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Larvicidal property of the extracts of the seaweeds; Sargassum wightii, S. ilicifolium and Gelidiella acerosa vis-a-vis Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus

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Abstract

Toxicity potential of the 3-solvent extracts of *Sargassum wightii*, *S. ilicifolium* and *Gelidiella acerosa* – seaweeds against the 4th larval-stage of 3 mosquitos was evaluated. The ethyl acetate extracts of the brown seaweeds (*S. wightii* & *S. ilicifolium*) and the red seaweed (*G. acerosa*) exerted the highest larvicidal effect. The *S. wightii*-ethyl acetate extract (SW-EA) exerted the huge death-rates of *Anopheles stephensi* with the lethal concentrations of (µg/mL);3.98 - LC50 and 12.17- LC90 followed by 4.43 & 24.44 on *Culex quinquefasciatus* and 9.26 & 52.00 on *Aedes aegypti*, respectively. The *S. ilicifolium* - ethyl acetate (SI-EA) caused the deaths of *Anopheles stephensi* with the lethal (LC-50&LC-90) doses (µg/mL) of; 18.934 & 371.753, followed by 34.104 & 423.012 on *Aedes aegypti*, and 40.728 & 683.813 on *Culex quinquefasciatus* respectively. Corresponding values for the *G. acerosa*-ethyl acetate-extract (GA-EA) (that caused maximum death-toll of *Anopheles stephensi* were); 4.59& 15.91, followed by 14.18& 78.77 on *Culex quinquefasciatus* and 23.261& 354.903 for *Aedes aegypti*. The algal-analyses showed the common phytochemical - constituents, aliphatic amines, alkynes and alkenes - groups and predominantly the Phytol acetate, OCTADECANOIC ACID and n-Hexadecanoic acid. The generated data form important basis for further investigation towards antimalarial drug development.

Introduction

Malaria is a dreaded insects transmitting disease which is largely occurring in the tropical countries of the world. Malarial - disease is transmitted through the female *Anopheles* mosquitos. And this *Anopheles*- mosquito has become formed to be the main carrier of the disease-causing parasite, *Plasmodium falciparum* and the progressive severe infection of this parasite would lead to severe illnesses in the human beings, that finally leads to death (Paton et al. 2019). The African countries were found to record unusually more malarial- infections with the 94% of death-toll of such infected persons in 2019 itself (WHO,2019). Likewise, the WHO (2020) recently reported the global occurrence of 229 million malarial-cases with the 409,000 related deaths in the year, 2020 itself. Out of the 400 different existing species of *Anopheles* mosquitos, around 30 species are considered to be the potent malaria-causing vectors (WHO, 2020). The intensity of transmission of disease depends on the factors like the nature of the parasite, the vector, the human host, and the environment at large.

Dengue fever is also a mosquito transmitting viral infection in humans which has become mainly recorded in the warmer countries of the globe. Dengue-virus is being primarily carried through the *Aedes aegypti*-mosquito. During the last few decades, some 390 million cases of dengue infections were recorded globally and India alone recorded 34% (33 million infections) of dengue- infections in 2013 (Bhatt et al.2013; Chakravarti et al., 2012; Kakkar et al., 2012). Such insect-transmitted diseases are reported to be spreading alarmingly recently. And the crustacean-mosquitos are the main causative agent. In view of failure of eradication of disease- spreading insects through the presently employed chemical-based insecticides, more efficient and ecofriendly pesticides have to be developed. In this backdrop, innovative and eco-friendly, plant-based pesticide formulations have been attempted through the combination of many compounds (Beckeret al., 2020). Lakshmi et al., (2021) have investigated the anti-mosquito property of the extracts of the *Croton*- plant individually as well as in combination with the pyrethroids, Lambda and Cypermethrin, and they found their good lethal-potentiality via synergic mechanism.

The abundantly occurring seaweeds along the coastal environments are considered to be the valuable sources of biologically active compounds, like polysaccharides, antioxidants, minerals, fatty acids, vitamins, and amino acids, that could be potentially used as an antitumor, anticancer, antithrombin and functional ingredients. Incorporation of seaweeds in food could solve the various health problems viz; protein, mineral, and carbohydrate deficiencies (Kumar et al., 2021). Phaeophycean-algae are reportedly possess an excellent antibiotic value vis-à-vis cancers, microbes and larvae (Kim et al., 2013; 2018). Moreover, they are also useful to treat the skin complications and to eradicate the problematic larvae of coastal environs besides mosquitos. Therefore, there is a scope for the use of such brown algae in pharmaceutical and eco-friendly larvicidal formulations (Negara et al., 2021).

Recently, Kilic-team (2021) has found the good anti-enzymatic activity and anti-mosquito-effects by the ethanolic extracts (200 mg/mL) of about fifty macro-algae from three seas. The large-scale applications of chemically-formulated pesticides were found to be ineffective against the unwanted insects but are detrimental to useful organisms and to the ecosystems at large. In view of the commonly available largely untapped wild vegetations including the marine-macro-algae and their possession of various metabolites (possessing antibiotic property), there is an immense scope for the use of algal - components for formulating environmentally benign insecticides. And thus, with the availability modern scientific tools including spectroscopic analytical methods, scientists are evincing keen interest on plant-based pesticide formulations (Ghosh et al., 2012).

The current investigation focuses on the anti-mosquito efficacy of the macro-algae that are commonly occurring along the Mandapam coast, Southeast India.

Materials And Methods

Algal samples

The seaweeds sampled from the Mandapam coast, Gulf of Mannar region, India (9°16'15.5"N, 79°07'48.5"E) were identified as; *Sargassum wightii* Greville(Acc. No. MH178136*), Sargassum ilicifolium* (Turner) C.Agardh(Acc. No. MH178137)and *Gelidiella acerosa* (Forsskal) Feldmanna & Hamel (Acc. No. MH178138),with the help of theBotanical Survey of India-Southern Regional Centre (Government of India, Coimbatore). The algal-surface contaminants were removed through tap-water-washing. Fine powder was obtained from the shade-dried weeds through an electrically operated blender, that was subsequently used for the extraction of metabolites.

Preparation of Seaweed - extracts

100 g of powdered seaweed sample taken in a Whatman No.1 paper- device was fixed to a Soxhlet apparatus-chamber of extraction, which was in turn joined to the solvent filled bowl (flask) and then it was provided with heat-supply constantly. The crude-extract was obtained after running the Soxhlet apparatus for about 8 hours. Paste-extract was obtained from the condensation of the algal-extracts, by employing a rotary evaporator. The air-dried and concentrated powder obtained from the paste-extracts of *S. wightii* hexane (SW-H), *S. wightii* ethyl acetate (SW-EA), *S. wightii* methanol (SW-M), *S. ilicifolium* hexane (SI-H), *S. ilicifolium* ethyl acetate (SI-EA), *S. ilicifolium* methanol (SI-M) and *G. acerosa* hexane (GA-H), *G. acerosa* ethyl acetate (GA-EA), *G. acerosa* methanol (GA-M) - extracts were kept ready for experimental purpose.

Eggs-samplings and larval raising

The instars of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* were obtained from the Institute of *Vector Control Research Centre (VCRC), ICMR, Madurai, Tamil Nadu*, India. Such eggs were transported immediately to the Bharathidasan University-lab and transferred to the 18 × 13 × 4-cm trays filled with adequate water. The raised young-ones were continuously fed with the dog biscuits + yeast until their attainment of 4^{th} instars (Kamaraj et al., 2009).

Test Organisms and Larvicidal toxicity Assay

The 4^{th}-stage mosquito-larvae were employed in the present investigation (Kamaraj et al. 2009). The *in vitro* toxicity - bioassays were performed under the optimum conditions of relative humidity (70–80%), temperature (28 ± 2 °C) and photoperiod (12:12 L/D). Sets of 25 larvae were introduced (individually) into the tap water filled 250ml experimental chambers, that contained various concentration of algal extracts (25 to 100 µg/mL) of SW-H, SW-EA, SW-M, SI-H, SI-EA, SI-M and GA-H, GA-EA, GA-M - extracts. Triplicate experiment was performed and the negative control was maintained. From the recorded values / data after 24 hours of treatment, the mortality rate was worked out through the standard formula of Abbot (1925),

i.e., Mortality (%) =X-Y/X*100;

where, X: control and Y: treated sample.

Toxicity bioassay on non-target organism

Hatching of brine shrimp

Brine shrimp eggs procured from the Marine Planktonology & Aquaculture Laboratory, Dept. of Marine Science, Bharathidasan University, Trichitrappalli-24, India., were hatched in a shallow rectangular container (60 x 30 cm) filled one fourth with artificial sea water (prepared with natural seawater and diluted with distilled water). Salinity of 30ppm was maintained. After 48 h the phototropic nauplii were collected from the lighted side.

Artemia-bioassay

The cysts were allowed to hatch in the marine water at laboratory condition. 20 healthy nauplii were transferred to the pre-labelled 24 well plate filled with 3 mL of seawater in each well and the test samples (3.125–100 lg/mL) were loaded in the respective wells (Kamaraj et al. 2018). The *A. salina* mortality rate was observed after 24 h. Three replicates were maintained along with negative control (seawater). The mortality rate was calculated using standard formula:

Mortality (%) =X-Y/X*100;

X: Survival in the untreated control and Y: Survival in the treated sample.

Histopathological investigation

For the histopathological analysis of mosquito larvae, 24 h post treated (and control) *A. stephensi, A. aegypti* and *C. quinquefasciatus* larvae were fixed with diluted formaldehyde for 2 h at 5^{o} C. The wax blocks were cooled at 25^{o}C up to 5 h and these waxes were cut into 8 µm thin 1.1mm ribbons with a microtome. And these larval gut sections were stained with haematoxylin and eosin stains (Vivekanandhan et al., 2018). The slides were air dried and then the slides - sections were viewed/photographed under a LED-light microscope (Labomed - LX-300_LED) at a magnification of 400X.

Phytochemical analysis

For the preliminary qualitative screening of phytochemicals in various solvent-extractions of three seaweeds, the standard protocol of Deyab et. al., (2016) was adopted.

FTIR analysis

The Fourier-transform infrared (FTIR) – analysis was performed to identify and characterize the various frequencies of different types of functional groups that are present in the seaweed extracts, by using the FTIR spectrometer (Bruker Tensor 37, Germany) at ATR mode.

Gas Chromatographic-Mass Spectrometric (GC-MS)-Analysis for Active compounds' purification & characterization

The seaweed-extracts GC-MS Shimadzu, Japan. analysis (software consolation) fractions were investigated through Mass spectrometry analysis of the three different solvent seaweeds-extracts (fractions) were characterized by using gas chromatograph mass spectrometer by employing DB-5 columns of size of 30 m × 0.25 mm & 0.25 µm thickness film. 1µl of extract was injected through an autosampler and carrier gas was used Helium (99.999 % purity) at a 1.65/ml/min column flow rate was followed. The standard temperatures of 280 (injector post), 280 (interface) and 230 (ion source) were maintained. The MS involved in electron ionization detector of (EI)-mode at 70cV6 with a current – emission of 60 mA. Complete data-scan mode were found in a range of Mass ratio of m/z 50-500. The obtained Mass-spectrum was interpreted by comparing them with library-data base and the spectrum of the unknown components were related with the spectra of known compounds (available in the library) (Selvaraj et al. 2015).

Statistical treatment of data

The generated data from the toxicity assay were used for Probit analysis (Finney, 1971) and various lethal concentrations were obtained. The related other values like 95 % confidence, F-values were obtained through the SPSS version 16.0 statistical software (2007).

Results

Lethal effects of algal extracts on mosquito larvae and histopathological study

The recorded lethal toxic values of various solvents – extracts of algae (*S. wightii* on the larvae of *A. stephensi*, *A. aegypti* and *C. quinquefasciatus*) are furnished in **Table** 1. The toxicity values of Hexane, ethyl acetate and methanol crude extracts of *S. ilicifolium* & *Gelidiella acerosa* against the *A. stephensi*, *A. aegypti* and *C. quinquefasciatus* larvae including the obtained lethal concentration (LC_{50} and LC_{90}) are presented in **Table 2**,**3**.

Table 1 Mosquito-larvicidal activities of *S. wightii* extract of *A. aegypti, C. quinquefasciatus* and *A. stephensi.*

Mosquito vector	Sample	LC_{50} µg/ml (LCL-UCL)	LC_{90} µg/ml (LCL-UCL)	χ^2
A. aegypti	SWH	12.42 (9.33±20.53)	58.74 (32.72±162.65)	1.172
	SWEA	9.26 (7.14±13.50)	52.00 (29.86±128.96)	3.110
	SWM	11.57 (8.04±19.98)	72.11 (49.04±398.42)	2.641
C. quinquefasciatus	SWH	6.38 (5.34±7.97)	42.94 (17.49±42.30)	1.332
	SWEA	4.43 (3.64±5.57)	24.44 (16.32±44.55)	4.373
	SWM	7.62 (6.20±10.03)	33.41 (21.80±64.97)	1.602
A. stephensi	**SWH**	**5.86 (1.41±13.36)**	**18.42 (11.52±39.15)**	**3.724**
	SWEA	**3.98 (2.14±10.66)**	**12.17 (4.81±29.69)**	**2.977**
	SWM	**4.28 (3.87±19.76)**	**13.24 (9.41±21.74)**	**2.691**

*SD standard deviation, LCL lower confidence limit, UCL upper confidence limit, χ^2 Chi square test, $p<0.05$, level of significance, values are mean ± SD of three replicates.

Table 2 Mosquito-larvicidal activities of *S. ilicifolium* extract of *A. aegypti, C. quinquefasciatus* and *A. stephensi.*

Mosquito vector	Sample	LC_{50} µg/ml (LCL-UCL)	LC_{90} µg/ml (LCL-UCL)	χ^2
A. aegypti	SIH	37.739 (29.396±53.161)	415.271 (371.282±772.821)	1.706
	SIEA	34.104 (12.853±58.838)	423.012 (169.842±521.523)	2.901
	SIM	34.733 (17.165±38.365)	462.729 (262.821±582.357)	2.201
C. quinquefasciatus	SIH	40.739 (29.649±89.820)	448.483 (342.218±619.342)	1.793
	SIEA	42.728 (36.052±98.452)	683.813 (520.327±720.318)	3.158
	SIM	44.305 (27.300±76.366)	631.380 (202.655±711.312)	2.606
A. stephensi	**SIH**	**26.069 (16.069±41.359)**	**451.285 (185.301±502.552)**	**1.111**
	SIEA	**18.934 (14.729±34.277)**	**371.753 (151.786±421.152)**	**2.935**
	SIM	**22.609 (15.666±25.472)**	**361.191 (133.226±421.152)**	**1.308**

*SD standard deviation, LCL lower confidence limit, UCL upper confidence limit, χ^2 Chi square test, $p<0.05$, level of significance, values are mean ± SD of three replicates.

Table 3 Mosquito-larvicidal activities of *G. acerosa* extract of *A. aegypti, C. quinquefasciatus* and *A. stephensi.*

Mosquito vector	Sample	LC_{50} µg/ml (LCL-UCL)	LC_{90} µg/ml (LCL-UCL)	χ^2
A. aegypti	GAH	27.866 (15.466-40.153)	309.943 (193.011-632.122)	2.803
	GAEA	23.261 (10.458-32.284)	354.903 (168.259-601.141)	1.768
	GAM	39.322 (6.112-25.947)	526.431 (168.108-529.221)	3.861
C. quinquefasciatus	GAH	17.82 (3.92-28.20)	109.23 (78.11-207.59)	1.022
	GAEA	14.18 (4.58-29.28)	78.77 (56.85-98.97)	3.183
	GAM	16.18 (4.14-32.88)	87.07 (61.74-102.83)	2.576
A. stephensi	**GAH**	**5.19 (2.71-10.9)**	**16.98 (10.33-42.97)**	**1.231**
	GAEA	**4.59 (2.19-5.056)**	**15.91 (8.31-28.87)**	**3.183**
	GAM	**4.81 (3.85-12.45)**	**15.88 (12.43-34.83)**	**2.252**

*SD standard deviation, LCL lower confidence limit, UCL upper confidence limit, χ^2 Chi square test, $p<0.05$, level of significance, values are mean ± SD of three replicates.

Presently the ethyl acetate extracts have caused high larval- death – toll in all the mosquito species. The *S. wightii* ethyl acetate (SW-EA) exhibited most-increased rate of toxicity on *A. stephensi* at the concentration (µg/mL) of 3.98 as LC_{50} - dose & 12.17 as LC_{90}-value, followed by 4.43 & 24.44 in *C. quinquefasciatus* and 9.26 & 52.00 in *A. aegypti*, respectively. The *S. ilicifolium* ethyl acetate - extract (SI-EA) exhibited most-increased rate of toxicity on *A. stephensi* at the concentration (µg/mL) of 18.934 as LC_{50}- dose & 371.753 as LC_{90} value, followed by 34.104 & 423.012 in *A. aegypti*, and 40.728 & 683.813 in *C. quinquefasciatus* respectively. The *G. acerosa* ethyl acetate (GA-EA) exhibited most-increased rate of toxicity on *A. stephensi* at the concentration (µg/mL) of 4.59 as LC_{50} - dose & 15.91 as LC_{90}-value, followed by 23.261 & 354.90 in *A. aegypti* and 14.18 & 78.77 in *C. quinquefasciatus* respectively. Among the different solvent extracts of *S. wightii, S. ilicifolium* and *G. acerosa*, the ethyl acetate - extract caused high toll **(Figs.** 1,2,3**).** The control did not show any mortality and the χ^2 value was noteworthy at $p < 0.05$ level.

The histopathology of SW-EA, SI-EA and GA-EA extracts treated larvae revealed disordered and broken epithelial cell layers with severely affected midgut, gut lumen, and muscles. And the control group mosquito larvae did not show any deformities. The malformations were observed in the bodies of the mosquito larvae treated by crude extracts. **(Fig.** 4**).** In addition to the swelling, the deformation /elongation of the epithelial cells were also found.

Toxicity on Non-target Brine Shrimp (A. salina)

The toxicity of SW-EA, GA-EA and SI-EA were assessed by using *A. salina*. at 24 h exposure, and the results are shown in **Table** 4**; Fig.** 5**.** The SW-EA, SI-EA and GA-EA did not exert any cytotoxicity in the *nauplii* stage of the *A. salina.* First the GA-EA exhibited minimum death-toll at the concentration (µg/mL) of LC_{50} and LC_{90} values as; 127.805 & 312.163; next to that SW-EA exhibited death-toll at the concentration (µg/mL) of LC_{50} and LC_{90} values as; 117.009 & 610.296, and SI-EA exhibited death rate at the concentration (µg/mL) of LC_{50} and LC_{90} values as; 114.512 & 305.366 respectively. The ethyl acetate extracts of GA-EA, SW-EA and SI-EA have exhibited 19.04%, 20.02% and 25.02 % mortality at 24 h. And also, the experiment revealed that the control and treated groups did not induce any lethal effect on *A. nauplii* and adult at 24 h exposure. These results have indicated that the percentage of mortality rates were significantly increased with increasing seaweeds – extracts - concentrations and exposure time.

Table 4 Non-toxicity bioassay of seaweed extract on *Artemia salina* (Brine Shrimp).

Mosquito vector	Sample	LC_{50} µg/ml (LCL-UCL)	LC_{90} µg/ml (LCL-UCL)	χ^2
A. salina	SW	117.009 (47.43-175.23)	610.296 (220.43-632.12)	1.703
	GA	127.805 (65.43-145.23)	312.163 (134.23-429.23)	2.268
	SI	114.512 (52.33-25.142.23)	305.366 (168.108-529.221)	1.361

Phytochemical-Profile

The recorded phyto-chemical constituents of various solvent - extracts of seaweeds are listed in **Table** 5**.** Invariably, the ethyl acetate extracts were found to possess high number of 18 compounds. Next, the methanol & hexane extracts revealed the presence of 17 compounds in each extract.

Table 5 Phytochemical profile of *S. wightii, S. ilicifolium* and *G. acerosa* - ethyl acetate crude - extracts.

FTIR findings

Through FTIR analysis of the seaweed – extracts many functional groups were determined. The FTIR spectrum of *S. wightii* showed seven peaks at the range of 3425.01 cm^{-1}, 2926.00 cm^{-1}, 2854.51 cm^{-1}, 1716.58 cm^{-1}, 1378.66 cm^{-1}, 1377.81 cm^{-1}, 1172.29 cm^{-1}, 1053.27 $cm^{-1}$and the obtained major peak at 3425.01 cm^{-1} could be related to the OH stretching vibration of alcohols and phenols. The spectrum of *S. ilicifolium* showed seven peaks at the range of 3395.58 cm-1, 2926.24 cm^{-1}, 2855.05 cm^{-1}, 1713.11 cm^{-1}, 1465.20 cm^{-1}, 1377.81 cm^{-1}, 1241.21 $cm^{-1}$and the major peak at 3395.58 cm^{-1}has to be related to the OH vibration-stretching of alcohols and phenols. And *G. acerosa* showed seven major peaks at the range of 2924.22 cm^{-1}, 2854.04 cm^{-1}, 1710.71 cm^{-1}, 1465.70 cm^{-1}, 1376.12 cm^{-1}, 1237.18 cm^{-1}, 1047.02 cm^{-1}, 721.87 cm^{-1}respectively and the major peak at 2924.22 cm^{-1} could be related to the C-H stretching vibration of alkenes **(Table** 6 **& Fig.** 6**)**.

Table 6 Structural features of the ethyl acetate extracts of *S. wightii, S. ilicifolium* and *G. acerosa* by FTIR spectra.

Samples		Alkaloids	Terpenoids	Steroids	Tannins	Saponins	Flavonoids	Phenols	Coumarins	Quinones	Glycosides
SW	Hexane	+	+	+	+	–	–	+	–	–	+
	Ethyl acetate	+	+	+	+	–	–	+	–	+	+
	Methanol	+	+	+	–	–	–	–	–	+	–
SI	Hexane	+	+	+	–	–	–	–	–	–	+
	Ethyl acetate	+	+	+	–	–	–	–	–	+	–
	Methanol	+	+	+	–	–	+	–	+	+	–
GA	Hexane	+	+	+	–	–	+	+	–	+	+
	Ethyl acetate	+	+	+	–	–	+	+	–	+	+
	Methanol	+	+	+	–	+	+	–	+	+	–

S.No	Frequency	Bond	Functional Group
SW	3425.01 cm^{-1}	OH – stretch H- Bonded	Alcohols, Phenols
	2926.00 cm^{-1}	C-H stretch	Aromatics
	2854.51 cm^{-1}	C-H stretch	Alkenes
	1716.58 cm^{-1}	C=O stretch	Esters
	1378.66 cm^{-1}	C-H stretch	Alkenes
	1172.29 cm^{-1}	C-H wag ($-CH_2X$)	Alkyl halides
	1053.27 cm^{-1}	C-N stretch	Aliphatic Amins
SI	3395.58 cm^{-1}	OH – stretch H- bonded	Alcohols, Phenols
	2926.24 cm^{-1}	C-H stretch	Alkenes
	2855.05 cm^{-1}	C-H stretch	Alkenes
	1713.11 cm^{-1}	C=O stretch	Aliphatic, Esters
	1465.20 cm^{-1}	C-H bend	Alkenes
	1377.81 cm^{-1}	C-H rock	Alkenes
	1241.21 cm^{-1}	C-N stretch	Aliphatic Amins
GA	2924.22 cm^{-1}	C-H stretch	Alkenes
	2854.04 cm^{-1}	C-H stretch	Alkenes
	1710.71 cm^{-1}	C=O stretch	Aldehydes, Ketones
	1465.70 cm^{-1}	C-H bend	Alkenes
	1376.12 cm^{-1}	C-H stretch	Alkenes
	1237.18 cm^{-1}	C-N Stretch	Aliphatic Amins
	1047.02 cm^{-1}	C-N stretch	Aliphatic Amins
	721.87 cm^{-1}	C-H rock	Alkanes

Compounds present

The GC-MS running time for n-hexane: ethyl acetate (50:50 v/v) extract(s) of *S. wightii grev, S. ilicifolium* and *G. acerosa* was 30 min and the obtained spectra are provided in **Fig.7**. The identified compounds (of seaweeds), their mass ions & retentions times are shown in **Table** 7. The results revealed that, *S. wightii* extracts contained 15 bioactive compounds viz; CYCLOPROPANE, NONYL-, Decane, Neodene, 1-HEXADECANOL, Phytol acetate, 1-Octadecyne, n-Hexadecanoic acid, Capraldehyde, Caprylaldehyde, Nonyl acetate, Neodene, Eicosane, Caproleic acid, Diphenylcyclopropane, thiadiazole; The *S. ilicifolium* possessed 11 compounds viz; Docosane-1,2-diol, isopropylidene deriv, Capric acid, 1-tridecene, 3-Tetradecene, (Z)-, Hendecane, 5-octadecene, (E)-, Phytol acetate, octadecanoic acid, Cinnamyl alcohol <alpha-amyl->, cis-2-Methyl-7-octadecene, Ethyl homovanillate, TMS derivative; *G. acerosa* contained 18 components viz; tetradecane, 5-Octadecene, (E)-, tetradecanoic acid, neophytadiene, 2-pentadecanone, 6,10,14-trimethyl-, cyclododecanol, 2-dodecenal, hexadecanoic acid, phytol, 9-octadecenoic acid, hexadecenoic acid, z-11-, octadecanoic acid, benzoic acid, 2,3,5-triiodo-, cholesta-4,6-dien-3-ol, androst-16-en-3-one <5-alpha->, cholest-5-en-3-ol, Cholestan-3-ol, Ergosta-5, 22-dien-3-ol.

Table 7 Identification of phytocompounds from ethyl acetate extracts of *S. wightii, S. ilicifolium* and *G. acerosa*

S.No (Peak #)	Compound name	R.Time	Area%	Base m/z
SW	CYCLOPROPANE, NONYL-	8.533	7.44	55.10
	Decane	8.687	3.60	57.10
	Neodene	12.365	7.89	55.05
	1-HEXADECANOL	15.996	6.21	55.05
	Phytol acetate	16.809	11.93	68.10
	1-Octadecyne	17.533	4.43	57.10
	n-Hexadecanoic acid	18.851	21.17	60.05
	Capraldehyde	18.942	3.40	57.10
	Caprylaldehyde	19.075	6.28	73.10
	Nonyl acetate	19.217	3.21	60.85
	Neodene	19.344	7.87	55.05
	Eicosane	19.440	4.23	57.10
	Caproleic acid	21.677	4.36	55.05
	Diphenylcyclopropane	30.423	3.91	95.45
	THIADIAZOLE	31.259	4.07	134.90
SI	Docosane-1,2-diol, isopropylidene deriv	5.075	7.57	50.05
	Capric acid	5.317	7.64	60.40
	1-TRIDECENE	8.537	10.92	55.05
	3-Tetradecene, (Z)-	12.361	12.66	55.10
	Hendecane	12.500	5.03	57.10
	5-OCTADECENE, (E)-	16.000	7.19	55.05
	Phytol acetate	16.811	7.73	68.10
	OCTADECANOIC ACID	18.932	17.98	57.10
	Cinnamyl alcohol <alpha-amyl->	19.183	8.19	71.20
	cis-2-Methyl-7-octadecene	19.351	9.26	57.15
	Ethyl homovanillate, TMS derivative	28.858	5.83	73.05
GA	TETRADECANE	8.691	0.84	57.10
	TETRADECANE	12.500	0.45	57.05
	5-Octadecene, (E)-	14.214	0.27	55.05
	TETRADECANE	14.352	4.86	57.05
	TETRADECANOIC ACID	15.517	2.04	60.00
	Neophytadiene	16.808	1.88	68.10
	2-Pentadecanone, 6,10,14-trimethyl-	16.906	2.23	58.05
	Cyclododecanol	17.531	0.65	81.05
	2-DODECENAL	18.188	0.65	57.10
	HEXADECANOIC ACID	19.162	44.76	60.05
	Phytol	21.250	3.24	71.05
	9-OCTADECENOIC ACID	21.688	3.22	55.05
	Hexadecenoic acid, Z-11-	21.742	1.50	55.05
	OCTADECANOIC ACID	22.023	1.92	55.05
	BENZOIC ACID, 2,3,5-TRIIODO-	29.371	3.61	341.90

CHOLESTA-4,6-DIEN-3-OL	31.347	1.40	366.20
Androst-16-en-3-one <5-alpha->	33.638	0.87	55.05
CHOLEST-5-EN-3-OL	34.100	23.70	55.05
Cholestan-3-ol	34.183	1.18	55.05
Ergosta-5,22-dien-3-ol	34.544	0.73	55.10

Discussion

The effective control of rapidly growing mosquito-population is a critical need of the day since they are responsible for the occurrence of diseases like dengue, filariasis and malaria. The complete eradication of diseases-transmitting insects is, therefore, an important goal of many countries. Hence, of late, the scientists have been focusing on the eco-friendly way of formulation of anti-mosquito agents by using the wild plant-metabolites (Garcez et al. 2009; Kanis et al. 2011). Earlier, Kovendan et al. (2012) have found the mosquito-larvi and pupicidal property of leafy-extracts of the plant, *Morinda citrifolia*, which is in line with the eco-friendly-approach towards insecticide formulation.

The current toxicity study has generated some interesting data on the lethality of various solvents-extracts of seaweeds, *S. wightii, S. ilicifolium* and *G. acerosa.* Among them, the SW-EA exerted the highest death-rate (of toxicity) on *A. stephensi*, at the concentration (µg/mL) of 3.98 as LC_{50} - dose and 12.17 as LC_{90}-value, followed by 4.43 & 24.44 in *C. quinquefasciatus* and 9.26 & 52.00 in *A. aegypti*, respectively. The GA-EA exhibited most death-toll on *A. stephensi* at the concentration (µg/mL) of 4.59 as LC_{50}- dose and 15.91 as LC_{90-} value, followed by 14.18 & 78.77 in *A. aegypti*, and 23.261 & 353.903 in *C. quinquefasciatus* respectively. The SI-EA exhibited most death-toll on *A. stephensi* at the concentration (µg/mL) of 18.934 as LC_{50}- dose & 371.753 as LC_{90-} value, followed by 34.104 & 423.012 in *A. aegypti*, and 40.728 & 683.813 in *C. quinquefasciatus* respectively. And these findings have revealed that the ethyl acetate-extracts of all the seaweeds might possesses some important metabolites that might have caused the highest larval mortality. Earlier, Sayono et al. (2020), who have evaluated the toxicity-effects of the components of the *Derris elliptica*-plant found potential toxic effect on the *A. Aegypti*-larvae.

Kumari et al. (2020) have demonstrated that the brown alga, *Turbinaria conoides*, collected from Mandapam coast (Southeast India) exhibited the effective mosquito larvicidal and bactericidal effect against the *C. quinquefasciatus* larvae and *S. aureus* bacterium respectively. They have also found that diethyl ether and acetone extracts of *Turbinaria conoides* were superior in mosquito-larvicidal effect (LC_{50} 104 mg/L, 469 mg/L) and antibacterial activity (4 mm ZoI at 0.1mg and 1.2mg extract), respectively. Such recorded toxicity was related to the presence of major algal - compounds viz; 1,2, dicarboxylic derivatives of benzene, Phthalic acid, Pentane 2,2,4-trimethyl, and titanium 1,3,5,7 cyclooctatetraene 2,4 cyclopentadien 1-Yl in the acetone extract of the *Turbinaria conoides*. The seaweeds have been found to possess many bioactive compounds. Trivedi et al. (2021) reported the industrial applications like anti-mosquito & antimicrobial agents by the AgNPs synthesized from the brown seaweed, *Sargassum muticum* of Red sea. Amutha et al. (2019) have documented the effective mosquitocidal property of the nanoparticles that they synthesized through the seagrass, *Cymodocea serrulate* species. However, they have found only least toxic effect on non-target forms like *Artemia* and *Oithona* (by those *Cymodocea serrulate*-AgNps). Likewise, African tulip tree - extract (*Spathodea campanulata*) was tested against the *Aedes aegypti*-instars and found the drastically changed shape of the young-ones (Saranya et al., 2013). Bagavan et al. (2011) have studied the larvicidal properties of some medicinal plants and have recorded highest larval mortality through the seed - ethyl acetate extracts of *Abrus precatorius* and leaf extracts of *Croton bonplandianum*. Saadawi et al. (2021) have assessed the effects of species of the genera; *Thymus, Mentha, Pelargonium, Syzygium* besides peels of *Citrus* and have found that the methanolic extracts exhibited lethality on *Culex* species. In another study, Prakash et al. (2021) have evaluated the toxicity of the solvent extracts of root of *Decalepis hamiltoniithe* on the mosquito-instars and found the dose-dependent death rate. And the histopathological study by Vijayakumar et al. (2015) on the treatment of the *lectranthus amboinicus* leaf extract mediated synthesis of zinc oxide NPs, against the *A. stephensi* and *C. quinquefasciatus* larvae revealed presence of damaged cells and tissues. Similarly, our present investigation revealed that the SW-EA, SI-EA and GA-EA extracts treated mosquito larvae viz; *A. stephensi, A. aegypti* and *C. quinquefasciatus* exhibited the deformations of the gut lumen, epithelial layers, mid gut tissues and muscles of all the mosquito larvae. Thus, the findings underlined the potentiality of the extracts of the seaweeds against the mosquito vectors.

Wangrawa et al. (2016) have assessed the impact of various solvent-extracts of the species of *Lantana, Hyptis* and *Ocimum* on the ovary-position and larval-lethality vis-à-vis *An. Gambiae* strains. And they reported a most effect of *Lantana's* extract by recording 20.19 ppm as LC_{50}-lethal dose and 49.29 ppm for LC_{90}-dose. Hassan et al. (2021) have found the good efficacy of the extracts of *Amphora* and *Scenedesmus* - algae on the *Culex*-mosquito, as they recorded lethal doses (µg/mL) of 513.63 LC-50-value & 855.66 as LC-90-value (in relation to the 173.16 value due to the impact of commercial cyhalothrin).

Many research findings on the phytochemicals of salt-water algae are already available and the present investigation on the selected macro-algae showed the presence of important secondary metabolites like alkaloids, coumarins, flavonoids, glycosides, quinones, phenols, saponins, steroids, tannins and terpenoids. An earlier investigation on the effect of diverse solvent-leaf-extracts of *Leucaena sp.* revealed some interesting antibiotic/biochemical characteristics like antimicrobial/antitumor values due to their secondary metabolites (Zayed et al. 2016). And recently, Mani et

al. (2021) have focused on the phytoconstituents of the tropical macro- algae representing the genera; *Chaetomorpha, Halimeda* and *Ulva* and found the flavonoids, phenols, saponins and terpenoids in them, with an excellent antibiotic property by the metabolites of *Halimeda* and *Ulva* - algae.

Artemia (brine shrimp) is a minuscule halophilic invertebrate belonging to class Crustacean, and it is highly valued for application in the area of toxicity detection such as ecology, physiology, ecotoxicology, aquaculture and genetics (Ntungwe et al. 2020). Aqueous and dichloromethane:methanol (7:3) extracts of twenty-seven species of seaweeds collected from the Gulf of Mexico and Caribbean coast of the Yucatan Peninsula (Mexico) were evaluated for non-toxicity assay in *Artemia salina.* And it was observed with LC_{50} ranging from 119 to ≥ 1000 µg/ml (Freile-Pelegrin et al. 2008). The brine shrimp toxicity bioassay was used to screen 211 methanol extracts from 128 species of Pacific Northwest plants and there are 139 extracts from 99 species exhibited non-toxic with the value of LC_{50} > 1000 µg/ml (Karchesy et al. 2016). Ethanol extracts of the red seaweed *Gracilaria fisheri* was subjected to non-toxicity assay of brine shrimp - first instar larvae showed no mortality with the concentrations lower than 1.0 mg ml^{-1} for 24 h. And increasing mortality was observed at increasingly higher concentrations of ethanol extracts and the resultant LC_{50} value was 4.29 ± 0.5 mg ml^{-1} (Kanjana et al. 2011). Similarly, in our assay the extracts; SW-EA, SI-EA and GA-EA did not exhibited cytotoxicity on the nauplii stage of the *A. salina.* The SW-EA exhibited death-toll at the concentration (µg/mL) of LC_{50} and LC_{90} values as; 117.009 & 610.296, GA-EA exhibited death-toll at the concentration (µg/mL) of LC_{50} and LC_{90} values as; 127.805 & 312.163 and SI-EA exhibited death rate at the concentration (µg/mL) of LC_{50} and LC_{90} values as; 114.512 & 305.366 respectively. And it is concluded that the ethyl acetate extract of *S. wightii, S. ilicifolium* and *G. acerosa* was not benign to *A. nauplii* at elevated level.

An infra-red spectrum revealed the molecular configuration vis-à-vis different functionality groups that were present in the SW-EA, SI-EA and GA-EA - extracts. In SW-EA, the peak at 3425.01 cm^{-1} corresponds to the OH stretching vibration of alcohols and phenols. In SI-EA, the peaks at 3395.58 cm-1 could be related to the vibration of OH stretching of alcohols and phenols and in GA-EA, the peak at 2924.22 cm^{-1} could be related to the vibration of C-H stretching of alkenes. Thus, the three seaweeds revealed the presence of aliphatic amines, alkynes and alkenes groups with polymeric association, and similar findings were earlier reported by Sowmiya et al. (2017) in, *Jania rubens*, *Turbinaria ornata* and *Ulva fasciata*.

The Mass-spectral results of the ethyl acetates' crude extracts of *S. wightii*, revealed 15 compounds with 11.93% of Phytol acetate and 21.07% of n-Hexadecanoic acid. And the larger-presence of this Hexadecanoic acid and Phytol acetate (in *S. wightii*) could be related to the presently recorded large-scale death of mosquito-larvae. Recently, Anjum Mobeen Syeda et al. (2020) have found the compounds like Phytol acetate in the methanolic extracts of *Moringa oleifera*. Harada et al. (2002) have also reported the presence of such common plant-based palmitic acid (. n-Hexadecanoic acid) in the leaves of *Kigelia pinnata*-plant, that has the capacity to prevent the proliferation of human cancer (through its selective inhibitory ability on the topoisomerase- I of DNA). And some earlier researchers have studied the *Kalanchoe pinnata* based N-hexadecanoic acid, an intermediate product during the biosynthetic pattern of sexual hormones by insects, that was found to possess various biocide features including anti-vectors value (Kumar et al. 2010; Bharathy et al. 2013; Zayed et al. 2014; Trabalon et al. 2005). Presently, fraction of ethyl acetate extract of *S. ilicifolium* had the 11 compounds; among them, 3-Tetradecene, (Z)- has the highest peak area percentage of 12.66% and next to that, the 5-OCTADECENE, (E)- has the highest peak area percentage of 7.19%. And these compounds may be responsible for the exerted high mortality by the *S. ilicifolium* - extracts. Earlier, Hossain et al. (2013) have reported that the *Datura metel* extracts has 3-Tetradecen, (Z) – compound and they underlined that crude-extract is potential as a natural healer of human-ailments. Pauldasan at al. (2020) reported, through their chromatographic process of the *Cyperus corymbosus*- rhizome extracts revealed the presence of twenty six chemical compounds, with the major ones being; n-Hexadecanoic acid and Cyclopropanepentanoic acid, 2-undecyl-, methyl ester (21.45%) followed by Oleic acid (13.02%), Z-8- Methyl-9- tetradecenoic (10.16%), 4,5-di-epi-aristolochene (7.388), Trans,-13-octadecenoic (4.877%), and the least chemical compounds were 5-octadecene, Caryophyllene oxide (2.363), alfa-copaene (0.411 and ledene oxide-(II) (0.007). And these secondary metabolites were found to possess various valuable antibiotic characteristics pertaining to microbes, parasites, insects, nematodes, anesthetic, arthritis and cancer. Out of the 20 compounds of *G. acerosa's* ethyl acetate-extracts, the predominant ones being the CHOLEST-5-EN-3-OL (23.70% peak area) and HEXADECANOIC ACID (46.76% peak area). The presence of these two compounds might have been largely responsible for the recorded *G. acerosa* based lethal effect. And these crude extracts had the 20 compounds; among them, CHOLEST-5-EN-3-OL has the highest peak area percentage of 23.70% and next to that HEXADECANOIC ACID has the highest peak area percentage of 44.76%. Hence, this compound might have been responsible for the recorded mortality of mosquito larvae. The compound Cholest-5-en-3-ol formate was first time isolated from the red alga *Grateloupia sp*., its used for the chemotaxonomic classification of algae (Erwan Plouguerne et al. 2006). The presence of such hexadecanoic acid was reported by Mahesh et al. (2016) and they have also found its anti-feedant property vis-à-vis

Spodoptera litura. And the compounds like, 14-methyl-pentadecanoic acid, 9- octadecanoic acid, among others, were reported in *Asparagopsis taxiformis* by Manilal et al. (2010) and they have underlined the synergistic bioactivity of such compounds. And presence of these bioactive compounds might play a vital role for antibacterial, antioxidant, ant inflammatory insecticidal, cytotoxic activities and potent mosquito larvicide.

Conclusion

The current investigation revealed that the ethyl acetate extracts of the seaweeds, *S. wightii, S. ilicifolium* and *G. acerosa* possess high toxicity potential against the mosquito larvae, and those extracts were found to contain important compounds viz; Phytol acetate, OCTADECANOIC ACID and n-Hexadecanoic acid. Further- more studies are needed, for the formulations of eco-friendly insecticides in future.

Declarations

Authorship contribution statement

Pachiappan Perumal: Methodology, Investigation, Conceptualization, Writing – review & editing – original, draft, Supervision, Project administration. Sundaramoorthy Dhanasundaram: Writing – review & editing, Formal analysis, Resources, Data curation. Annamalai Aravinth: Writing – review & editing, Data curation, Formal analysis. Vadivel Amutha: Writing – review & editing, Data curation. Perumal Santhanam: Writing – review & editing, Data curation. All authors reviewed the manuscript."

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Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

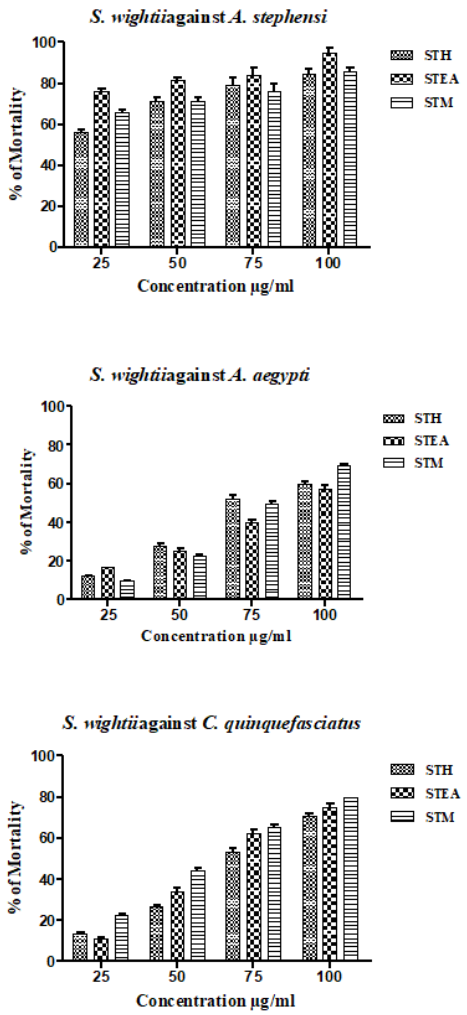


Figure 1

Percentage of Mortality in *S. wightii* crude extracts against the *A. stephensi*, *A. aegypti* and *C. quinquefasciatus*.

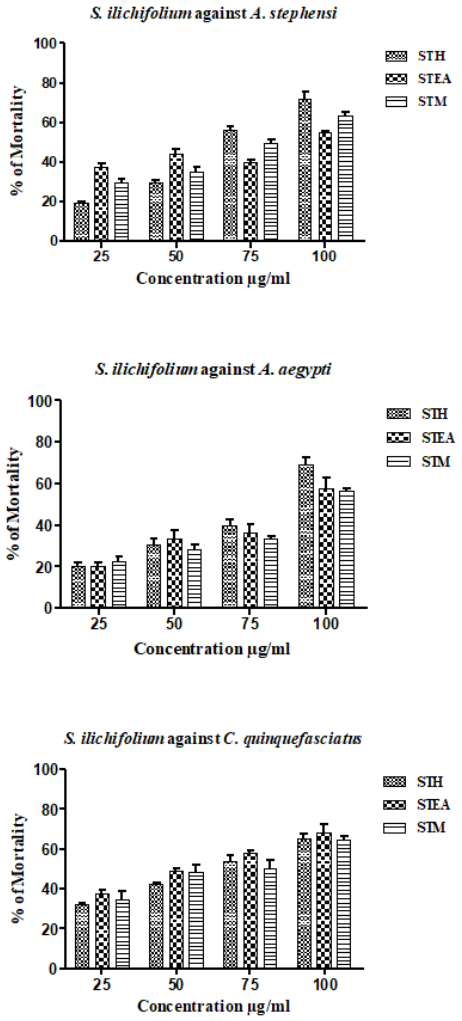


Figure 2

Percentage of Mortality caused by the *S. ilicifolium* crude extracts on the *A. stephensi*, *A. aegypti* and *C. quinquefasciatus.*

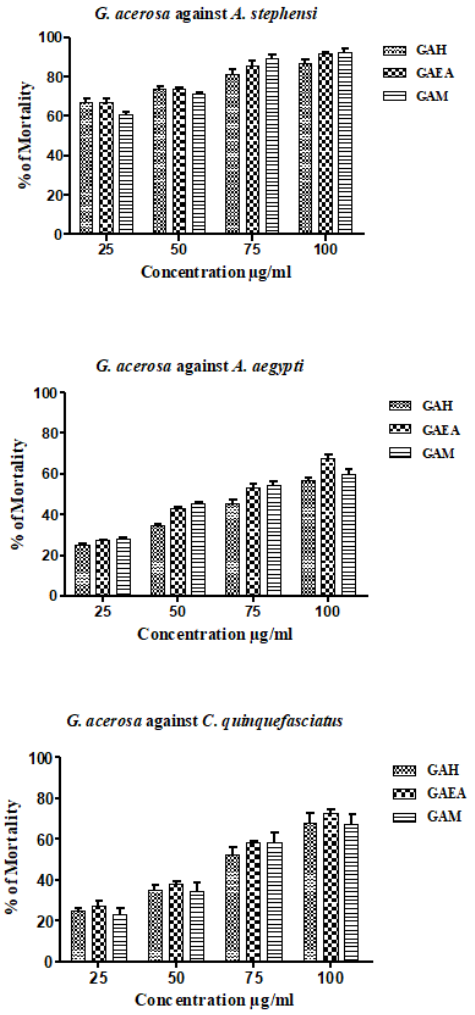


Figure 3

Percentage of Mortality caused by the *G. acerosa* crude - extracts against *A. stephensi*, *A. aegypti* and *C. quinquefasciatus*.

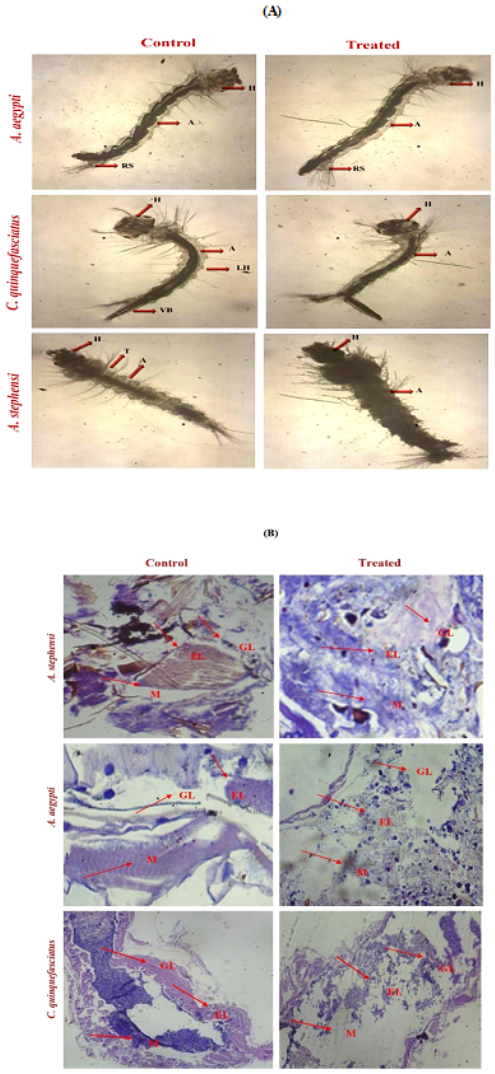


Figure 4

Control & Treated mosquito larvae (A), (B): No deformities were witnessed in the control group of mosquito larvae. The malformations were observed in the bodies of the larvae treated with SW-EA, GA-EA and SI-EA crude extracts, tested against different mosquito vectors viz; *A. stephensi*, *A. aegypti* and *C. quinquefasciatus* were, (M) muscles, (EL) epithelium layer and (GL) gut lumen.

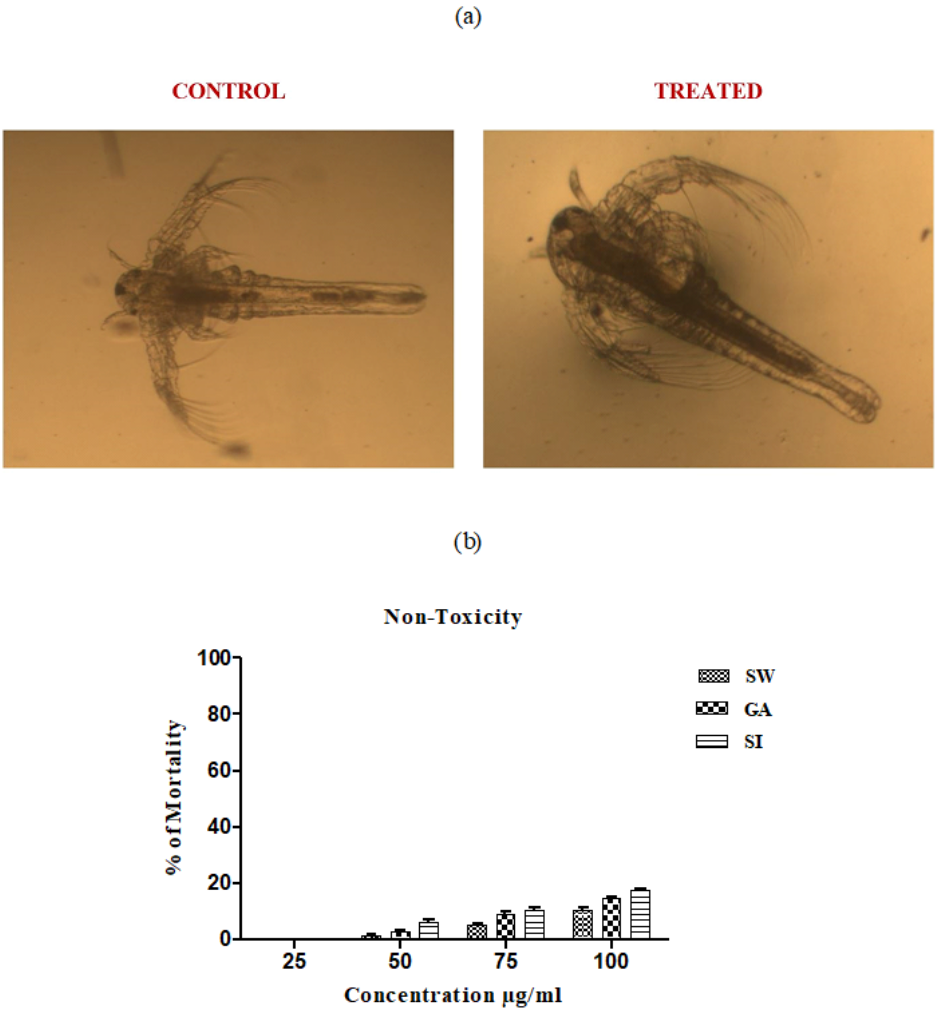


Figure 5

(a)Toxicity assessment of SW-EA, SI-EA and GA-EA extracts against nauplii larvae of *A. salina.* (b) Percentage mortality of SW-EA, SI-EA and GA-EA - extracts treated against nauplii larvae of *A. salina*.

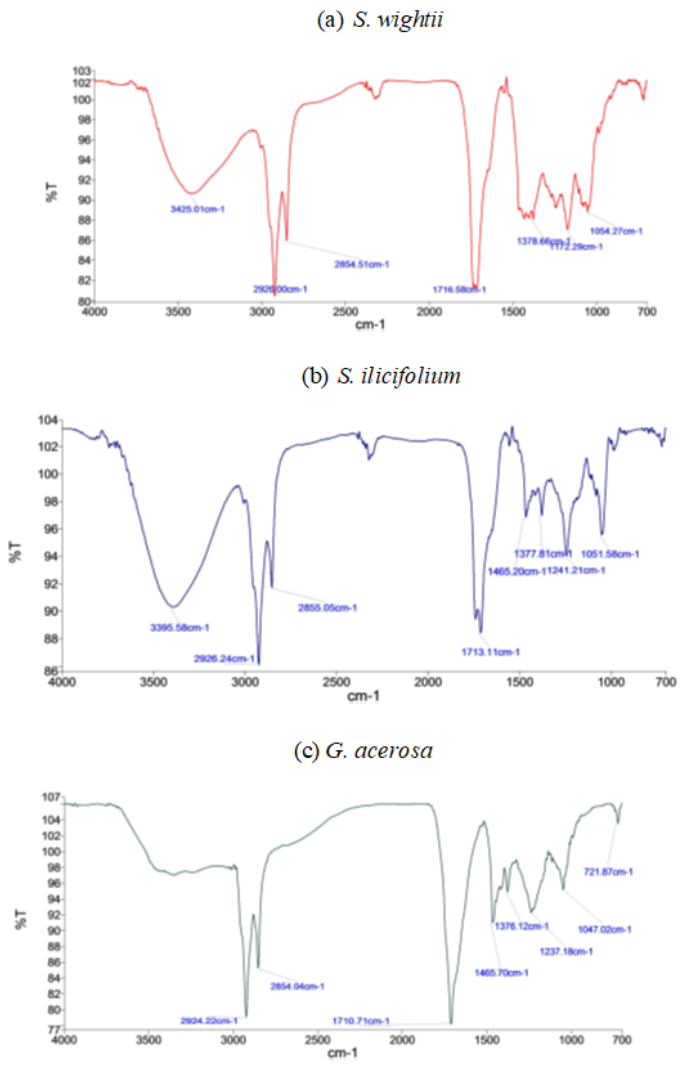


Figure 6

FTIR spectra of ethyl acetate extracts of (a) *S. wightii,* (b) *S. ilicifolium* and (c) *G. acerosa.*

Figure 7

GC-MS chromatogram for the ethyl acetate extracts of (a) *S. wightii,* (b) *S. ilicifolium* and (c) *G. acerosa*.