Antimicrobial and antifouling activities achieved by extracts of seaweeds from Gulf of California, Mexico

Actividades antimicrobiana y anti-incrustante obtenidas de los extractos de algas marinas del Golfo de California, México

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ABSTRACT

Six species of common seaweed extracts were tested in laboratory assays: *Dictyota flabellata*, *Padina concrescens*, *Laurencia johnstonii*, *Gymnogongrus martinensis*, *Ulva lactuca* and *Codium fragile* for potential industrial applications through evaluation of the antibacterial activity against pathogenic bacteria (5 strains) and the antifouling potency against the growth of key species of marine colonisers (7 bacteria, 5 fungi and 11 microalgae). The organic extract of *L. johnstonii, U. lactuca* and *D. flabellata* have bacterial antibiosis. The ethereal extracts were more active in comparison with buthanol extracts against the bacterial strain *Staphylococcus aureus*. The best antifouling results were obtained with *U. lactuca* and *L. johnstonii* (0.1-1 µg ml^{-1}) against all strains tested. *C.fragile* exhibited significant antifouling activity with minimum inhibitory concentration (MIC) between 1-10 µg ml^{-1} against marine microalgae *Rhodosorus magnei, Neorhodella cyanea* and *Prymnesium calathiferum*.

Key words: Antibacterial, antifouling, Gulf of California, bioactivity, seaweeds.

RESUMEN

Se analizaron seis especies de macroalgas comunes del Golfo de California: *Dictyota flabellata, Padina concrescens, Laurencia johnstonii*, *Gymnogongrus martinensis*, *Ulva lactuca* y *Codium fragile* para determinar su potencial aplicación industrial, a través de la evaluación de la actividad antibacteriana frente a bacterias patógenas (5 cepas), y el potencial anti-incrustante como inhibidores de crecimiento de especies colonizadoras en ambientes marinos (7 bacterias, 5 hongos y 11 microalgas). Los extractos orgánicos de *L. johnstonii*, *U. lactuca* y *D. flabellata* presentaron antibiosis bacteriana. Los extractos etéreos fueron más activos en comparación con los extractos de butanol frente a la cepa bacteriana *Staphylococcus aureus*. Los mejores resultados de actividad anti-incrustante se obtuvieron con *U. lactuca* y *L. johnstonii* (0.1-1 µg ml^{-1}) frente a todas las cepas probadas. *C. fragile* mostró una significativa actividad anti-incrustante, presentando una concentración mínima inhibitoria (MIC) entre 1-10 µg ml^{-1}, frente a las microalgas marinas *Rhodosorus magnei, Neorhodella cyanea* y *Prymnesium calathiferum*.

Palabras clave: Antibacterial, anti-incrustante, Golfo de California, bioactividad, macroalgas.

INTRODUCTION

The great varieties of organisms that inhabit the seas and oceans, and also the possible pharmacological properties of pure extracts compounds obtained from them, represent a potential source of new drugs and an open field for research (De Lara-Isassi, 1991). Seaweeds are a promising source of bioactive compounds that can be used in the treatment of human diseases or to control the colonization of fouling organisms on man-made surfaces. These organisms are sessile, without any physical defenses and exposed to various environmental conditions, and thus have generated a number of important physiological adaptations that promote defense, one of these consisted of the synthesis of bioactive compounds (Duffy & Hay, 1990; Charzeddine & Fariñas, 2001). These compounds have specific chemical structures that confer biological defense capabilities against grazers and/or the installation of epiphytes and fouling organisms (Duffy & Hay, 1990; Hay 1996; Magallanes *et al*., 2003; Hellio *et al.,* 2004). Many of the substances obtained from seaweeds, such as alginates, carrageenan and agar have been used for decades in traditional medicine, pharmacology and industry (Barsanti & Gualtieri, 2006). Other compounds isolated from seaweeds have bacteriostatic or antibacterial, antiviral, antitumor, anti-inflammatory and antifouling (AF) activities (Hellio *et al*., 2009; Tuney *et al*., 2006; Smit, 2005; Lima-Filho, 2002). Research showed that the antibacterial activity of algae is due to their ability to synthesize respectively nitrogen compounds and diterpenes in Chlorophyceae, mixed halogenated terpenes in Rhodophyceae and metabolites of aromatic origin in Phaeophyceae (Bhakuni & Rawat, 2005).

An interesting line of research is inspired by biomimetic solutions and a better understanding of the avoidance of epibiosis (Ralston & Swain, 2009). Although, some algae are heavily covered by epiphytes, other species within the same habitat would have developed mechanisms to keep their surfaces free of colonizers, which provide an indication of potential defense chemical mechanisms (Hellio *et al*., 2009; Nylund & Pavia, 2003). Seaweeds have the ability to synthesize secondary metabolites, presumably whose primary function is to protect against the colonization by epibionts. This feature not only represents an important ecological role, also aroused the interest in the potential applications of these chemicals from a business perspective (Marechal *et al*., 2004).

With the development of marine biotechnology, it is important to increase the research to identify promising algae species, characterize and identify substances of interest to pharmaceutical and marine industries.

We decided to work on two main applications: a) the search for new antibiotics and b) for new antifouling compounds.

Infectious diseases caused by bacteria are the world's biggest killers of children and young adults (Abdelmohsen, 2010). Due to the indiscriminate use of antibiotics, microorganisms have developed new strategies to evade the action of drugs. This has led to results in multi-resistant bacteria strains such as methicillin-resistant *Staphylococcus aureus,* which is the most commonly encountered as multiple drug resistant organisms in patients residing in non-hospital healthcare facilities (Lindberg *et al*., 2004). The increase in the current problems of growing resistance towards numerous pathogens together with new emerging infectious diseases also the toxic effects of some currently used drugs, leads to research for novel drugs with the goal to find new compounds with antimicrobial activity (McMichael, 2000).

Another potential utilization of bioactive compounds from seaweeds is within the sector of marine antifouling paints. Indeed, all surfaces immersed in the marine environment are a potential site for the establishment of biofouling communities (Wahl, 1989). If we consider that not only natural areas are subject to this process, but also those from anthropogenic origin are potentially affected by the settlement and development of fouling organisms. This natural phenomenon then becomes one of the biggest problems to be faced by the global maritime domain (Marechal & Hellio, 2009; Yebra *et al*., 2004). In the past, shipping companies have used paints containing toxic compounds such as arsenic and mercury to counteract the effects of fouling organisms (Jones, 2009). From 1960´s, there was a wide spread use of TBT (tributyltin) in commercial marine paints formulation. However due to the adverse side effects of TBT-based paints, worldwide pollution of the marine environment and food-chain, potential causes of genetic mutations in populations of exposed animals, this compound was banned in the manufacture of products for antifouling from 2008 (Hellio & Yebra, 2009).

The Gulf of California was chosen as sampling site as this is an area of exceptional biodiversity. This is indeed a transition zone: in the summer, tropical seaweed species will be present. During the winter there is a shift to temperate species (Cruz-Ayala *et al*., 1998). Some species as *Codium, Dictyota, Ulva* or *Laurencia* are found all year long and are very abundant (Cruz-Ayala *et al*., 1998). All these species face very high environmental pressure from herbivorous fishes and other predators (Duffy & Hay, 1990).

It has been stated that several seaweeds did exhibit significant variation of the production of bioactive compounds (Hellio *et al*., 2004; Marechal *et al*., 2004) concomitant with the seasonal variation of the fouling pressure. A higher production of bioactive compounds in spring and summer developed during the algae and invertebrates spawning season. In this study we describe the antibacterial and antifouling activities of crude extracts of seaweeds collected from the Gulf of California.

MATERIALS AND METHODS

Biological samples: The seaweeds studied were two Heterokontophyta, Phaeophyceae: *Dictyota flabellata* (Collins) Setchell

et Gardner and *Padina concrescens* Thivy, two Rhodophyta, Florideophyceae: *Laurencia johnstonii* Setchell *et* Gardner, and *Gymnogongrus martinensis* Setchell *et* Gardner, and two Chlorophyta, Ulvophyceae: *Ulva lactuca* Linnaeus and Bryopsidophyceae: (*Codium fragile* (Suringar) Hariot). The specimens were collected in two sites located in Baja California Sur , Mexico; Fig. 1: The rocky reef of Punta Arena de la Ventana (24° 02'-24° 08' N and 109° 49'-109° 53' W) is an area of high biodiversity and transition, with tropical waters species in summer and temperate species in winter. Second location was San Juan de la Costa (24° 22'-24° 29' N and 110° 40'-110° 45' W) characterized by the presence of large beds of *Sargassum* and other abundant seaweeds. The collection was by SCUBA diving between 2 and 6 m depth in May 2008. In the laboratory, specimens were washed and cleaned of epiphytes, sand, necrotic parts, etc. and air-dried at room temperature in the shade. Specimens were stored at –20 °C prior to extraction.

Extraction: The extraction was performed using a mixture of acetone/MeOH mixture (1:1 400 ml, FISHER) at room temperature and macerated by grinding. The solution was filtered (filter paper Whatman No. 3) and the solvent was concentrated under reduced pressure to give a first crude extract. Subsequently, the crude extract was suspended in 50 ml of distilled water and extracted three times with 50 ml of ethyl ether for 30 minutes at room temperature. The ether phase was concentrated under reduced pressure to give the ether extract. The aqueous phase was extracted again with 50 ml of buthanol for 30 minutes and evaporated under reduced pressure to obtaining the buthanol extract. Yields of the extracts were calculated by dry weight of the extract over dry weight of the seaweed sample (expressed as percentage; Table 1). The extracts were stored at –20 °C until the bioactivity assays.

Figure 1. Locations of collection of seaweeds in Baja California Sur, Mexico.

Bioassays toward pathogenic bacteria. The bioactivity was performed using an agar diffusion test (NCCLS, 1988) against five strains of human pathogenic bacteria, one Gram negative: *Escherichia coli* (ATCC BAA-196), and four Gram positive: *Staphylococcus aureus* (ATCC BAA-42), *Bacillus cereus* (ATCC 10987), *Bacillus subtilis* (ATCC 10774) and *Staphylococcus epidermidis* (ATCC 11249). Petri dishes (8 cm of diameter) containing Müeller-Hinton medium (agar 3%) were inoculated with the strains to test at the concentration of 10^8 cells ml^{-1}. Ether and buthanol extracts were diluted (65 mg ml^{-1}) and 30 µl of this solution was added to filter paper discs (6 mm diameter). In each plate two discs were placed with extract plus one positive control (erythromycin 30 µg) and one negative control (carrier solvent). Every assay was performed in triplicate. Prior to incubation, Petri dishes were placed in the refrigerator for 40 min, in order to retard microbial growth while the antibiotic substance spreader on the agar. Then the plates were incubated at 35 °C for 24 h (Jensen *et al*., 1996). The results were expressed as the diameter of inhibition zone around the discs in mm.

Towards antifouling organisms. For the bioassays, initial extract concentration was 1 mg ml^{-1}, with dilutions to obtain the following concentrations: 0.01, 0.1, 1, 10 and 50 µg ml^{-1}.

Antibacterial assay. The antibacterial assay was evaluated using seven strains of marine bacteria: *Halomonas marina* (ATCC 25374), *Polaribacter irgensii* (ATCC 700398), *Pseudoalteromonas elyakovii* (ATCC 700519), *Rosevarius tolerans* (DSM 11457), *Vibrio aestuarianus* (ATCC 35048), *Vibrio anguillarum* (ATCC 19264) and *Vibrio pomeroyri* (CAIM 578). These strains are involved in the colonization of immersed surfaces. The extracts were incubated with 100 µL of bacterial solution (2×10^8 cells ml^{-1}) in 96 wells (VWR), in LB medium (Luria Hinton Broth) supplemented with NaCl (35 g l^{-1}) at 30 °C for 72 h. Each experiment was run in six replicates and with two batches of microorganisms. Control consisted of seawater. The minimum inhibitory concentrations (MIC) were determinate following the conventional method of Amsterdam (National Committee for Clinical Laboratory Standars, 1996). MIC represents the lowest concentration that inhibits the organism's growth.

Antimicroalgal activity of extracts were evaluated using benthic phase of six temperate microalgae species: *Halamphora coffeaformis* (Agardh) Levkov (Bacillariophyceae) (AC713), *Cylindrotheca closterium* (Ehrenberg) Reimann *et* Lewin (Bacillariophyceae) (AC170), *Navicula jeffreyae* Hallegraeff *et* M. A. Burford

Table 1. Antimicrobial activity of seaweeds extracts against pathogen bacteria.

Species	Dry weight (g)	Extract	Yield extract (%)	*Staphylococcus aureus*
Rhodophyta *Laurencia johnstonii* Setchell *et* Gardner	50.83	E	0.77	18.7±1.5
		B	0.02	—
Gymnogongrus martinensis Setchell *et* Gardner	23.67	E	0.25	—
		B	0.03	—
Phaeophyceae *Padina concrescens* Thivy	29.29	E	0.65	—
		B	0.02	—
Dictyota flabellata (Collins) Setchell *et* Gardner	59.02	E	0.61	7.66±0.28
		B	0.01	9.5±1
Chlorophyta *Ulva lactuca* Linnaeus	55.69	E	0.27	8.3±0.7
		B	0.02	—
Codium fragile (Surigar) Hariot	8.14	E	2.40	—
		B	0.07	—

Diameter of inhibition zone of bacterial growth in mm. (–) = Negative result, E = Either fraction; B = Butanol fraction. No activity was recorded against *Escherichia coli*, *Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus epidermidis* (data not shown).

(Bacillariophyceae) (AC181), *Pleurochrysis roscoffensis* (Dangeard) Fresnel *et* Billard (Prymnesiophyta) (AC32), *Exanthemachrysis gayraliae* Lepailleur (Prymnesiophyta) (AC15), *Chlorarachnion globosum* Ishida *et* Hara (Chlorarachniophyta) (AC132), and four tropical microalgae *Rhodosorus magnei* Fresnel *et* Billard (Rhodophyta) (AC130), *Neorhodella cyanea* (Billard *et* Fresnel) Scott, Yokoyama, Hara *et* West (Rhodophyta) (CCAP 1346), *Prymnesium calathiferum* Chang *et* Ryan (Prymnesiophyta) (MLB298), *Ochrosphaera neapolitana* Schussnig (Prymnesiophyta) (CCMP 593) and the marine dinoflagellate (Dinophyta) *Gambierdiscus toxicus* Adachi *et* Fukuyo (373098). Extracts were transferred in 96 well plates using methanol as carrier solvent. After evaporation of the solvent, microalgae were added following the method of Tsoukatou *et al*. (2002). After 48 h incubation at 18 °C for temperate strains and at 25 °C for tropical strains, optical density was measured at 600 nm with a spectrophotometer as a probe for growth evaluation. Results are expressed as MICs values in $\mu g\ ml^{-1}$.

The antifungal activity test was performed with five strains of marine fungi obtained from the culture collection of the University of Portsmouth (UK): *Halosphaeriopsis mediosetigera* (Cribb *et* Cribb) Johnson, *Asteromyces cruciatus* Moreau *et* M. Moreau ex Hennebert, *Lulworthia uniseptata* Nagakiri, *Zalerion* sp. and *Monodictys pelagic* (Johnson) Jones. Microorganisms were placed in a liquid medium containing the extracts for testing. Extracts were diluted in 5% dimethyl sulfoxide and filtered (Millex-GV 0.22 mm pore size, Millipore, Watford, UK) and were transferred in 6 ml of corn meal agar, pH 6 (Sigma). After incubation for 2 days at 25 °C, MIC was determinated by microdilution method.

RESULTS

Extraction: The yield of crude extraction varied significantly among the different species collected. Thus, the highest yield was obtained with *Codium fragile* and the minimum was to *Gymnogongrus martinensis* and *Ulva lactuca* (Table 1).

Towards pathogenic assay: The results indicated that only *Laurencia johnstonii, Dictyota flabellata* and *Ulva lactuca* showed activity against pathogenic bacteria in humans. The ether extracts for this species being the most active against *Staphylococcus aureus*. Only the buthanol fraction from *D. flabellata* was active against the same strain. In the case of tests against *Escherichia coli, Bacillus cereus, Bacillus subtilis and Staphylococcus epidimidis* extracts showed no activity (Table 1).

None of the negative control discs presented growth inhibition, thus ruling out the influence of solvents on the bioactivity filed. The inhibition zone around the positive control disc with commercial antibiotic presented a diameter of 20 mm, similar to that of the ether extract of *L. johnstonii*.

Table 2. Minimum Inhibitory Concentration (μg ml^{-1}) of seaweed extracts against some species of marine bacteria.

Species	*Halomonas marina*	*Polaribacter irgensii*	*Pseudoalteromonas elyakovii*	*Rosevarius tolerans*	*Vibrio aestuarianus*	*V. anguillarum*	*V. pomeroyri*
Dictyota flabellata	>50	>50	>50	>50	>50	>50	>50
Padina concrescens	10	>50	25	>50	>50	25	25
Laurencia johnstonii	0.1	1	1	0.1	1	1	1
Gymnogongrus martinensis	>50	>50	>50	>50	>50	>50	>50
Ulva lactuca	1	10	1	1	10	1	1
Codium fragile	>50	>50	>50	>50	>50	>50	>50

Table 3. Minimum Inhibitory Concentration (μg ml^{-1}) of seaweed extracts against some species of temperate marine microalgae.

Species	*Halamphora coffeaformis*	*Cylindrotheca closterium*	*Navicula jeffreyae*	*Pleurochrysis roscoffensis*	*Exanthemachrysi gayraliae*	*Chlorarachnion globosum*
Dictyota flabellata	> 50	> 50	> 50	> 50	> 50	> 50
Padina concrescens	> 50	> 50	> 50	> 50	> 50	> 50
Laurencia johnstonii	25	10	10	25	50	25
Gymnogongrus martinensis	10	10	> 50	10	> 50	> 50
Ulva lactuca	10	10	10	10	10	25
Codium fragile	> 50	> 50	> 50	> 50	> 50	> 50

Towards antifouling organisms. The activity assay showed that the algae *Ulva lactuca* and *Laurencia johnstonii* showed the highest activity against all organisms tested (Tables 2-5). So against strains of marine bacteria inhibited by growth with MIC values of 0.1 to 1 μg ml^{-1} while the rest of the species had low activity with values greater than 50 μg ml^{-1} (Table 2). The strains most sensitive to the extract from *L. johnstonii* were *Halomonas marina and Rosevarius tolerans* with MIC values of 0.1 μg ml^{-1}.

Ulva lactuca and *L. johnstonii* were active against all strains of temperate microalgae with inhibitions ranges between 10 and 25 μg ml^{-1} (Table 3). With tropical microalgae, *U. lactuca* and *L. johnstonii* showed higher levels of activity against all strains with values between 0.1 and 1 μg ml^{-1}. *Codium fragile* presented activity against *Neorhodella cyanea* and *Prymnesium calathiferum* with values of 1 μg ml^{-1}, whereas *Padina concrescens* presented activity against three tropical strains although with higher concentrations (10 μg ml^{-1}). However, it is interesting that activity against tropical strains was increases and species such as *C. fragile* and *P. concrescens* showed activity against *Rhodosorus magnei, Neorhodella cyanea* and *Prymnesium calathiferum* (Table 4).

Ulva lactuca and *L. johnstonii* showed the highest activity (MIC 0.1-1 μg ml^{-1}) against all marine fungi assayed, except with *Lulworthia uniseptata* strain in which the activity was good but at a higher MIC (10 μg ml^{-1}). This test results also showed considerable activity of *P. concrescens* against *Zalerion* sp. and *Monodictys pelagic* (10 μg ml^{-1}; Table 5).

DISCUSSION

There are numerous reports of compounds obtained from seaweeds with a wide range of biological activities such as antibiotic, antiviral, anticoagulant, antitumor and anti-inflammatory (Albuquerque *et al.*, 2004; Charzedinne & Fariñas, 2001; De Lara-Issasi, 1991; Vlachos *et al.*, 1997), however, few studies on the activity of seaweeds of the Gulf of California (De Lara-Issasi, 1991; Castro-Reyes, 1997) and none of them refer to antifouling activity. This work represents the initial study which describes the antifouling activity.

The main objective of this study was to evaluate the activity of different species of seaweeds from the Gulf of California against pathogenic bacteria and antifouling organism. As for the tests with pathogenic bacteria, the extracts showed differences in their activity, depending on the solvent used in the extraction. As the extracts obtained from the less polar solvent (ethyl ether) were the most active, similar results have already been reported by other authors (De Lara-Issasi *et al.*, 1989, 1993, 1996, 1999). This is possibly due to the active compounds which may be comprised by low polarity. Ethyl ether extract of *Laurencia johnstonii* showed the highest activity, with inhibition zones comparable to those obtained with commercial antibiotic erythromycin (positive control). Different species of *Laurencia* have shown antibacterial activity and from this isolated sesquiterpene-type compounds have been found which have led scientist to consider this genus as a promising source for antibacterial compounds (Bansemir *et al.*, 2004).

Table 4. Minimum Inhibitory Concentration (µg ml^{-1}) seaweed extracts against species of tropical marine microalgae.

Species	*Rhodosorus magnei*	*Neorhodella cyanea*	*Prymnesium calathiferum*	*Ochrosphaera neapolitana*	*Gambierdiscus toxicus*
Dictyota flabellata	> 50	> 50	> 50	> 50	> 50
Padina concrescens	10	10	10	> 50	> 50
Laurencia johnstonii	1	1	1	1	0.1
Gymnogongrus martinensis	> 50	> 50	> 50	> 50	> 50
Ulva lactuca	1	0.1	1	1	0.1
Codium fragile	10	1	1	> 50	> 50

Table 5. Minimum Inhibitory Concentration (µg ml^{-1}) seaweed extracts against species of marine fungi.

Species	*Halosphaeriopsis mediosetigera*	*Asteromyces cruciatus*	*Lulworthia uniseptata*	*Zalerion* sp.	*Monodictys pelagica*
Dictyota flabellata	>50	>50	>50	>50	>50
Padina concrescens	25	25	50	10	10
Laurencia johnstonii	1	1	10	0.1	1
Gymnogongrus martinensis	>50	>50	>50	>50	>50
Ulva lactuca	1	1	10	1	1
Codium fragile	>50	>50	>50	>50	>50

In the case of *Dictyota flabellata* (Phaeophyceae), both extracts (ether and buthanol) were active. Other studies showed slight antibacterial activity of species of the genus *Dictyota* against strains of Gram positive and Gram negative (Nair *et al*., 2005).

None of the extracts tested were active against *Escherichia coli* (Gram negative). Similar results have been observed in other studies, where certain algae extracts were active against Gram-positive bacteria but not against Gram-negative (Nair *et al*., 2005). Possibly indicating that the compounds of these species have restrictive reactions and therefore, its antibiotic activity is reduced to the most sensitive bacteria, such as Gram-positive bacteria. *Laurencia johnstonii* extracts showed good activity also against *E. coli*. In other studies the extract of this species it had shown activity (Castro-Reyes, 1997). *L. johnstonii* has been reported as endemic to the Gulf of California and studies from Bahía de La Paz where its raw extracts presented antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus* and *E. coli*. This activity reconfirms that this species is a potential source of bioactive compounds (Castro-Reyes, 1997).

The ethyl ethanol extracts of *Ulva lactuca* (Chlorophyta) showed antimicrobial activity. Pérez *et al*. (1990) and Tuney *et al*. (2006) reported that this species does not present any relevant activity against pathogenic bacterial strains that were tested. This difference may have been due to algal species variation, because in other studies, *U. lactuca* showed inhibitory effects against different pathogenic bacteria (Fareed & Khairy, 2008). Kandhasamy and Arunachalam (2008) showed that *U. lactuca* extract inhibited all of the test organisms except *E. coli*. Abd El-Baky *et al*. (2008) reported in more detail the chemical composition of *U. lactuca* and based on their observations suggest that the antibacterial activity of this genus would be related directly to the lipophilic content in crude organic extracts and in particular the presence of steroids from fatty products and high levels of acrylic acid. This species showed the highest activity against all fouling organisms tested contrary to reported by other authors. Hellio *et al*. (2000, 2001, 2004) tested extracts of different species of seaweed from the coast of France, including *U. lactuca* and found no activity against the majority of fouling organisms strains tested, only in the case of diatoms they observed inhibition of less than 59%.

Laurencia johnstonii showed the highest antifouling activity against all organisms tested. Antifouling studies against *Balanus amphitrite* (Darwin, 1854) have shown that species of *Laurencia* have potential activity against these organisms due to the presence of sesquiterpenes (Pawlik, 1992). Vairappan *et al*. (2008), isolated from *Laurencia* species brominated sesquiterpenes, acethylmajapolenes, halogenated sesquiterpenes, some halogenated acetogenins and bromoalenes. All these compounds displayed antibacterial activity against some marine bacterial strains.

The results presented here confirm that the seaweed *Laurencia johnstonii, Ulva lactuca* and *Dictyota flabellata* are rich in antibacterial or antifouling compounds, thus require further studies to identify the compounds responsible for these activities.

REFERENCES

Abd El-Baky, H. H., F. K. El Baz & G. S. El-Baroty. 2008. Evaluation of marine algae *Ulva lactuca* L. as a source of natural preservative ingredient. *American-Eurasian Journal of Agricultural and Environmental Science* 3: 434-444.

Abdelmohsen, U. R. 2010. Antimicrobial activities from plant cell cultures and marine sponge-associated actinomycetes. PhD. Thesis. Julius Maximilians University Würzburg, Germany. 172 p.

Albuquerque, I. R. L., K. C. S. Queiroz, L. G. Alves, E. A. Santos, E. T. Leite & H. A. O. Rocha. 2004. Heterofucans from *Dictyota menstrualis* have anticoagulant activity. *Brazilian Journal of Medical and Biological Research* 37: 167-171.

Amsterdam, D. 1996. Susceptibility testing of antimicrobials in liquid media. *In:* Loman, V. (Ed.). *Antibiotics in Laboratory Medicine*. Williams and Wilkins, Baltimore, pp. 52-111.

Bansemir, A., N. Just, M. Michalik, U. Lindequist & M. Lalk. 2004. Extracts and sesquiterpene derivatives from the red alga *Laurencia chondrioides* with antibacterial activity against fish and human pathogenic bacteria. *Chemical Biodiversity* 1: 463-467.

Barsanti, L. & P. Gualtieri. 2006*. Algae: Anatomy, Biochemistry and Biotechnology*. CRC Press, Taylor & Francis Group, Boca Raton, Florida, Estados Unidos. 301 p.

Bhakuni, D. S. & D. S. Rawat. 2005. *Bioactive Marine Natural Products*. Springer-Anamaya Publisher, Nueva Delhi, India. 382 p.

Castro-Reyes, M. A. 1997. Actividad antibacteriana de *Sargassum sinicola* (Sargassaceae, Phaeophyta) y *Laurencia johnstonii* (Rhodomelaceae, Rhodophyta) de Bahía de La Paz, B.C.S. México. Tesis de Maestría (Manejo de recursos marinos). CICIMAR-IPN, La Paz, Baja California Sur, México. 97 p.

Charzedinne, L. & M. Fariñas. 2001. Propiedades bioactivas de algas marinas del nororiente de Venezuela. Universidad de Oriente, Cumaná, Venezuela. *Boletín oceanográfico* 40: 49-54. También disponible en la página web http://bibliotecadigital.udo.edu.ve/boletinoceanografico/documentos/Vol.%2040%202001/.

Cruz-Ayala, M. B., M. Casas-Valdez & S. Ortega-García. 1998. Temporal and spatial variation of frondose benthic seaweeds in La Paz Bay, B.C.S., Mexico. *Botanica Marina* 41(1-6): 191-198.

De Lara-Issasi, G., A. Sobrino-Figueroa, C. Lozano Ramírez, M. Ponce-Márquez & E. Dreckman. 1989. Evaluación de la actividad antibiótica de las macroalgas de la costa de Michoacán, México. *Boletín del Instituto Oceanográfico*. Venezuela, Univ. Oriente 28: 99-104.

De Lara-Isassi, G. 1991. Propiedades antibióticas de algunas especies de algas marinas bentónicas. *Hidrobiológica* 1: 21-28.

De Lara-Isassi, G., M. E. Ponce, N. Hernández & A. Aguilar. 1993. Actividad antibiótica de las algas marinas de las costas de Nayarit, Jalisco y Colima, México. Facultad de Ciencias del Mar, Universidad Católica del Norte, Coquimbo, Chile, *Serie Ocasional* 2: 43-46.

De Lara-Isassi, G., S. Álvarez-Hernández & C. Lozano-Ramírez. 1996. Actividad antibacteriana de algas marinas de Oaxaca, Pacífico Tropical Mexicano. *Revista de Biología Tropical* 44: 895-898.

De Lara-Isassi, G., S. Álvarez-Hernández & C. Lozano-Ramírez. 1999. Nuevas adiciones al conocimiento de la actividad antibiótica de macroalgas marinas mexicanas. *Hidrobiologica* 9: 159-169.

Duffy, J. E. & M. E. Hay. 1990. Seaweed adaptations to herbivory. *American Institute of Biological Science* 40(5): 368-375.

Fareed, M. F. & H. M. Khairy. 2008. *In vitro* antimicrobial activities of seaweeds collected from Abu-Qir Bay Alexandria, Egypt. *World Applied Science Journal* 5: 389-396.

Hay, M. E. 1996. Marine chemical ecology: what´s know and what´s next? *Journal of Experimental Marine Biology and Ecology* 200 (1-2):103-134.

Hellio, C., G. Bremer, A. M. Pons, Y. Le Gal & N. Bourgougnon. 2000. Inhibition of the development of microorganisms (bacteria and fungi) by extracts of marine algae from Brittany (France). *Applied Microbiology and Biotechnology* 54: 543-549.

Hellio, C., D. De La Broise, L. Dufosse, Y. Le Gal, & N. Bourgougnon. 2001. Inhibition of marine bacteria by extracts of macroalgae: potential use for environmentally friendly antifouling paints. *Marine Environmental Research* 52: 231-247.

Hellio, C., J. Marechal, B. Verón, G. Bremer, A. Clare & Y. Le Gal. 2004. Seasonal variation of antifouling activities of marine algae from the Brittany Coast (France). *Marine Biotechnology* 6: 67-82.

Hellio, C & D. M. Y. Yebra. 2009. Introduction. *In:* Hellio C. & D. M. Y. Yebra (Eds.). *Advances in Marine Antifouling Coatings and Technologies*. Woodshead Publishing: Cambridge, UK., pp. 1-15.

Hellio, C, J. P. Maréchal, B. A. P. Da Gama, R. C. Pereira & A. S.Clare. 2009. Natural marine products with antifouling activities. *In:* Hellio C., D. M. Y. Yebra (Eds.). *Advances in Marine Antifouling Coatings and Technologies*. Woodshead Publishing: Cambridge, UK., pp. 572-622.

Jensen, P. R., C. D.Harvell, K. Wirtz, & W. Fenical. 1996. Antimicrobial activity of extracts of Caribbean gorgonian corals. *Marine Biology* 125: 411-419.

Jones, G. 2009. The battle against marine biofouling: a historical review. *In:* Hellio C., D. M. Y. Yebra (Eds.). *Advances in Marine Antifouling Coatings and Technologies*. Woodshead Publishing: Cambridge, UK., pp. 19-45.

Kandhasamy, M. & K. D. Arunachalam. 2008. Evaluation *in vitro* antibacterial property of seaweeds of southeast coast of India. *African Journal of Biotechnology* 7: 1958-1961.

Lindberg, E., I. Adlerberth & A. E. Wold. 2004. Antibiotic resistance in Staphylococcus aureus colonising the intestines of Swedish infants. *Clinical Microbiology Infection* 10: 890-894.

Lima-Filho, J. 2002. Antibacterial activity of extracts of six macroalgae from the northeastern brasilian coast. *Brazilian Journal of Microbiology* 33: 311-313.

Magallanes, C., C. Cordova & R. Orozco. 2003. Actividad antibacteriana de extractos etanólicos de macroalgas marinas de la costa central de Perú. *Revista Peruana de Biología* 10: 125-132.

Marechal, J. P., G. Culioli, C. Hellio, H. Thomas-Guyon, M. E. Callow, A. S. Clare, & A. Ortalo-Magné. 2004. Seasonal variation in antifouling activity of crude extracts of the brown alga *Bifurcaria bifurcata* (Cystoseiraceae) againts cyprids of *Balanus amphitrite* and the marine bacteria *Cobetia marina* and *Pseualteromonas haloplanktis*. *Journal of Experimental Marine Biology and Ecology* 213: 47-62.

Marechal, J. P. & C. Hellio. 2009. Challenges for the Development of New Non-Toxic Antifouling Solutions. *International Journal of Molecular Sciences* 10: 4623-4637.

McMichael, A. J. 2000. The changing global context of public health. *The Lancet* 356 (9228): 495-499.

Nair, R., T. Kalariya & S. Chanda. 2005. Antibacterial activity of some selected Indian medicinal flora. *Turkish Journal of Biology* 29: 41-47.

National Committee for Clinical Laboratory Standards. 1988. *Performance standards for antimicrobial disk susceptibility test*. Tentative standard, M2-T4, vol. 8. National Committee for Clinical Laboratory Standards, Villanova, Pa.

Nylund, G. M. & H. Pavia. 2003. Inhibitory effects of red algal extracts on larval settlement of the barnacle *Balanus improvises*. *Marine Biology* 143: 875-882.

Pawlik, J. R. 1992. Chemical ecologic of the settlement of benthic marine invertebrates. *Oceanographic and Marine Biology: Annual Review* 30:273-335.

Pérez, G. R. M., A. J. G. Ávila, G. S. Pérez, C. A. Martínez & C. G. Martínez. 1990. Antimicrobial activity of some American algae. *Journal of Ethnopharmacology* 29: 111-116.

Ralston, E. & G. Swain. 2009. Bioinspiration-the solution for biofouling control?. *Bioinspiration & Biomimetics* 4: 1-9.

Smit, A. J. 2005. Medicinal and pharmaceutical uses of seaweed natural products: A review. *Journal of Applied Phycology* 16: 245-262.

Tsoukatou, M., C.Hellio, C. Vagias, C. Harvala & R. Roussis. 2002. Chemical defense and antifouling activity of three Mediterranean sponges of the genus *Ircinia*. *Verlag der Zeitschrift für Naturforschung* 57: 161-171.

Tuney, I., B. H. Cadirci, D. Unal & A. Sukatar. 2006. Antimicrobial activities of the extracts of marine algae from the Coast of Urla (Izmir, Turkey). *Turkish Journal of Biology* 30: 171-175.

Vairappan, C., M. Susuki, T. Ishíi, T. Okino, T. Abe, & M. Masuda. 2008. Antibacterial activity of halogenated sesquiterpens from Malaysian *Laurencia* spp. *Phytochemistry* 69: 2490-2494.

Vlachos, V., A. T. Critchley & A. Von Holya. 1997. Antimicrobial activity of extracts from selected southern African marine macroalgae. *South Africa Journal of Science* 93: 328-332.

Wahl, M. 1989. Marine epibiosis I. Fouling and antifouling: some basic aspects. *Marine Ecology Progress Series* 58: 175-189.

Yebra, D., S. Kiil & K. Dam-Johansen. 2004. Antifouling technology: past, present and future steps towards efficient and environmentally friendly antifouling coatings. *Progress in Organic Coatings* 75: 104-109.

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