Effect of seaweeds occurring at Karachi coast on mosquito larvae and liver function in rats

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Abstract: Seaweeds have been consumed as human food from thousands of years. In this study ethanol extract of 16 different seaweeds were tested for mosquito larvicidal activity against 4th instar larvae of *Aedes aegyptii*. The ethanol extracts of *Padina pavonia* and *Sargassum ilicifolium* caused 50% mortality at 1200ppm concentration. However other seaweeds *Halimeda tuna*, *Ulva lactuca* (Chlorophyta), *Dictyota dichotoma* var *intricata*, *Jolyna laminariodes*, *Sargassum binderi* (Phaeophyta), *Melanothamnus afaqhusainii* and *Solieria robusta* (Rhodophyta) showed LC50 at ≈1500 ppm concentration. The n-hexane fraction of *Padina pavonia* was most potent and produced lethality at minimum concentration (LC50 at 250ppm).The effect of ethanol and water extracts of *S. binderii* was also examined on liver function of healthy rats. The ethanol extract of *Sargassum binderi* given orally to rats @ 200mg/kg for 14 days slightly increased the concentration of liver enzymes (ALT, AST, ALP and LDH) and urea level as compared with normal control rats, but did not increase bilirubin, glucose, triglycerides, cholesterol and creatinine. Whereas water extract of *S. binderi* affected ALT while other biochemical parameters were near normal or slightly decreased as compared to normal control.

Keywords: Seaweeds, larvicidal, *Aedes aegyptii*, ethanol extract, liver function, rats.

INTRODUCTION

Research and utilization of marine algae have been increased markedly from last several decades (Jimenez-Escrig and Goni, 1999; Jimenez-Escrig and Sanchez-Muniz, 2000; Mayer and Lehmann, 2001). More than 200 seaweeds are utilized commercially world-wide of which 65% are used as human food (Zemek-White and Ohno, 1999). The most common seaweeds used as food include brown algae such as *Laminaria*, *Undaria, Sargassum fusiformis (*Hijiki*)* and *Porphyra*. Seaweeds are the rich source of dietary fiber, proteins, polysaccharides, vitamins, minerals and trace elements as well as minor compounds such as phytosterols (Bouzidi *et al.,* 2008; Jimenez-Escrig and Goni, 1999: Ruperez and Saura-Calixto, 2001). Algal fat content is low but consist of *n*-3 fatty acids (Jeong *et al.,* 1993) whereas, dietary fiber differ chemically from those of land plants (Jimenez-Escrig and Sanchez-Muniz, 2000).

Seaweeds have also been used as drugs or drug sources over a large number of years going back into folk medicine. Various biological activities including cytotoxic (Ara *et al*., 1999; Ayesha *et al*., 2010), hypolipidaemic (Ruqqia *et al.,* 2015), antioxidant (Tariq *et al.,* 2011), antimicrobial (Ara *et al*., 2002) and mosquito larvicidal (Hira *et al*., 2010) have been reported from Karachi coast. Since consumption of seaweeds as human food or animal feeds is increasing rapidly, it is necessary to evaluate the toxic effect of seaweeds, before it is consumed as food. The present report is in continuation of our previous report (Hira *et al*., 2010) and describes the mosquito larvicidal effect of some new seaweeds occurring at Karachi coast. The report also describes the impact of seaweed extracts on liver function in rats.

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MATERIALS AND METHODS

Collection of seaweeds

Sixteen seaweed species, belonging to Chlorophyta, Phaeophyta and Rhodophyta were collected from the coast of Karachi (Buleji) at low tide (table 1) and washed thoroughly in laboratory under tap water, followed by air drying under shade and grinded in order to make fine powder. Herbarium sheets of each seaweed were also prepared along with formalin fixed samples. The powered seaweeds were stored in polyethylene bags at room temperature until used.

Extraction

Each seaweed (500g) were soaked in ethanol (2L), separately for a week. The extracts were pooled and filtered through cotton wool, concentrated to semisolid state, solvent refluxed and used for extraction 3 times on rotary vacuum evaporator at 35ºC. Water extract was prepared after vigorous shaking of 100g of each seaweed species with distilled water using magnetic stirrer for 1 hour. The filtrate was pooled and lyophilized and dry powder was stored at -20ºC.

Table 1: Mosquito larvicidal effect of ethanol extract of seaweeds

S.#	Seaweeds	500 ppm	1000 ppm	1200 Ppm	1400 ppm	1500 ppm	2000 ppm	2500 ppm	3000 ppm	LC_{50}
	Control (water)	0	0	0	0	0	0	0	0	--
	Control (ethanol)	0	0	0	0	0	0	0	0	--
Chlorophyta										
1	*Caulerpa chemnitzia*	0	5	10	15	20	30	50	100	2500
2	*Caulerpa scalpelliformis*	5	15	20	30	40	50	70	100	2000
3	*Caulerpa taxifolia*	0	5	10	15	30	60	90	100	1900
4	*Halimeda tuna*	10	20	30	40	50	80	100	---	1500
5	*Ulva fasciata*	0	10	20	30	40	60	70	80	1750
6	*Ulva lactuca*	20	30	40	50	65	80	100	---	1400
Phaeophyta										
7	*Dictyota dichotoma var intricata*	10	20	30	40	50	80	100	---	1500
8	*Jolyna laminariodes*	10	20	30	40	50	80	100	---	1500
9	*Padina pavonia*	15	35	50	60	75	90	100	---	1200
10	*Sargassum binderi*	5	10	25	35	50	80	100	---	1500
11	*S. ilicifolium*	5	20	50	75	100	---	---	---	1200
12	*S. lanceolatum*	0	5	10	15	20	40	60	100	2250
13	*Spatoglossum variabile*	5	15	20	25	30	50	100	---	2000
Rhodophyta										
14	*Halymenia porphyroides*	5	10	15	20	25	45	70	100	2100
15	*Melanothamnus afaqhusainii*	0	5	15	25	40	70	90	100	1600
16	*Solieria robusta*	10	15	30	50	65	80	100	---	1400

Table 2: Mosquito larvicidal effect of solvent fractions of ethanol extract of seaweeds

S.#	Seaweeds	125 ppm	250 ppm	500 ppm	750 ppm	1000 ppm	1250 ppm	1500 ppm	3000 ppm	6000 ppm	LC50 ppm
	Control (water)	0	0	0	0	0	0	0	0	0	
	Control (ethanol)	0	0	0	0	0	0	0	0	0	
1	*Ulva lactuca*										
i	Hexane	--	--	--	--	--	--	--	--	--	--
ii	Chloroform	10	30	50	70	100	---	---	---	---	500
iii	Methanol	10	30	50	80	100	---	---	---	---	500
2	*Padina pavonia*										
i	Hexane	30	50	80	100	---	---	---	---	---	250
ii	Chloroform	0	10	25	35	40	50	70	90	100	1250
iii	Methanol	0	0	0	5	10	15	20	50	100	3000
3	*Sargassum ilicifolium*										
i	Hexane	10	20	40	70	90	100	---	---	---	600
ii	Chloroform	10	25	45	65	90	100	---	---	---	560
iii	Methanol	0	0	10	20	30	40	50	80	100	1500
4	*Solieria robusta*										
i	Hexane	10	30	50	70	90	100	---	---	---	500
ii	Chloroform	10	20	35	50	70	90	100	---	---	750
iii	Methanol	0	0	0	0	0	0	0	10	20	--

Fractionation of seaweeds

A portion (20gm) of ethanol extract of *Ulva lactuca*, *Padina pavonia*, *Sargassum ilicifolium* and *Solieria robusta* was dissolved in hexane in separating funnel. The hexane soluble fraction was separated and residual fraction was dissolved in chloroform, the chloroform soluble fraction was separated and insoluble portion was finally mixed with methanol and separated. All three fractionated portions were concentrated using rotary vacuum evaporator at 35ºC and stored in airtight vials at room temperature.

Table 3a: Effect of ethanol extract of *Sargassum binderi* on liver/cardiac enzymes and bilirubin in normal rats

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	LDH (U/L)	Bilirubin Total (mg/dl)	Bilirubin Direct (mg/dl)
Normal control	27.6^{b} ±2.5	85^{b} ±7	50.5^{b} ±2.3	322.6^{b} ±21.6	0.46^{a} ±0.059	0.166^{a} ±0
S. binderi	31.6^{ab} ±2.8	104.3^{a} ±4.5	61^{a} ±0.54	353.3^{a} ±15	0.53^{a} ±0.057	0.2^{a} ±0
Normal range (Johnson-Delaney, 1996)	17.5-30.2	45.7-80.8	56.8-128	61.0-121	0.2-0.55	--

Table 3b: Effect of ethanol extract of *Sargassum binderi* on blood glucose, lipid profile and kidney markers in normal rats

Groups	Glucose (mg/dl)	Triglycerides (mg/dl)	Total Cholesterol (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)
Normal control	113.6^{a}±4	101.3^{a} ±4.1	91.3^{a} ±1.5	0.9^{a} ±0	26.3^{b} ±2
S. binderi	112^{a} ±6	95.6^{b}±3	90.3^{a}±4.6	0.86^{a} ±0.05	32.6^{a} ±2
Normal range (Johnson-Delaney, 1996)	50-135	26-145	40-130	0.2-0.8	15-21

Table 4a: Effect of water extract of *Sargassum binderi* on liver/cardiac enzymes and bilirubin in normal rats

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	LDH (U/L)	Bilirubin Total (mg/dl)	Bilirubin direct (mg/dl)
Normal control	28^{b}±2	93.3^{a}±4	63.3^{ab}±5	238.3^{a}±36	0.56^{a}±0.051	0.168^{a}±0
S.binderi	41^{a}±1	98.6^{a}±7.3	70.6^{a}±1.5	240^{a}±6	0.53^{a}±0.059#	0.16^{a}±0.057

Table 4b: Effect of water extract of *Sargassum binderi* on blood glucose, lipid profile and kidney markers in normal rats

Groups	Glucose (mg/dl)	Triglycerides (mg/dl)	Total Cholesterol (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)
Normal control	115^{a}±2.6	79.3^{ab} ±2.5	67.3^{b} ±0.57	0.83^{b} ±0.051	34.6^{b} ±1.5
S. binderi	94.3^{b} ±4	80.3^{a}±1.5	75.3^{a}±3	0.96^{a} ±0.05	39.6^{a} ±3.2

The values having the same superscript within the column are not significantly ($p<0.05$) different according to Duncan's multiple range test.

Larvicidal activity

The mosquito larvacidal activity of seaweeds was determined following the method of Hira *et al.,* (2010), where young fourth instar larvae of *Aedes aegypti* were transferred in to 50mL beaker containing 20mL of tap water. A dosages of 0.05mL, 0.1mL, 0.2mL, 0.3mL, 0.4mL, 0.5mL, 0.6mL, 0.8mL, 1.0mL, 1.2mL, 2.4mL of ethanol extract of seaweed were transferred to 20 ml of water to give a concentrations of 125ppm, 250ppm, 500ppm, 750ppm, 1000ppm, 1250ppm, 1500ppm, 2000ppm, 2500ppm, 3000ppm and 6000ppm. The control (only water) and (only ethanol) were also prepared for comparison. Each experiment was repeated 5 times with three replicates. LC_{50} was calculated according to Abbot's formula (1925).

Effect of seaweed extracts on liver function

Healthy male albino rats of *Wistar* strain (140-170gm), purchased from Dow University of Health Sciences, Karachi were housed in prebedded polyethylene cages (3 rats/cage) with standard laboratory conditions (12 light/dark cycle and 25±2°C with relative humidity), fed with standard pellet diet and had free access to water. All the animals were kept in laboratory for 1 week and then randomly divided into two groups of 6 rats in each group. Group 1 (normal control group) were given distilled water (orally) daily for 14 days. While Group 2 rats were given ethanol extract of *Sargassum binderi* (EESB) orally @ 200mg/kg, b.w. daily for 14 days. On day fifteen all the animals were fasted overnight, decapitated and serum was collected for biochemical testing. In another similar experiment rats were given water extract of *S. binderi* (WESB) instead of ethanol extract at same dose. All experimental details were same as mentioned for ethanol extract.

Biochemical analysis

Serum samples were analyzed for aspartate aminotransferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), urea, creatinine, bilirubin (total and direct), glucose, cholesterol and triglycerides by using kits from Merck (France) and Ecoline (Germany).

RESULTS

Effect of seaweed on mosquito larvae

Out of sixteen seaweed viz., *Caulerpa chemnitzia*, *C. scalpelliformis*, *C. taxifolia*, *Halimeda tuna*, *Ulva fasciata* and *U. lactuca* (green), *Dictyota dichotoma var intricate*, *Jolyna laminariodes*, *Padina pavonia*, *Sargassum binderi*, *S.ilicifolium*, *S.lanceolatum* and *Spatoglossum variabile* (brown), *Halymenia porphyroides, Melanothamnus afaqhusainii* and *Solieria robusta* (red) tested for mosquito larvicidal effect, ethanol extract of two green species showed mortality of larvae at higher concentration with LC50 >1500ppm. On contrary the ethanolic extracts of most brown seaweeds showed good activity against the 4th instar larvae of *Aedes aegyptii*. Ethanol extract of brown species *P. pavonia* and *S. ilicifolium* showed larvicidal activity at minimum concentration of 1200ppm. While a red seaweed *S. robusta* showed LC50 at 1400 ppm concentration (table 1).

Among solvent fractions highest mortality was found in n-hexane fraction of *P. pavonia* with LC_{50} at 250 ppm. The larvicidal activity of chloroform and methanol fractions of *U. lactuca* and hexane extract of *S. robusta* was found more than 50% at 500ppm concentration, followed by chloroform, n-hexane extracts of *S. ilicifolium* and chloroform extract of *S. robusta* with LC_{50} at 560 & 600 and 750ppm concentration respectively (table 2).

Impact of seaweed extracts on liver function in rats

The ethanol extract of *S. binderi* slightly increased the concentration of liver enzymes (AST and LDH) and urea level in serum when administered to rats for 14 days, as compared with normal control rats. While it did not affect bilirubin (total & direct), glucose, triglycerides, cholesterol and creatinine levels when compared with normal control group (table 3a, 3b). Whereas water extract of *S. binderi* increased the ALT concentration upto 41±1 as compared to 28 in normal group, with no significant effect on AST, ALP and LDH levels as compared to control. The other biochemical parameters, bilirubin (total & direct), triglycerides level were also found near to normal, but creatinine and urea levels were found slightly elevated in rats pretreated with water extract of *S. binderi* (table 4a, 4b).

DISCUSSION

The marine environment is incomparable reservoir of bioactive natural products (Samee *et al*., 2009). In the present study ethanol extracts of 16 seaweeds were screened against 4th instar larvae of *Aedes aegyptii*. The crude ethanolic extract of 7 (2 green, 5 brown and one red) showed LC_{50} near to 1500ppm. Bianco *et al*., (2013), reported that out of 15 species tested, dichloromethane and methanol (2:1) extracts of four species showed LC50 at 300 ppm against fourth instar larvae of *A. aegyptii*. Similarly Manilal *et al*. (2011), reported the larvicidal activity against third instar larvae of *Culex pipens.* However in the present study crude extracts of seaweeds showed LC_{50} at higher concentrations. While some solvent fractions caused 50% mortality at low concentration. The n-hexane extract of *Padina pavonia* exhibited 50% lethality at minimum concentration of 250 ppm. Bianco *et al.,* (2013) reported that n-hexane, dichloromethane, ethyl acetate and methanol extracts of red seaweed *Laurencia dendroidea* caused 100% larval mortality against 4th instar larvae of *Aedes aegyptii* at concentrations of 50 ppm but hexane extract was found to be most effective having LC_{50} at 10 ppm. The polyhalogenated monoterpenes, diterpenes, aplysiaterpenoid A and telfairine isolated from seaweeds have been reported to possess insecticidal activity (El Gamal, 2010; Watanabe *et al.,* 1989).

A large number of epidemiological studies have been reported regarding seaweeds consumption and their health benefits (Hiqashi *et al*., 1999; Cassolato *et al*., 2008; Tariq *et al.,* 2011). The seaweeds are known to cause a lowering effect on elevated lipid profile in triton induced hyperlipidaemic rats (Ruqqia *et al.,* 2015), hypoglyceamic effect in alloxan diabetic rats (Akhtar, 2001), hepatoprotective effect in CCl_4 and acetaminophen intoxicated animals (Ross *et al.,* 2012). In the present study administration of ethanol and water extracts of *S. binderi* in normal rats slightly increased AST, ALP and LDH level in serum while normal ranges of bilirubin (total & direct), glucose, triglycerides, cholesterol and creatinine levels were found. Although seaweeds contains various biological activity but their use as dietary supplement needs toxicological study and their effect on healthy rats should be examined before suggesting as food.

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