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Original article

Screening and evaluation of different algal extracts and prospects for controlling the disease vector mosquito *Culex pipiens* L.

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ABSTRACT

Continual application of synthetic insecticides in controlling mosquito larvae has resulted in several problems as build-up of mosquito resistance beside to negative impacts on human health and environment. Discovering new and affordable bio-insecticidal agents with high efficiency, cost effective and target specific become a crucial need. The current study assessed the larvicidal activity of eight methanolic algal extracts belong to three different algal divisions against the 3rd larval instar of *Culex pipiens* L. (Diptera: Culicidae). Comparative studies showed that four species of red and green algal extracts exhibited good larvicidal activity. *Galaxaura elongata* and *Jania rubens* (Rhodophyta), *Codium tomentosum* and *Ulva intestinales* (Chlorophyta) showed higher larvicidal potencies than *Padina boryana*, *Dictyota dichotoma*, and *Sargassum dentifolium* (Phaeophyta) and *Gelidium latifolium* (Rhodophyta). The maximum level of toxicity was achieved by exposure to *G. elongata* extract with LC_{50} (31.13 ppm), followed by *C. tomentosum* (69.85 ppm) then *J. rubens* (84.82 ppm) and *U. intestinalis* (97.54 ppm), while the lowest toxicity exhibited by *G. latifolium* (297.38 ppm) at 72 h post- treatment. The application of LC_{50} values of *G. elongate, J. rubens, C. tomentosum*, and *U. intestinalis* extracts affected the activities of antioxidant enzymes *viz.* superoxide dismutase, catalase and glutathione peroxidase as oxidative stress markers. An increase of antioxidant enzymes activities was recorded. Therefore, a significant elimination of free radicals, causing toxic effects. Overall, this study casts light on the insecticidal activity of some algal extracts, suggesting the possibility of application of these bio- agents as novel and cost- effective larvicides.

1. Introduction

Mosquitoes act as vectors of pathogens and parasites that cause dreadful diseases (malaria, lymphatic filariasis, dengue, chikungunya, yellow fever, Zika virus and Japanese encephalitis) that threaten worldwide (WHO, 2020). *Culex pipiens* L. primarily considered the vector of lymphatic filariasis (*Wuchereria bancrofti*) to more than 100 million people of which about 43 million are seriously infected (Vinoth et al., 2019; Hamama et al, 2022). To control the mosquito borne diseases, traditional chemical insecticides have been developed in the last 30 years (Ravikumar et al., 2011a,b). The chemical insecticides cause undesirable consequences in human beings and thus affect the ecosystem causing negative impact on non-target organisms (human and beneficial organisms and insects). In addition to, the development of insect resistance, contamination of natural resources therefore food chain and reduction of biodiversity (Benelli et al., 2017). Therefore, researchers focused on novel and potentially eco-friendly control tools. Algae are a group of photosynthetic organisms which inhabit a wide range of environments even extreme habitats vary in their sizes from microalgae to seaweeds (Seckbach, 2007). Although most algae

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are nutritious food for mosquito larvae, some species kill the larvae when ingested in large quantities. Phytochemicals derived from seaweeds offer a natural source of compounds to develop new insecticides and antimicrobials (Suganya et al., 2019). Algae generally have higher antioxidant activity due to their high contents of antioxidant components such as ascorbic acid, reduced glutathione, phenols and flavonoids (Tabakaeva and Tabakaev, 2016). The number of bioactive compounds from algae is increasing today due to the improvement of extraction methods (Michalak and Chojnacka, 2015). Seaweeds are important natural alternatives to insecticides, as phytochemicals extracted from them may act against mosquitoes as toxicant, growth regulators, repellents and ovipositional deterrent (Ghosh et al., 2012; Kannan and Priya, 2019). No doubt, algae are safe and promising agent not only in public health but also in agriculture for insect control and crop protection (Singh et al., 2016). Finally, the development of bioinsecticides such as algal extracts represent safe, applicable, and low-cost alternatives for synthetic pesticides, which negatively affect the environment and health. They contain several active compounds with potential biopesticidal activity for pest control and contributing to sustainable agriculture (Costa et al., 2019). Algae- derived bio- insecticides have been reported as safe and cost- effective alternative for mosquito management (Elbanna and Hegazi, 2011). This study aims to screening different algae to develop an eco-friendly algal extracts and evaluation the bioactivity of these extracts against selected mosquito vectors larvae e.g.,*Culex pipiens.*

2. Material and methods

2.1. Plant materials

Fresh eight samples of seaweeds were collected from the coastal of Red and Mediterranean Sea in Egypt (Table 1). Collected samples comprised three algal species: *Sargassum dentifolium*, *Padina boryana* and *Dictyota dichotoma* belonged to Phaeophyta, three Rhodophyta: *Gelidium latifolium*, *Jania rubens* and *Galaxaura elongata*, in addition, two algal taxa: *Ulva intestinalis* and *Codium tomentosum* belonged to Chlorophyta. The collected algal samples were immediately washed with seawater to remove the sand particles and epiphytes. Then they were kept in an ice box and immediately transported to the laboratory. The collected algae were washed gently four times with tap water and five times with distilled water to remove any adhered residues, salts, or small animals. Then, the species were identified according to Zinova, (1967) and Aleem, (1993).

2.2. Extract preparation

The washed seaweeds were dried under shade conditions for six days then grinded by an electric blender (Moulenix, France) to a coarse powder (Yogarajalakshmi et al., 2020). About 100 gm of seaweeds powder homogenized in 300 ml of methanol. After dark incubation for two weeks, the samples were extracted four times and filtered. The methanol was concentrated by evaporation in a rotary evaporator (Labo-Rota C311) in water bath at (40 °C) for (2– 3) h. The crude methanolic extracts were weighed and kept in deep freezer (-4°C) until further use in experiments (Gonzalez-Castro et al., 2019).

2.3. Mosquito larval culture

The egg rafts of susceptible strain of *C. pipiens* were obtained from the Research and Training Center on Vectors of Diseases (RTC), Ain Shams University. The mosquitoes were reared in the insectary of the Entomology department. The newly hatched larvae were reared in plastic trays containing distilled water, 0.5 g of sterilized Tetramine were added for feeding (Farag et al., 2020). Pupae were collected and introduced into mosquito cages until emergence. The emerged adults were fed on 10% sucrose solution then females allowed for blood meal using a pigeon. All the experiments were held under controlled laboratory conditions at 27 ± 2 °C, 70 ± 5 % RH, and a 14:10 h light/dark photoperiod (Abdel-Haleem et al., 2020).

2.4. Larvicidal activity

The larvicidal efficiency of methanolic extracts of seaweeds were evaluated against 3rd instar larvae of *C. pipiens* according to the (WHO, 2005) standard method. Each algal extract was dissolved in ethanol to prepare the stock solution. Then, batches of 25 early 3rd larval instar of *C. pipiens* were transferred to small disposable plastic cups containing five different concentrations of each algal extract prepared in water. Three replicates were conducted for each experiment and control with distilled water only. Mortality data were recorded after 24, 48 and 72 post-treatment (Emam et al., 2021; Saad et al., 2021) and the mortality percentages were corrected according to Abbott's formula (Abbott, 1925).

$$\%\text{corrected mortality} = \frac{\%\,\text{test kill} - \%\text{control kill}}{100 - \%\,\text{Control kill}} \times 100$$

2.5. Preparation of larval samples for biochemical assay

The 3rd instar larvae of *C. pipiens* were exposed to the LC_{50} of each tested algal extract and the samples were collected 48 h post treatment. The treated and control larvae were weighted, counted then mechanically homogenized in phosphate buffer pH 7.3 and EDTA, using Dounce Tissue Grinders. The homogenized larvae were centrifuged at 4,000 rpm for 15 min at 4 °C and the supernatant used for enzyme estimation. The absorbance of colored substances was measured by double beam ultraviolet /visible spectrophotometer (Sectronic 1201, Milton Roy Co., USA) at Biochemistry unit,

Table 1
The mean yield of methanolic extracts from seaweed species.

Name of species	Algal division	Algal family	Sampling sites	Mean yield (gm) from 100 gm of the algae ± SE.
Sargassum dentifolium	Phaeophyta (Brown algae)	Sargassaceae	Hurghada, Red Sea	0. 65 ± 0.08 ^{d}
Dictyota dichotoma		Dictyotaceae	Hurghada, Red Sea	0. 53 ± 0.03 ^{e}
Padina boryana		Dictyotaceae	Hurghada, Red Sea	0. 45 ± 0.06^{f}
Gelidium latifolium	Rhodophyta (Red algae)	Gelidiaceae	Alexandria, Mediterranean Sea	0. 73 ± 0.05^{c}
Jania rubens		Corallinaceae	Alexandria, Mediterranean Sea	0. 88 ± 0.03^{b}
Galaxaura elongata		Galaxauraceae	Hurghada, Red Sea	1.02 ± 0.01 ^{a}
Ulva intestinales	Chlorophyta (Green algae)	Ulvaceae	Alexandria, Mediterranean Sea	0. 67 ± 0.06 ^{d}
Codium tomentosum		Codiaceae	Hurghada, Red Sea	0. 74 ± 0.02^{c}

Means with the same letters are not significantly different.
SE = Standard error.

Faculty of Medicine, Ain shams University according to Rup et al., (2006).

2.6. Enzyme activities

2.6.1. Catalase

Catalase activity was estimated by Biodiagnostic, Kit No. CA 25 17, Egypt, according to the method given by Fossati et al., (1980) and Aebi, (1984). The absorbance of a formed chromophore was inversely proportionate to the amount of catalase in the tested sample (Rup et al., 2006). Briefly, 0.05 ml of the sample, 0.50 ml of phosphate buffer (pH 7) and 0.10 ml of chromogen-inhibitor were mixed and incubated exactly one min. at 25 °C then 0.20 ml chromogen- inhibitor and 0.50 ml H_2O_2 were added to the mixture then incubated for 10 min at 37 °C. The decrease in absorbance was recorded at 510 nm.

2.6.2. Glutathione peroxidase

Glutathione peroxidase activity was estimated by using Biodiagnostic, Kit No. GP 25 24, Egypt, according to Paglia and Valentine, (1967). The decrease in NADPH absorbance during the oxidation of NADPH to $NADP^+$ is an indicator of glutathione peroxidase activity in the tested sample (Rup et al., 2006). 0.01 ml of larval sample was added to 2 ml of cold buffer (50 mM potassium phosphate buffer, pH 7, 5 mM EDTA and one mM 2-mercaptoethanol), then centrifuged to obtain the supernatant. 0.01 ml of supernatant was added to one ml of glutathione peroxidase buffer, and 0.1 ml of the NADPH reagent in a quartz cuvette at 25 °C. The cuvette and were mixed by inversion. The decrease in absorbance was recorded at 340 nm.

2.6.3. Superoxide dismutase

Superoxide dismutase was measured by Biodiagnostic, Kit No. SD 25 21, Egypt, according to Nishikimi et al., (1972) method. The homogenized larvae mixed with 5 ml of cold buffer [(100 mM potassium phosphate buffer (PMS), (pH 7)] and 2 mM EDTA). 2 ml of 10% w/v homogenate was centrifuged at 4,000 rpm for 15 min at 4 °C and the supernatant was used for superoxide dismutase enzyme assay. 10 ml of phosphate buffer, 1 ml of nitro blue tetrazolium (NBT) and 1 ml of PMS were mixed to prepare the working reagent. 1 ml of working reagent and 0.1 ml of the sample were mixed well. The reaction was initiated by adding 0.1 ml of PMS to the mixture. The increase in absorbance was measured at 560 nm for 5 min. at 25° C.

2.7. Statistical analysis

The larval mortality data were analyzed by the probit analysis (Finney, 1971) using the statistics package (LDP-line) to calculate LC_{25}, LC_{50}, LC_{90} with 95% fiducial limits of upper and lower confidence limit, Chi-sequre, slope, standard error, and correlation coefficient. The biochemical results were analyzed by One – way analysis of variance (One- way ANOVA). The means were compared by the Duncan's multiple range test Duncan, (1955). Results with $p < 0.05$ were considered statistically significant.

3. Results

3.1. Extraction

Data presented in Table 1 indicates that the mean yields of the methanolic extracts were varied between algae species. The methanolic extract of *G. elongata* gave the maximum yield (1.02 gm) followed by extract of *J. rubens* and the lowest were the extracts of *P. boryana* (0.45 gm). Generally, the higher methanolic extracts yields were obtained from red algae (Rhodophyta).

3.2. Larvicidal activity

The data represented in Table (2, 3 and 4) revealed that the toxicity of the algal extracts increase gradually over time. The mortality of *C. pipiens* larvae initiated from the 1st day of exposure and increased up to 3rd day. The maximum level of toxicity was achieved by exposure to *G. elongata* extract with LC_{50} (78.52 ppm), followed by *C. tomentosum* (87.44 ppm) then *J. rubens* (97.54 ppm) and the lowest toxicity achieved by *G. latifolium* (348.33 ppm) at 24 h post treatment (Table 2). The activity of algal extracts was arranged as follows: *G. elongata* > *C. tomentosum* > *J. rubens* > *U. intestinales* > *D. dichotoma* > *P. boryana* > *S. dentifolium* > *G. latifolium* (Table 3 and 4). It was noticed that the activity of *G. elongata* greatly increased with 6.84 and 9.55 folds greater than *G. latifolium* at 48 and 72 h, respectively. In general, *S. dentifolium, D. dichotoma* and *P. boryana* showed convergent, low toxicity, while *J. rubens*, *U. intestinalis* and *C. tomentosum* exhibited moderate activity against *C. pipiens* larvae. The extracts of *J. rubens*, *U. intestinalis* and *C. tomentosum* showed high levels of toxicity index values at 24 h post treatment, which gradually decrease at 48 h and 72 h, respectively. The low values of the slope indicated that the tested population of *C. pipiens* larvae is homogenous.

3.3. Biochemical analysis

The effect of treatment with the LC_{50} values of *J. rubens*, *G. elongata U. intestinales* and *C. tomentosum* extracts on the activities of various antioxidant enzymes *viz.* catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) was studied and the results data are presented in Fig (1, 2 and 3). The *C. pipiens* larvae treated with *G. elongata* and *C. tomentosum* extracts showed significant ($p < 0.05$) increase in activity of SOD as compared to control (Fig. 1). Although, all exposures with the tested extracts slightly raised the CAT activity, *G. elongata* extract showed non-significant ($p > 0.05$) increase as compared to its control (Fig. 2). Maximum CAT activity was observed when *C. pipiens* larvae were treated with *J. rubens* extract. While, convergent upsurge was observed in CAT activity by treatment with *U. intestinales* and *C. tomentosum* extracts.

Glutathione peroxidase activity in all treatments was significantly elevated with respect to control (Fig. 3), despite, *J. rubens*, and *C. tomentosum* extracts are convergent in their effect on its activity. *G. elongata* extract (652.8 units/mg protein/min) exhibited slight increase, while *U. intestinalis* extract (1003.1 units/mg protein/min) showed the greatest increase in glutathione peroxidase activity compared with control. In general, the activities of all tested enzymes differently increased after treatment with the algal extracts.

4. Discussion

There is global trend to reduce the continual use of chemical insecticides to overcome the problems of food and environmental pollution. Several studies were conducted to develop affordable new insecticidal agents from natural sources with desirable environmental characteristics (Ahmad et al., 2001; Ali et al., 2013; Hasaballah and El-Naggar, 2017; El-Naggar and Hasaballah, 2018; Farag et al., 2020). Seaweed widely distributed all over the shores of seas and oceans (John, 1994; Subba, 2012), so it considered a cheap and available alternative to synthetic insecticides (Cetin et al., 2010; Murugan et al., 2015; Gowthish and Kannan, 2019a). Algal extracts have larvicidal activity against mosquito

Table 2
Larvicidal activity of methanolic extracts of algae against *Culex pipiens* at 24 h post treatment.

Species name	LC_{25}(*F.l. at 95%)	LC_{50} (*F.l. at 95%)	LC_{90} (*F.l. at 95%)	*Slope ± SE	P	*χ^2	Toxicity index	Relative potency
S. dentifolium	158.08 (128.53–183.32)	306.86 (273.56–347.47)	1082.13 (829.23–1622.94)	2.34 ± 0.26	0.34	3.34	25.58	1.13
D. dichotoma	133.72 (104.95–158.28)	266.85 (236.05–301.19)	991.71 (764.78–1472.53)	2.24 ± 0.25	0.16	5.05	29.42	1.31
P. boryana	151.04 (121.69–176.09)	295.52 (263.04–334.17)	1057.78 (811.91–1582.16)	2.31 ± 0.26	0.31	3.61	26.56	1.17
G. latifolium	183.46 (153.13–209.59)	348.33 (311–397.82)	1177.73 (894.09–1796.81)	2.42 ± 0.27	0.45	2.59	22.54	1
J. rubens	33.2 (23.99–42.49)	97.54 (80.22–118.7)	755.61 (521.13–1274.76)	1.44 ± 0.13	0.104	6.16	80.50	3.57
G. elongata	27.98 (20.18–35.9)	78.51 (64.57–94.57)	557.51 (402.84–869.83)	1.51 ± 0.33	0.05	7.66	100	4.43
U. intestinales	39.73 (29.44–49.99)	111.62 (92.59–135.45)	794.65 (549.76–1337.03)	1.50 ± 0.14	0.15	5.25	70.34	3.12
C. tomentosum	29.76 (21.30–38.33)	87.43 (71.69–106.15)	677.58 (473.71–1116.45)	1.44 ± 0.13	0.09	6.29	89.08	3.98

* (F.l.) Fiducially Limits.
*(χ^2) Chi square value.
*Slope of the concentration-inhibition regression line ± standard error.

Table 3
Larvicidal activity of methanolic extracts of algae against *Culex pipiens* at 48 h post treatment.

Species name	LC_{25}(*F.l. at 95%)	LC_{50} (*F.l. at 95%)	LC_{90} (*F.l. at 95%)	*Slope ± SE	P	*χ^2	Toxicity index	Relative potency
S. dentifolium	166.74 (139.42–190.34)	302.42 (272.40–337.76)	937.37 (748.39–1304.52)	2.60 ± 0.27	0.72	1.29	15.37	1.05
D. dichotoma	122.65 (94.62–146.67)	246.33 (216.65–277.79)	926.62 (720.53–1356.55)	2.22 ± 0.25	0.12	5.72	18.88	1.29
P. boryana	137.97 (109.13–162.59)	273.40 (242.37–308.50)	1002.53 (773.38–1486.98)	2.27 ± 0.25	0.24	4.19	17.01	1.16
G. latifolium	165.79 (136.12–191.19)	318.59 (284.44–361.22)	1102.10 (844.15–1653.86)	2.37 ± 0.26	0.38	3.03	14.59	1
J. rubens	30.41 (21.70–39.24)	90.98 (74.49–110.86)	729.62 (503.29–1229.50)	1.41 ± 0.13	0.08	6.64	51.12	3.50
G. elongata	14.45 (9.29–19.98)	46.51 (36.24–57.72)	428.42 (306.4–678.05)	1.32 ± 0.12	0.05	7.718	100	6.84
U. intestinales	39.49 (13.98–57.58)	113.78 (65.73–212.89)	849.64 (614.13–5174.19)	1.46 ± 0.14	0.04	8.03	40.87	2.80
C. tomentosum	25.21 (17.65–32.96)	75.90 (61.68–92.39)	615.92 (432.79–1005.20)	1.41 ± 0.13	0.06	7.37	61.27	4.19

* (F.l.) Fiducially Limits
*(χ^2) Chi square value.
*Slope of the concentration-inhibition regression line ± standard error.

Table 4
Larvicidal activity of methanolic extracts of algae against *Culex pipiens* at 72 h post treatment.

Species name	LC_{25}(*F.l. at 95%)	LC_{50} (*F.l. at 95%)	LC_{90} (*F.l. at 95%)	*Slope ± SE	P	*χ^2	Toxicity index	Relative potency
S. dentifolium	144.24 (115.11–169.09)	284.31 (252.57–321.16)	1032.19 (793.75–1539.25)	2.28 ± 0.25	0.27	3.91	10.95	1.04
D. dichotoma	113.01 (85.63–136.40)	227.33 (198.58–256.47)	857.90 (674.00–1233.78)	2.22 ± 0.25	0.09	6.47	13.69	1.30
P. boryana	123.94 (94.97–148.65)	253.88 (223.00–287.37)	991.63 (758.92–1495.74)	2.16 ± 0.25	0.07	6.82	12.26	1.17
G. latifolium	155.15 (126.38–179.80)	297.38 (265.56–335.14)	1023.71 (794.42–1500.03)	2.38 ± 0.26	0.40	2.92	10.46	1
J. rubens	27.96 (19.72–36.36)	84.82 (69.15–103.45)	698.36 (482.71–1172.06)	1.39 ± 0.13	0.06	7.08	36.70	3.50
G. elongata	8.11 (4.38–12.42)	31.13 (22.27–40.58)	400.86 (275.59–681.27)	1.15 ± 0.12	0.53	2.16	100	9.55
U. intestinales	33.21 (23.99–42.49)	97.54 (80.22–118.70)	755.61 (521.13–1274.7)	1.44 ± 0.13	0.104	6.16	31.91	3.04
C. tomentosum	23.43 (7.17–34.21)	69.85 (35.86–119.45)	556.54 (388.14–2523.35)	1.42 ± 0.13	0.04	8.09	44.57	4.25

* (F.l.) Fiducially Limits.
*(χ^2) Chi square value.
*Slope of the concentration-inhibition regression line ± standard error.

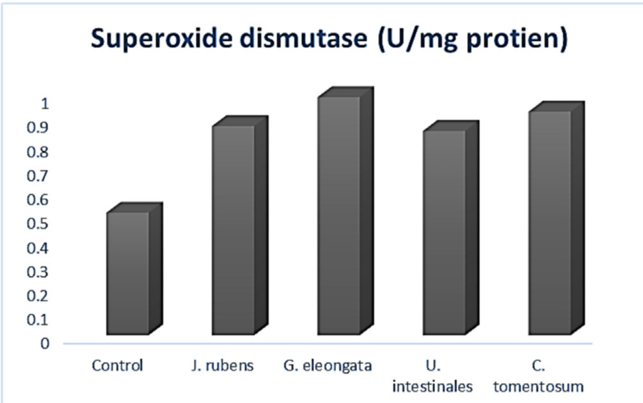

Fig. 1. Effect of *Jania rubens*, *Galaxaura elongata Ulva intestinales* and *Codium tomentosum* extracts on the superoxide dismutase activity of 3rd larval instars *Culex pipiens* at 48 h post treatment.

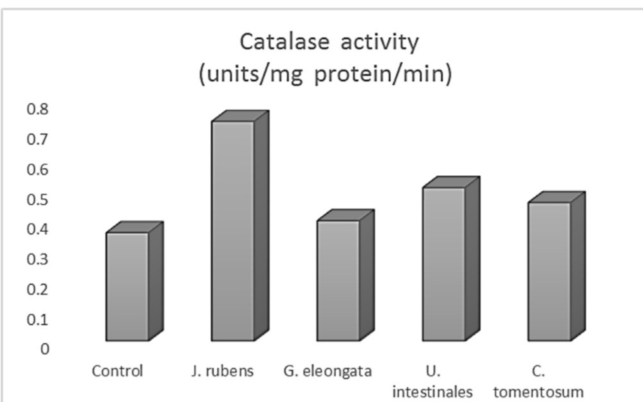

Fig. 2. Effect of *Jania rubens*, *Galaxaura elongata Ulva intestinales* and *Codium tomentosum* extracts on the catalase activity of 3rd larval instars *Culex pipiens* at 48 h post treatment.

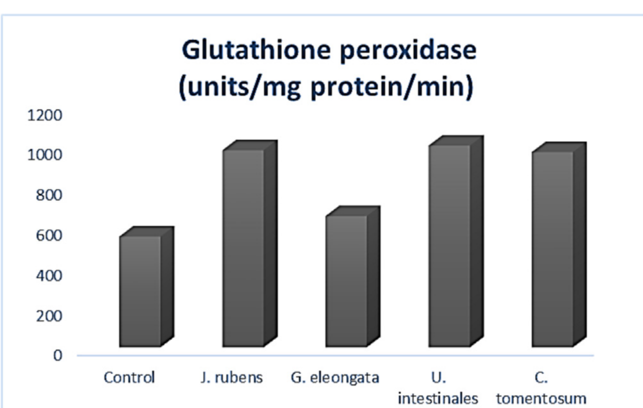

Fig. 3. Effect of *Jania rubens*, *Galaxaura elongata Ulva intestinales* and *Codium tomentosum* extracts on the glutathione peroxidase activity of 3rd larval instars *Culex pipiens* at 48 h post treatment.

larvae because they are source of bioactive compounds, selective, biodegradable and easily applied to mosquito breeding sites as traditional insecticides (Manilal et al., 2009; Samidurai et al., 2009; Ravikumar et al., 2011a,b). Simply, certain species of green algae, abundant in nature, kill larvae consuming them by precluding of other food consuming and then starve. Therefore, these indigestible algae might be introduced into the larval habitat to render it inadequate for mosquito production. Moreover, the algae can persist for several years, under periodical drought conditions (Marten, 2007). The methanolic extracts of three groups of seaweed like brown, green and red algae have strong larvicidal activity against *Aedes albopictus* and *Aedes aegypti* (Ahmad et al., 2016). The current study aimed to evaluate the larvicidal activity of eight methanolic algal extracts against the 3rd larval instar of *C. pipiens* to recommend new alternatives to traditional insecticidal agents.

Both red and green algal extracts exhibited higher toxic actions against *C. pipiens* larvae than brown algal extracts. Therefore, *G. elongata J. rubens* (Rhodophyta), *C. tomentosum*, and *U. intestinalis* (Chlorophyta) showed higher larvicidal potencies than *P. boryana, D. dichotoma* and *S. dentifolium* (Phaeophyta). However, *G. latifolium* (Red algae, Rhodophyta) has low potency than other red algae (Gopu et al., 2021). The bioactivity variations between the species of the same division might be due to ecological and geographical factors besides to seasonal variations which affect the chemical composition of the bioactive metabolites and their production (Manilal et al., 2009; Stengel et al., 2011; Yu et al., 2015).

The phenolic compounds in natural materials' extracts have various biological activity (El-Saadony et al., 2021b,a, Saad et al., 2015, 2021). In this study, the larvicidal activity of these algal extracts might be due to various bioactive compounds, including phlorotannins, flavonoids, phenolics, amino acids, alkaloids, polysaccharides, terpenoides, saponins and halogenated compounds existing in algae (Yu et al., 2014). Though, the content of marine algae varies with species, locality, environmental factors and season (Ali et al. 2013). The metabolites extracted from algae not only responsible for larval mortality but also, interfered their development and normal transformation to the pupae. Larval mortality and abnormalities were observed in the *A. aegypti* population (Bibi et al. 2020). The red algal extracts have high potency may be a result of presence toxic compounds *viz.* farnesyl acetone and plastoquinones, which inhibit the AChE enzyme. Also, the red algae are rich with polyphenolic and terpenes compounds compared to green and brown algae these triterpenes inhibit the protein responsible for the cholesterol transportation during the larval development resulting in larval mortality (Blunt et al., 2011; Bibi et al., 2020). In addition, halogenated sesquiterpene (−)-elatol and (+)-obtusol, brominated-oxygenated heterocyclic and halogenated, polyhalogenated compounds obtained from red algae exhibited larvicidal activity against mosquitoes (Abou-Elnaga et al., 2011; Salvador-Neto et al., 2016). Deepak et al., (2019) reported that the presence of alkyl halides, carboxylic acid, alkynes, and amides in the methanolic extract of red algae are responsible for its larvicidal effect. Methanolic extract of green algae has high content of terpenoids, alkaloids, tannin, saponins, glycosides and nitrogenous compounds with larvicidal activity against mosquitoes (Elbanna and Hegazi, 2011; Suganya et al., 2019). While, terpenoids, alkaloids, tannins, glycosides, quinones and saponins were identified in the methanolic extract of brown algae (Suganya et al., 2019). The methanol extract of both green and brown algae contains high rates of saponins which block the uptake of sterols, therefore, enhance insect mortality because the insects can't produce sterol by themselves (Gowthish and Kannan, 2019b).

Insects have complex enzymatic antioxidant systems to overcome reactive oxygen generated during stress (Lomate et al., 2015). At normal physiological conditions, there is an equilibrium between the antioxidant defence enzymes and oxygen free radicals released, the organisms use antioxidant enzymes for deactivation and protection against the toxicity by free radical (Kiran and Aruna, 2010). The antioxidant enzymes are major components of the insect's antioxidant defence system *viz.* superoxide dismutase, catalase and glutathione peroxidase used as oxidative stress markers. The SOD catalyzes the conversion of superoxide into hydrogen peroxide and oxygen, then CAT detoxifying hydrogen peroxide into water and oxygen, thereby, prevent oxidative injury of cells (Kiran and Prakash, 2015). The phytotoxins and insecticides elevate the free radicals level and induce antioxidant defense mechanism in cells (Rajapakse and Walter, 2007). Insecticides cause oxidative stress by stimulating protein oxidation and lipid peroxidation, therefore induce production of reactive oxygen species (ROS) that highly harm the biological tissues Adamski et al. (2003); Otitoju, (2005). ROS include mainly the free radicals' superoxide anion ($O_2^{\ -}$), hydroperoxyl (HO_2), hydroxyl (OH), and hydrogen peroxide (H_2O_2) (Swelum et al., 2020). These free radicals are unstable and highly reactive molecules also their metabolism may release other ROS (Renault et al., 2016). Plant prooxidants such as a flavonoid, a furanocoumarin and a β-carboline alkaloid are responsible for toxic ROS production in the insect gut (Lomate et al., 2015; El-Tarabily et al., 2021). The variation in SOD, CAT and GPx activities depend on the type, intensity and duration of stress conditions (Sharma et al., 2012). The results showed significant increase SOD activity as adaptive response to produced ROS, therefore, generation high levels of hydrogen peroxide and oxygen radical. The SOD activity elevated gradually with the degree of algal extracts potencies, these agree with many studies that reported increased expression and activities of SOD in insects by exposure to toxic agents (Mamidala et al., 2012; Nardini et al., 2013; Kaur et al., 2021). The elevated release of superoxide anion radical might be result in a non– significant increase in CAT activity (Sreejai and Jaya, 2010; Jia et al., 2016) in addition to accumulation of toxic hydrogen peroxide causing peroxidation of membrane lipids and oxidative damage to the cell (Nikolić et al., 2016).

The glutathione system, including glutathione peroxidase (GPx), catalyzes the reduction of hydrogen peroxide and other organic hydroperoxides as lipid hydroperoxides to non– toxic compounds using reduced glutathione as a substrate and protection against oxidative stress (Maheshwari et al., 2011; Cen et al., 2020). The GPx activity significantly elevated by application of tested algal extracts, this agrees with Aslanturket al., (2011); Murali and Prabakaran, (2018) who reported that GPx activity induced with exposure to toxins, as oxidative stresses, to reduce variety of hydroperoxides (Rup et al., 2006). In addition, GPx can catalyze detoxification of hydrogen peroxides at low concentrations more efficiently than CAT. Consequently, the GPx had high affinities to the hydrogen peroxides substrate than CAT (Renault et al., 2016). These finding suggested that the toxicity of tested algal extracts to *C. pipiens* larvae might be related to release of ROS which induced SOD activity which produced high level of superoxides accompanied by low CAT activity leading to the oxidative imbalance and the excessive accumulation of reactive molecules (Kiran and Prakash, 2015). These reactive molecules oxidize the membrane-associated proteins and lipids causing destruction of membrane integrity and functions, also damage the nucleic acids and DNA, thereby loss of cellular function and leading to insect death (Wu et al., 2017).

5. Conclusion

The methanolic extracts of eight algae species were evaluated against 3rd larval instar of *Culex pipiens*. The extracts of *G. elongate* and *J. rubens* (red algae), *C. tomentosum*, and *U. intestinales* (green algae) exhibited good larvicidal activities with low LC_{50} values than *P. boryana*, *D. dichotoma*, *S. dentifolium* (brown algae) and *G. latifolium* (red algae). The activities of antioxidant enzymes were estimated, SOD showed significant activity and GPx while CAT exhibited non– significant activity. Thereby, accumulation of hydrogen peroxide and other reactive compounds leading to denaturation of cell membrane lipid and protein causing loss of cellular function and leading to insect death.

Declaration of Competing 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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