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Eco toxicity of plant based mosquito larvicidal, repellent and adulticidal activities of *Sargassum polycystum* extract against dengue and filaria vectors

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Abstract
The present study was conducted to analyze the mosquito larvicidal, repellent and adulticidal activities by ethanol extract of *Sargassum polycystum* against *Aedes aegypti* and *Culex quinquefasciatus.* The larvicidal and repellent activity was tested by various concentrations of the extract at 100, 150, 200, 250 and 300µL/cm^2 against batches of 20 early fourth-instar larvae of dengue and filariasis vector. Mortality was observed 24h after treatment. Adulticidal activity of *S. polycystum* extract was tested against 4-5 old female adults of both mosquitoes and mortality was observed 24h under laboratory conditions. The maximum larvicidal and adulticidal activity was observed at 200 µL concentrations of LC_{50} were (52.2±2.3), (53.2±2.3) and (50.2±0.7), (51.2±0.9) µL/ml. At 300 µL, extracts of *S. polycystum* showed maximum repellency of 93% and 95.1% against *Aedes aegypti* and *Culex quinquefasciatus*. The present study identifies active insecticidal compounds from *S. polycystum* by GC-MS and can be novel source against dengue and filariasis mosquitoes.

Keywords: *S. polycystum, Aedes aegypti, Culex quinquefasciatus,* mosquito larvicidal, repellent and adulticidal activity

1. Introduction
Currently, most insecticides are non-selective and can be harmful to other organisms and to the environment [1]. The activity of crude plant extracts is often attributed to the complex mixture of active compounds [2]. Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations [3]. In view of the residue problems in the environment and development of insect resistance to synthetic insecticides like DDT and chlorinated hydrocarbons [4]. Development of resistance to commercial acaricides by parasites has stimulated the search for new control strategies [5]. Natural products are generally preferred because of their less harmful nature to target organisms and due to their innate biodegradability [6]. Extracts or essential oil from plants may be an alternative source of mosquito control agents [7]. Plant parts have been provided as a good source of novel drug compounds [8]. However, mosquitoes have successfully adapted to most insecticides by becoming physiologically or behaviorally resistant to them [9]. Marine biodiversity provides important sources of chemical compounds, which have many therapeutic applications such as antimicrobial, infertility and anticancer activities [10, 11]. Seaweeds are one of the most important marine resources of the world and being used as human food, animal feed and raw material for many industries. For centuries, seaweed has been of botanical, industrial and pharmaceutical interest [12]. Various surveys have shown that seaweeds are an excellent source of parts such as polysaccharides, tannins, flavonoids, phenolic acids, bromophenols and carotenoids have exhibited different biological activities [13]. *Sargassum polycystum* is brown (Phaeophyceae) macro algae belongs to the family named *Sargassaceae*, under *Fucales* [14]. *Sargassum polycystum* used as medicine for ailments, antibacterial fatty acids, anti-oxidants used as animal feed, fertilizer and insect repellent [15]. The objective of this present study was to evaluate larvicidal, repellent and adulticidal activities of *Sargassum polycystum extracts* against *Aedes aegypti* (*Ae. aegypti*) and *Culex quinquefasciatus* (*Cx. quinquefasciatus*) and identify the compounds by using gas chromatography–mass spectrometry (GCMS).

2. Materials and Methods

2.1 Plant Materials

This study was conducted from May 2017 to Jan 2018. Fresh sample of brown seaweed *Sargassum polycystum* was collected from Mandapam, Ramanathapuram District, Tamil Nadu of south east coast of India (Latitude 9° 45′ N and longitude 79° 13′ E). The collected samples were washed in seawater to remove sand, mud and all epiphytes, thrice with tap water and twice with distilled water to remove the adhering salts. The samples were dried at room temperature and were ground separately into powder using a miller before extraction of the crude seaweed extract.

2.2 Extract Preparation

The algal powder was boiled in ethanol and distilled water mixture (7:3 v/v) at 55°C for 2 h using a soxhlet apparatus under reduced pressure. The filtrate was condensed by evaporating to a minimal volume at 45ºC and then freeze-dried (-80ºC). The extract obtained was referred to as crude seaweed extract. The extract preparation was done by following the method of Syed Ali *et al* [12], with slight modification. The percentage of extraction was calculated by using the following formula,

$$\text{Percent of extraction} = \frac{\text{Weight of the extract}}{\text{Weight of the plant material}} \times 100$$

2.3 Mosquito Larval Culture

Mosquito larva of *Aedes aegypti and Culex quinquefasciatus* were collected from our college (Mohamed sathak college of arts and science, sholinganallur, Chennai) sewage water, placed in dechlorinated water in separate plastic trays. They were reared indoors at (28±2) °C and 75-85 % relative humidity under 14:10 light and dark period cycle. The larvae were fed with powdered mix of dog biscuits and brewer's yeast powder in 3:1 ratio. Pupae were transferred from the trays to a cup containing tap water and were maintained in cages (45×45×40 cm) where adults emerged. After five days emergence, female mosquitoes were moved into a mosquito cage where the emerging adults were fed with a 10% sucrose solution in air- tight cylindrical glass container with a cotton wick. Glass Petri dishes with 50 ml of tap water lined with filter paper were kept inside the cage for oviposition. The Mosquito larval culture was done by following with slightly modified the method of Syed Ali *et al,* [15].

2.4 Larvicidal Activity

The test for the larvicidal effect of ethanolic extract derived from *S. polycystum* against mosquito larvae (*Ae. Aegypti and Cx. quinquefasciatus*) was conducted with slightly modified as per the method of Syed Ali *et al,* [15]**,** in accordance with the standard WHO guidelines [16]. Batches of IV instar, 20 larvae of two mosquitoes (*Ae. aegypti, Cx. quinquefasciatus*) were transferred to 100 ml glass beaker containing 50mL of distilled water and various concentrations (100µL, 150µL, 200µL, 250µL, 300µL) of extracts. Each experiment was conducted with three replicates and a concurrent control group. A control group consisted of 50 ml of distilled water only. After treatment, symptoms in treating larvae were observed and recorded immediately with at time intervals and no food was offered to the larvae. At the end of 24 h, the larvae were considered dead, they showed no sign of swimming movements even after gentle touching with a glass rod, as described in the WHO's technical report series. Later, the lower concentration of ethanolic seaweed extract that had successfully produced more than 75-80% larval mortality rate was applied in a toxicity test on a non-target organism. The percentage of mortality was calculated by with Abbott's formula [17],

$$\text{Corrected Percent mortality} = \frac{\text{Percent of test mortality - Percent of control mortality}}{\text{100-Percent of control mortality}} \times 100$$

2.5 Repellent Activity

The repellent activity was conducted with slightly modified as per the method of Syed Ali *et al.,* [10] and was determined by the percentage protection time in relation to dose method WHO [26]. Repellency bioassays were carried out in a 10x10x3m room at 27-35ºC and 60-80% RH. The target *Ae. aegypti and Culex quinquefasciatus*, the testing period was run between 0-6 h. Three to four days old blood-starved 100 adult females of *Ae. aegypti* and *C. quinquefasciatus* mosquito was randomly selected and placed in an experimental cage (30 x 30 x 30 cm) and left to acclimatize for 1h. The arm tested person was cleaned with ethanol. After air drying the arm of the test person, only 25 cm^2 dorsal side of the skin on each arm was exposed and the remaining area being covered with rubber gloves. The ethanolic seaweed extract of *S. polycystum* with different concentration (100µL, 150µL, 200µL, 250µL, 300µL) was applied. The control and treated arms were introduced simultaneously into the cage. The first bite by the *Ae. aegypti and Cu. quinquefasciatus* was noted to 5 minutes for every 1 h from the 6 h. Subsequently, the test arm was introduced into the cage for the same period of time and the numbers of mosquitoes that landed and attempted to feed were recorded. The experiment was conducted for three times. It was observed that there was no skin irritation by the extracts of *S.polycystum*. The percentage protection was calculated by using the following formula (Syed Ali *et al),*

$$\text{Percentage Protection} = \frac{\text{No. of bites received by control} - \text{No. of bites received by treated}}{\text{No. of bites received by control}} \times 100$$

2.6 Adulticidal activity

The repellent activity was conducted with slightly modified as per the method of Govindarajan *et al* [13] and was determined by the percentage mortality rate in relation to dose method WHO [18]. The toxicity of the ethanolic extract derived from seaweed against four to five days old female adults of *Aedes aegypti and Culex quinquefasciatus* was examined. Glass tubes of 20ml capacity were used as exposure chambers during the fumigation test. The ethanolic seaweed extract was applied on Whatman no. 1 filter paper (12×15 cm^2) placed inside the glass tubes at concentrations of (100µL, 150µL, 200µL, 250µL, 300µL) of extracts. The extract papers were

rolled and placed in exposure tubes, the hole on the top of the tube sucrose fed and blood starved 20 mosquito adults was allowed inside and plugged with cotton. They kept for acclimatize for 1hr and fumigated adults were observed and tabulated. A pre dried Whatman no. 1 filter paper consists ethanol added was served as control. At the end of exposure the mosquitoes were placed in holding glass tube. Cotton pads soaked in 10% sugar solution with vitamin B complex were placed during the holding period of 24h. The number of dead mosquitoes and mortality percentages were determined after 24 hrs of treatment. Triplicates of each treatment and control were set up.

2.7 GC-MS Analysis

GC-MS technique was used to examine the constituents of extracts from *S. polycystum* was carried out in IITM, Tamil Nadu. It was performed using Agilent and Jeol GC mateII (Mass spectrometry) by HP-5 column capillary equipped with a high temperature column (DB-5mm 30 × 0.25 mm × 0.25 µm) was used and works with 70eV. The injector and detector temperature can set at 250˚C. A 1µL sample volume was injected into the column and employed using split less mode. High pure Helium is carrier gas was programmed to maintain a constant flow rate of 1ml/min. The column oven temperature was initially kept at 80°C for 2 min, and then programmed at 200°C/min, which was held at 20 min. Identification of organic compound was matching their recorded spectra with the data bank mass spectra of NIST library provided by the instrument.

2.8 Statistical Analysis

The average mortality data were subjected to profit analysis to calculate LC_{50} and 95% fiducial limits of upper confidence limit (UCL) and lower confidence limit (LCL), regression equation, Chi-square and analysis variation values were calculated using the Stat plus 2009 software. Results with $P \leq 0.05$ were considered to be statistically significant.

3. Results

Results revealed that the extract of *S. polycystum* showed various ranges of larvicidal, repellent and adulticidal activities. The LC_{50} value of *S. polycystum* extract ranged from 100 to 300µL with different concentrations. Table 1 and 2 show IV instars larvae of *a. aegypti* and *Cx. quinquefasciatus* showed maximum activity with a minimum concentration of LC_{50}=52. 2±2.3 and 53.2±2.3 at 200 µL of extract more effective than the other concentration (100, 150, 250 and 300 µL**)** Lower confidence and upper confidence level value showed (50.8-54.8 and 52.8-54.8) and the regression value of *Ae. aegypti* and *Cx. quinquefasciatus* showed R^2 = 0.98 and 0.981 and analysis of variable values was significant at $P \leq 0.05$ levels. *S. polycystum* showed the maximum percentage of *Ae. aegypti* mosquito protection (93%) and protection time (4.1hrs) was observed at the 300µL of concentration of extract in Table 3. Moreover, the *S. polycystum* showed protection percentage of *Cx. quinquefasciatus* mosquito protection (95.1%) and protection time (4.4hrs) was observed at the 300 µL of *S. polycystum* extract. The results were presented in Table 4. However, bites were observed between 10.00-16.00 hrs with the remaining concentration of extract treated arms. The result of the adulticidal activity from *S. polycystum* against *Ae. aegypti* and *Cx. quinquefasciatus* are presented in Tables 5 and 6. Among the five concentrations of seaweed extract tested, the highest adulticidal activity was observed in 200µL against *Ae. aegypti* and *Cx. quinquefasciatus* with the LC_{50}=50.9±0.6, 51.2±0.9 and LCL-UCL values of 46.2-54.2, 50.2-56.8 respectively, and the regression value R^2 = 0.99, 0.959 and analysis of variation was significant at $P \leq 0.05$ level. The relative percentage of identifying compounds from the ethanolic extract of *S. polycystum* were depicted in Table 7 were Hexadecanoic acid, followed by Glucobrassicin, Ethanol, 2-(9-octadecenyloxy)-,(Z), Ethanone, 1,1´-(1,4-dihydro-2,4,6-trimethyl-3,5-pyridinediyl) bis-, Tridecanoic acid, methyl ester, 1,2,4-Trioxolane-2-octanoic acid, 5-octyl-, methyl ester, Pentetic Acid and 9-Hexadecenoic acid .

Table 1: Larvicidal activity of ethanolic seaweed extracts of *S. polycystum* against *Ae.aegypti*

S.no	Concentration (µL)	LC_{50} (µL/ml)		R^2	$P \leq 0.05$
		Mean±SE	(LCL-UCL)		
1.	100	33.5±0.8	(32.5-34.6)	1.89	2. 05
2.	150	41.6±1.8	(40.8-43.6)	3. 05	0.89
3.	200	53.2±2.3	(52.8-54.8)	0.98*	0.032*
4.	250	70.8±1.28	(69.8-71.7)	1.12	1.28
5.	300	73.6±0.8	(71.7-74.5)	2.3	1.68

LCL means lower confidence level and UCL means upper confidence level. *Significant at P< 0. 05 level Analysis variation between lethal dose and concentration.

Table 2: Larvicidal activity of ethanolic seaweed extracts of *S.polycystum* against *Cx. quinquefasciatus*

S.no	Concentration (µL)	LC_{50} (µL/ml)		R^2	$P \leq 0.05$
		Mean±SE	(LCL-UCL)		
1.	100	33.5±0.82	(32.5-34.6)	1.8	1.81
2.	150	42.8±1.1	(40.8-43.6)	1.92	2. 08
3.	200	53.2±2.3	(25.8-54.8)	0.987*	0. 04*
4.	250	70.8±2.8	(69.8-71.8)	1.56	1.9
5.	300	73.6±0.8	(71.1-74.5)	2.8	1.8

LCL means lower confidence level and UCL means upper confidence level. *Significant at P< 0. 05 level Analysis variation between lethal dose and concentration.

Table 3: Repellent activity of ethanolic seaweed extracts of *S.polycystum* against *Ae. aegypti*

Concentration (%)	Treatments Period (hrs)												% of protection	Protection Time (hrs)
	10-11		11-12		12-13		13-14		14-15		15-16			
	T	C	T	C	T	C	T	C	T	C	T	C		
100	0	0	0	2	1	3	2	5	3	7	5	10	59.2	1.1
150	0	0	0	1	1	4	2	7	4	9	5	11	62.5	1.7
200	0	0	0	3	1	6	2	8	3	10	5	11	71.7	2.1
250	0	0	0	4	0	7	1	9	2	10	3	12	85.7	3.2
300	0	0	0	3	0	5	0	8	1	12	2	15	93.0	4.1

T-Treatment, C-Control

Table 4: Repellent activity of ethanolic seaweed extracts of *S. polycystum* against *Cx. quinquefasciatus*

Concentration (%)	Treatments Period (hrs)												% of protection	Protection Time (hrs)
	10-11		11-12		12-13		13-14		14-15		15-16			
	T	C	T	C	T	C	T	C	T	C	T	C		
100	0	0	0	0	3	5	4	7	3	9	3	10	59.3	1.2
150	0	0	0	1	2	5	2	7	3	9	4	10	65.6	1.5
200	0	0	0	1	1	4	2	6	2	9	3	11	74.1	2.1
250	0	0	0	3	0	5	1	8	2	10	3	11	83.7	3.4
300	0	0	0	4	0	5	0	7	1	12	1	13	95.1	4.1

T-Treatment, C-Control

Table 5: Adulticidal activity from seaweed extracts of *S. polycystum* against *Ae. aegypti*

No. of Mosquito	Concentration (µL)	LC_{50}(mg/ml)		R^2	*P*≤0.05
		Mean ± SE	LCL - UCL		
25	100	25 ±3.2	22.6-28.9	1.048	2.87
25	150	35.1±1.1	25.1-39.1	1.125	1.26
25	200	50.2±0.7	46.2-54.2	0.99*	0.024*
25	250	55.9±0.6	48.3-54.1	2.98	0.80
25	300	64.1±1.1	62.8-79.8	1.135	0.9

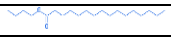
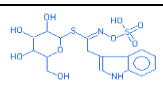
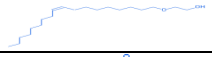
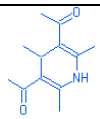
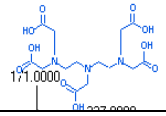
*Significant at P<0.05 level, LCL: Lower confidence level, UCL: Upper confidence level, R^2 – Regression equation of significant level of *P*≤0.05

Table 6: Adulticidal activity from seaweed extract of *S. polycystum* against *Cx. quinquefasciatus*

No. of Mosquito	Concentration (µL)	LC_{50}(mg/ml)		R^2	*P*≤0.05
		Mean ± SE	LCL - UCL		
25	100	32.6 ±0.58	29.6-34.9	1.048	2.87
25	150	45.1±0.25	43.1-46.1	1.125	1.26
25	200	51.2±0.9	50.2-56.8	0.959 *	0.032*
25	250	65.9±0.9	62.3-69.1	2.98	0.80
25	300	69.1±1.5	67.4-79.8	1.135	0.9

*Significant at P<0.05 level, LCL: Lower confidence level, UCL: Upper confidence level, R^2 – Regression equation of significant level of *P*≤0.05

Table 7: Identification of compound from seaweed extr act of *S. polycystum* using GCMS

Retention time	Compound	NIST Structure	Molecular weight	Peak Area
15.67	Hexadecanoic acid		256.42	15272544
13.63	Glucobrassicin		447.46	5986464
17.33	Ethanol, 2-(9-octadecenyloxy)-,(Z)		312.53	7423168
10.45	Ethanone, 1,1´-(1,4-dihydro-2,4,6-trimethyl-3,5-pyridinediyl)bis-		165.18	7269504
15.07	Tridecanoic acid, methyl ester		228.37	7721632
56.23	1,2,4-Trioxolane-2-octanoic acid, 5-octyl-, methyl ester		344.48	6614032
73.33	Pentetic Acid		393.35	5986464
56.23	9-Hexadecenoic acid		254.40	8790688

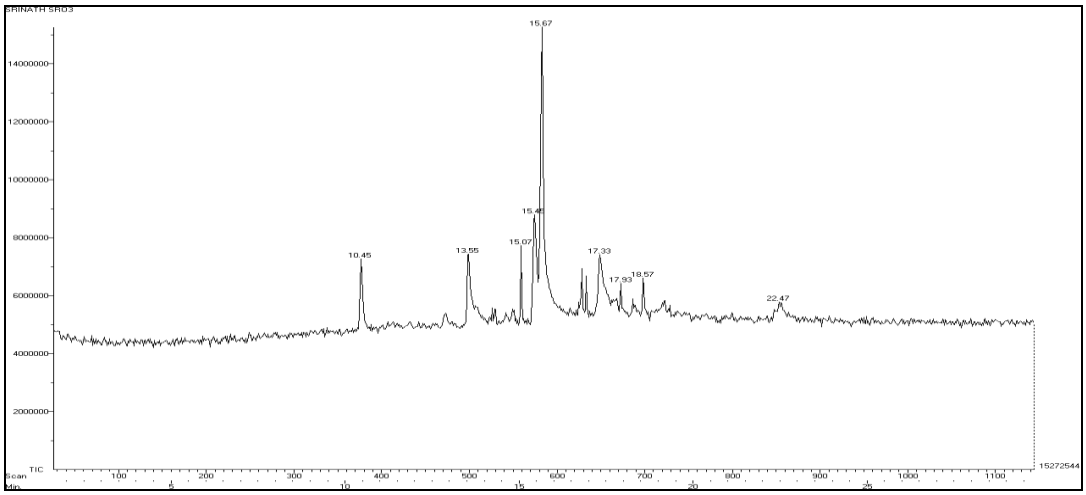
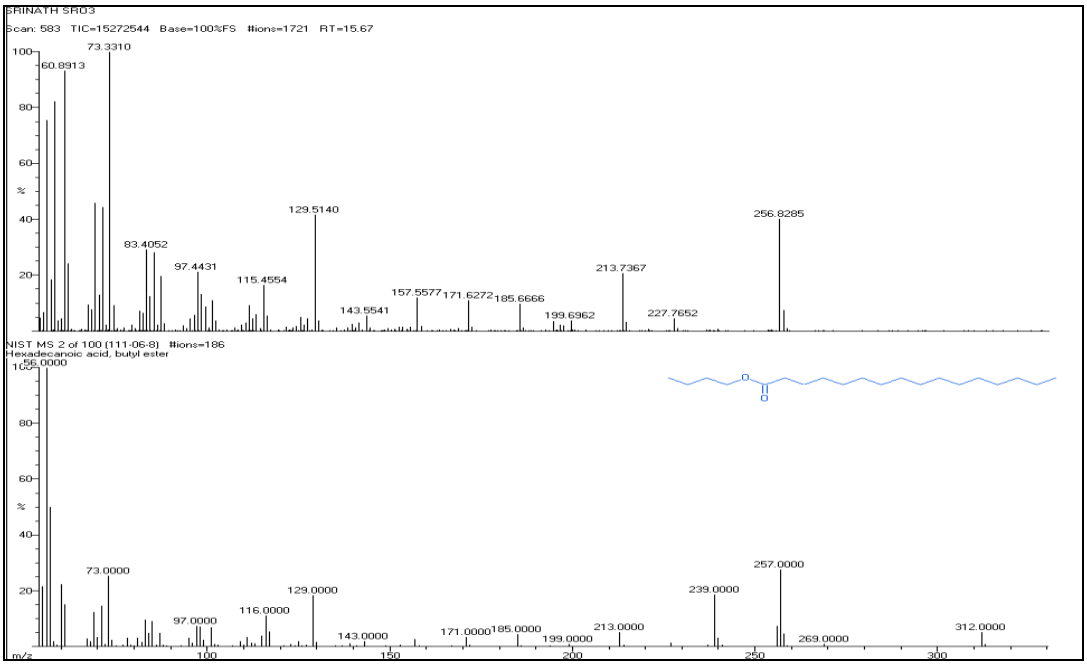
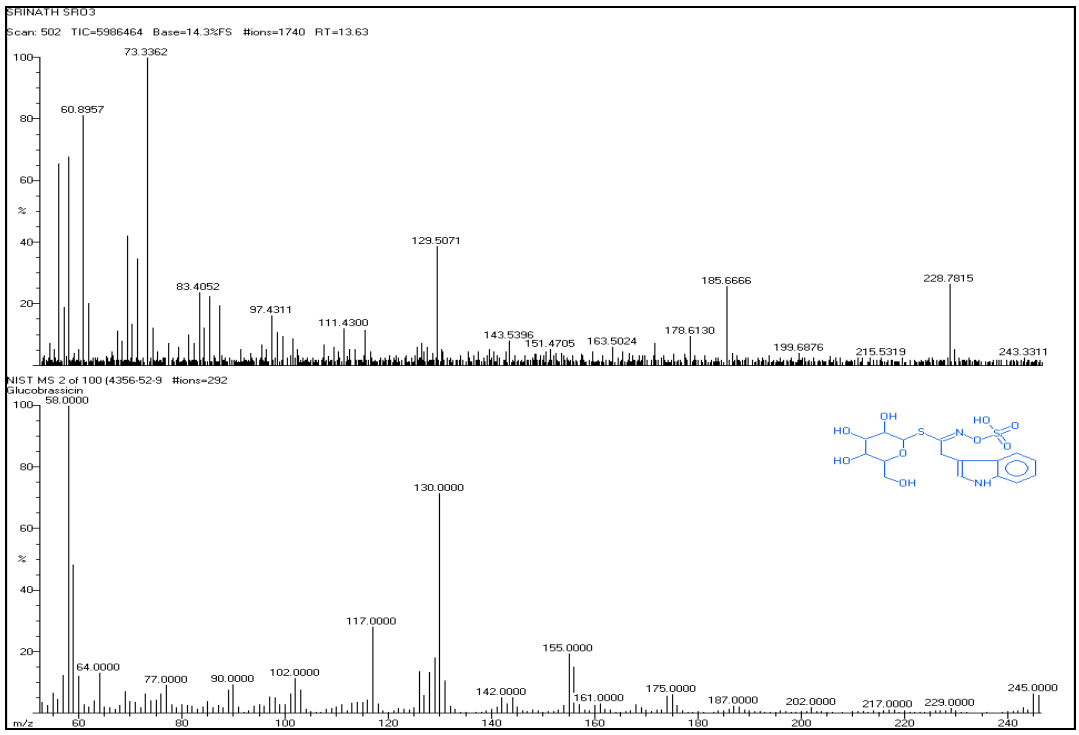
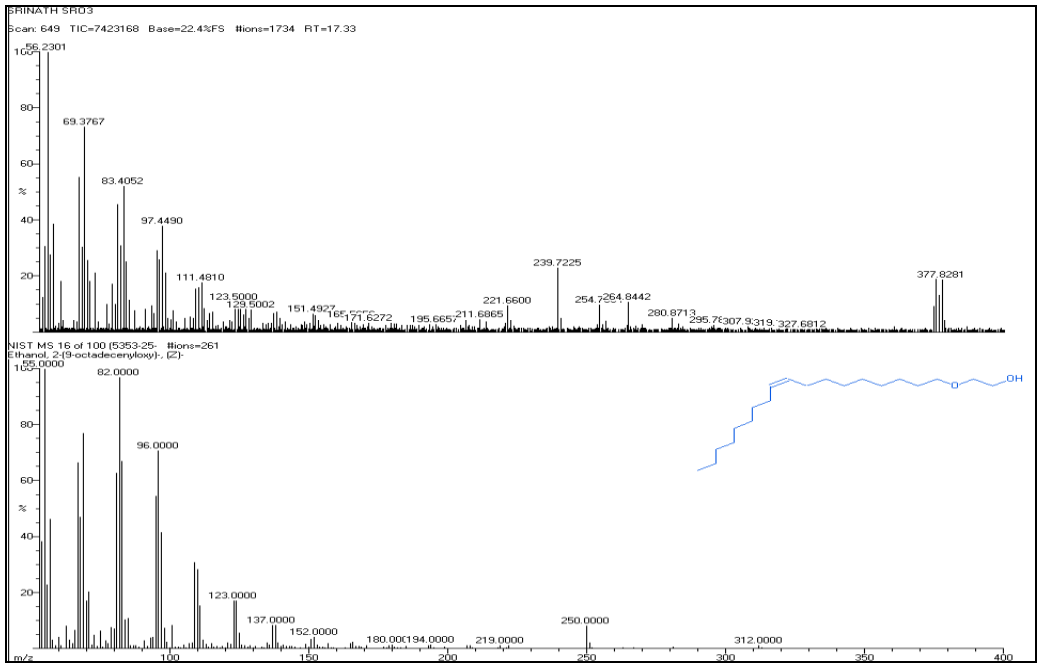
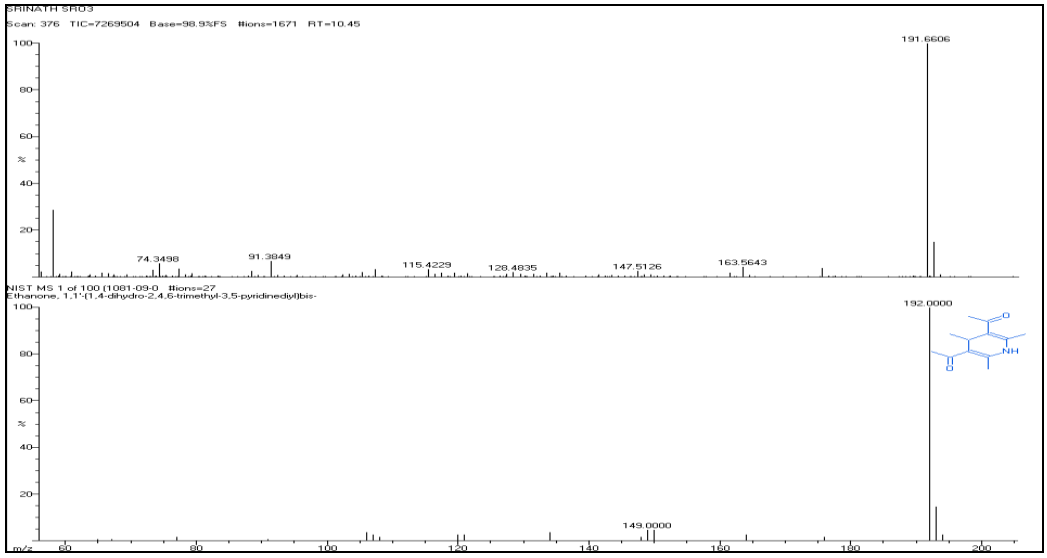


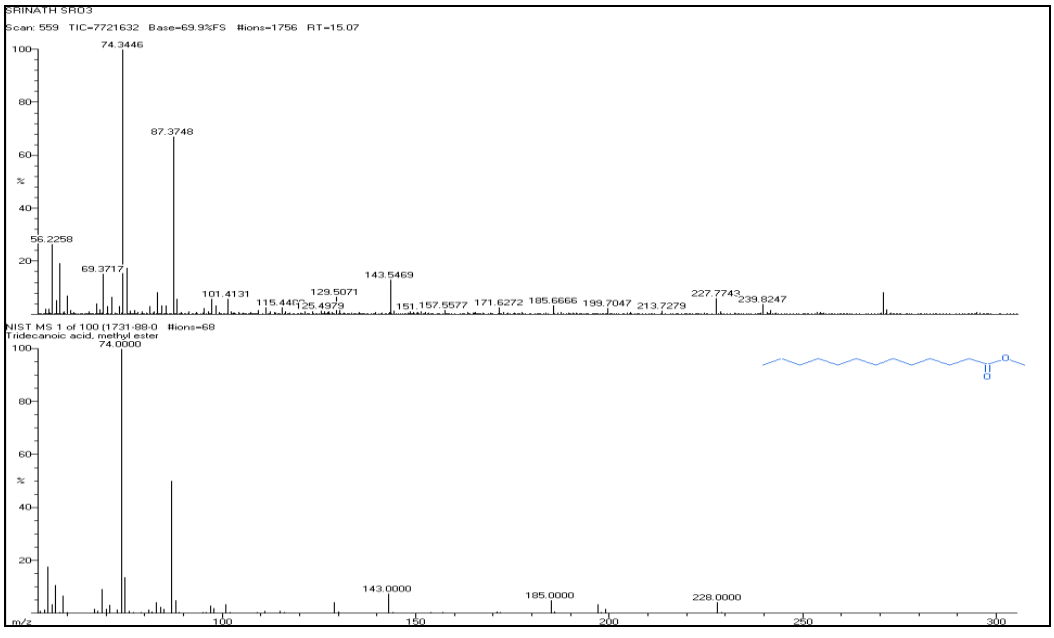
Fig 1: GCMS analysis of seaweed extract of *Sargassum polycystum*






SRINATH SR03
Scan: 649 TIC=7423168 Base=22.4%FS #Ions=1734 RT=17.33
56.2301
69.3767
83.4052
97.4490
111.4810
123.5000
129.5002
151.4927
171.6272
195.6657
211.6865
221.6600
239.7225
254.7264.8442
280.8713
295.7307.9319
327.6812
377.8281
NIST MS 16 of 100 (5353-25- #Ions=261
Ethanol, 2-[(9-octadecenyloxy)-, (Z)-
55.0000
82.0000
96.0000
123.0000
137.0000
152.0000
180.0019
194.0000
219.0000
250.0000
312.0000
OH
m/z


SRINATH SR03
Scan: 376 TIC=7269504 Base=98.9%FS #Ions=1671 RT=10.45
191.6606
74.3498
91.3849
115.4229
128.4835
147.5126
163.5643
NIST MS 1 of 100 (1081-09-0 #Ions=27
Ethanone, 1,1'-(1,4-dihydro-2,4,6-trimethyl-3,5-pyridinediyl)bis-
192.0000
149.0000
NH
m/z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115.4402
125.4979
129.5071
143.5469
151.1575577
171.6272
185.6666
199.7047
213.7279
227.7743
239.8247
NIST MS 1 of 100 (1731-88-0 #Ions=68
Tridecanoic acid, methyl ester
74.0000
143.0000
185.0000
228.0000
m/z

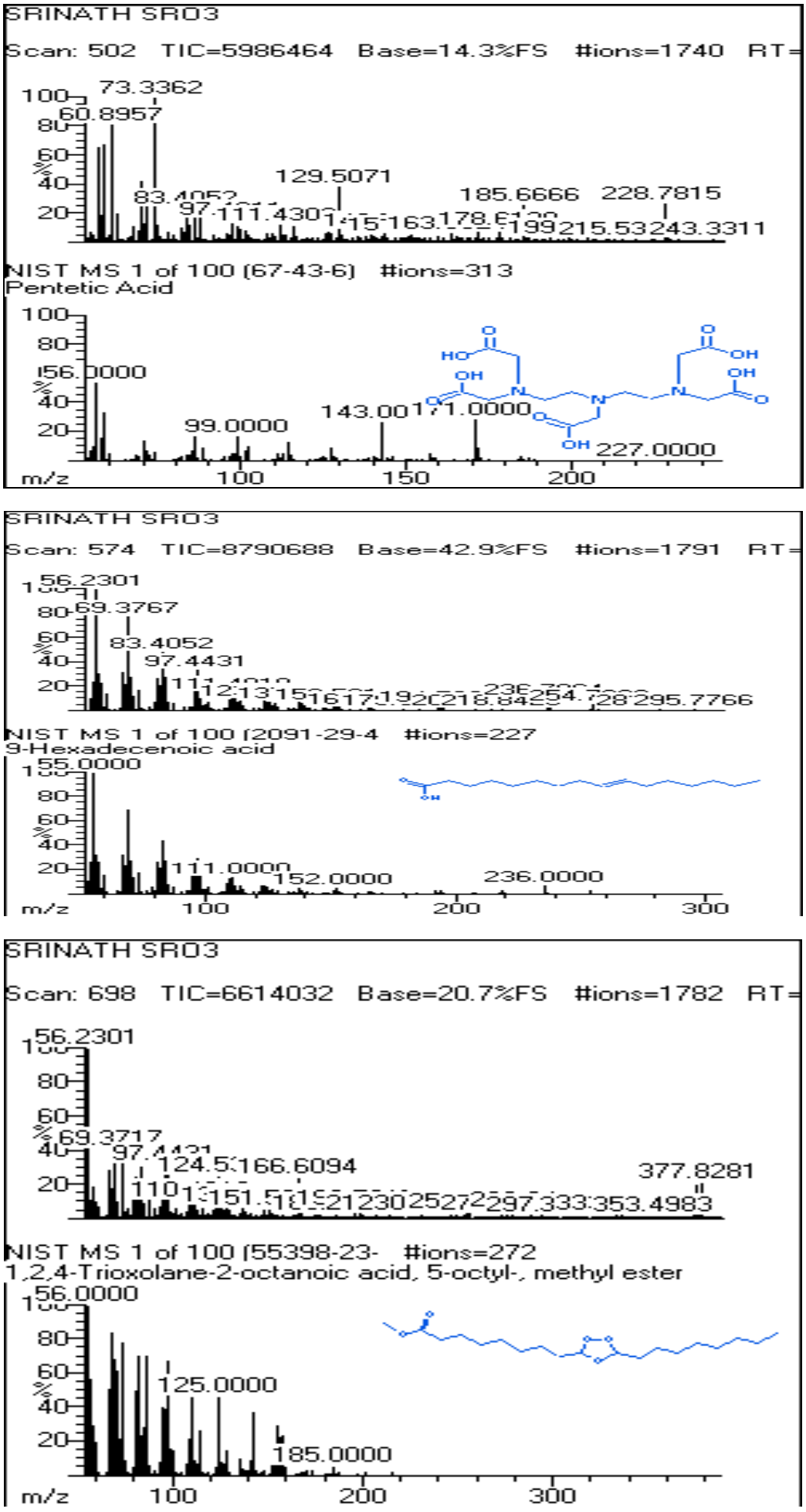

Fig 2: Mass spectrometric analysis of seaweed extract of *Sargassum polycystum*

Plant products have been used traditionally by the human communities in different parts of the world against vectors and species of insects. Marine algae are found to be a vital source of useful bioactive substance since two decades. The studies on mosquito larvicidal activities with seaweed extracts are too restricted. The seaweed extract of *S. polycystum* showed significant larvicidal, repellent and adulticidal activities. The results were comparable with early studies of Syed Ali *et al*, reported the seaweed extract, *C. racemosa* showed toxicity against 4th instar larvae of *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles stephensi* with equivalent (LC_{50}=0.0556±0.0103 µg/mL, 0.0675±0.1360 µg/mL and 0.0661 ±0.0076 µg/mL) respectively [12]. Previous reports reveal environmentally safe and relatively inexpensive methods for controlling the mosquitoes are botanical extracts have been tried as larvicides and adult repellents. Baranitharan and Dhanasekaran examined the highest larvicidal activity was observed in diethyl ether extract of *C. aromaticus* against *Ae. aegypti* with the LC_{50} and LC_{90} values being 73.49 and 80.16 ppm [7]. Early on inspecting of Rahuman *et al*, stated the larval mortality was found in stem-bark hot water, acetone, and methanol extracts of *C. deodara* (LC_{50}=133.85, 141.60, and 95.19 ppm, LC_{90}=583.14, 624.19, and 639.99 ppm) and leaf hot water, acetone, methanol, and chloroform extracts of *N. tabacum* (LC_{50}=76.27, 163.81, 83.38, and 105.85 ppm, LC_{90}=334.72, 627.38, 709.51, and 524.39 ppm) against the larvae of *C. quinquefasciatus*, respectively [8]. Most of the study was phytochemical based on herbs and other medicinal plants. This is because the historical experiential knowledge and some scientific studies have shown them to be particularly active against certain organisms. Syed Ali *et al*, [10] reported that column chromatographic fractions of *R. mucronata* bark extracts (E1) showed maximum larvicidal activity (LC_{50} = 0.0496 ± 0.0085 µg/ml and LC_{90} = 0.1264 ± 0.052 µg/ml), acetone extract (LC_{50} = 0.0564 ± 0.0069 µg/ml and LC_{90} = 0.1187 ± 0.05 µg/ml), ethanolic fraction (E4) of *R. mucronata* stilt root extracts showed maximum larvicidal activity (LC_{50} = 0.0484 ± 0.0078 µg/ml and LC_{90} = 0.1191 ± 0.025 µg/ml) and acetone fraction (A3) (LC_{50} = 0.0419 ± 0.0059 µg/ml and LC_{90} = 0.0955 ±0.069 µg/ml). Kovendan *et al* [11] evaluated LC_{50} values of hexane, chloroform, ethyl acetate, acetone and methanol extract of *O. thymiflorus* third instar larvae of *An. stephensi* were LC_{50}= 201.39, 178.76, 158.06, 139.22 and 118.74 ppm; *Cx. quinquefasciatus* were LC_{50}=228.13, 209.72, 183.35, 163.55 and 149.96 ppm and *Ae. aegypti* were LC_{50}=215.65, 197.91, 17505, 154.80 and 137.26 ppm respectively. Several authors have investigated that ethanolic extract show mosquito larvicidal activity [10, 12]. Similarly, the present study was made an attempt and LC_{50} values (52.2±2.3) and (53.2±2.3) µL/ml against *Aedes aegypti* and *Culex quinquefasciatus* were observed at maximum activity of minimum concentration at 200µL.

4. Discussion

Today, worldwide consumption of synthetic repellents has increased to prevent losses in store foodstuff materials. Unlike the larvicides, mosquito repellents only reduce the bites and cannot be considered as a control measure. Utpal Adhikari investigated Chloroform: methanol of mature leaf extract of *S. mahagoni* exhibits 100% repellency up to 2 h 15 min as no mosquito bites up to that time periods in the treated hands [28]. Syed Ali *et al*, [10] reported repellency of *R. mucronata* done in stilt root and bark extracts (A3) showed maximum protection (97.5%) with 9.1 h protection time at 4 mg concentration and ethanolic fraction of the stilt root (E4) extract showed maximum (100%) with 10 h protection time at 4 mg concentration. Plant based insecticides are non toxic, easily available and show target specific activities. Kamaraj *et al*, [29] observed maximum repellent activity was observed at 500 ppm in methanol extracts of *N. nucifera*, ethyl acetate and methanol extract of *P. nigrum* and methanol extract of *T. ammi* protection time from 30 to 150 min. Chemical repellents are not secure for public consume due to their apparent toxicity. Many researchers proved phytochemical constituents such as n-Hexadecanoic acid, Furfural, Glucobrassicin acts as insecticidal agents. Marimuthu Govindarajan *et al* evaluated the methanol extract of *E. alba* and *A. paniculata* was produced maximum repellency against *An. Stephensi* [30]. Skin repellent test by Pushpanathan *et al*, at 1.0, 2.5 and 5.0 mg/cm^2 concentration of *C. citratus* gave 100% protection up to 3.00, 4.00 and 5.00 hours respectively. The total percentage of protection of this essential oil was 49.64% at 1.0 mg/cm^2, 62.19% at 2.5 mg/cm^2 and 74.03% at 5.0 mg/cm^2 for 12 hours [31]. Likewise, the repellency percentage of 93% and 95.1% against *Aedes aegypti* and *Culex quinquefasciatus* at 300 µL of concentration extract of *S.polycystum*.

The most widely used synthetic compounds, DEET is most effective and in various commercial formulations such as solutions, lotions, gels, creams and aerosols. However, recent publications suggest that DEET poses some concerns in reproductive and developmental toxicity. The plant produces a great array of secondary metabolites as a result of metabolic activities. These compounds either alone or in combination are responsible for the specific therapeutic action administered as a medicament or a health supplement. Kovendan *et al*, [6] reported the adult mortality was found in ethanol extract of *C. sinensis* with the LC_{50} and LC_{90} values of 272.19 and 457.14 ppm, *A. stephensi*; 289.62 and 494.88 ppm, *Ae. aegypti*; and 320.38 and 524.57 ppm, respectively. Marimuthu Govindarajan *et al*, observed the LC_{50} and LC_{90} values of *E. alba* and *A. paniculata* against adults of *An. stephensi* were 150.36, 130.19 ppm and 285.22, 244.16 ppm, respectively [30]. Dua *et al*, investigated LD_{50} values of the oil were 0.06, 0.05, 0.05, 0.05 and 0.06 mg/cm^2 while LD_{90} values were 0.10, 0.10, 0.09, 0.09 and 0.10 mg/cm^2 against *Ae. aegypti*, *Cx. quinquefasciatus*, *An. culicifacies*, *An. fluvialitis* and *An. stephensi* [32] respectively. Maharaj *et al*, [33] stated the highest activity was observed in *Ptaeroxylon obliquum* and *Pittosporum viridiflorum*, exhibited more than 50% mortality with (81.63%) of the extracts tested. Bekele *et al*, [34] examined the maximum adult mortality was detected in the leaf extract of *Oreosyce africana* (LC_{50}=18.74 and LC_{90}= 39.66 ppm) followed by fruit extract of *Piper capense* (LC_{50}=24.30 and LC_{90}=46.32 ppm). Comparatively, the adulticidal activity was observed in 200µL concentration against *Ae. aegypti* and *Cx. quinquefasciatus* with LC_{50} were (50.2±0.7) µL/ml and (51.2±0.9) µL/ml. Many novel control agents from botanicals previously have proven to be a significant potential mosquito killer as they are relatively safer, cost effective, less toxic and easily degradable.

The present study have shown larvicidal, repellent, and adulticidal activity might be due to the presence of phytochemicals constituents such as Hexadecanoic acid, Glucobrassicin, Ethanol, 2-(9-octadecenyloxy)-(Z), Tridecanoic acid, 1,2,4-Trioxolane-2-octanoic acid 5-octyl-methyl ester, Ethanone, 1,1´-(1,4-dihydro-2,4,6-trimethyl-3,5-pyridinediyl)bis-, Pentetic Acid and 9-Hexadecenoic acid which may cause inhibition of poly (ADP-ribose) polymerase

enzyme which is involved in the DNA repair in adult mosquito, alterations in the siphon [36] and toxicity of prothoracic glands in instar larvae [37]. Hexadecanoic acid, Tridecanoic acid and Glucobrassicin are natural insecticides and nematicides. Similarly *Sargassum polycystum* showed a lethal effect in mosquitocidal activity on *Ae. aegypti* and *Cx. quinquefasciatus*. Earlier reports [33] showed the essential oil compounds Eucalyptol, Caryophyllene, Germacrene-D and α-humelene showed significant adulticidal activity where as Cyclopentane, Hydrazinecarboxamide, Benzamide, Pentadecanoic acid, Cyclopentanone, Hexanedioic acid, 2-Hydroxy-1-(Hydroxymethyl) ethyl ester and mono (2-ethylhexyl) ester showed larvicidal and repellent activity [10]. Comparing earlier authors evaluation our results revealed that the experimental *S. polycystum* extract was effective to mosquito vectors.

5. Conclusion

From the present study it has been concluded that *Sargassum polycystum* has many unique phytochemical constituents and also proved to be a larvicidal, repellent and adulticidal activity against *Ae. aegypti* and *Cx. quinquefasciatus.* Further, studies on identification of active compounds, toxicity and field trials are needed to recommend the active fraction of these plant extracts for development of eco-friendly chemicals of insect vectors.

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