Antimicrobial activity of some macroalgae of the Veracruzano Reef System (SAV), Mexico

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The study of macroalgae antimicrobial agents is limited to Mexico and scarce in the Veracruzano Reef System (SAV). It is necessary to devote efforts towards this field of applied phycology. The aim was to evaluate the antimicrobial activity of some phyla of Rhodophyta, Chlorophyta and Ochrophyta from SAV. Methanolic extracts from 23 marine macroalgae species (7 Chlorophyta, 4 Phaeophyta and 12 Rhodophyta) from the Veracruzano Reef System (SAV) (Mexico) were evaluated for antimicrobial activity. Antibacterial and antifungal activity were assessed by agar diffusion and agar dilution methods. The differences between mean values obtained for experimental groups was done by analysis of variance (ANOVA multifactorial model), p-values of 0.001 or less were considered statistically significant. Two new records are recognized for SAV (Laurencia gracilis and Sebdenia flabellata) and Compsothamnion thuioides for the Gulf of Mexico coasts. 16 species showed antibacterial activity, of which Caulerpa sertulareoides, Ulva lactuca and Laurencia obtuse had significant activity on Gram-positive bacteria. 43.7% belong to the phyla Chlorophyta (7 species), 50% Rhodophyta (8 species) and 6.25% Ochrophyta (1 species). This indicates that the extracts of the algae of the Rhodophyta and Chlorophyta are the ones that showed the greatest activity. Regarding the yeasts, 16.6% of the total algae collected were active in the different yeast strains. 43.7% belongs to Chlorophyta species and for Rhodophyta were 60%. The macroalgae with the highest antifungal activity were: Cymopolia barbata, Ulva lactuca and Laurencia gracilis. The macroalgae of the Veracruzano Reef System present antimicrobial activity. This study is the first investigation of macroalgae’s bioactive components from SAV, where they could be sources for future medical applications.

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1. Introduction

Marine resources produce a wide variety of metabolites of therapeutic interest due to their biological properties (Pérez et al., 2016). Resistance is a global health problem (OMS, 2017). In this sense, macroalgae are ideal candidates for the search for new antimicrobials. It is estimated that the global diversity of microalgae and macroalgae is around 164,000 species, of which approximately 9,800 are marine algae (El-Beltagi et al., 2022).

The antibacterial activity of seaweed has been studied in different parts of the world. Extracts from 13 seaweed from South Florida and Puerto Rico have been documented by Hodgson (1984) and Ballantyne et al. (1987), among the species studied are Caulerpa prolifera (Forsskål) J.V. Lamouroux, Caulerpa mexicana Sonder ex Kützting, C. cupressoides (Vahl) C. Agardh, C. racemosa (Forsskål) J. Agardh, Anadyomene menziesii Harvey, A. stellata (Wulfen) C. Agardh, Halimeda gracilis Harvey ex J. Agardh (Chlorophyta), Sparaglossum schoederi (C. Agardh) Kützing, Dictyota diversicata J. V. Lamouroux. Likewise, Beaulieu et al. (2015) isolated peptides from Saccharina longicurta (Phaeophyta) with antibacterial activity against Staphylococcus aureus.

Abdalla et al. (2018) evaluated the antimicrobial activity of hexane, chloroform, ethyl acetate and water extracts of six marine macroalgae collected from the intertidal area Sudanese Red Sea, Saudi Arabia. The species studied were (Phaeophyta), Asparagopsis taxiformis (Delile) Trevisan de Saint Leon, Gracilaria verrucosa (Hudson) Papenfuss, Laurencia poiteaui (J.V. Lamouroux) Howe e Hypnea cervicornis J. Agardh (Rhodophyta). The tested algal extracts showed antimicrobial effects in Gram-positive and Gram-negative bacteria, among others.

On the Mexican coasts of the Caribbean Sea and the Gulf of Mexico has been reported 651 species of macroalgae (Ortega et al., 2001). The applied phycology in Mexico arose with the contributions of Guzmán del Próo (1963), who recognized the macroalgae beds of the coasts of Baja California for the extraction of alginates and carrageenan. There are few studies, so it is necessary to investigate the pharmacological potential of the bioactive secondary metabolites of Mexican algae.

In Mexico, studies have demonstrated the potential of macroalgae as antimicrobial De Lara-Issasi (1991), De Lara-Issasi et al. (1996), De Lara-Issasi et al. (1999), conducted studies on the coast of Oaxaca, Tamaulipas, Veracruz, Campeche and Yucatan, finding that more than 54% of the 57 species of algae studied had antimicrobial activity on strains of clinical importance. Likewise, Freile-Pelegriñ and Morales (2004) evaluated the antibacterial activity of hexanolic and lipid-soluble extracts from 21 seaweeds from the coast of Yucatan. As it was, Zubia et al. (2007) valued the antioxidant activities of some marine macroalgae from the coasts of Quintana Roo and Yucatan; they have high value and therefore could be considered for future applications in medicine, food production or in the cosmetic industry.

Other uses on seaweeds of the coasts of Yucatan and Quintana Roo are Antileishmanial properties (Freile-Pelegriñ et al., 2008), anti-trichomonal activity (Moo-puc et al., 2008), antioxidant activities (Zubia et al., 2014), antitrypanosomal activity (León-Denizet et al., 2009), source of antiprotozoal compounds (Cantillo-Ciau et al., 2010), antitumor activity (Moo-Puc et al., 2009; Moo-Puc et al., 2011a, Moo-Puc et al., 2011b) and hepatoprotective effect of the fucoidan, a sulfated polysaccharide isolated from algae (Chale-Dzul et al., 2015).

The potential of macroalgae species makes them ideal candidates to meet new bioactive compounds. The study of macroalgae antimicrobial agents is limited to Mexico and scarce in the Veracruzano Reef System (SAV). It is necessary to devote efforts towards this field of applied phycology. Therefore, the aim was to evaluate the antimicrobial activity of some phyla of Rhodophyta, Chlorophyta and Ochrophyta from SAV.

2. Materials and methods

2.1. Study site

The SAV is the most complex reef system in the Southern Gulf of Mexico; it is located in the port area of Veracruz and Anton Lizardo, consisting of 15 reef structures, some of them well developed. This SAV is a protected natural area made up of two groups of reefs divided by the Jamapa River. The northern group consists of reefs: Punta Gorda, Punta Majahua, Galleguilla, Gallega, Aneilda de Adentro, Blanquilla, Pájaros, Isla Verde, Hornos, Isla Sacrificios and Punta Mocambo. While, the southern group is formed: Aneilda de Afuera, Topatillo, Santiago, Aneilda Blanca, Chopolos, Rizo and Cabezo (Tunell, 1988). Of these, six were selected reefs: Gallega (19°12'00" N & 96°7'60" W), Blanquilla (21°31'00" N & 97°16'60" W), Isla Verde (19°13'00" N & 96°4'60" W), Grote (19°4'00" N & 96°0'60" W), Isla de En medio (19°06'N & 95°56'W) and Santiagouro (19°09' N & 95°48'00" W) (Fig. 1) (Hernández-Candelario et al., 2015).

For the study area, some authors classify the year into three well-established climatic seasons. The first call of “dry” that covers from March to May with an average temperature of 24 °C, wind speeds of 6.1 m/s minimum precipitation of 6.0 mm. The second is the “rainy” season, which runs from June to October. The highest temperatures are recorded, on average 26.8 °C, minimum wind speeds of around 3.8 m/s and maximum rainfall on average 200 to 400 mm. Finally, there is the “northern” season from November to February, characterized by low temperatures with averages of 20 °C, high wind speeds of 7 m/s and precipitation of 24.0 mm on average (Tunell, 1988).

2.2. Algal samples

Macroalgae was collected at SAV during May 2013 (Table 1). The algal material was separated from the substrate with a spatula or otherwise extracted manually, once collected rinsed with seawater to remove sand, waste, and associated fauna, and at −20 °C until compound extraction. A total of 23 species were collected and identified according to Littler & Littler (2000); voucher specimens were deposited in the IZTA herbarium of Universidad Nacional Autónoma de México (Thiers, 2020).

2.3. Preparation of algal extracts

Once the material was in the laboratory, the part preserved for the microbiological tests was cleaned manually with the help of a stereoscopic microscope. This in order to eliminate all epiphytic algae and/or other marine organisms. After being cleaned, they were sonicated to eliminate epiphytes, small pieces of sand and salt that could remain. Once the material was clean, it was placed on absorbent paper in order to remove excess water.

The macroalgae samples (10 g dry weight) were shade-dried at room temperature, ground into powder and sequentially extracted with 30 mL methanol (for three days). The extracts were filtered (degreased cotton and filter paper Whatman grade 1) successively concentrated under low pressure; the excess salts were removed from the extracts by partitions with acetone. Finally, they were kept in the dark at 4 °C until tested.
2.4. Microbial strains

The following bacteria strains were used: Staphylococcus aureus ATCC 29213, Escherichia coli ATCC 25922, Salmonella typhi ATCC 19430, S. epidermidis donated by the Clinical Analysis Laboratory of FES-Iztacala. These strains were maintained at 4°C in Mueller Hinton Broth (Bioxon), submitted to sensitivity tests (multidisc Bigaux), and subculturally every month.

The yeasts tested were Candida albicans ATCC 14065, C. albicans isolated from a clinical case donated by the Clinical Analysis Laboratory of FES-Iztacala, C. glabrata, C. tropicalis isolated from a clinical case and donated by Hospital Angeles (Metropolitano). The stock culture was maintained in Czapek Dox Agar (Sigma).

2.5. Antibacterial activity

The antibacterial activity was measured by the disc-diffusion method (Vander & Vlietinck, 1991; CLSI, 2012). The microorganisms were grown overnight at 37°C in 10 mL of Mueller Hinton Broth (Bioxon). The cultures were adjusted with sterile saline solution to obtain turbidity comparable to that of McFarland no. 0.5 standard (1.0 × 10^8 CFU/mL). Petri dishes containing Mueller Hinton agar (Bioxon) were inoculated with these microbial suspensions. Solutions of 200 mg/mL of each extract were prepared, filter paper discs (Whatman no. 5) of 5 mm diameter were impregnated with 10 lL of each one (final doses per disc: 2 mg of methanolic extract) and placed on the agar surface. The discs impregnated with methanol and another with chloramphenicol (25 lL g) were used as negative and positive controls, respectively. The plates were incubated overnight at 37°C, and the resulting inhibition zones were measured with a vernier. Each experiment was performed in triplicate.

The Minimal Inhibitory Concentration (MIC) estimation was carried out by the broth dilution method (Vander & Vlietinck, 1991; CLSI, 2012). Dilutions of extracts from 2 to 0.06 mg/mL were evaluated. The tubes were inoculated with 10^5 CFU/mL microorganism suspensions. MIC values were taken as the lowest extract concentration that prevents visible bacterial growth after 24 h of incubation at 37°C. Chloramphenicol was used as a reference, and appropriate controls with no extract were used. Each experiment was made three times.

2.6. Antifungal activity

The evaluation of antifungal activity in yeast for each extract was evaluated following the same methodology as for the antibacterial test, but with potato dextrose agar and nystatin as a positive control (Vander & Vlietinck, 1991; CLSI, 2012). Dilutions of extracts from 2 to 0.06 mg/mL were evaluated. The tubes were inoculated with 10^5 CFU/mL microorganism suspensions. MIC values were taken as the lowest extract concentration that prevents visible fungal growth after 24 h of incubation at 37°C. Chloramphenicol was used as a reference, and appropriate controls with no extract were used. Each experiment was made three times.

2.4. Microbial strains

Table 1
Macroalgal species collected in SAV.

| Species                              | Locality       | Voucher number |
|--------------------------------------|----------------|----------------|
| Phylum Chlorophyta                   |                |                |
| Caulerpa chemnitzia (Esper) J. V. Lamouroux | S 1813        |                |
| Caulerpa racemosa (Forsskål) J. Agardh | BL, IV 1824, 1817 |                |
| Caulerpa sertularioides (S. G. Gmelin) M. Howe | GA 1823       |                |
| Cymopolia barbata (Linnaeus) J. V. Lamouroux | IE 1812       |                |
| Dictyosphaeria cavernosa (Forsskål) Bergesen | IE 1810       |                |
| Ulva intestinalis Linnaeus           | GA 1835       |                |
| Ulva lactuca Linnaeus               | GA 1829       |                |
| Phylum Rhodophyta                    |                |                |
| Amphirooa fragilissima (L.) J. V. Lamouroux | IE, IV 1822, 1837 |                |
| Ceramium sp.                         | GI 1818       |                |
| Composhamnion thiooides (Smith) Nägeli* | BL 1836       |                |
| Galaxaura comans Kjellman           | BL, IV 1826, 1827 |                |
| Hypnea spinella (C. Agardh) Kützing  | GA 1834       |                |
| Laurencia gracilis J. D. Hooker y Harvey** | GA, GI 1819, 1815 |                |
| Laurencia obtusa (Hudson) J. V. Lamouroux | GI, IE 1811, 1808 |                |
| Titanophysus validus (Harvey) Huisman G. W. et A. R. Sherwood | GA 1820 |                |
| Sebdenia flabelata (J. Agardh) P. G. Parkinson** | IE 1809       |                |
| Tricleocarpa cylindrica (J. Ellis et Solander) Huisman et Borowitzka | BL 1825 |                |
| Tricleocarpa fragilis (Linnaeus) Huisman et R. A. Townsend | IE 1821 |                |
| Wrangelia bicuspidata Bergesen       | GI 1807       |                |
| Phylum Ochrophyta                    |                |                |
| Dictyota dichotoma (Hudson) J. V. Lamouroux | IE 1828 |                |
| Padina boergsenii Allender & Kraft   | IE 1806       |                |
| Sargassum fluitans (Bergesen) Bergesen | S 1816       |                |
| Sargassum natans (Linnaeus) Gaillon  | IE 1814       |                |

BL: Blanquilla, GA: Gallega, IE: Isla de Enmedio, IV: Isla Verde, S: Santiaguillo, GI: Giote.
* New registration for Mexico,
** New registration for the SAV.
The macroalgae collected in the six reefs (Blanquilla, Gallega, Giote, Isla de Enmedio, Isla Verde and Santiaguillo) were determined. Table 1 shows the list of SAV marine algae by division. 23 species of macroalgae were collected. The Chlorophyta phylum presented 7 species. The genera Caulerpa and Ulva were recorded in more than one reef (Table 1). In the Rhodophyta, 12 species were determined. The genera Laurencia and Tricleocarpa were the ones with the highest incidence in the different reefs. It is important to mention that two new records were found from SAV: Laurencia gracilis (Gallega and Giote) and Sebdenia flavellata (Isla de Enmedio). In addition, Composothamnion thyoides collected in Blanquilla, is a new record for Mexico.

The Ochrophyta phylum was the one with the lowest number of species with only four records: Dictyota dichotoma, Padina boergenseni, Sargassum fluitans and S. natans. Most of the species were found on Isla de Enmedio.

The algae of the Rhodophyta phylum can be found in all the sampling sites. In the case of Chlorophyta in the only reef where they were not found is in Giote, this could be due to the fact that visibility in this place was poor at the time of collection. Finally, the Ochrophyta was only found in Isla de Enmedio and Santiaguillo.

The results obtained in the qualitative evaluation of the antibacterial activity of the algal methanolic extracts are shown in Table 2. Of the 23 algal methanolic extracts 16 had activity in at least one bacterial strain, which is equivalent to 53.3 % of active species, nine algae species had no activity: Caulerpa chemnitzia, Dictyota dichotoma, Padina boergenseni, Sargassum fluitans, Ceramium sp., Titanophyucus validus, Tricleocarpa fragilis, Sebdenia flavellata and Wrangelia bicuspidata.

On the other hand, the percentage of active macroalgae in both collection areas (north and south) was calculated. The North group presents 62.5 % of active species compared to the South group with 37.5 %. However, the statistical analysis does not show a significant difference ($F = 0.51, P < 0.05$) regarding the number of species that were active for each zone.

The results obtained in the quantitative determination of the antibacterial activity of the algae extracts are shown in Table 3. S. epidermidis was the most sensitive strain with the algae extract of L. obtusa (MIC = 0.125 mg/mL) and C. barbata (MIC = 0.5 mg/mL). Table 4 shows the average of the results obtained from the antifungal activity in yeasts. 16.6 % of the total macroalgae were active in the different yeast strains. The algae with the highest antifungal activity were: C. barbata, U. lactuca and L. gracilis.

When performing the ANOVA to compare the activity in both zones, it was obtained that there are no statistically significant differences ($F = 0.25, P < 0.05$) between the antifungal activity in yeasts for the North and South zones.

The yeasts were sensitive to the algal extract of C. barbata, this was the most active since it inhibited the growth of C. albicans cc with 0.0625 mg/mL, to C. albicans ATCC 14,065 at 0.125 mg/mL (Table 5).

The time-killing curves assay was carried out in S. aureus ATCC 29,213 and showed that in 0.5 MIC, MIC and MBC (0.25, 0.5 and 1.0 mg / mL respectively) there was a decrease in bacterial growth, becoming more evident at 6 h of exposure to a concentration of MBC, where the bacterial population decreases 1.5 logarithmic units (Fig. 2).

### 4. Discussion

In the qualitative evaluation of the antibacterial activity of the 23 algal methanolic extracts Caulerpa sertularioides showed the biggest inhibition zones in S. epidermidis (15.0 ± 0.0 mm) and S. aureus ATCC 29,213 (10.0 ± 0.0 mm). These activities were similar to Freile & Morales (2004) results for species from the Caulerpa coast, Yucatán, where the activity against Gram-positive bacteria was between 7.3 and 11.0 mm. This activity is probably due to the terpenoid compounds reported (Fenical & Paul, 1984; Ballantine et al., 1987). It should be noted that C. sertularioides also showed activity in Gram-negative bacteria.

While L. obtusa and U. lactuca had activity in two Gram-positive strains with 9.0 ± 0.0 and 8.0 ± 0.0 mm; the algae extracts show more inhibition zones Gram-positive strains than in the Gram-negative ones. This activity agrees with that reported by De Lara Isassi et al. (1996) and corroborates the knowledge of the structure of the wall of Gram-negative cells, which is more complex and, therefore, generally shows low activity (Lustigman & Brown, 1991).

In the quantitative determination of the antibacterial activity L. obtusa and C. barbata present the lowest MIC values (0.125–0.50 mg/mL) this is probably due to when they are subjected to stress, the production of secondary metabolites is altered (Dorta et al., 2002; El-Beltagi et al., 2022). Macroalgae are subject to stress because the sewage discharges near the port of Veracruz may be one of the main changes in the North group from SAV. Sewage dumping leads to nutrient enrichment of waters and may increase bacterial populations. Moreover, sewage also increases suspended solid sediments in the water (Gutiérrez-Ruiz et al., 2011; Landeros-Sánchez et al., 2012).

The yeasts were more sensitive to the algal extract of C. barbata present the lowest MIC values in C. albicans and C. glabrata strains (MIC 0.062–0.50 mg/mL), it is essential to emphasize the activity in yeasts since most infectious agents in mucosa and skin are caused by Candida species (Cecilia et al., 2012). The antifungal activity might be attributed to a wide range of chemical classes identified in the ethanolic extracts (Pérez et al 2016). For other side, the indiscriminate use of antifungal, yeasts have developed resistance, and C. albicans has been reported to have acquired resistance to many known antimicrobials (Cowen et al., 2002; Mickymaray and Alturaki, 2018). Our known that C. barbata (Dasycladales) is a lightly calcified green alga that produces antifungal compounds under conditions of physiological stress from Cuba (González del Val et al., 2001); similar to the studied samples.

In the time-killing curves assay there was a decrease in bacterial growth, Sánchez (2017) suggest that different processes can generally be happening when this effect is achieved: 1) inhibiting ribosomal function and therefore protein synthesis, 2) altering the cell membrane or 3) inhibiting the synthesis of nucleic acids. The antimicrobial activity of C. barbata can be explained by the fact that it has brominated compounds (Kazanjian & Fariñas, 2006), these substances belong to the group known as organic halides, which are present in these living beings due to the high content of fluorine, chlorine, iodine and bromine present in seawater. These are incorporated and therefore can synthesize compounds
with these atoms, unlike organisms in the terrestrial environment, where halides production is almost null. It is known that marine organisms produce them because they are used for various functions as they serve as repellents and appetite suppressants, antibacterial and anti-fouling agents, pheromones and hormones (Gribble, 2005; Hamed et al., 2015; Leandro et al., 2020).

Another important point is that macroalgae *C. barbata* synthesizes a compound called “cymopole” (Park et al., 1992; Gallimore et al., 2009), which has been reported to inhibit herbivory by the mollusk *Littorina littorea* and the sea urchin *Lytechinus variegatus*, Debromoisocymobarbatol has been reported to decrease herbivory levels by up to 50% of the *Lagodon rhomboides* fish and the amphipod *Hyale macrodactyla* (Park et al., 1992), thus indicating that the compound may serve a defensive role in this alga, that is, it is a rich source of pharmacologically active compounds, in addition to other biological properties defensive nature (Dorta et al., 2002). In addition to geography, seasonal variation can also influence antimicrobial activity by seaweed extracts. Studies performed by Deveau et al. (2016) showed greater antimicrobial activity of *U. lactuca* when algae were harvested in the fall and winter compared to the summer months, where the activity can be reduced.

### Table 2
Qualitative evaluation of the antibacterial activity of some macroalgae of the Veracruzano Reef System (SAV).

| Species                  | Inhibition zones (mm) |  |
|--------------------------|-----------------------|---|
|                          | *E. coli* cc | *S. typhi* | *S. epidermidis* cc | *S. aureus* ATCC 29,213 |
| *Caulerpa sertularioides* | 6.0 ± 0.0 | 6.0 ± 0.0 | 15.0 ± 0.0 | 10.0 ± 0.0 |
| *Ulva lactua*            | 6.0 ± 0.0 | 6.0 ± 0.0 | 8.0 ± 0.0 | 9.0 ± 0.0 |
| *Ulva intestinalis*      | na         | 6.0 ± 0.0 | 6.0 ± 0.0 | na |
| *Cymopolia barbata*     | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | na |
| *Dictyosphaeria cavernosa* | na | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 |
| *Caulerpa racemosa*     | 6.0 ± 0.0 | na | na | na |
| *Ochrophyta*             |           |           |           | |
| *Sargassum natans*      | na         | 6.0 ± 0.0 | 8.0 ± 0.0 | na |
| *Rhodophyta*            |           |           |           | |
| *Composthamnion thuioides* | na | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 |
| *Trichocarpa cylindrica* | na         | 6.0 ± 0.0 | 7.0 ± 0.0 | 6.0 ± 0.0 |
| *Galaxaura comans*      | 6.0 ± 0.0 | na | na | na |
| *Laurencia gracilis*    | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | na |
| *Titanophycus validus*  | na         | 6.0 ± 0.0 | 6.0 ± 0.0 | na |
| *Hypnea spinella*       | 6.0 ± 0.0 | na | 5.3 ± 4.6 | 7.0 ± 0.0 |
| *Laurencia obtusa*      | 6.0 ± 0.0 | 9.0 ± 0.0 | na | 8.0 ± 0.0 |
| *Amphiroa fragilissima* | na         | 6.0 ± 0.0 | 7.0 ± 0.0 | na |
| Control chloramphenicol | 15.7 ± 0.6 | 21.7 ± 1.5 | 20.0 ± 2.6 | 13.3 ± 0.6 |

Each bioassay was performed in triplicate. mean ± S. D. (n = 3). Positive control: Chloramfenicol 25 μg, algae extracts 2 mg, na: no activity.

### Table 3
Minimal inhibitory concentrations of some macroalgae of the Veracruzano Reef System (SAV) in bacterial strains.

| Species                  | *S. typhi* | *S. epidermidis* cc | *S. aureus* ATCC 29,213 |
|--------------------------|------------|---------------------|------------------------|
| *Composthamnion thuioides* | 4.0        | nd                  | nd                     |
| *Titanophycus validus*   | > 4.0      | > 4.0               | nd                     |
| *Laurencia obtusa*       | 0.5        | 0.125               | 0.5                    |
| *Cymopolia barbata*      | 4.0        | 0.5                 | 0.5                    |
| *Dictyosphaeria cavernosa* | > 4.0 | nd                  | nd                     |
| *Chlorophyllum (25 μg)*  | 0.002      | 0.002               | 0.008                  |

MIC (mg/mL), nd: no determinate.

with these atoms, unlike organisms in the terrestrial environment, where halides production is almost null. It is known that marine organisms produce them because they are used for various functions as they serve as repellents and appetite suppressants, antibacterial and anti-fouling agents, pheromones and hormones (Gribble, 2005; Hamed et al., 2015; Leandro et al., 2020).

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### Table 4
Qualitative evaluation of the antifungal activity of some macroalgae of the Veracruzano Reef System (SAV).

| Species                  | Inhibition zones (mm) |  |
|--------------------------|-----------------------|---|
|                          | *C. glabrata* cc | *C. albicans* ATCC 14,065 | *C. tropicalis* cc | *C. albicans* cc |
| *Chlorophyta*            |           |           |           |           |
| *Ulva lactua*            | 7.0 ± 0.0 | 6.0 ± 0.0 | 6.3 ± 0.6 | 6.0 ± 0.0 |
| *Cymopolia barbata*     | 7.0 ± 0.0 | 7.0 ± 0.0 | 7.3 ± 0.6 | 7.0 ± 0.0 |
| *Rhodophyta*            |           |           |           |           |
| *Composthamnion thuioides* | > 4.0 | nd | nd | nd |
| *Laurencia gracilis*    | > 4.0      | 1.00      | na        | na        |
| *Laurencia obtusa*      | 6.7 ± 1.2  | 9.0 ± 0.0  | 7.0 ± 0.0  | na        |
| *Nistatine (30 μg)*     | 7.3 ± 0.6  | na        | na        | na        |
| *Cymopolia barbata*     | 17.0 ± 0.1 | 25.1 ± 0.1 | 20.0 ± 0.1 | 25.1 ± 0.1 |

Each bioassay was performed in triplicate. mean ± S. D. (n = 3). Positive control: Nystatine 30 μg, algae extracts 2 mg, na: no activity.

### Table 5
Minimal inhibitory concentrations of some macroalgae of the Veracruzano Reef System (SAV) in yeast strains.

| Species                  | *C. glabrata* cc | *C. albicans* ATCC 14,065 | *C. tropicalis* cc | *C. albicans* cc |
|--------------------------|------------------|---------------------------|-------------------|------------------|
| *Ulva lactua*            | nd               | 0.25                      | nd                | 0.125            |
| *Laurencia obtusa*      | 1.00             | 0.125                     | 2.00              | 0.062            |
| *Cymopolia barbata*     | 0.50             | 0.125                     | 0.009             | 0.011            |
| *Nistatine*              | 0.008            | 0.011                     |                   |                  |

MIC (mg/mL), nd: no determinate.
extracts contain multiple antimicrobial compounds, which may act alone or synergistically to inhibit microbial growth.

5. Conclusion

The results indicate that extracts from marine algae from SAV exhibit antibacterial activity by two Chlorophyta: Caulerpa sertularioides, Ulva lactuca, and one Rhodophyta: L. obtusa were the ones that had more significant activity in bacterial strains Gram-positive. On the other hand, the methanolic extract of C. barbata recorded antifungal activity in C. albicans, C. glabrata and C. tropicalis. This is the first work on applied phycology, of this aspect with macroalgae from SAV, and they could be sources of natural products for future medical applications.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abdalla, E.O., Abdalla-Shigd, M.T., Khalid, H.E., 2018. Antimicrobial activity of solvent extracts of selected Red Sea Macrocystis against some pathogenic microorganisms. Saudi J. Pathol. Microbiol. (SPJM) Saudi J. Pathol. Microbiol. 3 (3), 93–98. https://doi.org/10.21276/spjm.20183.3.6.

Avila, J.G., De Liverant, J., Martínez, A., Martínez, G., Muñoz, J.L., Arciniegas, A., Romo de Vivar, A., 1999. Mode of action of Buddleja cordata verbascoside against Staphylococcus aureus. J. Ethnopharmacol. 66, 75–78. https://doi.org/10.1016/S0378-8741(98)00203-7.

Ballantine, D.L., Gerwick, W.H., Velez, S.M., Alexander, E., Guevara, P., 1987. Antibiotic activity of lipid-soluble extracts from Caribbean marine algae. Hydrobiology 151–152 (1), 463–469.

Beaulieu, L., Bondu, S., Douren, K., Rioux, L.E., Sylvie, L., 2015. Turgon Characterization of antibacterial activity from protein hydrolysates of the macroalga Saccharina longicruris and identification of peptides implied in bioactivity. J. Funct. Foods 17, 685–697. https://doi.org/10.1016/j.jff.2015.06.026.

Cantillo-Ciau, Z., Moo-Puc, R., Quijano, L., Freile-Pelegrín, Y., 2010. The tropical brown alga Lophophora variegata: a source of antiprotozoal compounds. Mar. Drugs 8, 1292–1304. https://doi.org/10.3390/md8041292.

Chale-Dzul, J., Moo-Puc, R., Robledo, D., Freile-Pelegrín, Y., 2015. Hepatoprotective effect of the fucoidan from the brown seaweed Turbinaria triplicata. J. Appl. Physiol. 27, 2123–2135. https://doi.org/10.1173/10811-014-0429-9.

CLSI (Clinical and Laboratory Standards Institute), 2012. Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement. Tech. Rep. M100-S22; Clinical and Laboratory Standards Institute (CLSI); Wayne, PA, USA. 184p.

Cowen, L.E., Anderson, J.B., Kohn, L.M., 2002. Evolution of drug resistance in Candida albicans. Annu. Rev. Microbiol. 56, 139–165. https://doi.org/10.1146/annurev.micro.56.012302.160907.

De Lara Isassi, G., 1991. Propiedades antibióticas de algunas especies de algas marinas bentónicas. Hidrobiología 1 (2), 21–28 http://www.redalyc.org/articulo.oa?id=37849746013.

De Lara Isassi, G., Álvarez-Hernández, S., Lozano-Ramírez, C., Hernández-Soto, N., 1999. Nuevas adiciones al conocimiento de la actividad antibiótica de macroalgas marinas mexicanas. Hidrobiología 9 (2), 159–169 https://hidrobiologia.uxm.uam.mx/index.php/revHidro/article/view/799.

De Lara Isassi, G., Álvarez-Hernández, S., Lozano-Ramírez, C., 1996. Actividad antituberculosa de algas marina de Oaxaca. Pacífico Tropical Mexicano. Revista Biología Tropical 44 (2), 895–898.

Deveau, A.M., Miller-Hope, Z., Lloyd, E.B., Williams, S., Bolduc, C., Meader, J.M., Weiss, F., Burkholder, K.M., 2016. Antimicrobial activity of extracts from macroalgae Ulva lactuca against clinically important Staphylococcus isococci is impacted by lunar phase of macroalgae harvest. Lett. Appl. Microbiol. 62, 363–371. https://doi.org/10.1111/lam.12563.

Dorta, E., Darias, J., San Martín, A., Cueto, M., 2002. New Preyneltad Bromoquinones from the Green Alga Cymopolia barbata. J. Nat. Prod. 65 (3), 329–333.

Durán, D.A., Vargas, V.A., Casneros, C.A.E., 2004. Bioestadística. Facultad de Estudios Superiores Iztacala; Universidad Nacional Autónoma de México: Mexico City, Mexico, pp. 100–114.

El-Beltagi, H.S., Mohamed, A.A., Mohamed, H.I., Ramadan, K.M.A., Barqawi, A.A., Mansour, A.T., 2022. Phytochemical and potential properties of seaweeds and their recent applications: a review. Mar Drugs. 20 (6), 342.

Fenic, W., Paul, V.J., 1984. Antimicrobial and cytotoxic terpenoids from tropical green algae of the family Udoteaceae. Hydrobiology 17, 137–170.

Freile-Pelegrín, Y., Morales, J.L., 2004. Antibacterial activity in marine algae from Yucatan Coast, Mexico. Bot. Mar. 47, 140–146. https://doi.org/10.1515/BOT.2004.014.

Freile-Pelegrín, Y., Robledo, D., Chan-Bacab, M.J., Ortega-Mora, B.O., 2008. Antileishmanial properties of tropical marine algae extracts. Fitoterapia 79 (5), 374–377. https://doi.org/10.1016/j.fitote.2008.02.006.

Gallimore, W.A., Sambo, T., Campbell, T., 2009. Dehormocymopolone from the green alga, Cymopolia barbata. J. Chem. Res. 2009 (3), 160–161.

González del Val, A., Platas, G., Basilio, A., Cabello, A., Gorrochategui, J., Suay, I., Vicente, F., Portillo, E., Jiménez del Río, M., García Reina, G., Peláez, F., 2011. Screening of antiparasitic activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). Int. J. Microbiol. 4, 35–40. https://doi.org/10.1155/2011/480156.

Gribose, G.W., 2005. Halogenogénos orgánicos. Investigación y Ciencia, 78–84.

Cecilia, E.A., Dos Santos, A.M.A., Pinheiro, S.A.K., Oliveira de Souza, L.I., De Barros, L., De Albuquerque, M.F.C., Goulart, S.A.E., 2012. Antifungal activities of different extracts of marine macroalgae against dermatophytes and Candida species. Mycopathologia 174 (1), 221–232.

Gutiérrez-Ruiz, C.V., Romás-Vives, M.A., Vergara, C.H., Badano, E.I., 2011. Impacto de perturbaciones antrópicas sobre la diversidad de corales pétreos super ciales en el Parque Nacional Sistema Arrecifal veracruzano. Revista Mexicana de Biodiversidad 82, 249–260.

Guzmán del Próo, S.A., 1963. Las algas marinas como un recurso natural renovable. El pescador 11, 32–37.
Hamed, I., Özogul, F., Özogul, Y., Regenstein, J.M., 2015. Marine bioactive compounds and their health benefits: a review. Comprehensive Review in Food Sci. Food Saf. 14, 446–453. https://doi.org/10.1111/1474-4138.12130.

Hernández-Candelario, I.C., Morteo, E., Heckel, G., Sosa-Nishizaki, G., Álvarez-Sánchez, L.G., Flores-Uzeta, O., Martínez-Serrano, I., 2015. Caracterización de la relación entre la distribución espacio-temporal de los tursiones (Turitopsis truncatus) y las actividades humanas en el Parque Nacional Sistema Arrecifal Veracruzano. E-Bios, Parque Nacional Sistema Arrecifal Veracruzano 2 (8), 34–52.

Hodgson, L.M., 1984. Antimicrobial and antineoplastic activity in some South Florida seaweeds. Bot. Mar. 27, 387–390.

Kazanjian, A., Fariñas, M., 2006. Actividades biológicas del extracto acuoso de la esponja Aplysia lacunosa (Porifera: Aplysinidae). Rev. Biol. Trop. 54 (3), 189–200.

Landeros-Sánchez, C., Lango-Reynoso, F., Castillo-Chávez, M.R., Galávez-Villa, L., Nikolski-Gavrilov, I., Palomar-García, M., Reyes-Velázquez, C., Mínguez-Rodríguez, M.M., 2012. Assessment of water pollution in different aquatic systems: aquifers, aquatic farms on the Jamapa River, and coastal lagoons of Mexico. J. Agric. Sci. 4 (7), 186–196.

Leandro, A., Pereira, L., Gonçalves, A.M.M., 2020. Diverse applications of marine macroalgae. Mar. Drugs 18, 17. https://doi.org/10.3390/md18010017.

León-Deniz, L.V., Dumonteil, E., Moo-Puc, R., Freile-Pelegrín, Y., 2009. Antiparasitonal in vitro activity of tropical marine algae extracts. Pharmaceutical Biol. 47 (9), 864–871. https://doi.org/10.1080/13880209092950777.

Littler, D.S., Littler, M.M., 2000. Caribbean reef plants: an identification guide to the reef plants of the Caribbean, Bahamas, Florida, and Gulf of Mexico. Offshore Graphics, Inc. Washington, D. C., U.S. A. 542 pp.

Lustgman, B., Brown, C., 1991. Antibiotic production by marine algae isolated from the New York and New Jersey coast. Bull. Environ. Contamination Toxicol. 46, 329–335.

Mickymaray, S., Alturaiki, W., 2018. Antifungal efficacy of marine macroalgae against fungal isolates from bronchial asthmatic cases. Molecules 23, 3032. https://doi.org/10.3390/molecules23113032.

Moo-Puc, R., Robledo, D., Freile-Pelegrín, Y., 2008. Evaluation of selected tropical seaweeds for in vitro anti-trichomonal activity. J. Ethnopharmacol. 120, 92–97. https://doi.org/10.1016/j.jep.2008.07.035.

Moo-Puc, R., Robledo, D., Freile-Pelegrín, Y., 2009. In vitro cytotoxic and antiproliferative activities of marine macroalgae from Yucatán, Mexico. Ciencia Marina 35 (4), 345–358. https://doi.org/10.1158/1940-6207.PREV-09-B78.

Moo-Puc, R., Freile-Pelegrín, Y., Robledo, D., 2011a. Enhanced antitumoral activity of extracts derived from cultured Udotea flabellum (CHLOROPHYTA). J. Evidence-Based Complementary Alternative Medicine 969275, 7 pages. https://doi.org/10.1155/2011/969275.

Moo-Puc, R., Robledo, D., Freile-Pelegrín, Y., 2011b. Improved antitumoral activity of extracts from cultured Penicillus dumetosus. Trop. J. Pharm. Res. 10, 177–185. https://doi.org/10.4314/tjr.v10i2.66561.

Ortega, M.M., Godínez, J.L., Garduño-Solórzano, G., 2001. Catálogo de algas béticas de las costas mexicanas del Golfo de México y Mar Caribe. Cuadernos 34. Instituto de Biología, UNAM. México, p. 594.

Park, M., Fenical, W., Hay, M.E., 1992. Debronoisoscyymobarbatol, a new chromanol from marine macroalgae. Mar. Drugs 18, 17. https://doi.org/10.3390/md18010017.

Further Reading

Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. http://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf?ua=1. [Accessed October 2, 2022].