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Estimation of Antibacterial Properties of Chlorophyta, Rhodophyta and Haptophyta Microalgae Species

Khawaja Muhammad Imran Bashir[1,2], Jae-Hyeon Lee[3], Maike Julia Petermann[4], Abid Ali Shah[1], Su-Jin Jeong[1], Moo-Sang Kim[1], Nam-Gyu Park[5], and Man-Gi Cho[1]*

[1]*Department of Biotechnology, Division of Energy and Bioengineering, Dongseo University, Busan 47011, Republic of Korea*
[2]*Research Center for Extremophiles and Microbiology, College of Medical and Life Sciences, Silla University, Busan 46958, Republic of Korea*
[3]*Department of Chemical and Biomolecular Engineering, Pusan National University, Busan 46241, Republic of Korea*
[4]*Institute of Biotechnology, Technische Universität Berlin, Germany*
[5]*Department of Biotechnology, Pukyong National University, Busan 48513, Republic of Korea*

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In this exploratory study, eight types of microalgae from different phyla (*Chlamydomonas reinhardtii*, *Chlorella* species, *Haematococcus pluvialis*, *Porphyridium purpureum*, *Porphyridium cruentum*, *Isochrysis* species, *Isochrysis galbana*, and *Pavlova lutheri*) were tested for their antibacterial activities against eight target pathogenic bacterial strains. The agar well diffusion method and broth micro dilution assay were conducted to estimate the antibacterial activity. Microalgae cell-free supernatants, exopolysaccharides (EPS), water, and organic solvent extracts were used for inhibition analysis. EPS extracted from *P. lutheri* showed activity against *Bacillus subtilis* and *Pseudomonas aeruginosa*. Inhibition zone diameters of 14–20 mm were recorded on agar plates, while the minimum inhibitory concentrations in the broth micro dilution assay were 0.39–25 mg ml^{-1}. During this study, haptophyte microalgae, *Isochrysis* species, and *P. lutheri* extracts showed the highest activity against most of the tested pathogenic bacterial strains, while most of the extracts were active against the important foodborne pathogen *P. aeruginosa*. This study showed promising results regarding important microalgae phyla, which will further aid research related to extracts and exploitation of bioactive metabolic compounds in the food and pharmaceutical industries.

Keywords: **Antibacterial activity, exopolysaccharides, microalgae extracts, natural compounds, pathogenic bacteria**

Introduction

Microalgae represent a rich source of potentially bioactive compounds and are thus of great interest [1]. From the very beginning, cyanobacteria and prokaryotic algae inhabited the earth. Through their ability to synthesize oxygen as a photosynthetic byproduct, they contributed in regulating the oxygen atmosphere of our planet, enabling life as we know it [2]. Environmental tolerance and high adaptability to conditions, ranging from extreme heat to extreme cold, drought, salinity and UV exposure, have enabled microalgae to survive in a large span of different ecosystems [2]. The conditions they were forced to adapt resulted in the biosynthesis of scientifically interesting chemical compounds [3]. Furthermore, microalgae cultivation is generally easy and cost-effective which is one of their biggest advantages as compound donors [4].

Marine microalgae are a rich source of vitamins, pigments, proteins and other substances providing benefits in cosmetics and skin care product composition [5, 6]. A lot of secondary metabolites from cyanobacteria have been associated with antimicrobial effects. Among different types of antimicrobial compounds isolated from

***Corresponding author**
Tel: +82-51-320-2662, Fax: +82-51-320-1547
E-mail: mgcho@gdsu.dongseo.ac.kr

microalgae, fatty acids and their derivatives have drawn special attention [7–9]. Different exometabolites and antifouling agents have also been explored from other microalgae species [8–11], including the bioactive exometabolites isolated from the filamentous marine cyanobacterium *Geitlerinema* sp. [12]. Microalgae such as *Arthrospira* and *Chlorella* species have been repeatedly used in cosmetics, skin care, and hair care products [13, 14]. Additionally, recent advancement in biotechnology has enabled the manufacturing of high-valued microalgae byproducts that are nearly free of contaminations.

Aqueous and organic extracts, as well as fatty acids, exopolysaccharides (EPS), and eicosapentaenoic acid (EPA) extracted from microalgae species have been reported active against several pathogenic bacteria [8, 15]. Eicosapentaenoic acid from *Phaeodactylum tricornutum* showed antibacterial activity against Multidrug-Resistant *Staphylococcus aureus* (MRSA), *Listonella anguillarum, Lactococcus garvieae* and *Vibrio* sp., [8, 16]. Pressurized liquid extracts obtained from *Haematococcus pluvialis* have been reported as active against *Escherichia coli* and *S. aureus* [17]. Organic extracts from *Euglena viridis* have shown activity against *Vibrio, E. coli, Pseudomonas, Aeromonas* and *Edwardsiella* [18]. Methanol and hexanol extracts from *Chlamydomonas reinhardtii* and *Chlorella vulgaris* were found active against *S. aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *E. coli,* and *Salmonella typhi* [19]. β-phycoerythrin extracted from *Porphyridium cruentum* was found active against *S. aureus*, *Streptococcus pyogenes* and *Salmonella typhimurium* [15].

Bacterial resistance to synthetic antibiotics emphasizes searching for the novel natural antibacterial compounds. Nevertheless, antibiotics are being discovered at a steady rate, but the consequences of this phenomenon are slow to be appreciated. At present, the paucity of the antimicrobial compounds coming into the market has led to the problem of antibiotic resistance, fast escalating into a global health crisis [20]. One approach to encounter antibiotic resistance is the discovery of novel natural antimicrobial compounds for clinical application [8]. Thus, the scientists are eager to explore the compounds and discover new drug leads [21, 22].

The use of microalgae in the medical and pharmaceutical industry is rapidly increasing, which shows the potential of this microorganism for the benefit of humankind. Despite the availability of several antibacterial compounds yet there is a lot more space for improvement. In the present study, green and red eukaryotic microalgae species from different phyla were explored for their antibacterial properties. Conditions to extract the viable natural antibacterial substances were practiced and the extracted compounds were checked against eight, gram-positive and gram-negative pathogenic bacterial strains through the agar well diffusion method and the broth microdilution assay.

Materials and Methods

Microalgae species and culture conditions

Eight microalgae species from different phyla including Chlorophyta, Rhodophyta and Haptophyta were obtained from the Korea Marine Microalgae Culture Center (KMMCC) Busan, Republic of Korea (Table 1). Stock cultures of each microalgae species were maintained on the appropriate agar slants. For antimicrobial activity analyses, non-axenic cultures were grown in 500 ml flask bioreactors with the respective medium, at 25 ± 2℃ under a continuous light intensity of 150 μmol photons m^{-2} s^{-1}. Aeration was provided by shaking at 110 rpm [26]. Both cell pellets and cell-free supernatants were used for sample preparation.

Bacterial strains and culture conditions

The tested bacterial strains include *Micrococcus luteus* KCTC 1071, *Staphylococcus aureus* RN 4220, *Bacillus subtilis* KCTC 1021, *Streptococcus iniae* FP 5228, *Escherichia coli* KCTC 1116, *Salmonella enterica* KCTC

Table 1. Microalgae species used under study.

Phylum	Species	Medium
Chlorophyta	*Chlamydomonas reinhardtii*	TAP [23]
	Haematococcus pluvialis	Modified BBM [24]
	Chlorella species	Modified ASW [25]
Haptophyta	*Pavlova lutheri*	Modified ASW [25]
	Isochrysis species	Modified ASW [25]
	Isochrysis galbana	Modified ASW [25]
Rhodophyta	*Porphyridium purpureum*	Modified ASW [25]
	Porphyridium cruentum	Modified ASW [25]

TAP: Tris-acetate-phosphate; BBM: Bold basal medium; ASW: Artificial seawater.

2514, *Pseudomonas aeruginosa* KCTC 2004 and *Vibrio parahaemolyticus* KCTC 2471. The inhibitory experiments were performed at the Biomedical Science Laboratory, Department of Biotechnology, Pukyong National University, Busan, Republic of Korea. All bacterial strains were stored at −80℃ in Tryptic Soy Broth (TSB: Merck, Germany), supplemented with 30% glycerol except *V. parahaemolyticus*, which was stored in Marine Broth (MB: MBCell, Korea). The cultures were streaked purified on agar plates with the respective medium and incubated overnight at 37℃ before the actual experiment. All the tested bacterial strains were cultured on the respective broth and agar medium for checking antibacterial activity. The bacterial strains with approximately adjusted to 1×10^5 CFU ml^{-1} were used for checking the activity of all extracts.

Extracellular sample preparation

Microalgae species were grown till pre-stationary phase and centrifuged at 3000 ×*g* for 15 min to separate cells and cell-free supernatant. Cell-free supernatants were further used for the preparation of extracellular samples.

Cell-free supernatant

Cell-free supernatant samples were prepared in two ways: 1) Twenty-five milliliters of cell-free supernatant from each species at pre-stationary phase was used as it is for checking the activity without further treatment and these samples were named as non-lyophilized cell-free supernatant (NLS); 2) Twenty-five milliliters of each cell-free supernatant was lyophilized at −85℃ (Ilshin Biobase, EU). The freeze-dried residues were weighed, dissolved in deionized double distilled water at a concentration of 25 mg ml^{-1} and sterilized by 0.22 μm syringe filter (Merck Millipore, USA). These samples were named as lyophilized and filtered cell-free supernatant (LFS).

Exopolysaccharide Preparation and Estimation

The exopolysaccharides were precipitated from cell-free supernatants. Briefly, a 50 ml cell-free supernatant was mixed with 99% ethanol at a ratio of 1:1 (v/v) and incubated at −20℃ for 48 h. Pellets were centrifuged at 6000 ×*g* for 20 min at 4℃ and rinsed twice with 70% ethanol to remove any possible contamination. Pellets were lyophilized at −85℃ and re-dissolved in deionized double distilled water at a concentration of 5 mg ml^{-1}. Total carbohydrates were determined by Phenol-sulfuric acid method [27].

Intracellular extract preparation

Three organic solvents, ethanol, methanol, and chloroform; deionized double distilled water (d_3) and TE-buffer (Tris-acetate, EDTA) were used to prepare the intracellular extracts according to the following methods.

Organic solvent extract preparation

Microalgae species grown till pre-stationary phase were centrifuged at 3000 ×*g* for 15 min to collect cell pellets. The cell pellets were further rinsed three times with distilled water and re-suspended separately in each solvent, i.e., ethanol (Et-OH), methanol (Me-OH) and chloroform at a ratio of 1 g biomass per 50 ml solvent (w/v). The pellets were vortexed and homogenized with ULTRA-turrex Homogenizer (IKA Works Inc., USA) at 22,000 rpm for 2–3 min. This enables the compounds to come out of the cells in the solution. The ethanol, methanol and chloroform samples were heated under reflux at the boiling temperature of the respective solvent for 120 min. In order to obtain the microalgal extracts, the samples were centrifuged at 4000 ×*g* for 10 min at 4℃, except the chloroform extracts that were filtered with 0.45 μm filter paper discs through vacuum filtration (Whatman, UK). The extracted supernatants were transferred into round bottom flasks while the cell pellets were discarded. Solvents were evaporated by a rotary evaporator (Hanshin Science Co., Japan). The dried extracts were dissolved in 100% DMSO (Dimethyl sulfoxide; Sigma-Aldrich, USA) at a concentration of 100 mg ml^{-1} and filter sterilized through 0.22 μm syringe filter (Merck Millipore, USA).

TE-Buffer and water extract preparation

Microalgae species grown till pre-stationary phase were centrifuged at 3000 ×*g* for 15 min to collect cell pellets. The pellets were further rinsed three times with distilled water and re-suspended separately in 60 mM TE-buffer (Tris-base with 0.1 mM EDTA, pH 6.8) [15], and deionized double distilled water (d_3). The pellets were vortexed and homogenized with ULTRA-turrex Homogenizer (IKA Works. Inc., USA) at 22,000 rpm for

2–3 min. TE-buffer and d_3 samples were heated separately under reflux at 90℃ for 60 min. Samples were centrifuged at 6000 ×*g* for 10 min at 4℃. The supernatants were lyophilized at –85℃ (Ilshin Biobase, EU). The dried residues were resuspended in deionized doubled distilled water at a concentration of 50 mg ml^{-1} and filter sterilized by 0.22 μm syringe filter (Merck Millipore, USA).

Estimation of antibacterial activity

Antibacterial activity was estimated by the agar well diffusion method of Spooner and Sykes [28] with slight modifications. Bacterial suspensions with approximately adjusted to 1×10^5 CFU ml^{-1} were plated on agar layers into the 90 × 15 mm Petri plates (Approx. 25 ml agar medium per plate). Six wells per plate with a diameter of 8 mm were prepared and 100 μl of each extracted sample was poured separately into each well. The plates were incubated at 37℃ and the inhibition zone diameters were measured after 48 h with the help of Vernier calipers (HEPT, Rep. of Korea). The extracts with the inhibition zone diameter of less than 13 mm were considered as lack of activity [15]. The respective organic or aqueous solvents (100 μl) were used as negative control. Ampicillin (Sigma-Aldrich, USA) and Kanamycin (Amresco, USA) were used as positive control. The inhibitory experiments were repeated at least three times.

Minimum inhibitory concentration

The minimum inhibitory concentration was measured by broth microdilution assay in the 96-well cell culture plate as described by Andrews [29], with slight modifications. Two-fold serial dilutions of 100 μl extracted samples were prepared and checked against 100 μl bacterial suspensions with approximately adjusted to 1×10^5 CFU ml^{-1}. The lowest concentration of the tested sample that limited the visible bacterial growth after 24 h incubation at 37℃ was considered as minimum inhibitory concentration (MIC) for the respective sample.

Results

The inhibition zone diameters of the intracellular compounds against the tested gram-positive and gram-negative bacterial strains ranged from 14 mm to 20 mm (Table 2). None of the cell-free supernatant samples showed antibacterial activity on the agar plates against the tested bacterial strains. The deionized double distilled water (d_3), TE-Buffer extracts and the extracted EPS as well did not show promising activity against most of the tested bacterial strains. The EPS extracted from *P. lutheri* with an inhibition zone diameter of 15 mm against *B. subtilis* and 14.5 mm against *P. aeruginosa* was the only exopolysaccharide which showed activity on agar plates. Whereas the EPS extracted from other species did not show promising activity on agar plates, thus the results were dropped out from Table 2. Water extract of *P. lutheri* was active against *M. luteus* (15 mm) and *P. aeruginosa* (14 mm), whereas the water extract of other haptophyte microalgae *Isochrysis* sp., was found active only against *S. aureus* (14 mm). TE-buffer extract of *P. lutheri*, showed activity against *S. aureus* (15 mm), *B. subtilis* (14 mm) and *P. aeruginosa* (17 mm), whereas those of *P. purpureum* and *Isochrysis* sp. were active against *B. subtilis* (14 mm) and *P. aeruginosa* (16 mm), respectively.

Ethanol (Et-OH) extracts of *P. lutheri* showed activity against all the tested bacterial strains except *E. coli* with the maximum inhibition zone diameter of 20 mm against the *V. parahaemolyticus*. Et-OH extracts of *P. purpureum* showed activity against *M. luteus* (15 mm), *S. enterica* (18 mm) and *P. aeruginosa* (16 mm). *C. reinhardtii* Et-OH extracts were active against *S. aureus, S. iniae* and *P. aeruginosa* with a maximum inhibition zone diameter of 17 mm against *S. aureus*. Ethanol extracts of *Isochrysis* sp. showed activity against *B. subtilis* (14 mm) and *P. aeruginosa* (14 mm). *I. galbana* Et-OH extracts showed activity only against *P. aeruginosa* whereas Et-OH extracts of *Chlorella* sp., *H. pluvialis,* and *P. cruentum* did not show activity against any of the tested bacterial strains on the agar plates.

Methanol (Me-OH) extracts of *P. purpureum* showed promising activity against *M. luteus* (15 mm), *P. aeruginosa* (16 mm) and *V. parahaemolyticus* (16.5 mm). *P. lutheri* Me-OH extracts were found active against most of the tested bacteria and this was the only methanol extract found active against *E. coli* (14 mm). *C. reinhardtii* Me-OH extracts showed activity against *S. aureus* (19 mm), *S. iniae* (20 mm) and *P. aeruginosa* (15 mm). *Chlorella* sp. Me-OH extracts were active against *B. subtilis* (14 mm) and *P. aeruginosa* (14 mm). *Isochrysis*

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Table 2. Inhibition zone diameters of screened extracellular and intracellular extracts.

Sample/ Microalgae species	Inhibition zone diameter/Test microorganism							
	Micrococcus luteus	*Staphylococcus aureus*	*Bacillus subtilis*	*Streptococcus iniae*	*Escherichia coli*	*Salmonella enterica*	*Pseudomonas aeruginosa*	*Vibrio parahaemolyticus*
EPS								
P. lutheri	-	-	15	-	-	-	14.5	-
Water Extracts								
P. lutheri	15	-	-	-	-	-	14	-
Isochrysis sp.	-	14	-	-	-	-	-	-
TE-Buffer Extracts								
P. purpureum	-	-	14	-	-	-	-	-
P. lutheri	-	15	14	-	-	-	**17**	-
Isochrysis sp.	-	-	-	-	-	-	**16**	-
Ethanolic Extracts								
C. reinhardtii	-	**17**	-	**16**	-	-	15	-
P. purpureum	15	-	-	-	-	**18**	**16**	-
P. lutheri	14.5	15	14	14	-	14.5	14.5	**20**
Isochrysis sp.	-	-	14	-	-	-	14	-
I. galbana	-	-	-	-	-	-	14	-
Methanolic Extracts								
C. reinhardtii	-	**19**	-	**20**	-	-	15	-
Chlorella sp.	-	-	14	-	-	-	14	-
P. purpureum	15	-	-	-	-	-	**16**	**16.5**
P. cruentum	-	-	14	-	-	-	-	-
P. lutheri	14	**16**	14	14	14	-	-	**19**
Isochrysis sp.	-	-	-	-	-	-	14	-
I. galbana	-	-	-	-	-	-	15	-
Chloroform Extracts								
C. reinhardtii	-	-	14.5	-	-	-	-	-
H. pluvialis	-	-	15	-	-	-	14	-
P. purpureum	-	-	14	-	-	-	14	15
P. cruentum	-	-	14	-	-	-	-	13.5
P. lutheri	**16**	-	15	-	15	**16.5**	-	**20**
Isochrysis sp.	-	14	**17**	-	-	-	15	-
I. galbana	-	-	-	-	-	-	14	-

EPS: Exopolysaccharides; TE-Buffer: Tris-Acetate EDTA buffer; (-): no activity and/or inhibitory zone diameter ≤ 13 mm; Unit = mm.
Only the extracts showing activity to at least one of the tested pathogenic bacteria are shown.

sp. and *I. galbana* Me-OH extracts were found active against *P. aeruginosa* with an inhibition zone diameter of 14 mm and 14 mm, respectively. *P. cruentum* methanol extracts were active only against *B. subtilis* with an inhibition zone of 14 mm, whereas Me-OH extracts of *H. pluvialis* did not show activity against the tested bacterial strains. None of the Me-OH extracts showed activity against *S. enterica*.

All of the chloroform extracts showed activity against *B. subtilis* with a maximum inhibition zone diameter of 17 mm (*Isochrysis* sp. extracts), whereas none of the chloroform extracts showed activity against *S. iniae*. Chloroform extracts of *P. lutheri* showed activity against *M. luteus* (16 mm), *B. subtilis* (15 mm), *E. coli* (15 mm), *S. enterica* (16.5 mm) and *V. parahaemolyticus* (20 mm). *P. lutheri* chloroform extracts were the only chloroform

Table 3. Minimum inhibitory concentration of extracellular and intracellular extracts.

Sample/ Microalgae Species	Minimum inhibitory concentration/ Test microorganism							
	Micrococcus luteus	*Staphylococcus aureus*	*Bacillus subtilis*	*Streptococcus iniae*	*Escherichia coli*	*Salmonella enterica*	*Pseudomonas aeruginosa*	*Vibrio parahaemolyticus*
LFS								
C. reinhardtii	-	-	**12.5**	-	-	-	-	**12.5**
P. cruentum	-	-	6.25	-	-	-	-	-
P. lutheri	-	-	**12.5**	-	-	-	-	-
Isochrysis sp.	-	0	0	-	-	-	**25**	-
Water Extracts								
P. cruentum	-	-	-	-	-	-	-	**25**
P. lutheri	-	-	6.25	-	-	-	-	0
Isochrysis sp.	-	-	6.25	-	-	-	3.13	-
I. galbana	-	**25**	-	-	-	-	**12.5**	-
TE-Buffer Extracts								
I. galbana	-	3.13	0	-	-	-	-	6.25
Ethanolic Extracts								
C. reinhardtii	-	-	1.56	-	-	-	-	3.13
H. pluvialis	-	6.25	6.25	-	0	-	6.25	3.13
P. purpureum	-	0.78	-	-	-	-	-	0
P. cruentum	-	0.39	0.78	-	-	-	-	6.25
P. lutheri	-	-	-	-	3.13	-	-	-
Isochrysis sp.	-	1.56	-	-	0	-	-	0.78
I. galbana	-	-	-	-	-	-	-	1.56
Methanolic Extracts								
C. reinhardtii	-	-	1.56	-	-	-	-	6.25
Chlorella sp.	0.78	0.78	-	-	-	**12.5**	-	-
H. pluvialis	-	**12.5**	**12.5**	-	-	**25**	**25**	-
P. purpureum	-	0	**12.5**	-	-	-	-	-
P. cruentum	0	6.25	-	-	-	-	-	**25**
P. lutheri	-	-	-	-	-	**12.5**	**12.5**	-
Isochrysis sp.	-	**25**	**12.5**	-	-	-	-	0
I. galbana	-	**12.5**	6.25	-	-	-	-	6.25
Chloroform Extracts								
C. reinhardtii	-	-	-	-	-	-	-	3.13
Chlorella sp.	-	**12.5**	6.25	**25**	-	-	3.13	**12.5**
H. pluvialis	-	**25**	-	-	-	**12.5**	-	6.25
P. purpureum	**12.5**	-	-	0	**12.5**	6.26	-	-
P. lutheri	-	-	-	**12.5**	-	-	3.13	-
Isochrysis sp.	-	-	-	-	-	**12.5**	-	**12.5**
I. galbana	-	**12.5**	-	-	-	-	-	**25**

LFS: Lyophilized and filtered cell-free supernatant; (-): Not tested; (0): No inhibitory effect; Unit = mg ml^{-1}.
Only the extracts showing activity to at least one of the tested pathogenic bacteria are shown.

extracts found active against *M. luteus, E. coli* and *S. enterica,* whereas *Isochrysis* sp., chloroform extracts were active only against *S. aureus*. The respective solvents used in this study i.e., d_3, 60 mM TE-Buffer and

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DMSO alone did not show antibacterial activity and were used as a negative control.

The extracted samples with inhibition zone diameters of less than 13 mm were further subjected to broth microdilution assay. The lyophilized and filtered cell-free supernatants (LFS) of *C. reinhardtii*, *P. cruentum* and *P. lutheri* were active against *B. subtilis* at a minimum inhibitory concentration (MIC) of 12.5 mg ml^{-1}, 6.25 mg ml^{-1} and 12.5 mg ml^{-1}, respectively (Table 3). The LFS of *C. reinhardtii* were also found active against *V. parahaemolyticus* at a MIC of 12.5 mg ml^{-1} and of *Isochrysis* sp. were active against *P. aeruginosa* (25 mg ml^{-1}). Water extracts of *P. cruentum* showed activity against *V. parahaemolyticus* (25 mg ml^{-1}), *P. lutheri* against *B. subtilis* (6.25 mg ml^{-1}), *Isochrysis* sp. against *B. subtilis* (6.25 mg ml^{-1}) and *P. aeruginosa* (3.13 mg ml^{-1}), whereas water extracts of *I. galbana* were active against *S. aureus* (25 mg ml^{-1}) and *P. aeruginosa* (12.5 mg ml^{-1}). TE-Buffer extracts of *I. galbana* showed activity against *S. aureus* (3.13 mg ml^{-1}) and *V. parahaemolyticus* (6.25 mg ml^{-1}).

Organic extracts of the tested microalgae species were found active against most of the tested bacterial strains and showed the MIC values ranging from 0.39 mg ml^{-1} to 25 mg ml^{-1} (Table 3). The strongest ones include: *P. cruentum* ethanol extracts against *S. aureus* (0.39 mg ml^{-1}); *C. vulgaris* methanol extracts against *M. luteus* (0.78 mg ml^{-1}) and *S. aureus* (0.78 mg ml^{-1}); *C. reinhardtii*, *Chlorella* sp., and *P. lutheri* chloroform extracts against *V. parahaemolyticus*, *P. aeruginosa* and *P. aeruginosa,* respectively. Most of the extracts with inhibition zone diameters of less than 13 mm on agar plates showed promising activity when checked by the broth microdilution assay.

Discussion

The potential of eight economically important marine microalgae species to produce substances suppressing the growth of the widely occurring foodborne pathogenic bacteria was studied and evaluated as a promising source of natural bioactive substances with pharmaceutical significance. Antibacterial screening of the extracted intracellular and extracellular samples was examined through the agar well diffusion method as well as by broth microdilution assay. It was interesting to note that the relatively higher concentrations of the extracted intracellular and extracellular samples showing no or small inhibition zone diameters (≤13 mm) on agar plates revealed promising inhibitory activities when subjected to broth microdilution assay and a minimum inhibitory concentration of up to 25 mg ml^{-1} was observed. The lack of appropriate inhibition zones on agar plates might be due to the low diffusion capabilities of the active substances [15, 30]. This was similar to the previously reported studies on the sensitivities of agar well diffusion method and broth microdilution assay reported for other natural compounds [15, 30]. However, both methods have their own significance in food and pharmaceutical industry.

The extracted samples showed activity to at least one of the tested pathogenic bacteria. The inhibition zone diameters of the intracellular and extracellular extracts against the tested gram-positive and gram-negative pathogenic bacterial strains ranged from 14 mm to 20 mm. None of the cell-free supernatants and lyophilized and filtered cell-free supernatants showed antibacterial activity on the agar plates, however when checked by broth microdilution assay, the lyophilized and filtered cell-free supernatants from *C. reinhardtii, P. cruentum, P. lutheri* and *Isochrysis* sp., showed a minimum inhibitory concentration ranging from 6.25 mg ml^{-1} to 25 mg ml^{-1}. The results of this experiment were similar to the previously reported studies by Medina-Jaritz *et al.* [4], and Najdenski *et al.* [15].

In the present study, water extracts and exopolysaccharides extracted from the haptophyte microalgae species (*P. lutheri* and *Isochrysis* sp.) were the only intracellular extracts which showed activity against *M. luteus* (15 mm), *S. aureus* (14 mm), *B. subtilis* (15 mm), and *P. aeruginosa* (14.5 mm) on agar plates. Contrary to this study, the exopolysaccharides isolated from *P. cruentum* showed potent antibacterial activity against *E. coli, S. enteritidis,* and *S. aureus* [31–33]. In addition, Li *et al.* [34] reported the inhibitory effect of a sulphated polysaccharide (fucoidan) isolated from the brown algae *Laminaria japonicus* against the *E. coli*. This may be due to the difference in the solvent, EPS extraction method, microalgae species, and in the polysaccharide itself [33]. The antibacterial activity may also be related to the anti-biofilm formation ability of the exopolysaccharides [33, 35]. The previously reported studies sug-

gest that the polysaccharides act by modifying the physical properties of the biotic surfaces [33, 36]. The differences in bacterial inhibition activity of polysaccharides reported by Raposo *et al.* [31–33], and Li *et al.* [34], and the present study might be due to the presence of different biotic surfaces on the gram-negative and gram-positive bacteria.

Chlorophyte microalgae species in the present study, showed maximum activity when the extracts were prepared by methanol or chloroform as an extraction solvent and a maximum inhibition zone diameter of 20 mm was recorded against *S. iniae*. Similarly, methanol extracts in Salem *et al.* [1], and Ghasemi *et al.* [19], also showed maximum activity among the tested solvents. Ghasemi and colleagues [19] were unable to report the inhibition of *P. aeruginosa* with methanol as a solvent. However, the ethanol, methanol and chloroform extracts in the present study showed noticeable results against one of the promising food-borne pathogen *P. aeruginosa.*

The red microalgae, *P. cruentum* ethanol extracts showed a minimum inhibitory concentration of 0.39 mg ml^{-1} against *S. aureus*. However, the ethanol, methanol and chloroform extracts failed to report activity against *E. coli, S. enterica* and *S. iniae*, respectively. Santoyo *et al.* [17], found prominent antimicrobial activity of the Et-OH and hexane extracts prepared from the red hematocysts of *H. pluvialis* against *E. coli* and *S. aureus*, whereas, in the present study, Et-OH extracts of *H. pluvialis* did not show activity by the agar well diffusion method. Similar to this study, a low activity was obtained when Santoyo *et al.* [17], used green motile cells of *H. pluvialis* instead of red hematocysts. This indicates that the compounds responsible for the antibacterial activity are found in the red phase of this microalga and thus, could be relatively polar compounds.

In conclusion, among all the tested species, haptophyte microalgae species, *Isochrysis* sp., and *P. lutheri* revealed the highest antibacterial activity against most of the tested pathogenic bacterial stains. The extracted samples in the present study showed noticeable results against one of the promising food-borne pathogen *P. aeruginosa.* Yet, the specific compounds responsible for the inhibition are still unknown which need to be further investigated. This study revealed the promising results of the economically important microalgae phyla and it will further aid in research studies aimed to extract and exploit the metabolic compounds for food and pharmaceutical industry.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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