INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 5, No. 12, p. 399-405, 2014

RESEARCH PAPER OPEN ACCESS

The effects of brown alga, *Sargassum glaucescens*as (Agardeh, 1948) against selected bacterial, fungal and yeast pathogens of shrimp

Abdolhamid Mahianeh[1], Babak Ghaednia[2], Maryam Mirbakhsh[2], Mohammad Velayatzadeh[3], Ehteram mohammadi[2], Mahdieh Jafari[1*], Ehsan Kamrani[4], Bahareh Ghaedifard[1]

[1]*Department of Agricultural Research, Education and Extension Organization, Bushehr, Iran*

[2]*Department of Microbiology, Shrimp Research Center, Bushehr, Iran*

[3]*Young Researchers and Elite Club, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran*

[4]*Department of Fisheries, Hormozgan University, Bandar Abbas, Iran*

Key words: Seaweed extract, *Sargassum glaucescens*as, Antimicrobial activity, *Vibrio harveyi*.

http://dx.doi.org/10.12692/ijb/5.12.399-405 Article published on December 27, 2014

Abstract

Use of antibiotics and chemical compounds com to destroy the bacteria that caused the against antibiotics show resistance of the microorganisms. Therefore, in this study, examined of anti-bacterial extract with ethanol, methanol, hexane, chloroform and hot water brown algae *Sargassum glaucescens*as. The extractions against shrimp selective pathogen bacteria including *Staphlilococus aureus*, *Vibrio harveyi*, *Escherichia coli*, *Bacillus cereus*, *Candida albicans*, *Aspergillus flavus* and *Fusarium solani* were used by disk diffusion agar method. According to the results that the hot water extract in *S. glaucesens* none of the strains studied have antibiotic effects. Ethanolic extract in compared to other extracts showed better antibacterial activity ($P<0.05$). Therefor, V. harveyi resistant to extract n hexane of S. glaucesens, Sub-critical to methanol and chloroform extract and sensitive to extract ethanol, but *S. aureus* sensitive to extracts n hexane, chloroform, methanol and ethanol, Also *B. cereus* sensitive to extract methanol and ethanol.

* **Corresponding Author:** mahdieh Jafari ✉ m.jafari97@ymail.com

Introduction

Marine organisms exhibit a rich chemical content that possess unique structural features as compared to terrestrial metabolites (Eom *et al*., 2012). Diseases caused by microorganisms have been in fish and other aquatic animals, causing significant economic losses in aquaculture, Including *Vibrio* species in many fish including salmon and crustaceans such as shrimp disease and other terms of the overuse of antibiotics in aquatic organisms are resistant because of the Fishery the new solutions are essential in preventing disease (Khairy and El-Kassas 2010; Aruna *et al*., 2010). Increasing resistance of clinically important bacteria to existing antibiotics is a major problem throughout the world (Kaplan and Mason 1998).

Seaweeds into three categories based paints are classified as red algae (4500 species), green algae (900 species), algae Brown (1000 species), which contain high levels of minerals, vitamins, essential amino acids and carbohydrates and as long as food, fertilizers and drugs have been used (Abd *et al*., 2009) and the defense mechanism macro algae in activity of viruses and bacteria has been observed in different time periods (Raja Kannan *et al*., 2010). The use of marine algae are cheaper than chemicals and antibiotics and have little effects on nature, humans and fish. Fishery production of the main challenges is important bacterial infections fungal every year causing significant losses in aquaculture centers are amplified. In addition, a major problem in the industry is the use of drugs and chemicals Fishery, physical and chemical parameters of water resources is various climates in different physicochemical conditions in different geographic locations, different toxic efficiency exhibited (Crasta *et al*., 1997; Elsie *et al*., 2011).

Concerns over human health and environmental safety due to some chemicals have prompted increasing interest in more "natural-green" alternatives such as antibiotics. Seaweeds are considered as potent source of 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; Dashtiannasab *et al*., 2012).

The development of shrimp aquaculture, in spite of its global necessity, is largely at stake as significant ecological and pathological problems are increasing in the vast majority of the shrimp producing countries (Ghaednia *et al*., 2011). Hot water extracts of brown algae contains sulfated polysaccharide that is the main sugar fucose. The sugar is unnatural monosaccharides present in a number of combinations mucopolysaccharides. Stimulatory effect of the extract on the immune system of medicine has been approved (Zhou and Yu 2004). Some investigators evaluated immunostimulant effects of hot water extracts of brown algae on the shrimp immune system (Yeh *et al*., 2005; Balasubramanian *et al*., 2008).

Seaweed *S. Glaucesens*, marine brown algae in the waters of the Persian Gulf, particularly of the coast from Boushehr province, is found in abundance. The aim of this study was to evaluate the antibacterial activity of a brown seaweed *Sargassum glaucescens*as obtained from Persian Gulf waters against three shrimp pathogen- bacteria named *V. harveyi*. There are many reports on the antibacterial activity of seaweeds against human pathogens, but little information was available for aquatic bacteria. Hence the present study was designed to study the effects of aquatic animals in marine brown algae antibiotic *S. Glaucesens* were examined on Gram-positive bacteria, gram-negative bacteria, yeast and molds.

Materials and methods

Collection and Extraction

Sargassum Glaucesens were collected for a Coastlines from the Persian Gulf (Jefreh and Jalali wharf of Bushehr port in southern Iran). In laboratory algae were rinse with water and remove algae and sand

particles. 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. This was done to remove salts from algae and to get better results several times with distilled water used was replaced with distilled water. Algae were spread over three days in the shade to dry completely and then drying to a powder mill was electric. Soxtec extraction condensing steam to the rotating column for each of the solvents was performed. Solvents used in this study include ethanol, methanol, chloroform, n-Hexane was distilled water. For extracts prepared from 150 g of dried algae *S. Glaucesens* were used in 1000 ml of solvent (Fazli *et al*., 2007; Sangeetha *et al*., 2014).

Microbial test

Strains of microbial (bacterial and fungal) used in this study contained *Bacillus cereus* (PTCC 1816), *Staphlilococus aureus* (PTCC 1337), *Escherichia coli* (PTCC 1397), *Vibrio harveyi* (PTCC 1755) *Candida albicans* (PTCC 5027), *Aspergillus flavus* (PTCC 5004), *Fusarium solani* (PTCC 5284), respectively.

Antibacterial effects were used of the modified disk diffusion method on agar antibiogram test (Agar Disk Diffusion) and minimum inhibitory concentration testing of sequential dilution method (Tube Dillution). For this purpose, suspensions of bacteria to antimicrobial susceptibility testing standards with a concentration equivalent to 0.5 McFarland was prepared from overnight cultures. The medium used for antibiogram Hinton agar (Muller Hinton Agar), with pH 7.2 and 5 mm in diameter, respectively. After providing a uniform culture suspensions were prepared aseptically using sterile swabs, Blank CDs antibodies Medicine Company (capacity 25 ml) were placed on media and inoculated with 20 ml each of the algal extracts prepared to help Sampler (with a concentration of 0.2 weight to volume). Pre-release pellets for 30 min at 4° C were then incubated at 35° C were transferred were to diameter of inhibition zone (mm) after 24-48 h using a digital caliper to measure and record. For Each instance replicated three times. Oxitetracycline antibiotics were used as positive controls (CLSI, 2008). To examine the sensitivity of yeast strains of the Potato broth (Potato Dextrose Broth) was used to perform the antibiogram. To determine the minimum inhibitory concentration, 1 to 9 of 9 test tubes and was named to the all-tube, 1 ml of culture medium was added to the liquid Hinton. 1 tube, 1 ml of sterile seaweed extract with filter 0.45 µ was added and mixed. Then 1 ml of tube 1 to tube 2 were added and removed, and this continued until the tube 7. Finally, 1 ml from tube 7 was removed and discarded. The tubes 8 and 9 were added to 1 ml of algal extracts. At the end of the tube all tubes except No. 9, was added to 1 ml of bacterial suspension was prepared. With the pipes 8 and 9, respectively, as positive and negative controls were considered. Where no growth (turbidity) was not observed, as the minimum inhibitory concentration was recorded (Murray *et al*., 1995; Ara *et al*., 2002).

Statistical analysis

The antibacterial activity of the data are expressed as means ±SD. Statistical analysis was performed by ANOVA with LSD test and Student's t-test. A value of P=0.05 was taken to indicate statistical significance.

Results and discussion

The results show that the hot water extract in *S. glaucesens* none of the strains studied have antibiotic effects (Table 1). Ethanolic extract in compared to other extracts showed better antibacterial activity ($P<0.05$). according to CLSI table, diameter of inhibition zone for the standard disk tetracycline, is interpreted inhibition zone less than more or equal 14 mm, resistant, between of 15 to 18 mm, Sub-critical and more than 19 mm, Sensitive to tetracycline (CLSI 2008). Therefor, *V. harveyi* resistant to extract n hexane of *S. glaucesens*, Sub-critical to methanol and chloroform extract and sensitive to extract ethanol, but *S. aureus* sensitive to extracts n hexane, chloroform, methanol and ethanol. Also *B. cereus* sensitive to extract methanol and ethanol.

In the present in vitro study we observed the potent antibacterial activity of the seaweed; *Sargassum Glaucesens* 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 n hexane, chloroform, methanol and ethanol extracts of *Sargassum Glaucesens* inhibited all of the selected bacteria. Highest antibacterial activity of ethanol extracts of *Sargassum Glaucesens*.

Table 1. Antimicrobial activity of ethanol, methanol, chloroform, n-hexane and hot water extract of *S. glaucescens* against eight species microorganisms

S. glaucescens extracts	Gram Positive				Gram negative				Yeast		Fungi			
	B. cereus		*S. aureus*		*E. coli*		*V. harveyi*		*C. albicans*		*F. solani*		*A. flavus*	
	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
ethanol extract	19.6±0.6	0.12	21.3±0.3	0.12	22.7± 0.2	0.25	21.5 ± 0.2	0.25	7.0 ± 0.5	1	*	0	11.0 ± 0.3	1
methanol extract	18.5 ± 0.6	0.12	20.3 ± 0.7	0.12	16.3 ± 0.6	0.50	18 ± 0.7	0.25	1.7 ± 0.2	0	*	0	8.2 ± 0.2	1
chloroform extract	14.2 ± 0.2	0.25	18.3 ± 0.6	0.12	17.2 ± 0.4	0.50	16 ± 0.8	0.50	6.2 ± 0.4	0	*	0	0	0
n-hexane extract	4.4 ± 0.3	1	18.0 ± 0.5	0.12	11.2 ± 0.3	1	11.7 ± 0.2	1	0	0	*	0	19.2 ± 0.4	0.50
hot water extract	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Zone of inhibition (mm), including of the agar – disk (5 mm), Mean value of three replicate ± SE.

0 ; no activity

*: no detected microbial growth.

Over two decades of rapid growth and prosperity of the shrimp industry in the world is going and this position as a supplier to the industry, among other sources of valuable protein, is explained. Growers to achieve greater profitability and respond to market demand for this product, be forced to increase the storage density of larvae in the pool that this paves the way for the emergence be provided and spread of infectious diseases such Vibrios.

Antimicrobial activity of the seaweed is depends on the solvents used for their extraction. Many researchers reported influence of different extraction solvents on the content of compounds in extracts. Solvents solubility efficiency is strongly dependent on material used for extraction (Zhou and Yu 2004; Grigonisa *et al*., 2005; Michiels *et al*., 2012).

The ethanol extract of seaweed species (*Halimeda tuna, Jolyna laminarioides, Melanothamnous afaqhusainii, Cystoseira indica, Sargassum ilicifolium, Sargassum lanceolatum, Sargassum tenerrimum, Sargassum swartzii* and *Ulva fasciata*) were tested for antifungal activity against root infecting fungi. *Sargassum ilicifolium* was effective at a concentration of 4 and 6 mg/disc with inhibition zone of 8 and 10 mm respectively against *Fusarium oxysporum* (Ambreen *et al*., 2012). Methanol has effectively soluble the compounds from the seaweeds than ethanol. Seaweeds are known to produce many secondary metabolites, including bioactive compounds with various activities (Newman *et al*., 2003). It is clear that using organic solvents provides a higher efficiency in extracting than water based methods (Lima-Filho *et al*., 2002; Sangeeth *et al*., 2014).

Emodi (1978) Several studies conducted on Algal and Concluded that algae can be used as a dietary supplement. Borowitzka and Borowitzka (1992) according to research conducted, declared that algae are rich sources of vitamins. Bhaskar *et al*. (2005) the antibacterial activity of brown algae found *Padinatetra tomatica*. In other research they found that the methanol extract of a species of Sargassum algae antioxidant and optimal antibacterial against gram positive and negative bacteria (Plaza *et al*., 2008).

Farmed shrimp larvae infected with the bacterium *V. harveyi* resulted in brilliant condition (*Luminous disease*) is a serious loss in the shrimp hatchery or in the early stages of storage provided. In addition to bacteria, a Gram-negative bacterium is *V. harveyi*, *Eschrechia coli* (gram-negative) and *Staphylococcus aureus* and *Bacillus cereus* (Gram-positive) were also examined that the results this study (table 1) showed that all extracts hot water extract had a positive effect on the growth of all three bacteria and finally it can be concluded that the use of extracts of algae, which reduces the use of antibiotics and chemicals.

Results this study showed that ethanol extract of the *S. glaucesens* can be used an alternative to antibiotics in the tetracycline which is now largely used in the hatchery and shrimp farms. The values obtained for the minimum inhibitory concentration (MIC), the organic extract of *S. glaucesens* with smaller amounts of antibiotic positive control in similar studies (Nariman *et al.*, 2007; Bansemir *et al.*, 2006). Should be observed that used solvents with different polarities and most of the polarity of the solvent can increase the extraction of other unwanted compounds did not the role in the antimicrobial activity of the extract, cause decreases the relative amount of the desired compounds in the extract. According to the polarity of methanol higher than chloroform, chloroform extracts were expected to show greater antibacterial activity However, the findings did not confirm this prediction. Hot water extracts of *S. glaucesens*, not was antifungal and antibacterial activity and *F. solani* compared to all the extracts studied showed resistance which pathogenic fungi from the shrimp industry is introduced.

References

Abd El, Mageid MM, Salama NA, Saleh MAM, Abo Taleb HM. 2009. Antioxidant and antimicrobial characteristics of Red and Brown algae extracts. 4th Conference on recent technology in Agriculture 818-827.

Ambreen A, Hira K, Ruqqia A, Sultana V. 2012. Evaluation of biochemical component and antimicrobial activity of some sea weeds occurring at Karachi coast. Pakistan Journal of Botany **44(5),** 1799-1803.

Ara J, Sultana V, Ehteshamul-Haque S, Athar M, Qasim R. 2002. Antibacterial activity of marine macro-algae from Karachi coast. Bulletin of the Polish Academy of Sciences Biological Sciences **50,** 199-206.

Aruna P, Mansuya P, Sridhar S, Kumar JS, Babu S. 2010. Pharmacognostical and antifungal activity of selected seaweeds from Gulf of Mannar region. Recent Research in Science and Technology **2(1),** 115-119. http://dx.doi.org/10.5829/idosi.wjfms.2014.06.01.76195.

Balasubramanian G, Sarathi M, Venkatesan C, Thomas J, Sahul Hameed AS. 2008. Studies on the immunomodulatory effect of extract of *Cyanodon dactylon* in shrimp, *Penaeus monodon*, and its efficacy to protect the shrimp from white spot syndrome virus (WSSV). Fish & Shellfish Immunology **25,** 820–828. http://dx.doi.org/10.1016/j.fsi.2008.09.002.

Bansemir A, Blume M, Schroder S, Lindequist U. 2006. Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. Aquaculture **252,** 79–84. http://dx.doi.org/10.1016/j.aquaculture.2005.11.051.

Bhaskar N, Miyashita K. 2005. Lipid composition of *Padina tetratomatica* (Dictyotales, Phaeophyta), a brown seaweed of the west coast of India. Indian Journal Fisheries **52,** 263-268.

Borowitzka MA, Borowitzka LJ. 1992. Vitamins and fine chemicals from microalgae. In: Microalgal Biotechnology. Cambridge University Press. Great Britain. p 179.

Crasta PJ, Raviraja NS, Sridhar KR. 1997. Antimicrobial activity of some marine algae of

southwest coast of India. Indian Journal Marine Science **26,** 201-205.

Dashtiannasab A, Kakoolaki S, Sharif Rohani M, Yeganeh V. 2012. In vitro effects of *Sargassum latifolium* (Agardeh, 1948) against selected bacterial pathogens of shrimp. Iranian Journal of Fisheries Sciences **11(4),** 765-775.

Elsie BH, Dhanarajan MS, Sudha PN. 2011. In vitro screening of secondary metabolites and antimicrobial activities of ethanol and acetone extracts from red seaweed *Gelidium acerosa*. International Journal of Chemistry Research **2(2),** 27-29.

Eom S, Kim Y, Kim S. 2012. Antimicrobial effect of phlorotannins from marine brown algae. Food and Chemical Toxicology **50,** 3251–3255. http://dx.doi.org/10.1016/j.fct.2012.06.028.

Emodi A. 1978. Carotenoid: properties and applications. Food Technology **32,** 38-42.

Fazeli MR, Amin G, Ahmadian Attari MM, Ashtiani H, Jamalifar H, Samadi N. 2007. Antimicrobial activities of Iranian sumac and avishan-e shirazi (*Zataria multiflora*) against some food-borne bacteria. Food Control **18,** 646-649. http://dx.doi.org/10.1016/j.foodcont.2006.03.002.

Ghaednia B, Mehrabi MR, Mirbakhsh M, Yeganeh V, Hoseinkhezri P, Garibi G, Ghaffar Jabbari A. 2011. Effect of hot-water extract of brown seaweed *Sargassum glaucescens* via immersion route on immune responses of *Fenneropenaeus indicus*. Iranian Journal of Fisheries Sciences **10(4),** 616-630.

Grigonisa D, Venskutonisa PR, Sivikb B, Sandahlb M, Eskilssonc CS. 2005. Comparison of different extraction techniques for isolation of antioxidants from sweet grass (*Hierochloe odorata*). Journal Supercritical Fluids **33,** 223–233. http://dx.doi.org/org/10.1016/j.supflu.2004.08.006.

Kaplan SL, Mason EO. 1998. Management of infections due to antibiotic resistant *Streptococcus pneumoniae*. Clinical Microbiology Reviews **11,** 628–644.

Khairy HM, El-Kassas HY. 2010. Active substance from some blue green algal species used as antimicrobial agents. African Journal of Biotechnology **9(19),** 2789-2800.

Lima-Filho JVM, Carvalho AFFU, Freits SM, 2002. Antibacterial activity of extracts of six macroalgae from the Northeastern Brazilian coast. Brazilian Journal of Microbiology **33,** 311-313. http://dx.doi.org/org/10.1590/S151783822002000400006.

Michiels JA, Kevers C, Pincemail J, Defraigne JO, Dommes J, 2012. Extraction conditions can greatly influence antioxidant capacity assays in plant food matrices. Food Chemistry **130(4),** 986-993. http://dx.doi.org/10.1016/j.foodchem.2011.07.117.

Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolke RH. 1995. Manual of clinical microbiology (6th ed.). Washington, DC: ASM.

Nariman F, Mobasheri F, Sohrabi Pur J. 2007. Antibacterial activities of four red and brown algae extracts from Hormozgan coasts. Journal Science Azzahra University **2,** 55-66.

Newman DJ, Craff GM, Snander KM. 2003. Natural products as a source of new drugs over the period 1981- 2002. Journal of Natural Products **66 (7),** 1022-1037. http://dx.doi.org/10.1021/np030096l.

Plaza M, Cifuentes A, Ibanez E. 2008. In the search of new functional food ingredients from algae.Ternds in food Science and Technology **19,** 31-39. http://dx.doi.org/10.1016/j.tifs.2007.07.012.

Raja Kannan RR, Arumugam RA,

Nantharaman P. 2010. Antibacterial potential of three sea grasses against human pathogens. Asian Pacific Journal of Tropical Medicine 890-893. http://dx.doi.org/10.1016/S1995-7645(10)60214-3.

Sangeetha S, Dhayanithi NB, Sivakumar N. 2014. Antibacterial activity of *Sargassum longifolium* and *Gracilaria corticata* from Gulf of Mannar against selected common shrimp pathogens. International Journal of Pharma and Bio Sciences **5(2),** 76-82.

Taskin E, Ozturk M, Taskin E, 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 BH, Unal D, 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.

Yeh S, Lee C, Chen J. 2005. Administration of hot-water extract of brown seaweed *Sargassum duplicatum* via immersion and injection enhances the immune resistance of white shrimp *Litopenaeus vannamei*. Fish and Shellfish Immunology **20,** 332-345. http://dx.doi.org/10.1016/j.fsi.2005.05.008.

Zhou K, Yu L. 2004. Effects of extraction solvent on wheat bran antioxidant activity estimation, LWT. Food Science and Technology **37(7),** 717-721. http://dx.doi.org/10.1016/j.lwt.2004.02.008.