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BIOTECHNOLOGY

# Antibacterial and cytotoxicity activity in macroalgae extracts: perspectives for the use against pathogenic bacteria from shrimp farms (*Litopenaeus vannamei*)

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**ABSTRACT.** Some infections caused by pathogenic microorganisms might shows high prevalence in farmed shrimp environments, compromising production and causing economic losses. Therefore, the search for compounds with antibiotic activity has become intensive, following the record of new antimicrobial-resistant bacteria. The study of those bioactive compounds in marine macroalgae has produced satisfactory results, such as the discovery of antibacterial activity against multiresistant strains. Accordingly, this study aims to research antibiotic activity in macroalgae extracts of Chlorophyta, Phaeophyta and Rhodophyta found in the coast of Ceará and also to evaluate the cytotoxicity activity against bacterial strains (*Vibrio* sp.) from shrimp farms (*Litopenaeus vannamei*). The extracts cytotoxicity was also evaluated. The results prove that there was antibacterial activity in ethanolic, acetonic, hexanic and methanolic extracts against bacterial strains of *Vibrio* with multiple resistance profile as well as displaying low cytotoxicity.

**Keywords:** bioactivity; antibiotic; cytotoxicity; resistance.

## Atividade antibacteriana e citotóxica em extratos de macroalgas: perspectivas de uso contra bactérias patogênicas de ambiente de cultivo de camarões (*Litopenaeus vannamei*)

**RESUMO.** Algumas infecções causadas por micro-organismos patogênicos podem apresentar alta prevalência em ambientes de cultivo de camarões marinhos, comprometendo a produção e causando prejuízos econômicos aos aquicultores. Assim, tem-se tornado intensa a busca por compostos com atividade antibiótica pelo registro cada vez mais frequente de bactérias com perfil de resistência a antimicrobianos. A presença desses compostos com bioatividade em macroalgas marinhas tem revelado resultados satisfatórios, como a descoberta de ação antibacteriana contra cepas multirresistentes. Desta forma, decidiu-se pesquisar as propriedades antibióticas dos extratos de macroalgas das classes Chlorophyta, Phaeophyta e Rhodophyta, coletadas no litoral cearense, bem como avaliar a citotoxicidade destes extratos, frente a cepas bacterianas (*Vibrio* sp.) isoladas e provenientes de ambientes de cultivo de camarões marinhos (*Litopenaeus vannamei*). Os resultados comprovaram que houve atividade antibacteriana dos extratos etanólicos, acetônicos, hexânicos e metanólicos contra cepas bacterianas de *Vibrio*, além de apontar que os extratos de todas as espécies apresentaram baixa citotoxicidade.

**Palavras-chave**: bioatividade; antibiótica; citotoxicidade; resistência.

## Introduction

Marine environment presents a large diversity of species. This environmental wealth stimulates the development of a variety of chemical structures, such as bioactive secondary metabolites, which hold a high potential for the discovery of new drugs (Molinski, Dalisay, Lievens, & Saludes, 2009; El Gamal & Ali A, 2010).

Among marine species, macroalgae are multicellular, eukaryotic autotrophs that lack a vascular system. They are classified in three groups, according to their pigmentation: green, red and brown; chlorophytes, rhodophytes and phaeophytes, respectively (Pádua, Fontoura, & Mathias, 2004). These aquatic organisms are the source of many substances used in various activities around the world, such as food, drugs, cosmetic and agro-industry (Vidotti & Rollemberg, 2004).

Marine macroalgae are rich in diverse kinds of chemical compound; they comprise most classes of natural products, including some that cannot be obtained from other sources. The interest in the biomedical potential of marine macroalgae is due to the

abundance and variety of their bioactive compounds (Maschek, Baker, & Amsler, 2008).Those organisms are rich in metabolites and compounds, such as lectins, terpenes, phenolic compounds and sulfated polysaccharides. Furthermore, marine macroalgae are used for various purposes including: biostimulants, fertilizers and control of pest and pathogenic microorganisms. Some polysaccharides have several applications in the pharmaceutical, food and biotechnology industries, among other uses (Dapper, Pujarra, Oliveira, Oliveira, & Paulert, 2014; Vasconcelos et al., 2015).

Moreover, the resistance to antibiotics is one of the main public health concerns worldwide. Resistance patterns are found in microorganisms pathogenic to humans as well as to animals. This status leads to increasing interest in new bioactive substances extracted from marine macroalgae that might work as an alternative to synthetic drugs (AL-Haj et al., 2010).

Among the many groups of compounds found in marine macroalgae, it is worth noting the presence of secondary metabolites, responsible for biological cytotoxic and antibacterial activities. Among the most prominent are terpenes, sesquiterpene, diterpenes, triterpenes and acetogenins (Machado, Kaiser, Costa, Gestinari, & Soares, 2010). According to Vairappan (2003), the halogenated compounds elatol and iso-obtusol were found and isolated from the red alga *Laurencia majuscula*, that inhibited the growth of the microorganisms *Staphylococcus epidermis*, *Klebsiella pneumoniae* and *Salmonella* sp.

Previous studies have raised attention to the relevance of isolates from marine macroalgae with antimicrobial potential (Chiheb et al., 2009; Kolanjinathan & Stella, 2009). Studies conducted with algae species have presented promising results in the detection, isolation and identification of molecules that interact with bacteria of both wall types: Gram-negatives (Cox, Abu-Ghannam, & Gupta, 2010; Silva et al., 2013;) and Gram-positives (Goecke, Labes, Wiese, & Imhoff, 2012; Pierre et al., 2011).

Gram-negative bacteria belonging to the *Vibrio* genus are well-known pathogens in marine shrimp farms, being related to infection events and consequent loss in production (Vieira et al., 2010; Vanmaele, Defoirdt, Cleenwerck, Vos, & Bossier, 2015). The use of antimicrobial agents in the treatment and prevention of these infectious diseases results in the selection of resistant strains, which accrue a series of losses to public health, environment and the activity itself.

Considering the need for alternatives for antimicrobial drugs generally used in human or animal health care, this research aimed to detect and evaluate the antimicrobial potential of macroalgae extracts from species present in the coast of Ceará state, as well as their cytotoxic effects. As indicators, Gram-positive and Gram-negative bacteria of the *Vibrio* genus with multi-resistant profile to commercial drugs were used.

## Material and methods

### Sampling

Samples were collected in natural banks during syzygy tides in the coastal area of Paracuru (Latitude 3°23'52"S, Longitude 39°0'46"W) and Pacheco (Latitude 03°41'06.8"S, Longitude 038°38'02.8"W) from the cities of Paracuru and Caucaia, Ceará State, respectively, in August 2013 (Figure 1). Species of red, green and brown macroalgae were collected according to local occurrence and evidence of biological activity in previous literature.

### Sample processing

After the samples were collected, they were transported in plastic bags to the Laboratory for Environmental and Fishing (*Laboratório de Microbiologia Ambiental e do Pescado* – LAMAP) at the Marine Sciences Institute (*Instituto de Ciências do Mar* – LABOMAR) and kept frozen until their identification and cleaning. The specimens were identified according to Littler and Littler (2000) and had their nomenclature updated according to Guiry and Guiry (2017) at the Macroalgae Laboratory (*Laboratório de Macroalgas* – LABOMAR), where the exsiccates are stored under the registry numbers 2152 (*Padina gimnospora*), 2145 (*Hypnea musciformes*), 2151 (*Lobophora variegata*), 2149 (*Ulva fasciata*), 2155 (*Caulerpa prolifera*) and 2146 (*Ulva lactuca*). Later, the algae were subjected to manual cleaning for the removal of epiphytes and impurities. Then, washed with distilled water and frozen again until lyophilization (lyophilizer EDWARDS, Brazil).

### Source of extracts and plate preparation

The lyophilized samples were grinded and, from the resulting powder, extraction with the organic solvents hexane, acetone, ethanol and methanol was performed, following the crescent polarity order and using a 1:10 proportion (p/v). The choice of the solvents was performed according to previous researches (Table 1). The samples were kept under stirring at 80 to 85 rpm for 48 hours in an incubator (TECNAL TE-420). Extracts were then filtered through a paper filter (WHATMAN) and put in a rotary evaporator (FISATOM®) until the production of a dense solution. The extract was kept in a desiccator for 48 hours in vacuum, for the complete remotion of the solvent. Afterwards it was weighted in an analytical scale and diluted in sterile distilled water. Then, white sterile disks (Laborclin®) in 6mm of diameter were put in contact to 40 $\mu$L of the algae extracts, amounting to 1 mg of the concentrate on each disk.

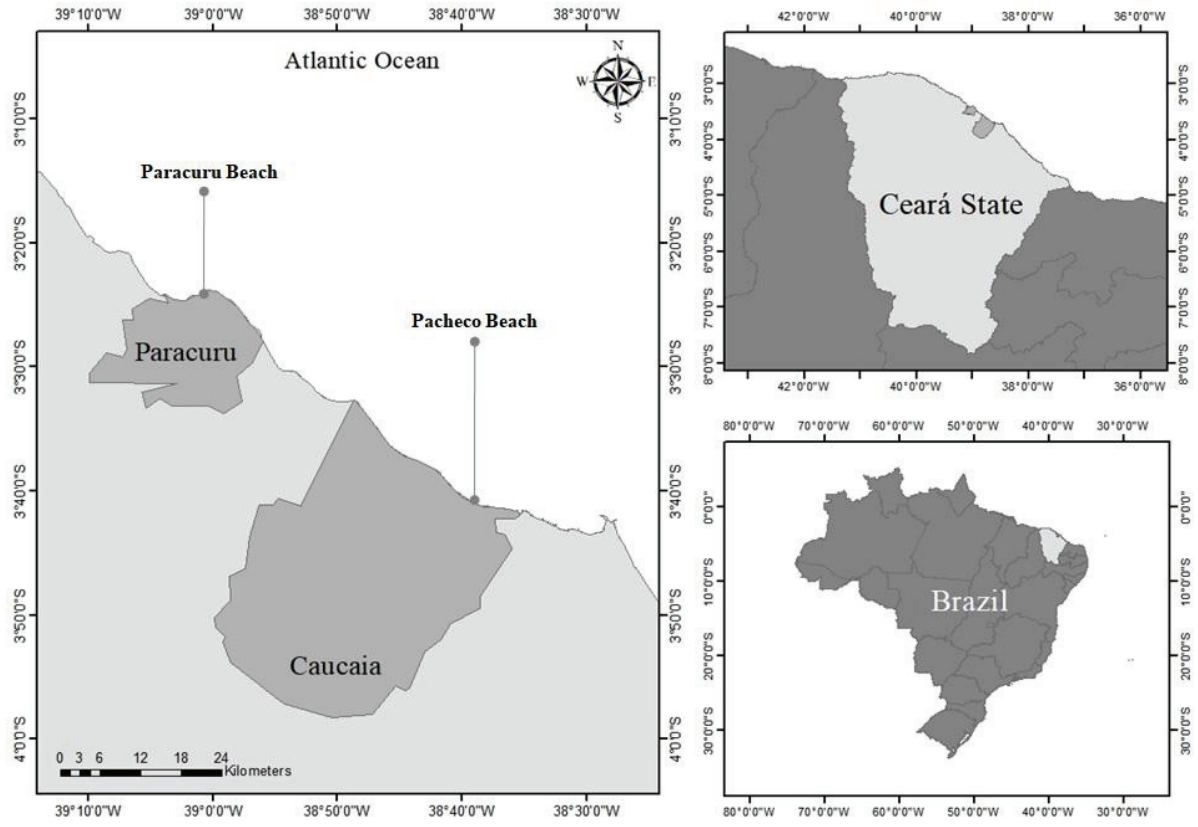


**Figure 1.** Sample location of marine macroalgae specimens from coast of Pacheco and Paracuru beaches, Ceará state.

**Table 1.** Solvents used to obtain macroalgal extracts.

| Solvent | Reference |
|---|---|
| Acetone | El-Din & El-Ahwany (2016) (adapted) |
| Ethanol | Al-Saif, Abdel-Raouf, El-Wazanani & Aref (2014) (adapted) |
| Hexane | Srikong, Bovornreungroj, Mittraparparthorn & Bovornreungroj (2017) (adapted) |
| Methanol | El Shafay, Ali, El-Sheekh (2016) (adapted) |

## Statistical analysis

Extract yielded was calculated from the total mass of grounded algae powder using the following equation. The Yield values, by marine macroalgae and by extractive substance, were submitted to analysis of variance (ANOVA-one way, $\alpha = 5\%$), in order to verify possible differences by groups (extractive substance or marine macroalgae). Statistical analyses were performed in R software (version 3.4.4).

$$Yield\ (\%) = \frac{Dry\ extract\ mass}{Alg\ ae\ powder\ mass} \times 100 \quad (1)$$

## Bioactivity test

The strains used were standard ATCC and *Vibrio* with multi-resistant profile to antimicrobials isolated from a shrimp farming environment, obtained from the Laboratory for Environmental and Fishing (*Laboratório de Microbiologia Ambiental e do Pescado* – LAMAP) at the Marine Sciences Institute (*Instituto de Ciências do Mar* – LABOMAR), according to the table below (Table 2).

**Table 2.** List of bacteria that indicate antimicrobial activity in algae extracts.

| Resistance profile | Species | Source |
|---|---|---|
| PEN – AMP – CFL | *Vibrio alginolyticus* | Farm sediment |
| | *Vibrio harveyi* | Estuary water |
| | *Vibrio parahaemolyticus* | Supply channel water |
| | *Vibrio coralliilyticus* | Estuary water |
| CHL – OTC – TET | *Vibrio mimicus* | Estuary sediment |
| AMP – PEN – TET – OTC | *Vibrio logei* | Estuary water |
| Nt | *Vibrio parahaemolyticcus* | ATCC 17802 |
| | *Staphylococcus aureus* | ATCC 25923 |
| | *Escherichia coli* | ATCC 25922 |

Nt: not tested, PEN: Penicilin, AMP: Ampicilin, CFL: Cefalotin, CHL: Chloramphenicol, OTC: oxytetracyclin, TET:tetracyclin.

Antibacterial effect of algae extract was tested by disk diffusion method (DD) in Muelle-Hinton agar (Clinical & Laboratory Standards Institute [CLSI], 2012). In the evaluation of antibacterial activity by the DD test, pure cultures grown on Triptic Soy Agar (TSA) with 1% of NaCl, were adjusted according to Hindler and Jorgensen (1995). After inoculation, duplicate discs in contact with the extracts were applied to the

medium surface with sterile tweezers. Afterwards, Petri dishes were kept in a bacteriological incubator at 35ºC for 48 hours. Disks that presented a ≥ 6 mm of diameter halo were considered indicative of antibacterial activity (Engel, Puglisi, Jensen, & Fenical, 2006). For the measurement of the halo diameter in millimeters, a digital caliper was used (Digimess). For the negative control, a blank disk, put in contact only with sterile distilled water was used. As positive control, commercial antimicrobial chloramphenicol (LABOCLIN) (30 $\mu$g) for *Vibrio alginolyticus*, *Vibrio harveyi* and standard *Vibrio parahaemolyticus* strains were used as control. For the standard *Staphylococcus aureus* and *Escherichia coli* strains, vancomycin and nalidixic acid were used.

### Cellular viability for MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide)

#### Preparation of cells with extracts

The cytotoxicity of the macroalgae extracts obtained by methanol and ethanol extraction was analyzed by the MTT assay (Silva et al., 2011). Cells of the Vero line were diluted to a 2.5 x $10^5$ cell $mL^{-1}$ concentration in a Leibovitz L-15 medium (Cultilab, Brazil) with 2% fetal bovine serum (FBS). An aliquot of 100 $\mu$L from this solution was then added to 96 wells plates. After a 24 hours extent in a $CO_2$ incubator at 37°C (Panasonic, MCO-18AC, USA & Canada), medium was removed and extracts were added (in quadruplicate) to the initial concentration of 2 mg $mL^{-1}$ in a Leibovitz L-15 medium with 2% FBS. A logarithmic dilution of $-\log^2$ of 10 dilutions was performed. The control wells did not receive any extract. The cells were incubated with the extracts for seven days in a $CO_2$ incubator at 37°C.

After this period, medium was removed, cells washed once with 200 $\mu$L of PBS 7,4 and 50 $\mu$L of 5 mg $mL^{-1}$ MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide) (Life Technologies, USA) was added. The plate remained for four hours in the $CO_2$ incubator at 37°C. Then, MTT was removed and formazan crystals dilluted in 50 $\mu$L of DMSO.

#### Cytotoxicity analysis

Plate was read at 540nm in the plate reader (Micronal B542). Cellular viability (CV) was calculated using the following formula: $CV(\%) = OD_{(a)}/(OD_{(c)} \times 100)$, where $OD_{(a)}$ was optical density to well contained extract with Vero cells and virus at 540 nm and $OD_{(c)}$ was optical density to well contained Vero cells and virus at 540 nm. For the determination of $CC_{50}$, a linear regression equation was made from the concentration and cellular viability results, using the equation model: $y = a+bx$.

| Marine Macroalgae | Fraction | Strains | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H | I |
| *H. musciformes* (18 positives) | Hx | + | + | - | - | - | - | + | - | + |
| | Ac | + | + | - | - | + | - | + | + | + |
| | Et | - | - | - | - | + | - | + | - | + |
| | Mt | + | - | + | - | - | + | + | - | + |
| *P. gymnospora* (15 positives) | Hx | - | - | + | + | + | - | + | - | + |
| | Ac | + | + | + | + | - | - | + | - | - |
| | Et | + | - | - | - | - | - | - | - | + |
| | Mt | + | - | - | - | + | - | - | - | + |
| *U. fasciata* (15 positives) | Hx | - | + | - | - | + | - | + | - | + |
| | Ac | - | - | - | - | + | - | + | - | + |
| | Et | - | - | - | - | - | - | + | - | + |
| | Mt | - | + | + | - | + | + | + | - | - |
| *L. variegate* (13 positives) | Hx | - | - | - | - | - | + | + | - | + |
| | Ac | - | - | - | - | + | - | - | + | + |
| | Et | - | - | - | - | - | - | + | + | + |
| | Mt | - | - | - | - | + | - | + | + | + |
| *C. prolifera* (12 positives) | Hx | + | - | + | + | + | - | + | - | + |
| | Ac | - | - | - | - | - | + | - | - | - |
| | Et | - | - | - | + | - | + | - | + | + |
| | Mt | - | - | - | - | - | - | - | - | + |
| *U. lactuca* (10 positives) | Hx | + | + | n.t | - | n.t | - | n.t | - | n.t |
| | Ac | + | - | n.t | - | n.t | - | n.t | - | n.t |
| | Et | + | + | n.t | - | n.t | - | n.t | - | n.t |
| | Mt | + | - | + | - | + | - | + | - | + |

A: *S. aureus* ATCC25923; B: *E. coli* ATCC 25922; C: *V. parahaemolyticus* ATCC 17802; D: *V. alginolyticus*; E: *Vibrio mimicus*; F: *Vibrio parahaemolyticus*; G: *Vibrio coralliilyticus*; H: *Vibrio logei*; I: *V. harveyi*; n.t = not tested; mm: halos measured in millimeters; - = lack of halo (resistant); + = halo presence (sensitive); Hx: hexanic extract; Ac: acetonic extract; Et: ethanolic extract; Mt: methanolic extract.

## Results

The antibacterial activity of macroalgae extracts against *Vibrio* and standard strains (*S. aureus* ATCC25923, *E. coli* ATCC 25922 and *V. parahaemolyticus* ATCC 17802*)* are shown on Table 3. The macroalgae extracts of *H. musciformes*, *P. gymnospora* and *U. fasciata* show a most frequent antibacterial activity against tested strains. Furthermore, all extracts of *H. musciformes* and *L. variegata* were effective against *V. harveyi* (I) (Table 3).

The evaluation of cytotoxicity comprised the use of cells from the Vero line for the extracts obtained with methanol and ethanol, analyzed by the MTT assay, as described in the methodology section. According to the results of $CC_{50}$ calculations (rate in which the cytotoxicity causes the death of 50% of bacteria), obtained in mg $mL^{-1}$, the extracts presented a cytotoxic effect from the 1.05 mg $mL^{-1}$ concentration, tested for the brown alga *Padina gymnospora*, up to the 5.92 mg $mL^{-1}$ concentration for *Ulva fasciata* (Table 4). The cytotoxicity tests were only possible for macroalgae alcoholic extracts (methanolic and ethanolic) due to solubility to that extractors and also the available quantity for the assays.

**Table 4.** Cytotoxic test results for the algae extracts.

| Extract | $CC_{50}$ |
|---|---|
| PG methanol | 1.32 mg $mL^{-1}$ |
| PG ethanol | 1.05 mg $mL^{-1}$ |
| UF methanol | 5. mg $mL^{-1}$ |
| UF ethanol | 1.48 mg $mL^{-1}$ |
| LV methanol | 1.32 mg $mL^{-1}$ |
| HM ethanol | 1.63 mg $mL^{-1}$ |
| CP methanol | 1.35 mg $mL^{-1}$ |
| CP ethanol | 1.60 mg $mL^{-1}$ |
| UL methanol | 2.53 mg $mL^{-1}$ |

PG= *P. gymnospora*, UF= *U. fasciata*, LV= *L. variegata*., HM PC= *H. musciformes* Pacheco, CP= *C. prolifera*, UL= *U. lactuca* and HM = *H. musciformes*.

The yield of the extracts using the organic solvents hexane, acetone, ethanol and methanol is shown on Table 5. No statistical difference was observed in the yields by extractive organic substance ($F = 0.5218$, $p = 0.6758$). However, by marine macroalgae group, it was verified that *Caulerpa prolifera* (CP) (Table 5) extracts had higher yields than all the others ($F = 48.709$, $p = 0.0057$).

## Discussion

The extracts of marine macroalgae *Lobophora variegata*, *Padina gymnospora*, *Hypnea musciformis*, *Ulva fasciata*, *Ulva lactuca* and *Caulerpa prolifera* inhibited the growth of tested bacteria (Table 3). Ability of marine algae of producing bioactive compounds with potential antimicrobial interest has been widely researched (Dussault, Dang Vu, Vansach, Horgen, & Lacroix, 2016). Antibacterial activity of seaweed is influenced by factors such as species, extraction efficiency and resistance to the bacteria tested (Seenivasan, Indu, Archana, & Geetha, 2010). Antimicrobial efficiency of marine macroalgae extracts was already observed in past studies, in which they were considered important growth inhibitors of pathogenic microorganisms, comprehending the algae of green, red and brown classes (Moubayed, Al Houri, Al Khulaifi, & Al Farraj, 2017; Rosaline, Sakthivelkumar, Rajendran, Janarthanan, & 2012; Al-Haj et al., 2010; Chiheb et al., 2009; Kolanjinathan & Stella, 2009).

Al-Saif, Abdel-Raouf, El-Wazanani, and Aref (2014) have shown that organic solvents always present a higher efficiency in the extraction of antimicrobial compounds in comparison to water. Among the four solvents used during the extract fraction process, the most efficient antibacterial activity was shown by *P. gymnospora* and *C. prolifera* when extracted with hexane, which inhibited multi-resistant *Vibrio* species (Table 3). Results obtained with hexanic, acetonic, methanoic, ethanolic extract from the brown macroalgae *P. gymnospora* have also shown activity against the multi-resistant *Vibrio* species tested. Among those, some bacteria are of particular interest to aquaculture, such as *V. parahaemolyticus* and *V. harveyi* (Zhang et al., 2014; Vanmaele, Defoirdt, Cleenwerck, Vos, & Bossier, 2015). In another approach to the antibacterial activity of algae against opportunistic bacteria of the genus *Vibrio*, it was verified that after introduction of extracts of *Gracilaria tenuistipitata* in the cultured shrimps diet there was a resistance increase in animals to the pathogen *Vibrio alginolyticus* (Sirirustananun et al., 2011 ).

**Table 5.** Yield of the organic extracts from marine macroalgae sampled in the coast of Paracuru and Pacheco beaches, in Ceará.

| Algae | Extract Yield (%) | | | | Mean ± SD⋆ |
|---|---|---|---|---|---|
| | HEX | ACET | ET | MET | |
| UF | 0.14 | 0.57 | 0.62 | 1.83 | 0.79 ± 0.72$^{c}$ |
| LV | 0.23 | 2.59 | 0.81 | 1.42 | 1.26± 1.00$^{c}$ |
| CP | 4.39 | 4.39 | 1.69 | 2.74 | 3.30± 1.32$^{b}$ |
| UL | 0.03 | 0.07 | 0.20 | 2.74 | 0.76± 1.32$^{c}$ |
| PG | 0.32 | 1.28 | 1.69 | 0.61 | 0.97± 0.62$^{c}$ |
| HM | 0.52 | 0.46 | 0.15 | 0.26 | 0.34± 0.17$^{c}$ |
| Mean ± SD⋆ | 0.93± 1.69$^{a}$ | 1.56± 1.64$^{a}$ | 0.86± 0.68$^{a}$ | 1.60± 1.04$^{a}$ | |

HEX= hexane, ACET= acetone, ET= ethanol and MET= methanol, UF= *U. fasciata*, LV= *L. variegata*, HM= *H. musciformes*, CP= *C. prolifera*, UL= *U. lactuca*, PG = *P. gymnospora* and HM = *H. musciformes*. ⋆Equal letters: no statistical difference to α=5%; Different letters: statistical difference to α=5%.

Extraction of *Hypnea musciformis* with the four solvents used in this research proved to be very efficient against *Vibrio harveyi* (Table 3). At least three of the *H. musciformis* extracts inhibited *S. aureus* as well, displaying their antibacterial activity in a wide range. It is also notable that, quantitatively, this was the algae species which inhibited the bacteria growth the most, from the ones analyzed in the bioguided assay. Likewise, Bouhlal, Riadi, Martínez, and Bourgougnon (2010) stressed the excellent activity of this species against Gram-negative and Gram-positive bacteria. In addition, the carrageenan of this macroalgae show antimicrobial, anticancer and neuroprotective activities. Furthermore, the aqueous extract of *H. musciformis* reduced the longevity and fecundity of the adult *Aedes aegypti*, considering the importance in the development of tools in pest control (Souza et al. al., 2018; Roni et al., 2015).

Hexanic extract of *C. prolifera* and acetonic extract of *H. musciformis* inhibited the largest amount of bacteria in comparison to other extracts. They prevented the growth of six out of nine bacterial species analyzed by the disk diffusion test. However, *L. variegata* and *U. fasciata* were the only species active only against Gram-negative bacteria. All other algae analyzed in this study were active against the Gram-positive bacteria *S. aureus* in at least one extract. The brown *Lobophora variegate* showed a decent activity against the multi-resistant vibrios tested. Thus, all extracts were able to produce compounds that inhibited the multi-resistant bacteria *Vibrio harveyi*, which is a great concern in shrimp farming.

The susceptibility of *V. harveyi* was reported when tested the extracts of *Sargassum glaucescenses* (Mahianeh et al., 2014). In another research, Kanjana, Radtanatip, Asuvapongpatana, Withyachumnarnkul and Wongprasert (2011) have shown that when the ethanolic extract of red algae *Gracilaria fisheri* was injected into juvenile of *Penaeus monodon* shrimps there was a significant increase in the total number of hemocytes, granulocytes and reduction of mortality to *V. harveyi* infection. Thus, the immunostimulating and antimicrobial activity that could protect *P. monodon* against *V. harveyi* was demonstrated.

*Ulva lactuca* was the only species from which the four extracts (with hexanic, methanolic, acetonic and ethanolic solvents) inhibited the species *S. aureus*. Antibacterial efficiency of the macroalgae genus *Ulva* sp. has previously been reported in several researches (Al-Saif et al., 2014; Dussault et al., 2016; Zbakh, Chiheb, Bouziane, Sánchez, & Riadi, 2012). According to Tan et al. (2012), *Ulva lactuca* extracts, using the solvents methanol, ethanol, acetone, ethyl acetate and hexane, inhibited the growth of bacteria like *S. aureus* methicillin resistant, *S. aureus*, *Bacilus subtilis* and *Enterococcus faecalis*.

For Cox et al. (2010), red and green algae improved the antimicrobial potential significantly when the solvent used was ethanol or acetone. As for the brown algae, they showed better activity when methanol was used in the extraction. Silva et al. (2013) have obtained similar results with extracts of *P. gymnospora,* verifying the inhibition of growth in all *Vibrio* species analyzed. Rosaline et al. (2012) have obtained, in the hexanic extract of *P. gymnospora*, notable activity against various Gram-negative bacteria, as *B. subtilis, E. faecalis, E. amylovora*, *E. coli* and *P. vulgaris*. As for the Gram-positive *S. aureus,* its growth was inhibited by the ethanolic extract of brown *Padina pavonica* (El Shafay, Ali, & El-Sheekh, 2015).

Toxicity of the inhibitory bacterial concentrations was evaluated considering the whole antibiotic activity from algae found in the present and in all aforementioned studies, aiming at the possible use of these extracts as inhibitory substances for potentially pathogenic bacteria. Studies have shown that methanolic extract of *P. gymnospora* has not been cytotoxic to the cellular line of fibroblasts in L929 common mice and to the line of human ovarian carcinome, OVCAR-3 up to the concentration evaluated of 110 μg $mL^{-1}$ (Baliano et al., 2016). Similar cytotoxicity results were obtained for Marques et al. (2015) to sulfated galactommanas from plants against DENV-2 virus. No cytotoxicity effect was observed until concentrations of 100 $\mu$g $mL^{-1}$ during 7 days. These results corroborate with the ones from Khanavi et al. (2010), who tested the cytotoxicity of methanolic extracts of phaeophyte algae and have found values higher than 1mg. According to Nunes et al. (2008), a low toxicity of substances originating from vegetable extracts is an important characteristic in the use for pharmacological ends. Thus, the low toxicity of the extracts points to the thorough search of the active substances in these extracts and, subsequently, a possible use as promising agents for the treatment of infections.

According to Cho, Lee, Kang, Won & You (2011), green algae extracts show higher yields in comparison with other classes. Some factors, such as extraction, weather, temperature, solvent and species influence the extract yield. However, in this study, extracts of *U. lactuca* produced the lowest yields, except those of the methanolic extract from this seaweed. Possibly, the low temperature used for extraction and the polarity of the

solvents were interference factors in the yield extract. According to Plaza et al. (2010) the higher the polarity of the solvent and the temperature employed in the extraction, the higher the yield extract.

## Conclusion

The extracts obtained from the macroalgae *Lobophora variegata*, *Padina gymnospora*, *Hypnea musciformis*, *Ulva fasciata*, *Ulva lactuca* and *Caulerpa prolifera* have shown antibacterial activities against *S. aureus* ATCC 25923, *E. coli* ATCC 259222 and strains of *Vibrio* isolated from shrimp-farming environments with a profile of multiple resistance to commercial antimicrobials. The bactericidal activity and low cytotoxicity verified in the extracts open perspectives for the purification and characterization of new molecules with therapeutic potential and may be used as an option for the treatment of bacterial diseases resistant to present day drugs.

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