In vitro effects of Sargassum latifolium (Agardeh, 1948) against selected bacterial pathogens of shrimp

Dashtiannasab A.1*; Kakoolaki S.2; Sharif Rohani M.2 Yeganeh V.1

Received: March 2011 Accepted: June 2011

Abstract

During the last decade, shrimp aquaculture has developed rapidly in many developing countries. Antibiotic resistance is a major problem in shrimp aquaculture. Seaweeds are considered as potent source of bioactive compounds that are able to produce biological activities. The aim of this study was to evaluate the antibacterial activity of ethanolic and chloroformic crude extracts of the brown algae, *Sargassum latifolium* derived from Persian Gulf waters. The extractions against shrimp selective pathogen bacteria including *Vibrio alginolyticus*, *V. parahaemolyticus* and *V. harveyi* were used by disk diffusion agar method. Results revealed that all mentioned bacteria were sensitive to both ethanolic and chloroformic crude extracts of *S. latifolium*. The minimum inhibitory concentration of chloroformic extract of *S. latifolium* against the bacteria was performed by tube dilution technique. MICs were 5, 10 and 10 mg ml⁻¹ for *V. alginolyticus*, *V. parahaemolyticus* and *V. harveyi*, respectively while minimum bactericidal concentrations (MBC) were 5, 15 and 20 mg ml⁻¹. This study recommended that *S.latifolium* isolated from Persian Gulf waters has antibacterial activity potential against shrimp pathogen bacteria.

Keywords: Antibacterial activity, *Sargassum latifolium*, Seaweed extract, *Vibrio*, Persian Gulf

¹⁻Iran Shrimp Research Center, P.O.Box:1374, Bushehr, Iran

²⁻Department of Aquatic Animal Health &Diseases, Iranian Fisheries Research Organization, Tehran-Karaj High way, Sarve Azad Ave. Nation herbarium, Tehran. Iran.

^{*}Corresponding author's email:adashtiannasab@gmail.com

Introduction

During the last decades, shrimp aquaculture has rapidly expanded in many developing countries (FAO, 2006). Shrimp culture has started in Iran in the 1990's in Bushehr and extended to other areas besides the Persian Gulf in southern Iran (Kakoolaki, 1997). Massive mortality has been reported from hatcheries due to diseases (Afsharnasab 2007: et al., Kakoolaki et al., 2011; Pazir et al., 2011). The origination of major shrimp mortalities is virus but the opportunistic bacterial infections also causing serious losses in marine shrimp hatcheries (Lightner and Redman, 1998). Antibiotics and chemicals were being used in some hatcheries without proper scientific investigation. The environmental pollution and development of antibiotic-resistant pathogens were being then (Karunasagar et al., 1994). Antibiotic therapy in shrimp hatcheries and farms has been used for many years. The antibiotic resistant bacteria in shrimp hatcheries and is a worldwide problem farms aquaculture that has caught the attention of many researchers (Tsoumas et al., 1989; McPhearson et al.1991; Song and Sung, 1993; Hektoen et al., 1995; Mahasneh et al., 1995; Herwing et al., 1997; Rahim et al., 1998; Miranda and Zemelman 2002).

It has been reported that luminous strains of *Vibrio harveyi* and *V. splendidus* isolated from shrimp larvae are resistant to some antibiotics such as kanamycin, penicillin, erythromycin and streptomycin (Baticados et al., 1990). The use of oxytetracyclin has caused an increase of bacterial resistance in shrimp farms (Nash et al., 1992) and also increasing resistance

to chloromphenicol has been reported in shrimp hatcheries in Ecuador and the **Philippines** (Baticados and Padihare, 1992). The occurrence of antibiotic resistance of pathogens in aquaculture necessitates new efficient antibiotics to treat maricultured species (Braithwaite and McEvoy 2005; Bansemir et al., 2006). A large variety of seaweeds has been assayed for antimicrobial activity worldwide and has showed different results due to the kind of seaweeds, season and area of collection, solvents and so on. Hornsey and Hide (1974) demonstrated that crude extracts of more than 150 seaweed species have antibacterial activity. Some efforts have been done to find new antibiotics organisms from marine such macroalgae to control bacterial pathogens (Bansemir et al., 2004; Immanuel et al., 2004; Vineela and Elizabeth 2005). Many types of seaweed (macroalgae) have potent defenseactivity against microbial epibionts, e.g. bacteria, viruses and fungi (Hellio et al., 2001; Bansemir et al., 2006). Concerns over human health and environmental safety due to some chemicals have prompted increasing more "natural-green" interest in alternatives such as antibiotics. Seaweeds considered as potent source bioactive compounds that are able to produce different important secondary metabolites described with great biological activities. Researchers have shown that these compounds have cytostatic, antiviral, anthelmintic, antifungal and antibacterial activities (Newman et al., 2003; Toney et al., 2006; Taskin et al., 2007). Srinivasa Rao and Parekh (1981) revealed that crude extracts of seaweeds from India waters were active only against Gram positive bacteria. Toney et al. (2006) examined antibacterial effects of 11 seaweeds from the Mediterranean Sea against E. faecalis, P. aeruginosa, E. coli and yeast and they found that only 6 seaweeds antibacterial effects. But only a few studies were conducted about the effects of mentioned compounds against aquatic (Mahaseneh pathogens et al.. 1995: Imanuel et al., 2004; Chouhury et al., 2005).

The aim of this study was to evaluate the antibacterial activity of a brown seaweed *Sargassum latifolium* obtained from Persian Gulf waters against three shrimp pathogen- bacteria named *V. harveyi*, *V. alginolyticus* and *V. parahaemolyticus*.

Materials and methods

63 Petri dishes including three groups (2 extractions and 1 antibiotic) along with 3 replicates were used against 3 selected bacteria to test the hypothesis of the study. The groups were 1-*Sargassum latifolium* in two form of extractions named ethanolic extraction and chloroformic extraction as three concentrations of 50,200 and 500 mgl⁻¹ 2- oxytetracyclin.

Sargassum latifolium were collected from a marine rocks area of the Persian Gulf coastal line named Alafdoon in north of Bushehr port in southern Iran during low tide November 2008.

Algal samples cleaned of epiphytes, debries and extraneous matters were removed along with the necrotics parts. The surface of algal samples were washed carefully with sea water and then in fresh water. Seaweeds were dried under

a shade for 5 days and cut to small pieces. Afterwards they formed powder in a mixer grinder. 30 g of that mixed in ethanol (300ml) and in chloroform (300ml) separately for 72 hr at room temperature. Then the different extracts were filtered through Whatman filter paper No. 1 and concentrated under vacuum condition on a rotary evaporate at 45°C to get ethanolic and chloroformic crude extracts. They were then stored at -20°C until use.

The *Vibrio spp*. including *V*. harveyi, *V*. parahaemolyticus, and *V*. alginolyticus were used in this study. Bacterial strains were cultured overnight at 30 °C in Triptic Soy agar (TSA, Merk, Germany) with supplement of 2% NaCl (Merk, Germany) at 4°C until use.

The method of disk diffusion was done for screening antibacterial activity of the ethanol-chloroform extracts. The minimal inhibitory concentrations (MICs) of the samples against the test microorganisms were determined by the tube dilution method, and minimum bactericidal concentration (MBC) was assayed according to Fazli et al. (2007).

The agar diffusion method was assayed as described by Murry et al. (1995)previously with some modifications. A 18-hr-old culture loopful isolated from the selected organisms that were previously diluted to 0.5 index of McFarland turbidity standard (in order to produce approximately 1.5×10^8 colony forming units (cfu) per ml) was introduced to Muler Hinton agar (MHA, Merck, Germany) along with a supplement of 2% NaCl (Merck, Germany). The ethanolchloroform extracts were dissolved in DMSO (dimethyl sulfoxide) to perform

different concentrations from 50 mg/ml to They were sterilized 500mg/m. filtration by 0.45 µm Millipore filters and then discs (6 mm in diameter) were impregnated with those and finally placed on the MHA agar plates. Negative controls were prepared using **DMSO** and Oxytetracyclin (20µg/disc) as positive reference standards to determine the sensitivity of each bacterial species. The inoculated plates were incubated at 25 °C for 24 hr. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organisms.

The minimum inhibitory concentration (MIC) of the ethanolchloroform extracts was determined for each of the selected organisms described by Doughary and Manzara (2008) with some modification, at 100, 80, 50, 30, 20, 15, 10 and 5 mg ml⁻¹. To achieve these concentrations, 1 ml of different concentrations of the extracts including double strength of the concentrations (200, 160, 100, 60, 40, 30, 20 and 10 mg ml⁻¹) in a test tube and 1 ml of nutrient broth with a supplement of 2% NaCl (Merck, Germany) were added and then a loopful of the selected organism previously diluted to 0.5 McFarland turbidity standard to produce 1.5×10^8 cfu per ml was introduced to the tubes. The procedure was repeated over the selected organisms using Oxytetracyclin. A tube containing nutrient broth was only used with the selected organisms to serve as negative control. All the tubes were then incubated at 30°C for 24 hr and then examined for growth by screening for The minimum bactericidal turbidity. concentration (MBC) of the ethanolchloroform extracts on the clinical bacterial isolates was performed according to Fazli et al. (2007). Briefly, 0.2 ml bacterial culture was pipetted from the highest dilution tubes that did not show any growth obtained for determination of MIC and subcultured on to MHA and incubated at 30°C for 24 hr. After incubation the concentration at which there was no single colony of bacteria was expressed as MBC (Fazli et al., 2007).

Statistical analysis

Student's *t*-test was applied to calculate the differences of diameter over inhibition zones performed in concentrations of 50 and 200 (mg ml⁻¹) in two forms of extractions (E.E. & C.E. extraction) and ANOVA test was used for the same aim between 3 groups (E.E. extraction & C.E. extraction in concentration of 500 mg ml⁻¹ and Oxytetracyclin) after exposure of each selected bacteria against them. Meanwhile ANOVA test was applied to show the differences of diameter over inhibition performed zones in the same concentrations within each group (E.E. extraction C.E. extraction and Oxytetracyclin) while P-values < 0.05were considered statistically to be significant.

Results

The average diameter of inhibition zone of chloroformic extracts of *S. latifolium* in concentration of 500 mgl⁻¹ was (n:3, 15 \pm 1.3 mm) significantly higher (P \leq 0.05) than that of the same concentration of ethanolic extracts of *S.latifolium* (n:3, 12 \pm 1.2 mm) meanwhile they were significantly lower (p \leq 0.05) than that of

Oxytetracyclin against V. alginolyticus. These results were repeated for these three groups at the same concentration against two other bacteria, V. parahaemolyticus and V.harveyi. The diameter of inhibition zone of chloroformic extracts of S. latifolium in concentration of 500 mgl⁻¹ (n:3, 15± 1.3 mm) against V. alginolyticus was greater than that of same substance against V. parahaemolyticus and V. harveyi (n:3,14±0.08 mm and n:3, 12±1.2 mm. respectively). The index chloroformic extracts of S. latifolium in concentration of 200 mgl⁻¹ (n:3, 10± 0.9

difference had no significant mm) comparison to that of $(P \ge 0.05)$ in ethanolic extracts (n:3, 10± 1.1 mm) V. alginolyticus but showed against significantly greater amounts within groups against V. parahaemolyticus and V. harvevi, respectively (n:3, 8±1.2 mm and 7.8±0.5 mm). The latter data was repeated for concentration of 50 mgl⁻¹ (Table 1). Antibacterial activity of ethanol and chloroform extracts of S. latifolium against the test bacteria are summarized in Table 1.

Table1: Antibacterial activity of ethanol and chloroform extracts of Sargassum latifolium against the selected bacteria

Bacteria	E. E. (mg ml ⁻¹) and diameter I.Z in mm (Mean±SD)			C.E. (mg ml ⁻¹) and diameter I.Z. in mm (Mean±SD)			Oxytetracyclin 20µg/disk
	50	200	500	50	200	500	diameter I.Z in mm (Mean±SD)
V. alginolyticus	^a 7±0.8 ^a	^a 10±1.1 ^a	c12±1.2a	^a 8±1.0 ^a	^a 10±0.9 ^a	^b 15±1.3 ^a	^a 18±0.4 ^a
V. parahaemolyticus	^a 6.2±0.6 ^b	^b 9±0.7 ^b	^c 11±0.9 ^b	^a 5.9±0.4 ^b	^a 8±1.2 ^b	^b 14±0.8 ^b	^a 19.2±0.5 ^b
V.harveyi	^a 5.8±0.5 ^b	^b 9.2±1.1b	^b 12±1.3 ^a	^a 6±1.1 ^b	^a 7.8±0.5 ^b	^b 12±1.2 ^c	^a 19.5±0.5 ^b

E. E: Ethanol extract, C. E: Chloroform extract, I.Z: Inhibition zone

The results also showed that minimum inhibitory concentration of chloroformic extract of *S. latifolium* for *V. alginolyticus*

was 5 mg ml⁻¹ and this amount (MIC) for *V. parahaemolyticus* and *V. harveyi* was 10 mg ml⁻¹ (Table 2).

Table 2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of chloroformic extracts against tested bacteria (mg ml⁻¹)

Test organisms	MIC	MBC
V. alginolyticus	5	5
V. parahaemolyticus	10	15
V. harveyi	10	20

Discussion

In the present in vitro study we observed the potent antibacterial activity of the seaweed; S. latifolium deriving from Persian Gulf waters against shrimp pathogen bacteria that could be applied in shrimp culture industries to control and treat such diseases as a substitute for antibiotics. Both crude ethanolic and chloroformic extracts of S. latifolium inhibited all of the selected bacteria. Highest antibacterial activity ofchloroformic extracts of S. latifolium was presented against alginolyticus and least one of the same solvent has effect against V. parahaemolyticus and V. harveyi, exception of 50 mg/l concentration.

It is revealed that the antibacterial activity of the chloroformic extracts of S. latifolium is more efficient than that of ethanolic extracts against V. alginolyticus and V. parahaemolyticus at concentration of 500 mg/l and caused better halo-zones but no significant difference dealing with the antibacterial effect of two extracts against V. harveyi was observed in the same concentration and showed that V. alginolyticus more sensitive chloroformic extracts of S. latifoliumin at concentration followed by parahaemolyticus and V. harveyi. Some findings about the efficiency of extraction

methods emphasized that methanolic gives higher antimicrobial extraction activity than n-hexane and ethyl acetate (Moreau et al., 1988; Tuney et al., 2006), while others informed that chloroform is better than methanol and benzene (Febles et al., 1995). Freile-Pelegr and Morales (2004) reported that the hexane extract has greater antibacterial activity. However the result showed the antibacterial effect of Oxytetracyclin at 20µg/disk is greater than of two selected extracts that in concentration up to 500 mg ml⁻¹.

According to our results, in contrary to V. parahaemolyticus and V. harveyi, no differences were observed between the effect ofantibacterial inhibition of both ethanolic and chloroformic crude extracts of S. latifolium against V. alginolyticus. The antibacterial effect of both ethanolic and chloroformic crude extracts of S. latifolium against V. parahaemolyticus and V. harveyi at 50 similar minimum mg/l is and concentrations. comparison other to Chellaram et al. (2004) observed that the obtained from extracts acetone and chloroform exhibit higher activity than that of butanol and toluene similar to our study in case of chloroform. It has been reported

that variety of solvents has different solubility for different capacities phytoconstituents (Marjorie, 1999). **Imanoel** et al. (2004)showed antibacterial properties of the n-butanolic extracts of four ayurvedic herbals and two seaweeds Ulva lactuca and wightii against shrimp Sargassum pathogen Vibrio parahaemolyticus. They reported that seaweeds (*U. lactuca and S.* wightii) have a good ability to inhibit shrimp pathogen producing with inhibitory zone 17.5 ± 0.81 and 16.3 ± 0.47 , respectively. These results were approximately similar to our investigation, particular at 500 mg/l concentration. Patar et al. (2008) reveald that the extract of Sargassum sp. was more effective against B. subtilis and E. coli, showing 18 mm and 16 mm zone of inhibition, respectively at 4000 µg100 μ l⁻¹ concentration in comparison to S. aureus showing 10 mm zone of inhibition at the same concentration. Also Choudhury et al. (2005) indicated that alcoholic extracts of marine algae can inhibit V. alginolyticus as a fish pathogen. The results of this work obviously revealed that the macroalgae S. latifolium contains bioactive which related compounds are antibacterial activities. Results of the present study revealed that minimum bactericide concentration (MBC) and MIC for V. alginolyticus was the same, but MBCs for V. parahaemolyticus and

V. harveyi were higher than their MICs (Table 2).

In addition this study revealed that both ethanolic and chloroformic crude extracts had bacteriostatic and bactericide properties against the examined *Vibrio spp*. The results of the present study showed that macroalgae can be a good substitute for antibiotics in shrimp aquaculture.

Acknowledgements

The authors are thankful to the director of Iranian Shrimp Research Center (ISRC) and personnels of Aquatic Animals Health Department of ISRC.

References

Afsharnasab, M., Dashtiannasab, A. and Yeganeh, V., 2007. Assessing pathogenesis of the white spot syndrome virus (WSSV). *Iranian Scientific Fisheries Journal*, 16(1), 1-8.

Bansemir, A., Blume, M., Schröder, S. and Lindequist, U., 2006.

Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. *Aquaculture*, 252, 79–84

Bansemir, A., Just, N., Michalik, M., Lalk, M. and Lindequist, U., 2004. Sesquiterpene derivatives from the red alga *Laurencia chondrioides* with antibacterial activity against fish and human pathogenic bacteria. *Chem. Biodiv*, 1, 463–467.

- R., Cruz-Lacicerda, E. R., Delapena, L. D. and Sunaz, N. A., 1990. Studies on the chemical control of luminous bacteria *Vibrio harveyi* and *V. splendidus* isolated from diseased *Penaeus monodon* larvae and rearing water. *Disease of Aquatic Organisms*, 9, 133-139.
- Baticados, M. C. L. and Padihare, J. 1992. **O..** The of use chemotherapeutic agent the in Philippines. In: Shariff, I.M., Subasinghe, R.P., Arthur, J.R. (Eds.), Disease in Asian Aquaculture. Fish Asian Fishery Society, Section. Manila, Philippines, pp. 531–546.
- **Braithwaite, R. A. and McEvoy, L. A., 2005**. Marine biofouling on fish farms and its remediation. *Advances in Marine Biology*, 47, 215–252.
- Chouhury, S., Sree, A., Mukherjee, S. C., Pattnik, P. and Bapuji M., 2005. *In Vitro* Antibacterial Activity of Extracts of Selected Marine Algae and Mangroves against Fish Pathogens. *Asian Fisheries Science*, 18, 285-294
- Diseases in Asian Aquaculture. I. Fish Health Section. Asian Fisheries Society, Manila, Philippines, pp. 143–155.
- Doughari, J. H. and Manzara, S., 2008. *In vitro* antibacterial activity of crude leaf extracts of *Mangifera*

- indica Linn. African Journal of Microbiology Research, 2, 067-072.
- **FAO, 2006.** Yearbook of fishery statistics, vol. 98/1& 2. Food and Agricultural Organisation of the United Nations, Rome.
- Fazli, M. R., Amin, G., Ahmadian, M. M., Ashtiani, H., Jamlifar, H. and Samadi, N., 2007. Antimicrobial activities of Iranian sumac and avishan-e shirazi (*Zataria multiflora*) against some food-borne bacteria. *Food Control*, 18, 646-649.
- **Febles, C. I., Arias, A. and Gil-Rodriguez, M. C., 1995**. In vitro study of antimicrobial activity in algae (Chlorophyta, Phaeophyta and Rhodophyta) collected from the coast of Tenerife (in Spanish). *Anuario del Estudios Canarios*, 34, 181-192.
- Freile-Pelegr, N. Y. and Morales, J.L., 2004. Antibacterial activity in marine algae from the coast of Yucatan, Mexico. *Bot. Mar*, (47), 140-146
- Hektoen, H, Berge, J.A., Hormazabal, V. and Yndeslad, M., 1995. Persistence of antibacterial agents in marine sediments. *Aquaculture*, 133, 175–84.
- Hellio, C., De La Broise, D., Dufosse,
 L., Le Gal, Y. and Bourgougnon,
 N., 2001. Inhibition of marine bacteria by extracts of macroalgae, potential use for environmentally friendly antifouling paints. *Marine*

- Environmental Research, 52 (3), 231–247.
- Herwing, R. P., Gary, J. P. and Weston, D. P., 1997. Antibiotic resistant bacteria in surfacial sediments near Salmon net cage farms in Puget Sound, Washington. *Aquaculture*, 14, 263–283.
- Hornsey, I. S. and Hide, D., 1985. The production of antimicrobial compounds by British Marine Algae. IV Variation of antimicrobial activity with algal generation. Br. Phycol. J. 20, 21-25.
- Immanuel, G., Vincybai, V. **C...** Sivaram V., Palavesam A. and Marian M. P., 2004. Effect of butanolic extracts from terrestrial herbs and seaweeds on the survival, pathogen growth and (Vibrio parahaemolyticus) load on shrimp Penaeus indicus juveniles. Aquaculture, 236, 53–65.
- **Kakoolaki, S., 1997.** A review on shrimp culture history of Iran and other countries. Ifro series publications, 20P. (In Persian)
- Kakoolaki S., Soltani M., Ebrahimzadeh Mousavi H. A., Sharifpour I., Mirzargar S., Afsharnasab M. et al., 2011. The different effect of salinities mortality and histopathological changes of **SPF** imported Litopenaeus vannamei. experimentally exposed to White

- Spot Virus and a new differential hemocyte staining method, *Iranian Journal of Fisheries Sciences*, 10(3), 447-460
- **Karunasagar, I., Pai, R. and Malathi, G. R. 1994.** Mass mortality of *Penaeus monodon* larvae due to antibiotic-resistant *Vibrio harveyi* infection. *Aquaculture*, 128, 203-209.
- **Lightner, D. V. and Redman, R. M., 1998.** Shrimp diseases and current diagnostic methods. Aquaculture, 164, 201-220.
- Mahasneh, I., Jamal, M., Kashashneh, M. and Zibdeh, M., 1995. Antibiotic activity of marine algae against multiantibiotic resistant bacteria. *Microbios*, 83, 23–26.
- **Marjorie, MC., 1999.** Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* 12(4), 564-582.
- McPhearson, Jr. R.M., DePaola, A., Zywno, S. R., Motes, Jr. M. L. and Guarino, A.M., 1991. Antibiotic resistance in Gram-negative bacteria from cultured catfish and aquaculture ponds. *Aquaculture*, 99(3/4), 203–11.
- Miranda, C. D. Zemelman, Raul., 2002. Bacterial resistance to oxytetracycline in Chilean salmon farming. *Aquaculture*, 212, 31–47.
- Baticados, M. C. L., Lavilla-Pitogo, C.
 R., Cruz-Lacierda, E. R., dela
 Pen~a, L. D. and Sun~az, N. A.,
 1990. Studies on the chemical control of luminous bacteria Vibrio harleyi

- and *V. splendidus* isolated from diseased *Penaeus monodon* larvae and rearing water. *Diseases of Aquatic Org*anisms, 9, 133–139.
- Moreau, J., Pesando, D. and Bernad P., 1988. Seasonal variations in the production of antifungal substances by some Dictyotales (brown algae) from French Mediterranean coast. *Hydrobiology*, 162, 157-162.
- Murray, P. R., Baron, E. J., Pfaller, M. A., Tenover, F. C. and Yolke, R. H., 1995. Manual of clinical microbiology (6th ed.). Washington, DC: ASM.
- Nash, G., Nithimathahoke, C., Tungmandi, C., Arkarjamon, A. and Rumathaversub, P., 1992. Vibriosis and its control in pond reared P. monodon in Thailand. In: Shariff, M., Subasinghe, R.P., Arthur, J.R. (Eds.),
- Newman, D. J., Cragg, G. M. and Snader, K. M., 2003. Natural products as sources of new drugs over the period 1981-2002. Journal of Natural Products, 66, 1022-1037.
- Patra, J. K., Rath, S. K., Jenerathud, V. K. and Tatoi. Н., 2008. Evaluation of Antioxidant and Antimicrobial Activity of Seaweed (Sargassum sp.) Extract: A Study on Inhibition of Glutathione-S-Transferase Activity. Turkish Journal of Biology, 32, 119-125.

- Pazir, M. K., Afsharnasab, M., Jalali Jafari, B., Sharifpour, I., Motalebi, A. A. and Dashtiannasab, A., 2011. Detection and identification of white spot syndrome virus (wssv) and infectious hypodermal and hematopoietic necrosis virus (ihhnv) of litopenaus vannamei from bushehr and sistan and baloochestan provinces (iran), during 2009-2010. *Iranian Journal of Fisheries Sciences*, 10 (4), 708-726.
- Rahim, Z., Sanyal, S. C., Aziz, K. M. S., Huz, M. I. and Chowdhury, A. 1998. **Isolation** A., enterotoxigenic, haemolytic and antibiotic resistant Aermonas hydrophila strains from infected fish Bangladesh. Environ. Appl. Microbiol. 48, 865–867.
- Song, Y. L. and Sung, H. H., 1993. Vibriosis resistance in tiger shrimp (*P. monodon*) induced by M. glucon treatment. From discovery to commercialization. *Eur. Aquac. Soc*, 9, 97 (Oostende, Belgium).
- **Srinivasa Rao, P. and Parekh, K. S., 1981.** Antibacterial activity of Indian seaweed extracts. *Botanica Marina*, 24, 577-582.
- Taskin, E., Ozturk, M., Taskin, E. and Kurt, O., 2007. Antibacterial activities of some marine algae from the Aegean Sea (Turkey). *African Journal of Biotechnology*, 6 (24), 2746-2751.

- Toney, I., Cadrici, B. H., Unal, D. and Suctar, A., 2006. Antimicrobial Activities of the Extracts of Marine Algae from the Coast of Urla (Izmir, Turkey). *Turkish Journal of Biology*, 30, 171-175.
- **Tsoumas, J. V., Alderman, D. J. and Rodgers, C. J., 1989.** Aeromonas salmonicida, development of resistance to 4- quinolone antimicrobials. *Journal of fish disease*, 12, 493–507.
- Vineela, CH. and Elizabeth KM., 2005. Antimicrobial activity of marine algae of Visakhapatnam city, Andhra pradesh. *Asian Jr. of Microbial. Biotech Env Sc.*, 7(2), 209-212.
- Chellaram, C., Gnanambal, K. Mary Elizabeth Gnanambal and Patterson Edward, J. K., 2004. Antibacterial activity of the winged oyster *Pteria chinensis* (Pterioida, Pteridae). *Indian Journal of Marine Sciences*, 33(4), 369-372.