 at room temperature. An absorbance spectrum was acquired the 4 cm^{-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.

3.2. Statistical analysis

Larval mortality data was exposed to probit analysis for calculating LC_{50}, LC_{90} and other statistical values at 95% confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL) values and chi-square test was calculated using SPSS16.0 (Statistical Package for Social Sciences) software version 16.0.

4. Results

4.1. Larvicidal bioassay

Larvicidal activity result exhibits the higher mortality rate was observed in acetone extract of *N. crenulata* at 24h post treatment against larvae of *Culex quinquefasciatus*, with very low LC_{50}, 1.020 mg/l (Table 1). Hexane, ethyl acetate and methanol extracts, 9.744 mg/l, 1.815 mg/l and 1.134 respectively. After 48h post treatment the acetone extracts were LC_{50} values were, 3.728 mg/l (Table 1). Larvicidal activity of bioactive fraction 9 exhibited the better mortality rate against larvae of *Culex quinquefasciatus* with $LC_{50,}$ 5.312 mg/l, 5.000 mg/l of 24 and 48hrs respectively (Table 4).

4.2. Phytochemical screening

Phytochemical analysis of *N. crenulata* crude extract clearly shows the presence of phenols, alkaloids, tannins, saponins in acetone and methanol extracts. Quinones and proteins were found in hexane and methanol extracts only. The carbohydrates were present in all the tested extracts. Glycosides were absent in anyone of the extract tested (Table 2)

4.3. GC–MS analysis

GC-MS analysis of *N. crenulata* acetone extracts shows presence of seventy-two phytochemical constituents. Five chemical constituent are major namely, Phenol, 2-Methyl-5-(1-Methylethyl)-(12.300%); Caryophyllene (15.076%); (−)-5-Oxatricyclo [8.2.0.0(4,6)] Dodecane,12-Trim (18.484%); Pentadecanoic Acid (24.680%) and 9,12-Octadecadienoic Acid (Z,Z)- (26.846%) these major chemicals maybe involved in larvicidal activity (Fig. 2; Table 3).

4.4. Thin-layer chromatography

Thin layer chromatography was done for partial purification of chemical components present in acetone crude extracts. Nine molecules were clearly separated using chloroform: methanol in ratio 9.7: 0.3 solvent system (Fig. 3).

4.5. High performance liquid chromatography analysis

A sharp single peak was obtained in the 9th fraction with retention time of 1.376min with purity of 99%. HPLC profile indicates the homogeneity of 9th fraction (Table 5 and Fig. 4).

Table 4
Larval toxicity effect of fraction 9th against larvae of *Cx. quinquefasciatus* for 24h and 48h treatments.

Species	Observation	LC_{50} (LCL-UCL) mg/ml	LC_{90} (LCL-UCL) mg/ml	χ^2	*df*
Cx. quinquefasciatus	24hrs	5.31 (2.45–15.02)	9.13 (4.41–17.00)	6.42	16
	48hrs	5.00 (1.56–19.85)	9.53 (5.43–22.67)	12.61	16

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, χ^2 Chi square, df degrees of freedom.

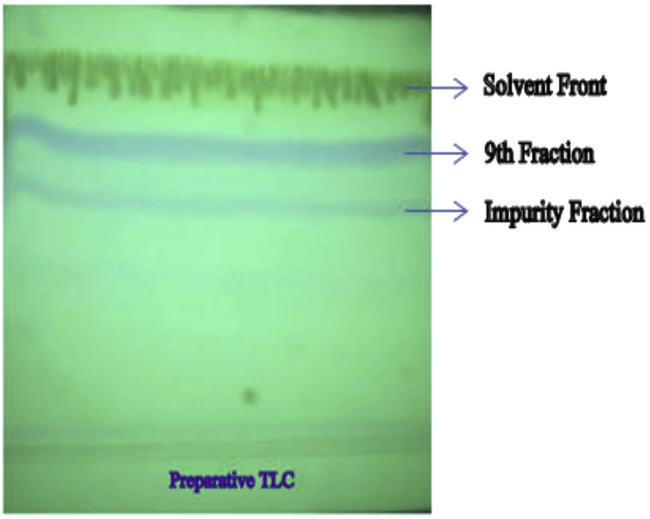

Fig. 3. Thin layer chromatography of *N. crenulata* acetone crude extract using different mobile phase for separation of different chemical constituents. Glass chamber was selected chloroform: methanol solvent system in the ratio of (chloroform 9.7: methanol 0.3).

Table 5
Fraction purity was evaluated using HPLC.

S. No	Ret. Time	Area	Height	Area %	Height %
1	1.376	4056091	812443	71.529	91.899
Total		5670534	884058	100.00	100.00

4.6. Fourier transmission-infra red analysis

Functional group analysis (FT-IR) of active fraction 9th from *N. crenulata* acetone crude extract shows that the presence of alkanes ($-CH_3$) alkynes (-C = C str), alcohols and phenols (O- Hstr, b), carboxylic acids (O- H str) stretching, wagging, bending of oxygenated bonding (O – H) (Fig. 5; Table 6).

5. Discussion

Synthetic chemical insecticides currently used in vector control programs in field level application, because chemical insecticides show fast in action, this is the only reason for using chemicals insecticides. Repeated use of chemicals produces several illnesses to green eco systems and non-target organisms (Ramkumar and Shivakumar, 2015). Botanicals has several chemical constituent, which is closely similar to synthetic chemicals these plant derived bioinsecticides are very cheaper, efficacy, target specific and lower toxicity to non-target organisms (Vivekanandhan et al., 2018a,b).

Our larvicidal activity result exhibits the higher mortality rate was observed in acetone extract of *N. crenulata* at 24h post treatment against larvae of *Culex quinquefasciatus*, with very low LC_{50} and LC_{90} values (Table 1). Phytochemical analysis of *N. crenulata* crude extract clearly shows the presence of phenols, alkaloids, tannins, saponins in acetone and methanol extracts. Quinones and proteins were found in hexane and methanol extracts only. The carbohydrates were present in all the tested extracts. Glycosides were absent in anyone of the extract tested (Table 2). Sharma et al. (2014) studied on the medicinal plants having many pharmacological active compounds like flavonoids, alkaloids, steroids, glycosides and phenols, which is stored in their specific parts of leaves, bark, flowers, seed, fruits, root etc.

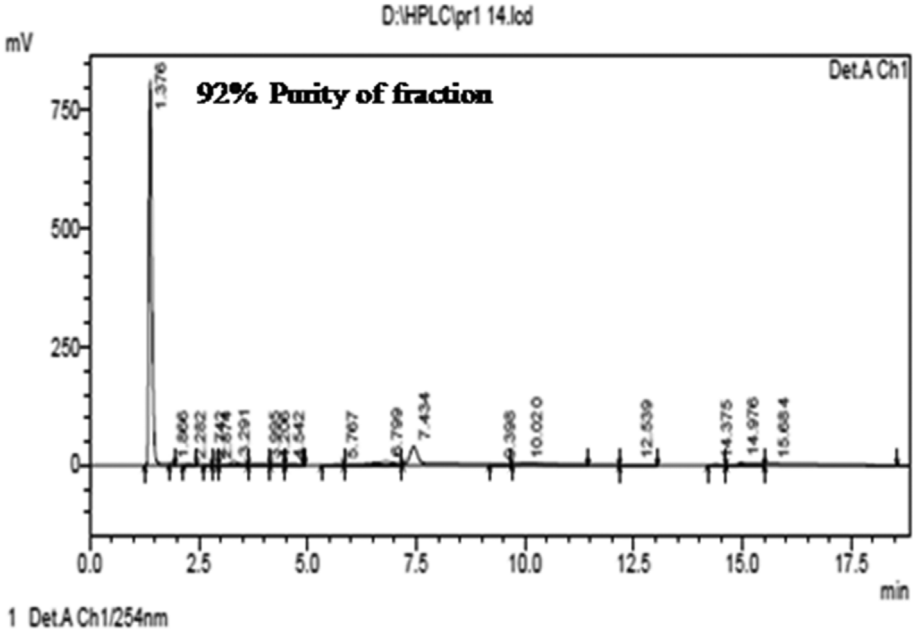

Fig. 4. HPLC chromatogram of fraction 9 from *N. crenulata* acetone extract.

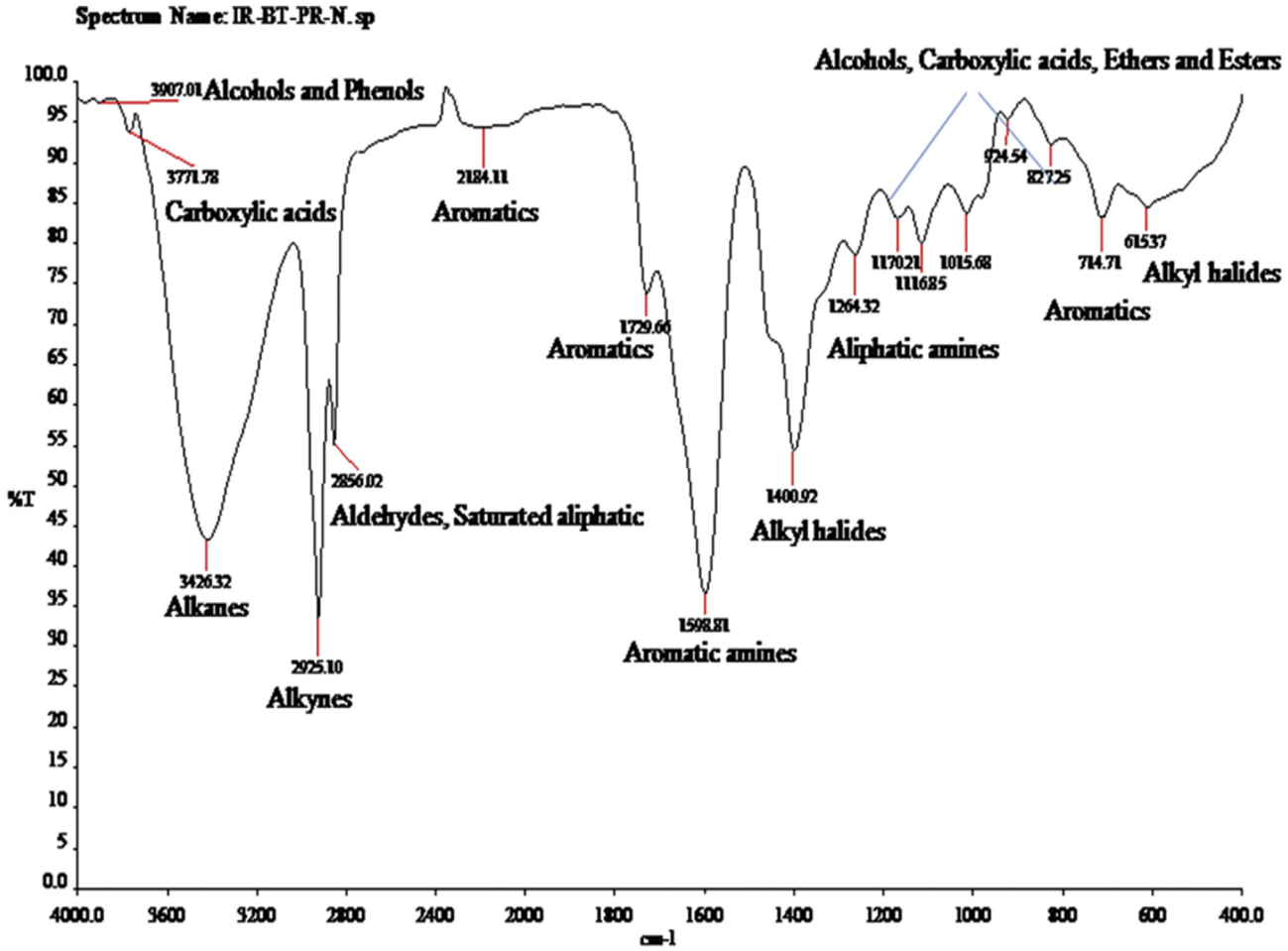

Fig. 5. FT-IR Spectrum of fraction 9 from *N. crenulata* acetone extract.

Table 6
FT- IR analysis of fraction 9 from *N. crenulata.*

S. no	Peak value	Functional groups	Bonding pattern
1	3426.32	O – H stretch,	Alcohols, Phenols
2	2925.10	O – H stretch	Carboxylic acids
3	2856.02	C =H stretch	Alkanes
4	2184.11	-C C- stretch	Alkynes
5	1729.66	C=O stretch	Aldehydes, Saturated aliphatic
6	1598.81	C-C stretch	Aromatics
7	1400.92	C-C stretch	Aromatics
8	1264.32	C-N stretch	Aromatic amines
9	1170.21	C-H wag	Alkyl halides
10	1116.85	C-N stretch	Aliphatic amines
11	1015.68	C-O stretch,	Ethers and Esters
12	924.54	O-H bend	Carboxylic acids
13	827.25	C-Cl stretch	Alky halides
14	714.71	C-H “oop”	Aromatics
15	615.37	C-Br stretch	Alkyl halides

This is the first report on *N. crenulata* leaf extracts and their fraction on larvae of *Cx.quinquefasciatus* vector. Similarly kind of research were done by Adaikala Raj et al. (2015) reported that the *Nigella sativa* essential oil show strong mosquitocidal activities on larvae of *Ae. aegypti, An. stephensi,* and *Cx quinquefasciatus* after 12 h and 24h of exposure periods. Jayasree et al. (2015) reported the methanol leaf extract from *N. crenulata* shows strong anti-bacterial activities against human pathogens. Similarly *N. crenulata* leaf chloroform, ethyl acetate and methanol extracts produced strong inhibition of multidrug resistant bacteria (Samundeeswari et al., 2012). Present study demonstrated that the same genous of plant crude extract obtained from *N. crenulata* as a potential larvicidal agent on *Cx. quinquefasciatus* at 24h and 48h post treatment. Similarly, *C. dentata*, *A. nilotica* and *A. hispidum* derived crude extracts and essential oils show strong larvicidal, pupicidal and adulticidal activities against *An.stephensi*, *Ae.aegypti* and *Cx.quinquefasciatus* mosquitoes at after 24h post treatment (Susitra Manjari et al., 2014; Vivekanandhan et al., 2018a,b).

Govindarajan and Sivakumar (2013) reported that *Erythrina indica* crude extracts shows remarkable larvicidal activities against *Cx. quinquefasciatus*. Kamaraj et al. (2010) found that the larvicidal properties from 8 medicinal plants crude extract against *Cx. gelidus* and *Cx. quinquefasciatus*. *Acacia nilotica* and *Mentha spicata* plant crude extracts and essential oils show remarkable larvicidal, pupicidal and adulticidal activities against *An.stephensi*, *Ae.aegypti* and *Cx.quinquefasciatus* mosquitoes (Govindarajan et al., 2011; Vivekanandhan et al., 2018b).

Ramkumar et al. (2015) reported that *C. dentata* plant leaf crude extracts show strong larvicidal and adulticidal activities against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes. Pradeepa et al. (2014) reported that *P. zeylanica* root extract derived larvicidal molecules had remarkable larvicidal activity against *A. stephensi*. In TLC and column fractionation show, nine fractions were separated and tested for larvicidal potential on *Cx. quinquefasciatus* mosquito. Fraction 9 had strong larvicidal activity on *Cx.quinquefasciatus* than other fractions with LC_{50} and LC_{90} 5.312, 9.130 mg/l, 5.000, 9.538 mg/l of 24 and 48hrs respectively (Table 4).

Present GC-MS analysis results show that presence of five major chemical constituents namely, Phenol, 2-Methyl-5-(1-Methylethyl)- (12.300%); Caryophyllene(15.076%); (−)-5-Oxatricyclo[8.2.0.0(4,6)] Dodecane,12-Trim (18.484%); Pentadecanoic Acid (24.680%) and

9,12-Octadecadienoic Acid (Z,Z)-(26.846%) (Fig. 2; Table .3). Vivekanandhan et al. (2018c) reported that 9,12-Octadecadienoic Acid (Z,Z) had mosquito larvicidal activity on *An.stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. The major signals at 2925.10, 1598.81 and 3426.32^{cm-1} indicates the presence of carboxylic acids O-H stretch, Aromatics C-C stretch in ring and Alcohols, Phenols O-H stretch, H banded (Table 6; Fig. 5). These FT-IR results supported the presence of the aromatic phenol ring and other functional groups of phenols show larvicidal activity in the purified fraction. A sharp single peak was obtained at the retention time of 1.376min with purity of about 94%. The single peak in HPLC profile indicates the homogeneity of pooled fraction (Table 5 and Fig. 4).

Similarly β-sitosterol compound was isolated from *Abutilon indicum*, which shows strong larvicidal activity against larvae of *Ae. aegypti*, *A. stephensi* and *Cx. quinquefasciatus* (Rahuman et al., 2008). Sanchez et al. (2000) reported that larvicidal flavonoids glabranine and 7-O-Methyle glabranine, extracted from some Mexican plants namely, *Tephrosia madrensis*, *T. viridiflora* and *T. crassifollia* that show significant antiviral activities against dengue virus.

Recently Zandi et al. (2011) has investigated the effect of four types of bioflavonoid such as quercetin, naringin, daidzein, and hesperetin produced strong antiviral activity on dengue virus. In present study clearly shows the acetone extracts of *N. crenulata* found to be potential extract which having mosquito control ability and proves higher mortality rates even at a lower concentration of plant extracts. In the present study, tells the acetone leaf extract and fraction of *Naringi crenulata* had strong larvicidal activity against *Cx.quinquefasciatus* and we further exposed to NMR and LC-MS further identification of bioactive compounds.

6. Conclusion

In conclusion, *N. crenulata* plant extract produced more than 90% mortality on larva of *Cx. quinquefasciatus*. GC-MS analysis results 9,12-Octadecadienoic Acid (Z,Z)- (26.846%) is a major chemical constituents maybe involved in larvicidal activity. A sharp single peak was obtained in the 9th fraction with retention time of 1.376min with purity of 99%. FT-IR analysis results clearly confirmed presence of alkynes, alcohols, phenols, carboxylic acids, stretching, wagging and bending of oxygenated bonding. The present study shows that chemical constituents obtained from *N. crenulata* offered alternative to synthetic chemical insecticides. The botanical derived extracts are cheaper, biodegradable, efficacy, target specific and low toxicity to non-target organisms.

Conflicts of interest

The authors declare that they have no conflict of interest.

Acknowledgements

We would like thankful to Mr. N. Muthukrishnan, Technician and National Centre for Diseases Control (NCDC), Mettupalayam, Tamil Nadu, for mosquito larvae. We thank Department of Biotechnology, Periyar University, Salem, Tamil Nadu for providing necessary infrastructural facilities for carryout this research work successfully and also thank the St. Joseph's College, Trichy for HPLC, FT-IR and Vellore Institute of Technology for GC-MS analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101137.

References

Adaikala Raj, G., Chandrasekaran, M., Krishnamoorthy, S., Jayaraman, M., Venkatesalu, V., 2015. Phytochemical profile and larvicidal properties of seed essential oil from *Nigella sativa* L. (Ranunculaceae), against *Aedes aegypti, Anopheles stephensi*, and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitol. Res. 114, 3385–3391.

Bashir, A., Ibrar, K., Shumaila, B., Sadiq, A., 2012. Chemical composition and antifungal, phytotoxic, brine shrimp cytotoxicity, insecticidal, and antibacterial activities of the essential oils of *Acacia modesta*. J. Med. Plant Res. 6, 4653–4659.

Benelli, G., 2015a. Research in mosquito control: current challenges for a brighter future. Parasitol. Res. 114, 2801–2805.

Benelli, G., 2015b. Plant-borne ovicides in the fight against mosquito vectors of medical and veterinary importance: a systematic review. Parasitoi. Res. 114, 3201–3212.

Benelli, G., 2016. Plant-mediated biosynthesis of nanoparticles as an emerging tool against mosquitoes of medical and veterinary importance: a review. Parasitol. Res. 115, 23–34.

Benelli, G., Duggan, M.F., 2018. Acta Trop. 182, 80–91.

Bernhard, L., Bernhard, P., Magnussen, P., 2003. Management of patients with lymphoedema caused by filariasis in north–eastern Tanzania: alternative approaches. Physiotherapy 89, 743–749.

Govindarajan, M., Benelli, G., 2016. Facile biosynthesis of silver nanoparticles using *Barleria cristata*: mosquitocidal potential and biotoxicity on three non-target aquatic organisms. Parasitol. Res. 115, 925–935.

Govindarajan, M., Sivakumar, R., 2013. Larvicidal, ovicidal, and adulticidal efficacy of *Erythrina indica* (Lam.) (Family: Fabaceae) against *Anopheles stephensi, Aedes aegypti*, and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitol. Res. 113, 777–791.

Govindarajan, M., Sivakumar, R., Rajeswari, M., Yogalakshmi, K., 2011. Chemical composition and larvicidal activity of essential oil from *Mentha spicata* (Linn.) against three mosquito species. Parasitol. Res. 110, 2023–2032.

Jaya Sree, S., Vijayakumar, N., Suseela Gomathi, K., Mary Helen, P.A., 2015. Study on antimicrobial activity and chemical profiling of *Pamburus missionis* (Wight) swingle. Int. J. Pharm. Pharm. Res. 3 (3).

Kamaraj, C., Abdul Rahuman, A., Mahapatra, A., Bagavan, A., Elango, G., 2010. Insecticidal and larvicidal activities of medicinal plant extracts against mosquitoes. Parasitol. Res. 107, 1337–1349.

Lakshmi, V., Viji Stella Bai, G., 2015. Determination of Biologically active compounds in *Clerodendrum phlomidis* (L.) leaf extract using GC/MS. Inter. J. Multidisci. Res. Devel. 2, 294–300.

Pradeepa, V., Sathish-Narayanan, S., Kirubakaran, S.A., Senthil-Nathan, S., 2014. Antimalarial efficacy of dynamic compound of plumbagin chemical constituent from *Plumbago zeylanica* Linn (Plumbaginaceae) against the malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). Parasitol. Res. 113, 3105–3109.

Pratheeba, T., Ragavendran, C., Natarajan, D., 2015. Larvicidal, pupicidal and adulticidal potential of *Ocimum gratissimum* plant leaf extracts against filariasis inducing vector. Inter. J. Mosqu. Res. 2, 01–08.

Rahuman, A.A., Gopalakrishnan, G., Venkatesan, P., Geetha, K., 2008. Isolation and identification of mosquito larvicidal compound from *Abutilon indicum* (Linn.) Sweet. Parasitol. Res. 102, 981–988.

Rajith, N.P., Ramachandran, V.S., 2010. Ethnomedicines of kurichyas, kannur district, Western Ghats, keral. Ind. J. Nat. Prod. Res. 2 249-25.

Ramani, R., Bindukarra, H., Madhavi, B., Ravinder, B., Anisetti, N., Banji, D., 2010. Pharmacognostical phytochemical and anthelmintic evaluation of *Naringi crenulata* (Roxb). Int. J. Pharm. Res. Dev. 2, 1–8.

Ramkumar, G., Shivakumar, M.S., 2015. Laboratory development of Permethrin resistance and cross-resistance pattern of *Culex quinquefasciatus* to other insecticides. Parasitol. Res. 114, 2553–2560.

Ramkumar, G., Karthi, S., Muthusamy, R., Natarajan, D., Shivakumar, M.S., 2015. Insecticidal and repellent activity of *Clausena dentata* (Rutaceae) plant extracts against *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes (Diptera: Culicidae). Parasitol. Res. 114, 1139–1144.

Samundeeswari, A., Chittibabu, C.V., Arumugam, P., 2012. In vitro investigation on the antibacterial activities of the leaves of Naringi crenulata (Roxb.) Nicols. Int. J. Curr. Sci. 94 (97) 2250-1770.

Sanchez, I., Gomez-Garibay, F., Taboada, J., Ruiz, B.H., 2000. Antiviral effect of flavonoids on the dengue virus. Pathol. Res. Pract. 14, 89–92.

Sarada, K., Jothibai Margret, R., Mohan, V.R., 2011. GC–MS determination of bioactive components of *Naringi crenulata* (Roxb) Nicolson. Inter. J. Chem. Tech. Res. 3, 1548–1555.

Sargunam, M.P., Viji Stella Bai, G., 2011. GC-MS studies on alcoholic leaf extract of *Limonia crenulata* Roxb. J. Basic Appl. Bio. 5, 60–71.

Saroj, K.M., Bimalendu, R., Ghosal, P.K., 2003. Isolation and characterization of pectic polysaccharides from the fruits of *Naringi crenulata*. Indian J. Chem. 42, 437–442.

Satheesh, K.B., Suchetha, K.N., Vadisha, S., Sharmila, K.P., Mahesh Prasad, B., 2012. Preliminary phytochemical screening of various extracts of *Punica granatum* peel, whole fruit and seeds. Nitte university. J. Health Sci. 2, 2249–7110.

Senthilkumar, M., Gurumoorthi, P., Janardhanan, K., 2006. Some medicinal plants used by Irular, the tribal people of Marudhamalai hills, Coimbatore, Tamilnadu. Nat. Prod. Rad. 5, 382–388.

Sharma, G., Kapoor, H., Chopra, M., Kumar, K., Agrawal, V., 2014. Strong larvicidal potential of *Artemisia annua* leaf extract against malaria (*Anopheles stephensi* Liston) and dengue (*Aedes aegypti* L.) vectors and bioassay-driven isolation of the marker compounds. Parasitol. Res. 113, 197–209.

Sudha, T., Chidambarampillai, S., Mohan, V.R., 2013. GC-MS analysis of bioactive components of aerial parts of *Fluggea leucopyrus* willd. (Euphorbiaceae). J. Appl. Pharm. Sci. 3, 126–130.

Susitra Manjari, M., Karthi, S., Ramkumar, G., Muthusamy, R., Natarajan, D., Shivakumar, M.S., 2014. Chemical composition and larvicidal activity of plant extracts from *Clausena dentata* (Willd) (Rutaceae) against dengue, malaria, and filariasis vectors. Parasitoi. Res. 113, 2475–2481.

Thamaraiselvi, P., Lalitha, P., Jayanthi, T., 2012. Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of *Eichhornia crassipes* (Mart.) *Solms*. J. Plant Sci. Res. 2, 115–122.

Torres, R.C., Garbo, A.G., Walde, R.Z.M.L., 2015. Characterization and bioassay for larvicidal activity of *Anacardium occidentale* (cashew) shell waste fractions against dengue vector *Aedes aegypti*. Parasitol. Res. 114, 3699–3702.

Udayan, P.S., Harinarayanan, M.K., Tushar, K.V., Indira, B., 2008. Some common plants used by kurichiar tribes of tirunelveli forest, Wayanad district, Kerala in medicine and other traditional uses. Indian J. Trad. Knowl. 7, 250–255.

Vivekanandhan, P., Senthil-Nathan, S., Shivakumar, M.S., 2018a. Larvicidal, pupicidal and adult smoke toxic effects of *Acanthospermum hispidum* (DC) leaf crude extracts against mosquito vectors. Phy. Mol. Plant. Pathol. 101, 156–162.

Vivekanandhan, P., Venkatesan, R., Ramkumar, G., Karthi, S., Senthil-Nathan, S., Shivakumar, M.S., 2018b. Comparative analysis of major mosquito vectors response to seed-derived essential oil and seed pod-derived extract from *Acacia nilotica*. Int. J. En. Res. Pub. Heal. 15, 388. https://doi.org/10.3390/ijerph15020388.

Vivekanandhan, P., Kavitha, T., Karthi, S., Senthil-Nathan, S., Shivakumar, M.S., 2018c. Toxicity of *Beauveria bassiana*-28 mycelial extracts on larvae of *Culex quinquefasciatus* mosquito (Diptera: Culicidae). Int. J. En. Res. Pub. Heal. 15, 440. https://doi.org/10.3390/ijerph15030440.

Vivekanandhan, P., Karthi, S., Shivakumar, M.S., Benelli, G., 2018d. Synergistic effect of entomopathogenic fungus *Fusarium oxysporum* extract in combination with temephos against three major mosquito vectors. J. Pat. Glob. Heal. https://doi.org/10.1080/20477724.2018.1438228.

Vivekanandhan, P., Deepa, S., Kweka, E.J., Shivakumar, M.S., 2018e. Toxicity of *Fusarium oxysporum*-VKFO-01 derived silver nanoparticles as potential insecticide against three mosquito vector species (Diptera: Culicidae). J. Clust. Sci. https://doi.org/10.1007/s10876-018-1423-1.0.

Vivekanandhan, P., Usha-Raja-Nanthini, A., Valli, G., Shivakumar, M.S., 2019. Comparative efficacy of *Eucalyptus globulus* (Labill) hydrodistilled essential oil and temephos as mosquito larvicide. J. Nat. Prod. Res. https://doi.org/10.1080/14786419.2018.1547290.

World Health Organization, 2005. In: Guidelines for Laboratory and Field Testing of Mosquito Larvicides. Communicable Disease Control, Prevention and Eradication, WHO Pesticide Evaluation Scheme, vol 13. WHO, Geneva, pp. 10–11.

Zandi, K., Teoh, B.T., Sam, S.S., Wong, P.F., Mustafa, M.R., Abubakar, S., 2011. Antiviral activity of four types of bioflavonoid against dengue virus type-2. J. Virol. 8, 560.

Zeinab, S.H., Abou-Elnaga, A., 2014. Insecticidal bioactivity of eco-friendly plant origin chemicals against *Culex pipiens* and *Aedes aegypti* (Diptera: Culicidae). J. Entomol. Zoo. Stud. 2, 340–347.