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 butanol 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 & Farías, 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, 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, Dicytota, 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: Dicytota 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 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 rigensii (ATCC 700398), Pseudoalteromonas elyakovii (ATCC 700519), Roseiarcus tolerans (DSM 11457), Vibrio aestuarianus (ATCC 35048), Vibrio anguillarum (ATCC 19264) and Vibrio pomeroyi (CAIM 578). These strains are involved in the colonization of immersed surfaces. The extracts were incubated with 100 µL of bacterial solution (2 × 10⁸ 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 determined following the conventional method of Amsterdam (National Committee for Clinical Laboratory Standards, 1996). MIC represents the lowest concentration that inhibits the organism’s growth.

**Antimicrobial activity of extracts were evaluated using bentthic phase of six temperate microalgae species:** Halamphora coffeiformis (Agardh) Levkov (Bacillariophyceae) (ACT13), Cylindrotheca closterium (Ehrenberg) Reimann et Lewin (Bacillariophyceae) (AC170), Navicula jeffreyae Hallegraeff et M. A. Burford
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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 µg 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 medioisetigera (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 determined 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 butanol fraction from D. flabellata was active against the same strain. In the case of tests against Escherichia coli, Bacillus cereus, Bacillus subtilis and Staphylococcus epidermidis 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 1. Antimicrobial activity of seaweeds extracts against pathogen bacteria.

<table>
<thead>
<tr>
<th>Species</th>
<th>Dry weight (g)</th>
<th>Extract</th>
<th>Yield extract (%)</th>
<th>Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhodophyta</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laurencia johnstonii Setchell et Gardner</td>
<td>50.83</td>
<td>E</td>
<td>0.77</td>
<td>18.7±1.5</td>
</tr>
<tr>
<td>Gymnogongrus martinensis Setchell et Gardner</td>
<td>23.67</td>
<td>E</td>
<td>0.25</td>
<td>—</td>
</tr>
<tr>
<td>Phaeophyceae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Padina concrescens Thivy</td>
<td>29.29</td>
<td>E</td>
<td>0.65</td>
<td>—</td>
</tr>
<tr>
<td>Dictyota flabellata (Collins) Setchell et Gardner</td>
<td>59.02</td>
<td>E</td>
<td>0.61</td>
<td>7.66±0.28</td>
</tr>
<tr>
<td>Chlorophyta</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulva lactuca Linnaeus</td>
<td>55.69</td>
<td>E</td>
<td>0.27</td>
<td>8.3±0.7</td>
</tr>
<tr>
<td>Codium fragile (Surigar) Hariot</td>
<td>8.14</td>
<td>E</td>
<td>2.40</td>
<td>—</td>
</tr>
</tbody>
</table>

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).
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 magni, 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).
In the case of *Dictyota flabellata* (Phaeophyceae), both extracts (ether and butanol) 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 Bahia 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 (Pawluk, 1992). Vairappan et al. (2008), isolated from *Laurencia* species brominated sesquiterpenes, acetethylajapolenes, 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.

### Table 4. Minimum Inhibitory Concentration (µg ml⁻¹) seaweed extracts against species of tropical marine microalgae.

<table>
<thead>
<tr>
<th>Species</th>
<th>Rhodosorus magnei</th>
<th>Neohodella cyanea</th>
<th>Prymnesium calthiferum</th>
<th>Ochrosphaera neapolitana</th>
<th>Gambierdiscus toxicus</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Dictyota flabellata</em></td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td><em>Padina concrescens</em></td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td><em>Laurencia johnstonii</em></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td><em>Gymnogongrus martinensis</em></td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td><em>Ulva lactuca</em></td>
<td>1</td>
<td>0.1</td>
<td>1</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td><em>Codium fragile</em></td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
</tbody>
</table>

### Table 5. Minimum Inhibitory Concentration (µg ml⁻¹) seaweed extracts against species of marine fungi.

<table>
<thead>
<tr>
<th>Species</th>
<th>Halosphaeriopsis medioisetigera</th>
<th>Asteromyces cruciatus</th>
<th>Lulworthia uniseptata</th>
<th>Zalerion sp.</th>
<th>Monodictys pelagica</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Dictyota flabellata</em></td>
<td>&gt;50</td>
<td>&gt;50</td>
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<td>&gt;50</td>
</tr>
<tr>
<td><em>Padina concrescens</em></td>
<td>25</td>
<td>25</td>
<td>50</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td><em>Laurencia johnstonii</em></td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td><em>Gymnogongrus martinensis</em></td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td><em>Ulva lactuca</em></td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Codium fragile</em></td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
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</table>
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