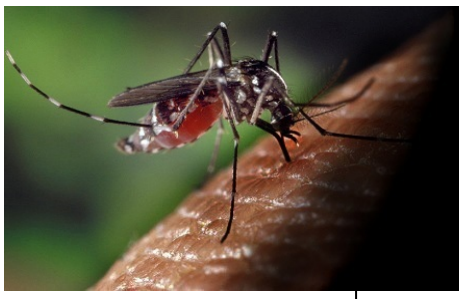

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Anjali Rawani
[1)] Department of Zoology, University of Gour Banga, Malda, West Bengal, India
[2)] Mosquito, Microbiology and Nanotechnology Research Units, Parasitology Laboratory, Department of Zoology, The University of Burdwan, Purba Bardhaman, West Bengal, India

Anupam Ghosh
[1)] Department of Zoology, Bankura Christian College, Bankura, West Bengal, India
[2)] Mosquito, Microbiology and Nanotechnology Research Units, Parasitology Laboratory, Department of Zoology, The University of Burdwan, Purba Bardhaman, West Bengal, India

Goutam Chandra
Mosquito, Microbiology and Nanotechnology Research Units, Parasitology Laboratory, Department of Zoology, The University of Burdwan, Purba Bardhaman, West Bengal, India

Corresponding Author:
Goutam Chandra
Mosquito, Microbiology and Nanotechnology Research Units, Parasitology Laboratory, Department of Zoology, The University of Burdwan, Purba Bardhaman, West Bengal, India

Evaluation of mosquito larvicidal activities of stem, root and flower of *Solanum nigrum* L. against filarial vector *Culex quinquefasciatus* Say

Anjali Rawani, Anupam Ghosh and Goutam Chandra

Abstract
Plant-based insecticides are eco-friendly, target-specific, less harmful to non-targets, biodegradable, and less prone to developing resistance. The present study was carried out to evaluate the mosquito larvicidal activity of crude and ethyl acetate solvent extracts of the stem, root, and flower of *Solanum nigrum* L. against immatures of *Culex quinquefasciatus* Say. Early third instar larvae of *Cx. quinquefasciatus* were exposed to 1, 1.5, 2, 2.5 and 3% of crude and 30, 50, 80, 100, and 120 ppm concentrations of ethyl acetate extracts of the stem, root, and flower. The highest mortality was observed at 3% crude and 120 ppm ethyl acetate extracts after 72 hours of exposure in all the plant parts. The lowest LC50 and LC90 values of crude and ethyl acetate extracts were observed to be 0.98% and 2.23%, and 30.38 ppm and 166.99 ppm respectively in the stem of *S. nigrum*. Apparent dose-dependent mortality was observed as a positive correlation between mortality (Y) and concentration (X) with a regression coefficient value close to one in each case. The IR and GC-MS analyses were carried out to find out the active ingredient. Chemical characterization revealed the presence of steroidal alkaloids in the stem as a primary active ingredient, which might be responsible for larval toxicity. The active ingredient of the stem did not cause any abnormality to non-target organisms. As a whole, different plant parts of *S. nigrum* showed varied larval mortality, so selectively, those can be employed to prepare cost-effective insecticides to control the mosquito population.

Keywords: *Solanum nigrum*, stem, root, flower, *Culex quinquefasciatus*, larvicidal activity, GCMS analysis

1. Introduction
In terms of public health concerns, mosquitoes are the most vital solitary group of vectors that transmit several deadly diseases like Malaria, Filariasis, Dengue, Japanese encephalitis in various tropical and subtropical countries. Worldwide in 91 countries, 216 million people are suffering from malaria alone [1]. While 859 million people in 50 countries worldwide remain threatened by lymphatic filariasis, among them, at least 25 million men were affected with hydrocele and over 15 million people with lymphoedema. However, 36 million people remain with these chronic disease manifestations [2]. Filariasis, a mosquito-borne disease, is principally caused by nematode *Wuchereria bancrofti* and transmitted by the female *Culex quinquefasciatus* mosquito. In India, the microfilaria carriers are estimated to be about 31 million people and suffer from filarial symptoms of over 23 million people [3]. Besides transmitting various diseases, mosquito bites may cause an allergic reaction such as local skin allergy and systemic sensitivity [4].
New control strategies have targeted to reduce the man-vector contact, primarily by reducing mosquito breeding sites and biting activity by using a blend of chemical-biological control methods to reduce the population of mosquitoes [5]. Various pesticides (chemical formulations) have been a matter of choice to control or eradicate mosquito populations. Although they are highly efficacious and show rapid action against the target species, these chemical pesticides are facing the threat of developing resistance [6]. The residues of these pesticides cause environmental pollution and mortality of other non-target organisms. Plant secondary metabolites [7-9] are considered a more potent option than chemical insecticides as they are rich in bioactive compounds that are environment-friendly, target-specific, readily available, cost-effective and biodegradable [10]. Insecticides of plant origin are not only responsible for larval

mortality [11], but they have also been employed as pupicide, adulticide, ovicide, repellent and insect growth regulators [12-13]. Besides mosquitocidal activity, plant products may act as anthelmintic, anti-bacterial, etc. [14-15].

Solanum nigrum L. is a cosmopolitan plant belonging to the family Solanaceae. The plant is commonly known as black nightshade. It is a small, erect, and delicate annual herb with soft and smooth stems and branches [16]. Several medicinal properties of this plant have been studied, including anti-inflammatory, antioxidant, antinociceptive, antipyretic, antitumor, antiulcerogenic, cancer chemopreventive, hepatoprotective and immunomodulatory effects [17, 18]. Previously, it has been reported that ethyl acetate solvent extract of mature leaves of this plant provides a very efficient mosquito larval control activity [19]. Active ingredient Glucosinolate has also been identified from leaf extract of *S. nigrum* [20]. The aromatic amide isolated from the berry also causes larval mortality against filarial vector *Cx. quinquefasciatus* [21].

The objective of the present study was to evaluate the efficacy of crude and ethyl acetate solvent extracts of different plant parts such as stem, root and flower of *S. nigrum* against the third instar larval form of *Cx. quinquefasciatus* and some non-target organisms and to find out the active ingredient, which is responsible for larval toxicity.

2. Materials and methods

2.1 Collection of mosquito larvae

Immatures of *Cx. quinquefasciatus* were collected by fine netting from some stagnant drains of Burdwan (23°16', 87°54'), West Bengal, India, during the study period (May', -2018 to May', -2019) and reared at 25-30 ºC with supplementary food consisting of a mixture of protein biscuit (60%) and dried Yeast powder (40%) in the insectary of Mosquito Research Unit, The University of Burdwan. India. Early third instar larvae were used in the experiments in the present study [22].

2.2 Collection and identification of plant Materials

Fresh mature stem, root, and flowers of *S. nigrum* were randomly harvested during the study period (May', -2018 to May', -2019) from plants growing on the outskirts of Burdwan (23°16' N, 87°54' E) having voucher specimen No. GCZAR-1 (the herbarium was deposited in the Department of Zoology, The University of Burdwan, West Bengal, India) and authenticated by Dr. Ambarish Mukherjee, Professor of Botany, The University of Burdwan, West Bengal, India.

2.3 Preparation of crude extract

The collected plant parts were initially rinsed with distilled water and dried on a paper towel. Crude extracts were prepared by grinding the small pieces of plant material in a mortar and pestle separately and passing the ground material through cheesecloth. The clear filtrate was used as a stock solution (100% concentration of crude extract) for bioassay experiments. The concentrations (1%, 1.5%, 2%, 2.5% and 3%) were prepared by addition with a suitable amount of sterilized distilled water to the stock solution [23].

2.4 Preparation of solvent extracts

Fresh stem, root and flowers of *S. nigrum* were collected during summer and monsoon and dried in the shade at room temperature. Then they were crushed to fine particle size and milled into a fine powder with Jankel and Kunkel model A10 mill. For solvent extraction, 50 g powder of each plant part was poured into three separate grease-free soxhlet apparatus. Each plant part was then subjected to ethyl acetate solvent extraction for 72 h at 40 °C. The extracts were collected separately and filtered through Whatman No. 41 filter paper. Each extract was collected in a beaker and subjected to evaporation in a rotary evaporator (RV8. IKA). Then the sample was lyophilized with the help of a lyophilizer (scan vac Cool Safe) to get the powdered sample. During the experiment, stock solutions of ethyl acetate solvent extract were prepared to have concentrations of 30, 50, 80, 100, and 120 ppm. From those stock solutions, 100 ml were taken from each during the experiment [23].

2.5 Larvicidal bioassay

The study was conducted according to standard test protocol [3] with slight modifications. Different concentrations of crude extract (1, 1.5, 2, 2.5 and 3%) and ethyl acetate solvent extract (30, 50, 80, 100 and 120 ppm) of each plant part were prepared and 100 mL of each extract was transferred into separate sterile Borosil glass beaker (120 ml capacity) to carry out the tests. In the laboratory, early third instar larvae (25) were introduced separately in each glass beaker. In each glass beaker, 20mg of larval food (powdered mixture of dog biscuits and dried yeast powder in the ratio of 3:1) was added. Percent mortality was recorded after 24, 48 and 72 h of post-exposure [3]. Dead larvae were identified when they did not show any movement after probing with a needle in their siphon or cervical region. The experiments were conducted for three days with three replicates on each day (n=9) at room temperature (30 ± 2 °C) and 80-90% relative humidity in a 12-h light/dark cycle. A set of control the experiment (without having any test solution) using only tap water was run parallel on each day of the experiment (n=3).

2.6 Thin layer chromatography (TLC) analysis

Ethyl acetate solvent extract of all plant parts was chromatographed by thin-layer chromatography on silica gel "G" (Merck, India) coated (0.5 mm thickness) plates using ethyl acetate and acetone (1:1 v/v) as mobile phase. After progress, plates were dried in the air and placed in an iodine chamber (21×21×9cm) for 1 min. The plates were removed, and the main band that appeared on the fractions of similar Rf values were mixed and used as a purified compound. Compounds having mosquito larvicidal effects were further subjected to one-dimensional preparative TLC using solvent system ethyl acetate and acetone (1:1 v/v) as mobile phase. The band containing bioactive principle was detected by keeping the plate in the iodine vapor chamber.

The distance of the run of the developing solvent from the bottom of the plate was measured, and the run of the sample spot was also measured. The Rf value was then calculated using the formula:

Rf = distance of the spot center from the start point/distance of the solvent run from the start point.

2.7 Bioassay with active ingredients

The said band was scraped from TLC plates and dissolved in 20 mL of absolute alcohol, and heated in a water bath (60-65 °C) for 5 min. Clear solutions were taken in separate conical flasks discarding the precipitate, including silica gel. After evaporation of alcohol, the solid mass present at the bottom of

the conical flask was scrapped and weighed. The fraction was dissolved in distilled water to make different concentrations with the help of a micropipette. During the experiment, stock solutions were prepared to have concentrations of 25, 50, 75 ppm. From those stock solutions, 100 ml were taken from each during the experiment. For the bioassay experiment, 25 third instar larvae of *Cx. quinquefasciatus* were introduced separately into different glass beakers containing graded nominal concentrations of active ingredient (25, 50, 75 ppm), and 20 mg of larval food was added per glass beaker. The experiment was kept in a 12 h light/dark setting. The mortality rate was recorded after 24, 48, and 72 h of post-exposure [3]. Dead larvae were identified when they did not move after probing with a needle in their siphon or cervical region. The experiments were conducted on three days with three replicates on each day (n=9) at 25-30 °C and 80-90% relative humidity. A set of control using only tap water was also run parallel on each experiment day (n = 3).

2.8 IR and GC-MS analyses of bio active principle

A portion of a dried sample containing active ingredients was subjected to infrared (IR) spectroscopy. For IR analysis, the sample was kept in vacuum desiccators over potassium hydroxide (KOH) pellets for 48 h, and then IR spectral analyses were carried out on a Perkin-Elmer FT-IR Spectrometer (Model: Spectrecee RX1) using potassium bromide (KBr) plates. GC-MS analysis was carried out a SHIMADZU QP 2010T, which comprised of an autosampler and gas chromatography interfaced to a mass spectrometer (GC-MS) instrument employing the following condition: capillary column-624ms (30m × 0.32mm × 1.8m) operating in an electronic mode at 70 eV; helium (99.999%) was used as the carrier gas at a constant flow of 1.491 mL/min and injection volume of 1.0 mL, injector temperature was 140 °C; ion source temperature of 200 °C. The oven temperature was programmed from 45 °C. Mass spectra were taken at 70 eV.

2.9 Effect on non-target organisms

The effect of bioactive compounds isolated from the stem of *S. nigrum* was tested against non-target organisms (NTO) like *Toxorhynchites splendens* and *Chironomus circumdatus*. For acclimation to the laboratory, they were kept in an environment similar to their natural habitats. As per the procedure used by Suwannee *et al*. [24], the non-targets were exposed to LC50 (at 24 h for third instar larvae) of the bioactive principle. Twenty-five *Toxorhynchites splendens* were placed in 200 ml pond water in a 500 ml beaker. Twenty-five third instar larvae of *Chironomus circumdatus* were kept in pond water in a plastic tray (12.6×10×6 inches). Abnormality or death, if any, of NTO was recorded. The experiments were conducted on three days with three replicates on each day (n=9). A set of control (without having the test solution) was run parallel on each day of the experiment (n=3) and average percent mortality was tabulated.

2.10 Statistical analysis

The percentage of larval mortality (M %) was corrected using Abbott's formula [25]. Statistical analysis of the experimental data was performed using the computer software Statplus 2007 and MS EXCEL 2003. Probit analysis was done to find the regression equations (Y = mortality; X = concentrations) and regression coefficient values and find out LC50 and LC90 values by Statplus 2007 software. Two-way factorial ANOVA using different plant parts and concentrations as variables for crude and solvent extracts was performed by SPSS 11.0.

3. Results

The percent mortality of test mosquito larvae at different concentrations of crude and ethyl acetate extracts is presented in Table 1. Probit analyses of mortality of both extracts of stem, root, and flower of *S. nigrum* have been presented in Table 2. Results of regression analysis revealed that the mortality (Y) was positively correlated with the concentration (X) having a regression coefficient value close to one in both extracts (Table 2). Multivariate ANOVA (Table 3a, b) revealed a significant effect of different instars and test concentrations on larval mortality in both the extracts tested. When the interactions of factors were considered, there was no significant difference.

During bioassay with active ingredients highest larval mortality (98.68%) was observed in the active ingredient of the stem at 75 ppm concentration, followed by flower (90.68%) and root (84.00%) (Table 4).

From IR spectroscopy, the vibration of C-H stretching, C=O stretching, N-H stretching and C-N stretching were observed in the stem; vibration of N-H stretching, C-H stretching, C=O stretching, and C-N structure, but H-bonded in aliphatic amide were observed in the root; vibration of N-H stretching, C-H stretching, C=O stretching, and H-bonded C-N structure was observed in flower. As the highest mortality was observed in the stem, the GC-MS analysis of ethyl acetate extract of the stem was done to identify the probable active ingredients responsible for larval mortality (Fig 1). GC-MS analysis detected the presence of 16 bioactive compounds. The identified compounds are presented in Table 5. No to significantly less abnormality or mortality was observed in non-target organisms after 72 h of exposure to the active ingredients isolated from the stem of *S. nigrum*.

Table 1: The activity of crude extract and ethyl acetate extract of other parts of *S. nigrum* on 3rd instar larvae of *Cx. Quinquefasciatus*

Plant parts	Concentration		% mortality					
	Crude (%)	EA (ppm)	24 h		48 h		72 h	
			Crude	EA	Crude	EA	Crude	EA
Stem	1	30	46.70 ± 0.33	36.70 ± 0.67	53.30 ± 0.33	40.00 ± 0.58	56.70 ± 0.33	56.70 ± 0.33
	1.5	50	53.30 ± 0.33	43.30 ± 0.33	50.00 ± 0.58	50.00 ± 0.58	66.70 ± 0.33	56.70 ± 0.33
	2	80	70.00 ± 0.58	63.30 ± 0.33	83.30 ± 0.33	66.70 ± 0.67	86.70 ± 0.33	70.00 ± 1.00
	2.5	100	73.30 ± 0.33	73.30 ± 0.33	86.70 ± 0.33	80.00 ± 0.58	90.00 ± 0.00	83.30 ± 0.33
	3	120	86.70 ± 0.33	76.70 ± 0.67	96.70 ± 0.33	83.30 ± 0.33	100.00 ± 0.00	90.00 ± 0.00
	Control		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
Root	1	30	33.30 ± 0.33	26.70 ± 0.33	43.30 ± 0.33	33.30 ± 0.33	50.00 ± 0.58	46.70 ± 0.67
	1.5	50	53.30 ± 0.33	36.70 ± 0.67	56.70 ± 0.33	43.30 ± 0.88	63.30 ± 0.33	53.30 ± 0.33

	2	80	60.00 ± 0.58	40.00 ± 0.58	66.70 ± 0.33	46.70 ± 0.33	70.00 ± 0.58	60.00 ± 0.58
	2.5	100	70.00 ± 0.58	53.30 ± 0.33	76.70 ± 0.33	63.30 ± 0.33	80.00 ± 0.58	70.00 ± 0.00
	3	120	73.30 ± 0.33	60.00 ± 0.58	80.00 ± 0.58	66.70 ± 0.67	83.30 ± 0.33	73.30 ± 0.33
	Control		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Flower	1	30	43.30 ± 0.33	36.70 ± 0.67	50.00 ± 0.58	40.00 ± 0.67	46.70 ± 0.33	50.00 ± 0.58
	1.5	50	46.70 ± 0.33	43.30 ± 0.88	53.30 ± 0.33	43.30 ± 0.33	70.00 ± 0.58	56.70 ± 0.33
	2	80	53.30 ± 0.33	60.00 ± 0.58	63.30 ± 0.33	70.00 ± 0.58	66.70 ± 0.33	76.70 ± 0.67
	2.5	100	63.30 ± 0.33	70.00 ± 0.58	66.70 ± 0.33	80.00 ± 0.58	76.70 ± 0.33	76.70 ± 0.33
	3	120	73.30 ± 0.33	76.67 ± 0.33	76.70 ± 0.33	83.30 ± 0.33	83.30 ± 0.33	86.70± 0.33
	Control		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 2: Probit and regression analyses of mortality rate in crude and ethyl acetate solvent extracts of stem, root and flowers of *S. nigrum*

Plant parts	Hours	Crude extract				Ethyl acetate extract			
		LC_{50} (%)	LC_{90} (%)	Regression equation	R value	LC_{50} (ppm)	LC_{90} (ppm)	Regression equation	R value
Stem	24	1.20	4.22	Y=20.00x+26.00	0.98	51.09	237.05	Y=0.48x+21.90	0.98
	48	1.12	2.67	Y=24.70x+24.60	0.93	44.04	179.68	Y=0.42x+28.98	0.98
	72	0.98	2.23	Y=21.98x+36.06	0.97	30.38	166.99	Y=0.41x+40.53	0.99
Root	24	1.48	5.65	Y=19.34x+19.30	0.96	91.32	752.22	Y=0.35x+16.24	0.97
	48	1.22	4.74	Y=18.68x+27.32	0.98	66.03	543.32	Y=0.37x+22.33	0.96
	72	1.02	4.27	Y=16.66x+36.00	0.98	39.25	478.58	Y=0.30x+37.64	0.99
Flower	24	1.48	9.26	Y=15.32x+25.34	0.98	52.95	270.49	Y=0.46x+21.91	0.99
	48	1.12	8.67	Y=13.36x+35.28	0.98	45.81	188.27	Y=0.55x+21.90	0.95
	72	1.03	4.51	Y=15.98x+36.72	0.91	33.48	177.74	Y=0.41x+38.10	0.97

Table 3: Two-way ANOVA on mortality of third instars larvae of *Cx. quinquefasciatus* in different test concentrations of crude extract (a) and ethyl acetate extract (b) of different plant parts of *S. nigrum*

3a: crude extract

Source of variation	Sum of Squares	df	Mean Square	F value	P value
Plant parts (P)	13.40	3	4.46	9.57	0.001*
Concentration (C)	94.33	4	23.58	50.53	0.001*
P * C	5.93	12	0.49	1.05	0.417 (N.S.)
Residual	18.66	40	0.46		
Total	132.33	59			

*Significant at $p<0.05$; N.S.: not significant.

3b: solvent extract

Source of variation	Sum of Squares	df	Mean Square	F value	P value
Plant parts (P)	25.20	3	8.40	17.37	0.001*
Concentration (C)	101.43	4	25.35	52.46	0.001*
P * C	6.96	12	0.58	1.20	0.315 (N.S.)
Residual	19.33	40	0.48		
Total	152.93	59			

*Significant at p<0.05; N.S.: not significant.

Table 4: Larvicidal activity of active ingredient of Stem, root and flower of *Solanum nigrum* against 3rd instar larvae of *Culex quinquefasciatus*

Plant parts	Concentration (ppm)	% mortality ± SE		
		24 h	48 h	72 h
Stem	25	74.68 ± 0.88	78.68 ± 0.88	81.32 ± 0.88
	50	82.68 ± 0.88	86.68 ± 0.33	92.00 ± 0.58
	75	92.00 ± 1.15	96.00 ± 0.58	98.68 ± 0.33
Root	25	58.68 ± 0.67	69.32 ± 1.20	72.00 ± 0.58
	50	66.68± 0.67	74.68 ± 0.88	77.32 ± 0.88
	75	73.32 ± 0.33	78.68 ± 0.33	84.00 ± 0.58
Flower	25	62.68 ± 0.67	68.00 ± 0.58	77.32 ± 1.20
	50	70.68 ± 0.67	74.68 ± 0.67	81.32 ± 0.67
	75	78.68 ± 0.33	82.68 ± 0.33	90.68 ± 0.33

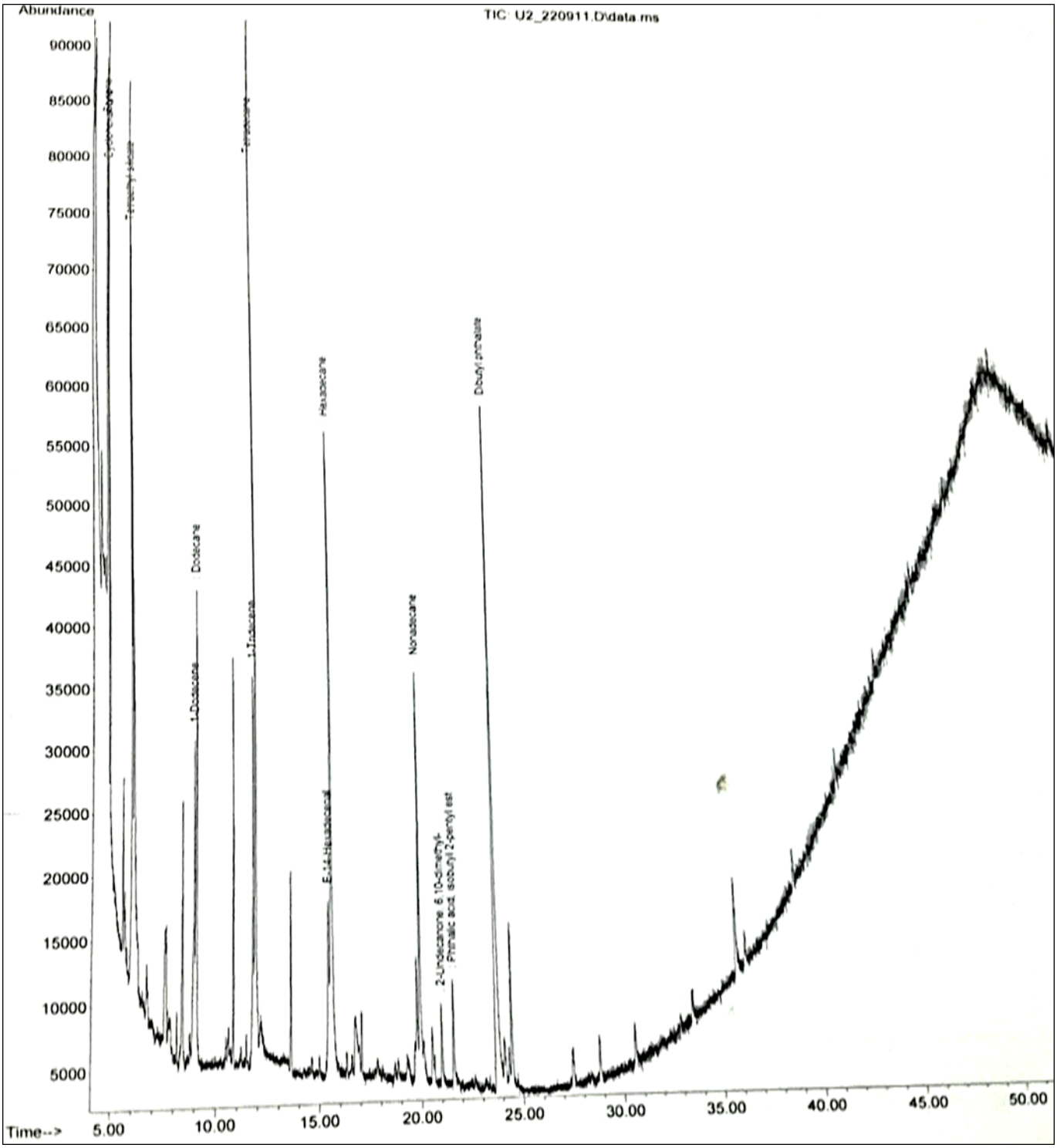

Fig 1: GCMS analysis of ethyl acetate extract of mature stem of *Solanum nigrum*

Table 5: List of identified compounds which were present in ethyl acetate extract of mature stem of *Solanum nigrum*

Peak	Retention Time (mins)	Area	Area%	Name of the compounds	Molecular weight
1	4.879	109346	6.302 %	Styrene	104.15
2	5.949	109937	6.336%	Cyclohexanone	98.15
5	8.977	79527	4.584%	1-Dodecene	168.32
10	13.610	40695	2.346%	Hexadecane	226.45
11	15.434	52972	3.053%	E-14-Hexadecenal	238.42
12	15.580	230142	13.265%	Nonadecane	268.52
13	19.926	115211	6.640%	2-Undecanone, 6,10-dimethyl	198.35
14	21.562	30447	1.755%	Phthalic acid, isobutyl 2-pen	166.14
15	23.690	30447	17.528%	Dibutyl phthalate	278.35

4. Discussion

Plant resources can be used as alternative mosquito larvicidal, pupicidal, ovicidal agents because they comprise a potential source of secondary metabolites. These secondary metabolites are naturally synthesized in the plant body for defense from their parasites and predators and to cope with environmental stress. More than 2,000 plant species have produced chemical compounds and metabolites of value in pest control [26]. The biological activity of the plant extract may be due to the deposition of secondary metabolites like alkaloids, terpenoids synthesized within the plants in varying magnitude [27-29]. These compounds, either singly or in combination, may be responsible for larval toxicity. The bioactive compound/s can thus be extracted by using solvents and used as mosquitocides and are considered to be the best alternatives to synthetic insecticides as they are relatively safe, target-specific, low-cost, biodegradable, and readily available [30]. The current strategy has opened up new prospects for large-scale extraction of active ingredients of plant origin for effective mosquito control. To take advantage of substantial plant resources, screening locally available medicinal plants for mosquito control efficacy is needed. The extract obtained from plant parts like leaves, roots, flowers, bark, seed, and fruits in their crude extracts can be used as conventional larvicide [31]. Crude and ethyl acetate extracts of different plant parts of *S. nigrum*, such as stem, root, and flower, show high mortality of third instar larvae of *Cx. quinquefasciatus*. At a 3% concentration of crude extract, the highest mortality has been observed in stem followed by flower and root. Ethyl acetate extract shows less activity than the crude extract. Several kinds of literature exist where crude plant extracts are effective such as the root of *Hemidesmus indicus* shows 80% mortality at 5% concentration against *Cx. Quinquefasciatus* [32], unripe and ripe fruits of *Piper retrofractum* have an LC50 value of 135 ppm against *Cx. quinquefasciatus* and 79 ppm against *Ae. Aegypti* [33], flower extract of *Myrtus communis* has an LC50 value of 16 mg/L against *Cx. pipiens molestus* [34].

In ethyl acetate extract, also the best result is found in stem followed by flower and root. From GC-MS analysis, sixteen active compounds have been identified in the mature stem of *S. nigrum*, having excellent larvicidal efficacy, and it appears that steroidal alkaloids may be responsible for larval mortality. These alkaloids are present in more than 350 plant species, especially the family Solanaceae [35]. In *Solanum* species, differential tissue and organ-specific syntheses and accumulation of steroidal alkaloids have been observed [36]. Synthesis commences during germination and peaks in the flowering period. The leaves are the first organs to attain a maximum concentration of secondary metabolites, followed by unripe fruits and flowers [37]. In potato tubers, the steroidal glycoalkaloids are concentrated in the peel, with the highest levels around the eyes constituting the periderm, cortex, and outer phloem [38]. Kumar *et al*. [39] reported the larvicidal activity of flavonoids, phenols, glycosides, and tannins from the whole plant extract of *Cassia occidentalis* against the filarial vector of *Cx. quinquefasciatus*. Many studies report a wide range of biological activities like saponin mixture isolated from *Cestrum diurnum* [40], steroid from *Solanum villosum* berry [23] and glucosinolate isolated from leaves of *Solanum nigrum* [20] have been reported before.

5. Conclusion

Crude and ethyl acetate extracts of the stem, flower and root of *S. nigrum* and isolated bioactive secondary metabolites from the stem showed good mosquito larvicidal efficacy, which can be used in stagnant water bodies i.e., in the breeding grounds for mosquitoes for vector control. The plant is readily available, the control method is cost-effective, and it does not cause environmental pollution or toxicity to non-target organisms tested. However, further studies on their mode of action and field/semi-field trials are needed before use on a commercial basis.

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7. Conflicts of interest/Competing interests

It is declared that the authors have no competing interests.

8. Availability of data and material

All data generated and analyzed for this study are presented in the manuscript.

9. Ethics approval

Not applicable.

10. Consent for publication

The authors agree to publish this paper. The data has not been published partially or completely in any other journal.

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