Journal of Aquatic Biology & Fisheries | 2023 | 11(1) | pp. 64-71
ISSN 2321–340X
OPEN ACCESS
http://www.jabf.in/
University of Kerala, INDIA

Insecticidal properties of brown seaweed *Padina gymnospora* (Kützing) Sonder (Dictyotaceae) on larval instars of dengue vector *Aedes aegypti* L. and filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae)

Vaithiyanathan Selvi[1], Subramanian Arivoli[2] and Samuel Tennyson.[3*]

[1]*Department of Microbiology, Sourashtra College, Madurai 625 004, Tamil Nadu, India*
[2]*Department of Zoology, Thiruvalluvar University, Vellore 632 115, Tamil Nadu, India*
[3]*Department of Zoology, Madras Christian College, Chennai 600 059, Tamil Nadu, India*
**E.mail: samtennyson@gmail.com*

ABSTRACT

Seaweeds are large libraries of chemical compounds and contain various bioactive substances with diverse insecticidal properties. In the present study, the brown seaweed, *Padina gymnospora* crude solvent (petroleum ether, chloroform, acetone and methanol) extracts were analysed for their chemical constituents, and tested for their biological control activity against the second and third instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* at concentrations of 100, 200, 300, 400 and 500mg/L for 24 hours. Qualitative chemical analysis revealed the presence of alkaloids, carbohydrates, flavonoids, phenols, proteins, saponins, steroids, tannins, terpenes and terpenoids. One hundred percent larval mortality was observed in the second instar of *Aedes aegypti* larvae by the chloroform and methanol extracts at 500mg/L; and in methanol extract only against the third instar at 500mg/L. The same solvent extracts exhibited one hundred percentage larval mortality at 500mg/L in the second and third instar of *Culex quinquefasciatus*, with the addition of acetone extract against the third instar. Overall, the results indicated that maximum larval mortality at the lowest concentration was observed in methanol extract against the second and third instars of *Aedes aegypti* and *Culex quinquefasciatus*. The respective LC_{50} values of methanol extract against the second and third instar larvae of *Aedes aegypti* were 143.802 and 170.266mg/L, and against *Culex quinquefasciatus* it was 148.250 and 182.867mg/L. The treated larvae showed signs of restlessness, wriggling, sluggishness, paralysis, sinking, leading to death. Larval mortality would have been responsible due to one or more of the active chemical constituents present in the methanolic extract of *Padina gymnospora*, *viz*., L-arabinitol, palmitic acid, oleic acid, dodecane 1-chloro, dodecane, 1-bromo, and tetradecane 1-chloro which was revealed through Gas chromatography-mass spectrometry.

ARTICLE HISTORY

Received on: 29-06-2023
Revised on: 27-11-2023
Accepted on: 04-12-2023

KEYWORDS

Padina gymnospora,
Brown seaweed,
Solvent extracts, GC-MS,
Chemical constituents,
Aedes aegypti,
Culex quinquefasciatus,
Larvicidal activity

1. Introduction

Vector control strategies are the primary methods for lowering the public health burden of the vast majority of diseases spread by mosquitoes. Chemical insecticides play a dominant role in vector control programmes, but the issues raised regarding environmental concern, human health, non-target organisms negatively impacted, and insecticide resistance have sparked interest in alternative control strategies (Aneha *et al*., 2022). This has stimulated the need for the discovery of effective substances from nature. Seaweeds are one of the important natural resources from marine ecosystems, as they are 'rich sources of bioactive compounds from aquatic wealth', producing a great variety of secondary metabolites with broad biological activities (Gupta and Abu-Ghannam, 2011). There is a renewed and increasing interest towards seaweeds in the past decades, primarily due to the vast array of their biomolecules (Ana *et al*., 2021). Seaweeds having a wide range of bioactive compounds have been proven to demonstrate several biological potentials (Chellappan *et al*., 2020). Seaweeds are large libraries of chemical compounds and their organic extracts are known to contain a variety of bioactive substances with diverse insecticidal properties. The long history of seaweeds in insecticide research on discovering new active agents is growing, and reports have revealed that the seaweeds have profound mosquitocidal properties (Yu *et al*., 2014).

Amongst the seaweeds, brown seaweeds (Phaeophyceae) are the largest and most complex type, broadly distributed from tropical to polar zones of ocean in the world (Bixler and Porse, 2011). The genus *Padina* housed under this group is well distributed throughout the tropics and very easy to recognize due to the resemblance of its structure with peacock tail. The thalli (fronds) of *Padina* species are flabellate, with a thin deposit of lime giving brown or whitish appearance (Geraldino *et al*., 2005; Ansari *et al*., 2019). Applications of *Padina* extracts showed significant insect mortality, and controlled the fecundity and hatchability in insects (Sahayaraj and Kalidas, 2011; Asaraja and Sahayaraj, 2013; Priya *et al*., 2022), thereby making *Padina* species an ecofriendly bioinsecticide, and as a good alternative in pest management programmes. *Padina gymnospora* is ecologically and biologically significant in Indian subcontinent, and is portrayed by the fan-shaped, greenish brown thick thallus, (Bipin *et al*., 2021; Grace *et al*., 2021). *Padina gymnospora* is a natural wound care product (Baliano *et al*., 2016); and possesses anticoagulant (Silva *et al*., 2005), antibacterial (Salem *et al*., 2011; Shiny *et al*., 2013; Sekar and Kolanjinathan, 2015), antifungal (Galal *et al*., 2011; Ibraheem *et al*., 2017), antimicrobial (Thamizharasan and Saravanan, 2017), hypolipidemic (Rajakani *et al*., 2018), antioxidant (Subramanian and Ravi, 2020; Bipin *et al*., 2021; Swaminathan *et al*., 2021), and antiviral (Swaminathan *et al*., 2022) properties. Scanty reports highlight *Padina gymnospora* extracts for its mosquito larvicidal activity (Guedes *et al*., 2014; Shameemrani, 2018; Yu *et al*., 2020; Rajagopal *et al*., 2021). Hence, this study was conducted to determine the biological control activity of *Padina gymnospora* extracts as an alternative to synthetic insecticides in managing mosquito larvae.

2. Materials and Methods

2.1. Seaweed collection

Brown seaweed, *Padina gymnospora* (Kützing) Sonder (Dictyotaceae) was collected by hand picking from the intertidal zone of Rameswaram, Tamil Nadu, India (8° 46 N, 78° 9 E and 9° 14 N, 79° 14°E). The collected seaweed was immediately rinsed in water to remove all kinds of epiphytes and other impurities like sand, molluscs, and sea grasses, and kept in sterilized zip lock bags, and transferred to laboratory for further studies. Taxonomical identification and confirmation of the seaweeds was done at the Marine Algal Research Station Mandapam, Central Salt & Marine Chemicals Research Institute, India with the help of morphological key characters and identification manual (Dhargalkar and Kavlekar, 2004; Ganesapandian and Kumaraguru, 2008; Rao, 2012).

2.2. Preparation of seaweed extracts

The cleaned brown seaweed was shade dried at room temperature for a week, and was powdered with the aid of a mixer grinder. The powdered seaweed (250g) was sequentially suspended in selective solvents (750mL each) ranging from non-polar to polar (petroleum ether, chloroform, acetone and methanol), for 72 hours, and then extracted in a soxhlet apparatus to obtain the extract (Vogel, 1989). Thereafter, each extracted sample was filtered using Whatman No.1 filter paper. The filtered sample was individually centrifuged at 5000 rpm for 10 minutes at 4°C, and the supernatant was collected in a separate flask. Each extract was then concentrated using a rotary vacuum evaporator (Puchi RII, Switzerland). The final concentrated crude solvent extracts obtained were individually stored in sterile air tight bottles and kept in a refrigerator until further use. Prior to this, the percentage of yield of extraction of the crude extracts were calculated.

2.3. Gas Chromatography-Mass Spectrometry (GC-MS) analysis

All the crude solvent extracts of *Padina gymnospora* were subjected to qualitative tests for the identification of various chemical constituents, *viz*., alkaloids, carbohydrates, flavonoids, phenols, proteins, saponins, steroids, tannins, terepenes and terpenoids as per standard procedures (Harborne, 1998). Thereafter, GC-MS analysis was carried out for the most promising larvicidal extract. Perkin-Elmer instrument Clarus 680 GC used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250 µm df) and the components were separated using helium as carrier gas at a constant flow of 1 mL/min. The injector temperature was set at 260 °C during the chromatographic run. The extract sample (1µL) injected into the instrument with the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min^{-1}; and 300 °C, where it was held for 6 min. The mass detector conditions were a transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 s, and a scan interval of 0.1 s. The fragments were from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in GC-MS – National Institute for Standards and Technology library.

2.4. Test vector mosquitoes

The eggs of *Aedes aegypti* and egg rafts of *Culex quinquefasciatus* were procured from Centre for Research in Medical Entomology (CRME), Indian Council of Medical Research (ICMR), Madurai, Tamil Nadu, India. Larvae of each test vector mosquito species were reared separately in enamel trays containing dechlorinated water, and were fed with finely powdered mixture of dog biscuits and dry yeast in the ratio 3:1.

2.5. Larvicidal bioassay

According to the guidelines of the World Health Organization (2005) with minor modifications, bioassays were performed on healthy F_1 generation of laboratory colonized larvae of *Aedes aegypti* and *Culex quinquefasciatus*. Serial dilution of 1.0% stock solution of each crude solvent seaweed extract yielded requisite test concentrations of 100, 200, 300, 400, and 500mg/L. Bioassays studies was done in triplicates on the early second and third instars of 20 numbers each added separately into glass beakers (250mL) holding distilled water and test concentration for each replicate. In parallel, control tests were performed with distilled water (250mL) as a positive control, and Tween 80 (1.0mL) dissolved in distilled water served as negative control. Larvae were fed with larval feed during the experiment. Larval mortality was observed 24 hours after treatment and larvae were scored dead when they displayed no signs of movement when probed by a needle at their respiratory siphon. The activity level of seaweed extracts tested based on the average percent mortality of larvae was reported as (>75%: highly active, 51–75%: moderately active, 25–50%: weakly active, and <25%: inactive) (Guedes *et al*., 2014). Besides these, the behaviour and movement of treated larvae were studied every two hours for 24 hours starting from the exposure time.

2.6. Data analyses

Percent larval mortality was calculated, and when control mortality ranged from 5-20% it was calculated as per Abbott's (1925) formula. Statistical analysis was conducted in IBM SPSS Statistics version 27 with significance set at 95% confidence (SPSS, 2021). Mortality data of larvicidal activity was based on probit, chi-square and regression analysis. Besides one-way analysis of variance and Duncan's multiple comparison significant difference post hoc tests were used to determine the mortality rate in treated bioassays compared to control at $P \leq 0.05$ significant level.

3. Results

The percentage yield of *Padina gymnospora* solvent extracts, *viz.*, petroleum ether, chloroform, acetone and methanol were 3.89, 3.74, 2.68 and 2.71, respectively. The active chemical constituents in the solvent extracts of *Padina gymnospora* were alkaloids, carbohydrates, flavonoids, phenols, polyphenols, proteins, saponins, steroids, tannins, terpenes and terpenoids. The GC-MS chromatogram of *Padina gymnospora* methanol extract revealed the presence of L-arabinitol, palmitic acid,

oleic acid, dodecane,1-chloro, dodecane, 1-bromo, and tetradecane 1-chloro.

The crude solvent extracts of *Padina gymnospora* showed activity against the larval instars of the tested vector mosquitoes. No larval mortality was observed in the positive and negative control. One hundred percent larval mortality was observed in the second instar of *Aedes aegypti* larvae by the chloroform and methanol extracts at 500mg/L; and in methanol extract only against the third instar at 500mg/L. The same solvent extracts exhibited one hundred percentage larval mortality at 500mg/L in the second and third instar of *Culex quinquefasciatus*, with addition of acetone extract against the third instar (Table 1; Fig. 1). Overall results indicated that maximum larval mortality at the lowest concentration was observed in methanol extract against the second and third instars of *Aedes aegypti* and *Culex quinquefasciatus*. The respective LC_{50} values of methanol extract against the second and third instar larvae of *Aedes aegypti* was 143.802 and 170.266mg/L; and against *Culex quinquefasciatus* it was 148.250 and 182.867mg/L (Table 2). The treated larvae showed signs of unnatural restlessness, wriggling, floating, sluggishness, paralysis, sinking, leading to death.

Table 1. Biological activity of *Padina gymnospora* extracts on the larval instars of vector mosquitoes

Concentration (mg/L)	Petroleum ether	Chloroform	Acetone	Methanol	Petroleum ether	Chloroform	Acetone	Methanol
	***Aedes aegypti* II instar**				***Aedes aegypti* III instar**			
PC	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}
NC	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}
100	11.33 ± 1.15^{b1}	13.33 ± 1.52^{b1}	15.33 ± 2.51^{b}	15.33 ± 2.51^{b1}	12.66 ± 1.15^{b2}	10.66 ± 0.57^{b1}	12.0 ± 1.0^{b12}	12.66 ± 1.52^{b1}
200	13.33 ± 1.52^{b1}	15.33 ± 2.51^{bc1}	16.0 ± 2.64^{b1}	16.0 ± 2.64^{b1}	12.66 ± 1.15^{b1}	12.0 ± 1.0^{b1}	12.66 ± 1.52^{b1}	14.0 ± 1.73^{bc}
300	13.33 ± 2.51^{b1}	16.33 ± 2.08^{c1}	16.66 ± 2.88^{b1}	16.66 ± 2.88^{bc1}	14.66 ± 2.08^{b1}	12.0 ± 1.0^{b1}	14.0 ± 2.0^{b1}	16.0 ± 2.64^{c1}
400	16.66 ± 1.52^{c1}	18.0 ± 2.0^{cd1}	17.0 ± 2.64^{b1}	17.0 ± 2.64^{bc1}	14.66 ± 3.05^{b1}	17.0 ± 2.64^{c1}	14.66 ± 2.51^{b1}	16.33 ± 3.21^{c1}
500	19.66 ± 0.57^{d1}	20.0 ± 0.0^{d1}	19.0 ± 1.0^{b1}	20.0 ± 0.0^{c1}	19.66 ± 0.57^{c1}	19.33 ± 0.57^{d1}	19.66 ± 0.57^{c1}	20.0 ± 0.0^{d2}
	***Culex quinquefasciatus* II instar**				***Culex quinquefasciatus* III instar**			
PC	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}
NC	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}
100	12.66 ± 1.52^{b1}	14.66 ± 2.08^{b1}	11.66 ± 1.52^{b1}	12.0 ± 1.0^{b1}	13.0 ± 4.35^{b1}	12.0 ± 1.0^{b1}	10.66 ± 2.08^{b1}	12.0 ± 1.0^{b12}
200	15.66 ± 0.57^{c12}	14.66 ± 2.08^{b12}	12.0 ± 1.73^{b1}	16.0 ± 2.64^{c2}	13.33 ± 1.52^{bc1}	13.33 ± 1.52^{b1}	10.66 ± 3.05^{b1}	12.0 ± 1.0^{b1}
300	17.33 ± 1.52^{cd1}	15.33 ± 2.30^{b1}	17.0 ± 2.64^{c1}	17.33 ± 2.08^{c1}	15.33 ± 0.57^{bc1}	14.0 ± 2.0^{bc1}	16.66 ± 2.88^{c1}	15.33 ± 2.30^{c1}
400	17.33 ± 1.52^{cd1}	17.33 ± 2.51^{bc1}	18.33 ± 1.52^{c1}	18.33 ± 1.52^{cd1}	16.0 ± 2.64^{bc1}	16.0 ± 2.64^{c1}	17.0 ± 2.64^{c1}	16.66 ± 2.88^{c1}
500	18.0 ± 1.0^{d1}	20.0 ± 0.0^{c2}	19.66 ± 0.57^{c2}	20.0 ± 0.0^{d2}	17.0 ± 1.0^{c1}	19.66 ± 0.57^{d2}	20.0 ± 0.0^{c2}	20.0 ± 0.0^{d1}

PC: Positive control; NC: Negative control; Data are mean±standard deviation of larval mortality of three replicates of three trials; Different numerical superscript in column indicate values significant than respective PC and NC, and different superscript alphabets in rows indicate values significant between the extracts at P≤0.05 level by one way ANOVA followed by Duncan multiple comparison post hoc test performed; Similarity in alphabetical and numerical superscripts in rows and columns indicate no significant variation.

Table 2. Probit analysis and associated statistical inferences of *Padina gymnospora* extracts against the larval instars of vector mosquitoes

Solvent extracts	LC_{50} (mg/L)	95% CL (LB-UB)	LC_{90} (mg/L)	95% CL (LB-UB)	Regression equation	R^2	χ^2	P value
***Aedes aegypti* II instar**								
Petroleum ether	191.225	144.606-237.212	393.046	332.046-493.344	Y=-0.555+0.203x	0.976	28.408*	0.001
Chloroform	151.791	101.161-200.100	319.259	260.919-422.966	Y=-11.05+0.205x	0.918	51.561*	0.001
Acetone	148.594	72.407-214.013	354.943	277.673-513.184	Y=-27.41+0.165x	0.871	39.344*	0.001
Methanol	143.802	73.287-206.366	327.016	254.984-475.942	Y=-1.688+0.217x	0.964	67.713*	0.001
***Aedes aegypti* III instar**								
Petroleum ether	192.052	132.167-249.982	413.827	339.088-553.121	Y=-8.122+0.160x	0.935	64.847*	0.001
Chloroform	206.327	161.748-251.362	413.744	353.036-512.374	Y=-1.022+0.224x	0.939	28.843*	0.001
Acetone	198.198	142.434-253.008	419.986	347.952-549.616	Y=-3.311+0.202x	0.99	31.998*	0.001
Methanol	170.266	112.835-224.854	360.62	293.822-483.707	Y=-0.611+0.201x	0.946	90.236*	0.001
***Culex quinquefasciatus* II instar**								
Petroleum ether	163.267	99.888-220.331	374.322	303.822-502.493	Y=-0.411+0.299x	0.908	46.799*	0.001
Chloroform	156.477	96.255-212.302	343.804	276.378-472.076	Y=-4.313+0.223x	0.946	51.561*	0.001
Acetone	170.023	128.436-211.294	337.141	285.183-421.055	Y=-13.82+0.202x	0.896	39.344*	0.001
Methanol	148.25	103.134-192.269	299.691	247.010-390.243	Y=-0.926+0.219x	0.977	67.713*	0.001
***Culex quinquefasciatus* III instar**								
Petroleum ether	192.359	121.670-258.166	446.788	360.294-618.019	Y=-0.266+0.191x	0.988	6.084*	0.031
Chloroform	188.585	136.355-239.450	396.863	330.745-511.822	Y=-17.65+0.189x	0.905	24.234*	0.004
Acetone	186.453	141.448-231.668	361.594	304.844-456.214	Y=-10.88+0.203x	0.93	37.665*	0.001
Methanol	182.867	134.210-230.797	371.978	311.026-476.126	Y=-0.523+0188x	0.989	36.458*	0.001

LC_{50} & LC_{90}: Lethal concentration that kills 50% and 90% of the treated larvae respectively; CL: Confidence limits; LB: Lower bound; UB: Upper bound; R^2: Coefficient of determination; χ^2: Chi-square value; *Values significant at P≤0.05 level

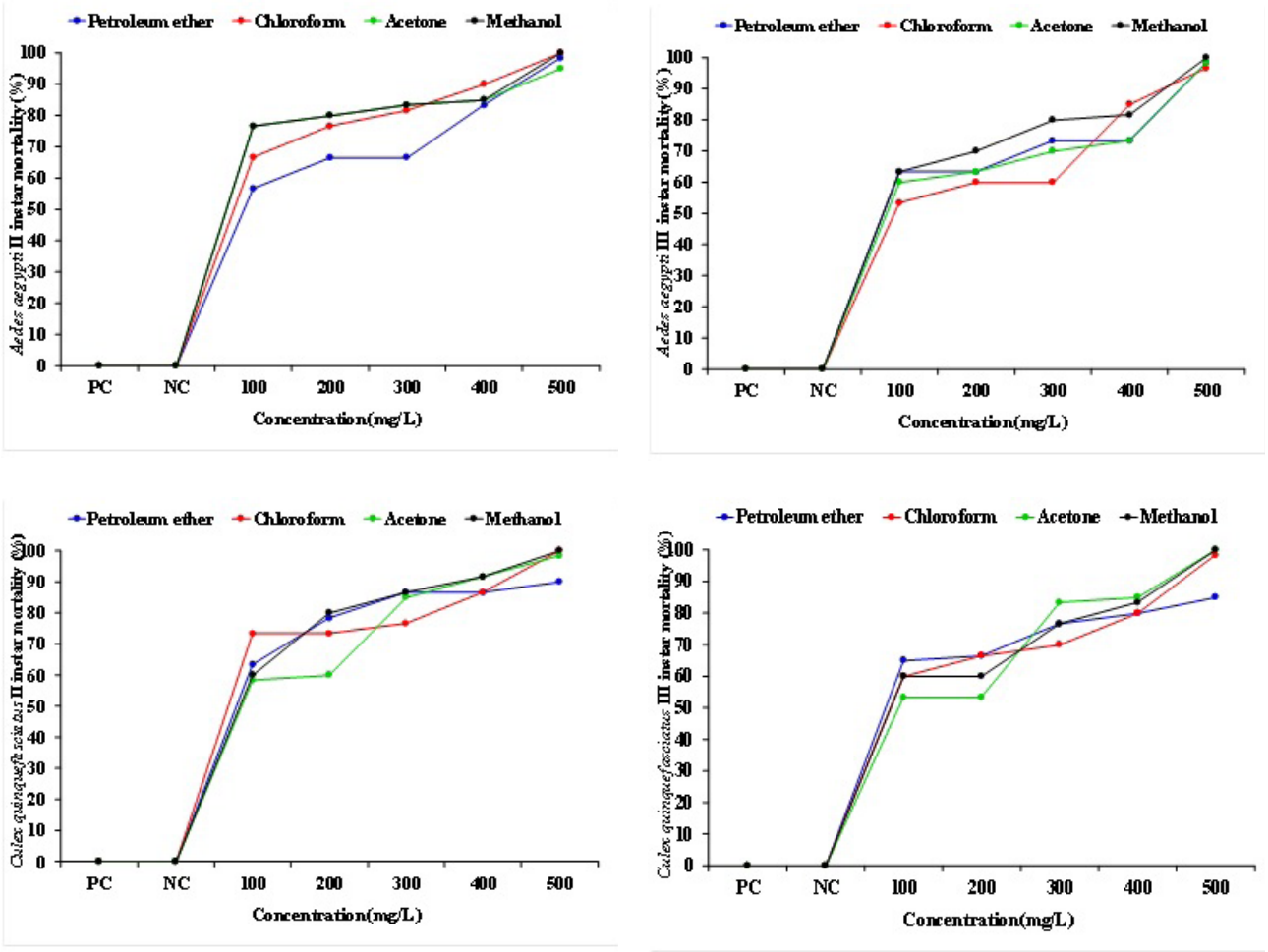

Fig. 1. Percent larval mortality of vector mosquitoes on exposure to *Padina gymnospora* extracts

4. Discussion

A broad spectrum of seaweed species has been reported for their property of biological control on mosquito larvae (Yu *et al*., 2014; Ahmad *et al*., 2016), and in the present study the crude solvent extracts of *Padina gymnospora* were reported for larvicidal action on *Aedes aegypti* and *Culex quinquefasciatus*. The results indicated that the methanol extract exhibited maximum activity against the larval instars of the vector mosquito species tested. This result was validated by Manilal *et al*. (2011) who reported on the larvicidal activities of methanol extracts of twenty seaweed species against the second instar larvae of *Aedes aegypti*. With regard to the vector mosquito species tested in the present study, *Aedes aegypti* was more susceptible than *Culex quinquefasciatus*, which corroborated with the reports of Manilal *et al*. (2011), and amongst the instars, second instar larvae were more susceptible than the third instar. Selvin and Lipton (2004) indicated that the second instar larvae were more susceptible than the fourth instar larvae, because the tolerance of larvae increases with age, as there is a higher mortality rate for younger larvae when compared to older larvae under the same concentration of treatment as reported by Alarif *et al*. (2010) and Abou-Elnaga *et al*. (2011).

The results of the present study were comparable with previous mosquito larvicidal activity of *Padina gymnospora*, wherein the hexane and chloroform fractions of its dichloromethane extracts exhibited LC_{50} values of 29.018 and 17.230µg/mL against *Aedes aegypti* (Guedes *et al*., 2014), the ethyl acetate extract against the second and third instar larvae of *Aedes aegypti* with LC_{50} values of 162.23 and 253.78mg/mL, and against *Culex quinquefasciatus* with respective LC_{50} values of 142.87 and 180.37mg/mL (Shameemrani, 2018), the methanol extract showed larvicidal potential and acetylcholinesterase inhibitory effects against *Aedes aegypti* and *Aedes albopictus* (Yu *et al*., 2020), and its aqueous extract showed LC_{50} value of 34.92µg/mL against *Aedes aegypti* third instar (Rajagopal *et al*., 2021). Other members of the *Padina* genus too have been reported for larvicidal activity, wherein, *Padina tetrastromatica* exhibited LC_{50} values of 97.94 and 97.41µg/mL against *Culex quinquefasciatus* and *Aedes aegypti*, respectively (Manilal *et al*., 2011). *Padina pavonica* exhibited larvicidal activity against *Culex quinquefasciatus* (Pucazhendi *et al*., 1995), and its ethanol extract showed LC_{50} value of 1200ppm against *Aedes aegypti* larvae (Hira *et al*., 2017). Bantoto and Dy (2013) reported that *Padina minor* ethanol extract showed a significant larvicidal activity with cent percent larval mortality (LC_{50} 50.80mg/mL) in *Aedes aegypti* suggesting terpenoid presence responsible for larvicidal action. *Padina australis* methanol, hexane, chloroform and aqueous extracts exhibited LC_{50} values of 400.46, 1029.47, 340.90

and 652.79µg/mL respectively against *Aedes aegypti* (Yu *et al*., 2015); its methanol extract exhibited LC_{50} values of 500.46 and 458.44µg/mL against *Aedes aegypti* and *Aedes albopictus* respectively (Ahmad *et al*., 2016), and possessed inhibition effect of acetylcholinesterase against *Aedes aegypti* and *Aedes albopictus* (Yu *et al*., 2020).

The bioefficacy of seaweed depends on its crude solvent extract, as solvents of different polarities extract different chemical constituents (Tiwari *et al*., 2011). Solvents with high polarity index extracts polar molecules. Chloroform, acetone, methanol, and ethanol extracts of *Padina gymnospora* displayed the presence of alkaloids, amino acids, anthraquinones, carbohydrates, coumarins, flavonoids, glycosides, phenolics, phenols, proteins, quinines, saponins, steroids, tannins and terpenoids (Kamenarska *et al*., 2002; El Shoubaky *et al*., 2014; Pradeep and Thatheyus, 2019; Grace *et al*., 2021; Rahman *et al*., 2021), and in the present study, one or more of these chemical constituents were present, which would have independently or jointly been responsible for larvicidal activity. Species of *Padina* exhibited mosquito larvicidal activity as they are prolific producers of secondary metabolites like phenolics, sterols, terpenes, terpenoids, fatty acids (Schnitzler *et al*., 2001; Zubia *et al*., 2008; De Paula *et al*., 2011; El Shoubaky and Salem, 2014), and polyphenolic compounds which retarded the growth rate of *Culex pipiens* larvae (Elbanna and Hegazi, 2011), and a specific type of tannin called phlorotannin (Zubia *et al*., 2008), which showed potential larvicidal activity, and exhibited LC_{50} values ranging from 0.0683 to 85.11µg/mL against *Aedes aegypti* and *Culex quinquefasciatus*, respectively (Thangam and Kathiresan, 1991; Beula *et al*., 2011; Manilal *et al*., 2011).

In the present study, the larvicidal effect caused by *Padina gymnospora* extracts would have been due to the rupture of treated larval midgut (Nagaraj and Osborne, 2014). The midgut of insect plays a vital role in the secretion of digestive enzymes and absorption of nutrients. The severe damage of midgut cells is suggested to disrupt function of midgut, leading to the death of larvae as reported by Yu *et al*. (2015). Further, the abnormal behaviour of treated larvae in the present study might be due to the effect of extracts that affect the neuromuscular coordination in chemical synapses (Warikoo and Kumar, 2013). The behavioral observation of the treated mosquito larvae in the present study also indicated the correlation of seaweed extract in affecting the nervous system and motor coordination of treated larvae. Manilal *et al*. (2011) described the abnormal before-death-behavior of *Culex quinquefasciatus* and *Aedes aegypti* larvae when treated with brown seaweed *Lobophora variegata*. Besides the killing effect, the insecticidal compounds of seaweeds are proven to influence the metabolism of mosquito larvae in a wide range of diverse ways, such as through toxicity, mortality, growth and development. This was proved in the study by Elbanna and Hegazi (2011), when they observed a longer larval duration for *Culex pipiens* on exposure to *Padina pavonica*. However, this did not occur in the present study.

Brown algae contains a broad spectrum of acid polysaccharides which constitutes alginic, palmitic, uronic and fatty acids acids (Kamenarska *et al*., 2002; Swaminathan *et al*., 2021), and *Padina gymnospora* is dominated by palmitic and oleic acids (Ibraheem *et al*., 2017). Genus *Sargassum* housed under brown seaweeds possess oleic, linoleic, linolenic, palmitic, and stearic acids and their respective methyl esters, were found to affect the metabolism and morphology of midgut of *Culex quinquefasciatus* fourth instar larvae (de Melo *et al*., 2018). The same can be corroborated to the present study, wherein, mortality to the larval instars of *Aedes aegypti* and *Culex quinquefasciatus* could be attributed to the presence of palmitic acid and oleic acid in *Padina gymnospora* extracts, for the reason that these chemical constituents work as stomach poison in mosquito larvae, as they enter the larvae body through the digestive tract, making it easier to be damaged, and functions as a stomach toxin.

5. Conclusion

The present research has highlighted *Padina gymnospora* methanolic extracts insecticidal activity owing to the action of its chemical constituents against the larval instars of *Aedes aegypti* and *Culex quinquefasciatus*. This information serves as fundamental data for further investigation of the active constituents that are responsible for biological action in this study, and on the possible mode of action too.

6. References

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18(2): 265-267. https://doi.org/10.1093/jee/18.2.265a

Abou-Elnaga, Z.S., Alarif, W.M. and Al-Lihaibi, S.S. 2011. New larvicidal acetogenin from the red alga *Laurencia papillosa*. *Clean-Soil Air Water*, 39(8): 787-794. https://doi.org/10.1002/clen.201000597

Ahmad, R., Yu, K., Wong, C. and Jantan, I. 2016. Larvicidal and adulticidal activity of Malaysian seaweeds against *Aedes aegypti* (L.) and *Aedes albopictus* Skuse (Diptera: Culicidae). *Southeast Asian Journal of Tropical Medicine and Public Health*, 47(4): 719-730. https://www.tm.mahidol.ac.th/seameo/2016-47-4/17-IMR0216p719.pdf

Alarif, W.M., Abou-Elnaga, Z.S., Ayyad, S.E. and Al-Lihaibi, S.S. 2010. Insecticidal metabolites from the green alga *Caulerpa racemosa*. *Clean-Soil Air Water*, 38(5-6): 548-557. https://doi.org/10.1002/clen.201000033

Ana, R., Michéle, B., Francesco, B., Atle, B.M., Leonor, C.M., Jens, C., Maria, C.F., Marta, C., Jerónimo, C., Meltem, C.D., Mercedes, C., Thanos, D., Irem, D., Ana, D.R., Dragana, D., Arita, D., Christine, E., Hjörleifur, E., Ayşegül, E., Tufan, E.O., David, E., Stefano, F., Richard, F.J., Laura, G.M., Susana, G.P., Marija, G.U., Nadica, I.D., Rósa, J., Marija, K., Katja, K., Jonne, K., Leila, K., Zrinka, L., Lada, L.B., Manolis, M., Alexia, M., Inga, M., Hanna, M., Mohamed, M., Laurentius, N.S., Lucie, N., Donata, O., Giuseppe, P., Praveen, R., Céline, R., Thorsten, R., Fernando, R., Baruch, R., Johan, R., Eric, R., Vita, R., Jerica, S., Ivo, S., Siret, T., Deniz, T., Xenia, T.S., Olivier, T.P., Anna, T., Cristina, V.G. and Marlen, V.I. 2021. The essentials of marine biotechnology. *Frontiers in Marine Science*, 16: 158. https://doi.org/10.3389/fmars.2021.629629

Aneha, K., Padmanaban, H., Bora, B., Sivaprakasam, M., Gangmei, K., Lukose, J., Vijayakumar, A., Mandodan, S., Ashokkumar, M., Krishnamoorthy, V. and Subbiah, P. 2022. A review on biological mosquito control measures-past, present and future. *World Journal of Advanced Research and Reviews*, 16(1): 302-310. https://doi.org/10.30574/wjarr.2022.16.1.1032

Ansari, A.A., Ghanem, S.M. and Naeem, M. 2019. Brown alga *Padina*: A review. *International Journal of Botany Studies*, 4(1): 1-3. http://www.botanyjournals.com/archives/2019/vol4/issue1/3-6-24

Asaraja, A. and Sahayaraj, K. 2013. Screening of insecticidal activity of brown macroalgal extracts against *Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae). *Journal of Biopesticides*, 6(2):193-203. http://www.jbiopest.com/users/lw8/efiles/vol_6_2_193-203.pdf

Baliano, A.P., Pimentel, E.F., Buzin, A.R., Vieira, T.Z., Romão, W., Tose, L.V., Lenz, D., de Andrade, T.U., Fronza, M., Kondratyuk, T.P. and Endringer, D.C. 2016. Brown seaweed *Padina gymnospora* is a prominent natural wound-care product. *Revista Brasileira de Farmacognosia*, 26: 714-719. https://doi.org/10.1016/j.bjp.2016.07.003

Bantoto, V. and Dy, D. 2013. The larvicidal activity of brown algae *Padina minor* (Yamada 1925) and *Dicyota linearis* (Greville 1830) against the dengue vector, *Aedes aegypti* (Linn 1762) (Diptera: Culicidae). *Journal of Vector Borne Diseases*, 50(1): 68-70. http://www.mrcindia.org/journal/issues/501068.pdf

Bipin, Arivarasu, L., Sivaperumal, P. and Thangavelu, L. 2021. Antioxidant potential from *Padina gymnospora* seaweed methanolic extracts. *Journal of Complementary Medicine Research*, 12(3): 40-46. doi:10.5455/jcmr.2021.12.03.05

Bixler, H.J. and Porse, H. 2011. A decade of change in the seaweed hydrocolloids industry. *Journal of Applied Phycology*, 23(3): 321-335. https://doi.org/10.1007/s10811-010-9529-3

Chellappan, D.K., Chellian, J., Leong, J.Q., Liaw, Y.Y., Gupta, G., Dua, K., Kunnath, A.P. and Palaniveloo, K. 2020. Biological and therapeutic potential of the edible brown marine seaweed *Padina australis* and their pharmacological mechanisms. *Journal of Tropical Biology and Conservation*, 17: 251-271. https://doi.org/10.51200/jtbc.v17i.2667

Dhargalkar, V.K. and Kavlekar, D. 2004. Seaweeds - a field manual. (1st edn). National Institute of Oceanography, Goa, India.

de Melo, A.R., Garcia, I.J.P., Serrão, J.E., Santos, H.L., dos Santos, L.A.R. and Alves, S.N. 2018. Toxicity of different fatty acids and methyl esters on *Culex quinquefasciatus* larvae. *Ecotoxicology and Environmental Safety*, 154: 1-5. https://doi.org/10.1016/j.ecoenv.2018.02.009

De Paula, J.C., Vallim, M.A. and Teixeira, V.L. 2011. What are and where are the bioactive terpenoids metabolites from Dictyotaceae (Phaeophyceae). *Revista Brasileira de Farmacognosia*, 21(2): 216-228. https://doi.org/10.1590/S0102-695X2011005000079

El Shoubaky, G.A. and Salem, E.A. 2014. Terpenes and sterols composition of marine brown algae *Padina pavonica* (Dictyotales) and *Hormophysa triquetra* (Fucales). *International Journal of Pharmacognosy and Phytochemical Research*, 6(4): 894-900. http://impactfactor.org/PDF/IJPPR/6/IJPPR,Vol6,Issue4,Article38.pdf

Elbanna, S.M. and Hegazi, M.M. 2011. Screening of some seaweeds species from South Sinai, Red sea as potential bioinsecticides against mosquito larvae, *Culex pipiens*. *Egyptian Academic Journal of Biological Sciences*, 4(2): 21-30.

Galal, H.R.M., Salem, W.M. and El-Deen, F.N. 2011. Biological control of some pathogenic fungi using marine algae extracts. *Research Journal of Microbiology*, 6(8): 645-657. https://scialert.net/abstract/?doi=jm.2011.645.657

Ganesapandian, S. and Kumaraguru, A.K. 2008. Seaweeds resource in the intertidal and subtidal regions of Pudumadam, Gulf of Mannar. *Seaweed Research and Utilization*, 30: 97-105.

Geraldino, P.J.L., Liao, L.M. and Boo, S.M. 2005. Morphological study of the marine algal genus *Padina* (Dictyotales, Phaeophyceae) from Southern Philippines: 3 species new to Philippines. *Algae*, 20(2): 99-112. https://doi.org/10.4490/algae.2005.20.2.099

Grace, M.P., Rajithra, M.R., Ilakkiya, V. and Dhanalakshmi, B. 2021. Qualitative analysis of phytochemical studies on *Sargassum wightii* and *Padina gymnospora*. *International Journal of Research and Development*, 6(5): 161-175. https://doi.org/10.36713/epra6784

Guedes, E.A.C., Carvalho, C.M.D., Junior, R., Lisboa, K.A., Ribeiro, L.T.F., de Barros, L.D. and Sant'Ana, G.A.E. 2014. Larvicidal activity against *Aedes aegypti* and molluscicidal activity against *Biomphalaria glabrata* of Brazilian marine algae. *Journal of Parasitology Research*, 501328. https://doi.org/10.1155/2014/501328

Gupta, S. and Abu-Ghannam, N. 2011. Bioactive potential and possible health effects of edible brown seaweeds. *Trends in Food Science & Technology*, 22(6): 315-326. https://doi.org/10.1016/j.tifs.2011.03.011

Harborne, J.B. 1998. Phytochemical methods: A guide to modern techniques of plant analysis. 3rd Edition. Chapman & Hall, London.

Hira, K., Tariq, R.M., Sultana, V., Ara, J. and Ehteshamul-Haque, S. 2017. Effect of seaweeds occurring at Karachi coast on mosquito larvae and liver function in rats. *Pakistan Journal of Pharmaceutical Sciences*, 30(2): 387-391. https://pubmed.ncbi.nlm.nih.gov/28649061/

Ibraheem, B.M.I., Hamed, S.M., Elrhman, A.A.A., Farag, M.F. and Abdel-Raouf, N. 2017. Antimicrobial activities of some brown macroalgae against some soil borne plant pathogens and *in vivo* management of *Solanum melongena* root diseases. *Australian Journal of Basic and Applied Sciences*, 11(5): 157-168. http://www.ajbasweb.com/old/ajbas/2017/April/157-168.pdf

Kamenarska, Z., Gasic, M., Zlatovic, M., Rasovic, A., Sladic, D., Kljajic, Z., Stefanov, K., Seizova, K., Najdenski, H., Kujumgiev, A., Tsvetkova, I. and Popov, S. 2002. Chemical composition of the brown alga *Padina pavonica* (L.) Gaill. from the Adriatic sea. *Botanica Marina*, 45(4): 339-345. https://doi.org/10.1515/BOT.2002.034

Manilal, A., Thajuddin, N., Selvin, J., Idhayadhulla, A., Kumar, R.S. and Sujith, S. 2011. *In vitro* mosquito larvicidal activity of marine algae against the human vectors, *Culex quinquefasciatus* (Say) and *Aedes aegypti* (Linnaeus) (Diptera: Culicidae). *International Journal of Zoological Research*, 7(3): 272-278. https://docsdrive.com/pdfs/academicjournals/ijzr/2011/272-278.pdf

Nagaraj, S.R. and Osborne, J.W. 2014. Bioactive compounds from *Caulerpara racemosa* as a potent larvicidal and antibacterial agent. *Frontiers in Biology*, 9: 300-305. https://doi.org/10.1007/s11515-014-1312-4

Pradeep, M.A. and Thatheyus, A.J. 2019. Screening phytochemical constituency profiling and antimicrobial activity of brown seaweed, *Padina gymnospora* extracts against chosen isolates. *Journal of Emerging Technologies and Innovative Research*, 6(6): 799-806. http://www.jetir.org/papers/JETIR1908D02.pdf

Priya, D.N., Raguraman, S., Bhuvaneswari, K., Lakshmanan, A. and Kumar, C.K. 2022. Comparative toxicity of aqueous and methanolic extracts of brown macroalgae against tobacco cutworm, *Spodoptera litura* Fabricius. *Journal of Current Crop Science and Technology*, 109(4-6): 1-8. https://doi.org/10.29321/MAJ.10.000620

Pucazhendi, N., Pucazhendi, S., Vaitheeswaran, M., Shanmucasundaram, R. and Lakshmanan, C. 1995. Biologically active saltern and marine algal extracts for control of *Culex quinquefasciatus* larvae. *Pestology*, 19(3): 291-301.

Rahman, M.F., Alim, M.A., Ahsan, T., Islam, T., Alam, M.M. and Hossain, M.N. 2021. Screening of potential bioactive compounds from *Padina gymnospora* found in the coast of St. Martin island of Bangladesh. *Journal of Marine Biology and Aquaculture*, 6(1): 1-7. DOI: 10.15436/2381-0750.21.3010

Rajagopal, G., Manivannan, N., Sundararajan, M., Kumar, A.G., Senthilkumar, S., Mathivanan, N. and Ilango, S. 2021. Biocompatibility assessment of silver chloride nanoparticles derived from *Padina gymnospora* and its therapeutic potential. *Nano Express*, 2(1): 010010. DOI 10.1088/2632-959X/abd965

Rajakani, M., Muthulingam, M. and Karuppasamy, R. 2018. Hepatichypolipidemic potential of seaweed, *Padina gymnospora* (Kutzing) on streptozotocin induced diabetes in male albino Wistar rats. *International Journal of Pharmacy and Biological Sciences*, 8(4): 845-850. https://www.ijpbs.com/ijpbsadmin/upload/ijpbs_5c264054ce829.pdf

Rao, S.G. 2012. Common seaweeds and seagrasses of India. Herbarium Volume 1, Central Marine Fisheries Research Institute, Kerala. India.

Beula, J.M., Ravikumar, S. and Ali, M.S. 2011. Mosquito larvicidal efficacy of seaweed extracts against dengue vector of *Aedes aegypti*. *Asian Pacific Journal of Tropical Biomedicine*, 1(2): S143-S146. https://doi.org/10.1016/S2221-1691(11)60143-3

Sahayaraj, K. and Kalidas, S. 2011. Evaluation of nymphicidal and ovicidal effect of a seaweed, *Padina pavonica* (Linn.) (Phaeophyceae) on cotton pest, *Dysdercus cingulatus* (Fab.). *Indian Journal of Geo-Marine Sciences*, 40(1): 125-129. http://14.139.47.50/bitstream/123456789/11381/1/IJMS%2040%281%29%20125-129.pdf

Salem, W.M., Galal, H. and El-deen, N.F. 2011. Screening for antibacterial activities in some marine algae from the Red sea (Hurghada, Egypt). *African Journal of Microbiology Research*, 5(15): 2160-2167. https://academicjournals.org/article/article1380122967_Salem%20et%20al.pdf

Schnitzler, I., Pohnert, G., Hay, M. and Boland, W. 2001. Chemical defense of brown algae (*Dictyopteris* spp.) against the herbivorous amphipod *Ampithoe longimana*. *Oecologia*, 126(4): 515-521. https://doi.org/10.1007/s004420000546

Sekar, D. and Kolanjinathan, K. 2015. Antibacterial activity of marine macroalgae *Padina gymnospora* and *Turbinaria conoides* collected from Mandapam coast of Tamilnadu, India. *International Journal of Advanced Research in Biological Sciences*, 2(8): 146-152. https://ijarbs.com/pdfcopy/aug2015/ijarbs19.pdf

Selvin, J. and Lipton, A.P. 2004. Biopotentials of *Ulva fasciata* and *Hypnea musciformis* collected from the peninsular coast of India. *Journal of Marine Science and Technology*, 12(1): 1-6. http://eprints.cmfri.org.in/6133/1/522.pdf

Shameemrani K. 2018. Efficacy of *Aedes aegypti* and *Culex quinquefasciatus* against *Padina gymnospora* and *Caulerpa racemosa*. *International Journal of Recent Scientific Research*, 9(8): 28656-28661. https://www.recentscientific.com/sites/default/files/11730-A-2018.pdf

Shiny, P.J., Mukherjee, A. and Chandrasekaran, N. 2013. Marine algae mediated synthesis of the silver nanoparticles and its antibacterial efficiency. *International Journal of Pharmacy and Pharmaceutical Sciences*, 5: 239-241. https://innovareacademics.in/journal/ijpps/Vol5Issue2/6523.pdf

Silva, T.M.A., Alves, L.G., De Queiroz, K.C.S., Santos, M.G.L., Marques, C.T., Chavante, S.F., Rocha, H.A.O. and Leite, E.L. 2005. Partial characterization and anticoagulant activity of a heterofucan from the brown seaweed *Padina gymnospora*. *Brazilian Journal of Medical and Biological Research*, 38(4): 523-533. https://doi.org/10.1590/S0100-879X2005000400005

SPSS. 2021. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp.

Subramanian, G. and Ravi, P. 2020. Determination of antioxidant potential of *Padina gymnospora* algal extract. *International Journal of Botany Studies*, 5(6): 603-606. http://www.botanyjournals.com/archives/2020/vol5/issue6/5-6-109

Swaminathan, R., Ali, M.S., Anuradha, V., Abinaya, R., Ananthalakshmi, J.S. and Yogananth, N. 2021. Antioxidant potential of fucose isolated from the marine macroalgae *Padina gymnospora*. *Bioscience Biotechnology Research Communications*, 14(3): 1302-1308. http://dx.doi.org/10.21786/bbrc/14.3.59

Swaminathan, R., Ali, M.S., Anuradha, V., Yogananth, N. and Saravanan, G.K. 2022. Antiviral effects of L-fucose from *Padina gymnospora* against various strains of dengue virus. *Journal of Advanced Scientific Research*, 13(4): 111-117. https://doi.org/10.55218/JASR.202213419

Thamizharasan, S. and Saravanan, N.A. 2017. Antimicrobials compounds synthesized from marine resources with special reference to *Padina gymnospora*. *International Journal of Zoology and Applied Biosciences*, 2(4): 162-165. https://doi.org/10.5281/zenodo.1311961

Thangam, T.S. and Kathiresan, K. 1991. Mosquito larvicidal effect of seaweed extracts. *Botanica Marina*, 34: 433-435. https://doi.org/10.1515/9783112328101-053

Tiwari, P., Kumar, B., Kaur, M., Kaur, G. and Kaur, H. 2011. Phytochemical screening and extraction: A review. *Internationale Pharmaceutica Sciencia*, 1(1): 98-106.

Vogel, A.L. (1989). Text book of practical organic chemistry. 5th Edition. London: The English Language Book Society and Longman.

Warikoo, R. and Kumar, S. 2013. Impact of *Argemone mexicana* extracts on the cidal, morphological, and behavioral response of dengue vector, *Aedes aegypti* L. (Diptera: Culicidae). *Parasitology Research*, 112: 3477-3484. https://doi.org/10.1007/s00436-013-3528-7

World Health Organization. 2005. Guidelines for laboratory and field testing of mosquito larvicides. WHO, Geneva, WHO/CDS/WHOPES/GCDPP/13 https://www.who.int/publications/i/item/WHO-CDS-WHOPES-GCDPP-2005.13

Yu, K., Ahmad, R., Wong, C. and Jantan, I. 2020. Mosquito larvicidal potential of tropical seaweeds: Acetylcholinesterase inhibitory effects of *Bryopsis pennata*, *Padina australis* and *Sargassum binderi* on *Aedes aegypti* (L.) and *Aedes albopictus* Skuse. *Malaysian Journal of Medicine and Health Sciences*, 16(Supp1): 125-130. http://medic.upm.edu.my/upload/dokumen/2020010215070220_MJMHS_0240.pdf

Yu, K., Wong, C., Ahmad, R. and Jantan, I. 2015. Larvicidal activity, inhibition effect on development, histopathological alteration and morphological aberration induced by seaweed extracts in *Aedes aegypti* (Diptera: Culicidae). *Asian Pacific Journal of Tropical Medicine*, 8(12): 1006-1012. https://doi.org/10.1016/j.apjtm.2015.11.011

Yu, K.X., Jantan, I., Ahmad, R. and Wong, C.L. 2014. The major bioactive components of seaweeds and their mosquitocidal potential. *Parasitology Research*, 113: 3121-3141. https://doi.org/10.1007/s00436-014-4068-5

Zubia, M., Payri, C. and Deslandes, E. 2008. Alginate, mannitol, phenolic compounds and biological activities of two range-extending brown algae, *Sargassum mangarevense* and *Turbinaria ornata* (Phaeophyta: Fucales), from Tahiti (French Polynesia). *Journal of Applied Phycology*, 20(6): 1033-1043. https://doi.org/10.1007/s10811-007-9303-3