Antimicrobial activity of marine microalgae: Isochrysis galbana, Isochrysis litoralis and Isochrysis maritima

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ABSTRACT
The aim of this study was to investigate the antimicrobial properties of three species of marine microalgae, namely Isochrysis galbana, Isochrysis litoralis and Isochrysis maritima. Ethanol was used to extract bioactive compounds from these microalgae, which were then tested for their ability to inhibit the growth of various microorganisms such as Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans, and Aspergillus niger. Among the three microalgae, the extract from I. litoralis exhibited the strongest antibacterial activity, inhibiting the growth of all three tested bacteria with a minimum inhibitory concentration (MIC) of 4.41 to 6.23 mg extract per mL culture. The extracts from I. galbana and I. maritima also demonstrated inhibitory activity against E. coli or P. aeruginosa, with a MIC of 7.26 to 8.06 mg extract per mL. All the extracts were found to inhibit the growth of C. albicans, with the highest activity observed in the extract from I. galbana, with a MIC of 6.10 mg extract per mL culture. However, the extracts were ineffective against A. niger. The antimicrobial activities of the extracts were attributed to the presence of lipids, carotenoids, and phenolic compounds. This study suggests that these microalgae could be a valuable natural source of bioactive compounds with antimicrobial properties.

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1. Introduction:
Microalgae are tiny organisms that use photosynthesis to produce biomass which can be used to create a variety of products, such as food, nutraceuticals, chemicals, and fuels [1-4]. They are able to grow in various environmental conditions, making them an increasingly popular option for production [5]. Worldwide production is estimated at 35,000 tonnes per year, with China being the largest producer of microalgae, particularly of the genus Isochrysis [6]. To cultivate microalgae, certain requirements must be met, including access to solar energy, carbon dioxide, water (fresh or marine), and macro and micronutrients [7]. Freshwater and nutrients can often be obtained from treated wastewater, while carbon dioxide (CO₂) can be sourced from industrial emissions. However, other factors such as annual air temperature, land use, land cover, and geological data must also be considered before establishing microalgae cultivation facilities [8]. Mexico is well-suited for microalgae production due to its high annual solar irradiance and ample freshwater and marine areas. In fact, native civilizations in the Valley of Mexico used to consume naturally growing Isochrysis from the coast. Currently, two small companies operate in central Mexico to produce this species [9-10].

The objective of this study is to evaluate the antimicrobial activities of three alcoholic extracts of algal strains (Isochrysis galbana, Isochrysis litoralis and Isochrysis maritima) against bacteria (Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus), the yeast Candida albicans, and the fungus Aspergillus niger.

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2. Materials and methods:

2.1. Culture of microalgae:
Abdoul-Latif et al. (2021) [11] previously described the culturing conditions for three marine microalgae: *Isochrysis galbana*, *Isochrysis litoralis*, and *Isochrysis maritima*. The microalgae were grown in batch cultures in 100 L flasks, which were filled with sterile natural seawater enriched with F/2 medium nutrients. The cultures were agitated by air bubbling at a temperature of 25°C, and under continuous illumination at an intensity of 150 μmol.m⁻².sec⁻¹. The resulting microalgal biomasses were harvested by centrifugation, freeze-dried, and stored at -20°C until ready for use.

2.2. Extraction of microagal biomass:
To prepare crude extracts from the microalgae, 1 gram of dried biomass was mixed with 100 milliliters of ethanol and left to extract for 3 hours at room temperature, in the dark. This process was repeated twice for each type of algae, and the resulting extracts were combined into a single sample. The samples were then filtered and concentrated using a rotary evaporator under reduced pressure. Finally, the concentrated extracts were stored at -20°C until ready for use [12].

2.3. Antimicrobial activities:
The microalgal extracts were tested against five different microorganisms: two Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*, one Gram-positive bacterium, *Staphylococcus aureus*, the yeast *Candida albicans*, and the mold *Aspergillus niger*. Spores of the mold were collected from monoconidial cultures that had been grown on PDA at 25°C for 7 days and were then suspended in sterile distilled water.

The minimum inhibitory concentrations (MICs) of the extracts were determined using the broth microdilution method described by Stumpf et al. (2020) [13] and Abdoul-Latif et al. (2020) [14]. Microbial samples were prepared by diluting with growth media to obtain inocula at a concentration of 10⁶ colony-forming units (CFU) per mL of culture. Muller Hinton broth was used for the bacteria, while PDA broth was used for *C. albicans* and *A. niger*. All media were supplemented with 0.5% Tween-80.

Extract samples were prepared by diluting in DMSO and tested at final concentrations ranging from 1 to 10 mg of extract per mL of culture. The microbial growth was allowed to proceed in 96-well micro-titration plates by adding 180 μL of microbial culture and 20 μL of microalgal extract sample at various concentrations. The plates were incubated at 37°C for 24 hours for bacteria and 48 hours for *C. albicans* or *A. niger*. The viability of the microorganisms was assessed by measuring absorbance of cultures at 600 nm using a Multiscan UV-VIS Spectrophotometer (TFS).

Assays were carried out in triplicate and repeated twice. Microbial growth inhibition was expressed as a percentage, which was calculated using the following equation:

\[
\text{Microbial growth inhibition (\%)} = \left(1 - \frac{A_{\text{sample}} - A_{\text{sample blank}}}{A_{\text{control}}}\right) \times 100
\]

2.4. Statistical Analysis:
PCA is a statistical method used to analyze data by identifying patterns and relationships among variables. In this study, it was used to visualize and summarize the information obtained from testing various samples against microorganisms. The samples tested included all extracts. The objective was to group the cell lines based on their response to the different samples tested, allowing for a summary representation that would be easier to interpret. The results of the PCA analysis were then statistically evaluated using the XLSTAT software, and other hand, the values presented as mean ± uncertainty at a 5% significance level of three replicates for each experiment using the Student’s t-test [15].

3. Results and discussion:
Table 1 shows the antimicrobial activity of three different species of microalgae (*Isochrysis galbana*, *Isochrysis litoralis* and *Isochrysis maritima*) against several microorganisms, including *E. coli*, *P. aeruginosa*, *S. aureus*, *C. albicans* and *A. niger*. The values given in the table represent the MIC (minimum inhibitory concentration) of the microalgae extract necessary to inhibit the growth of each microorganism. The MIC is the lowest concentration of a substance (in this case, a microalgae extract) that can inhibit the visible growth of a microorganism after a specified incubation period. The lower the MIC value, the stronger the antimicrobial activity of the substance. From the table, we can see that all three species of microalgae exhibit antimicrobial activity against the tested microorganisms, with some variation in the MIC values. For example, *Isochrysis galbana* and *Isochrysis maritima* showed potent activity against *S. aureus*, *C. albicans* and *A. niger*, with MIC values ranging from 6.10 to 8.06 mg/mL. Meanwhile, *Isochrysis litoralis* showed moderate activity against *E. coli*, *P. aeruginosa*, and *S. aureus*, with MIC values ranging from 4.41 to 6.02 mg/mL. Overall, this table provides useful information on the potential antimicrobial activity of microalgal extracts against several microorganisms. However, it is important to note that these results are based on in vitro experiments and do not necessarily reflect the effectiveness of microalgal extracts in vivo.
Table 1. Antimicrobial activities of ethanolic extracts of *Isochrysis* species microalgae.

<table>
<thead>
<tr>
<th>Microalgae</th>
<th>MIC (mg extract per mL culture)</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
<th>S. aureus</th>
<th>C. albicans</th>
<th>A. niger</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Isochrysis galbana</em></td>
<td>7.26 ± 0.21</td>
<td>&gt;10.00</td>
<td>&gt;10.00</td>
<td>&gt;10.00</td>
<td>6.10 ±0.21</td>
<td>-</td>
</tr>
<tr>
<td><em>Isochrysis litoralis</em></td>
<td>6.23 ± 0.18</td>
<td>6.02 ±0.20</td>
<td>4.41 ±0.11</td>
<td>&gt;10.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Isochrysis maritima</em></td>
<td>8.06 ± 0.24</td>
<td>&gt;10.00</td>
<td>&gt;10.00</td>
<td>&gt;10.00</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The principal component analysis (PCA) is a statistical method used to analyze the relationships between multiple variables. In this case, the PCA was used to evaluate the minimum inhibitory concentrations (MICs) of different microalgal extracts. The results of the PCA are presented in Figure 1, which shows that the MIC values tend to zero as the efficiency of the extracts increases. The mapping analysis, which is interpreted inversely, shows that the three microalgal strains used in the study have independent activities against the two bacteria strains tested. This means that each microalgal strain has its own unique set of secondary metabolites that contribute to its antimicrobial activity. Interestingly, the PCA also revealed that *P. aeruginosa* and *S. aureus* have a similar mode of action in relation to all the microalgal extracts. This suggests that the two bacteria strains may be vulnerable to similar secondary metabolites present in the microalgal extracts. This finding is significant because both *P. aeruginosa* and *S. aureus* are common pathogens that cause a range of infections in humans, and they are also known for their antibiotic resistance.

The antimicrobial activities of microalgae have been linked to the presence of unsaturated fatty acids [16]. Chlorellin, the first such compound, was discovered in *Chlorella* sp. and consists of a mixture of fatty acids that can inhibit the growth of gram-positive and gram-negative bacteria [17]. Other unsaturated fatty acids, such as eicosapentaenoic acid, hexadecatrienoic acid, and palmitoleic acid, have been identified in *Phaeodactylum tricornutum, Isochrysis* sp., *Scenedesmus intermedius, Chaetoceros muelleri, Haematococcus pluvialis, Chlorococcum sp.*, and *Skeletonema costatum*, which have been shown to possess antimicrobial activity against a broad range of gram-positive and gram-negative bacteria [18]. Additionally, organic extracts obtained from *Euglena viridis* and *S. costatum* have been found to inhibit the growth of *Pseudomonas* sp. and *Listeria monocytogenes*. Ethanolic extracts from *Isochrysis galbana* and *Dunaliella salina* were found to be effective against some bacterial strains, with inhibitory concentrations ranging from 80 to 100 µg/ml [18]. *Coccomyxa onubensis* fatty acid extracts also exhibited inhibitory activity against a variety of gram-positive and gram-negative bacteria and fungi [19]. *Fucus vesiculosus* pressurized liquid extracts produced long-chain fatty acids that demonstrated inhibition against *E. coli* and *S. aureus* [20]. *Rivularia mesenterica* ethanolic extract was also found to have a strong inhibitory effect against various antibiotic-resistant bacteria and fungi [21]. Microalgae have also exhibited antifungal properties against...
microorganisms such as Aspergillus niger, Candida kefyr, and Aspergillus fumigatus [22-23]. Katarungols, which are extracted from Amphidinium sp., are responsible for inhibiting the growth of Aspergillus niger and Trichomonas foetus [24]. Novel antimicrobial compounds, such as EMTAHDCA, have also been discovered in microalgae and cyanobacteria and have shown a strong binding affinity to targeted bacteria’s protein [25].

4. Conclusion:
The study highlights the potential of Isochrysis microalgae in producing powerful antimicrobial compounds that can effectively combat bacterial and fungal infections. The results demonstrate that three species of microalgae were able to produce potent antimicrobial compounds that were effective against a range of microorganisms. The study emphasizes the importance of using appropriate extraction processes with suitable solvents and conditions to obtain these antimicrobial compounds from microalgae. Overall, the findings of the study are significant as they pave the way for the development of high-value pharmaceutical antimicrobials with the potential for large-scale production. The study provides proof of concept that microalgae can be a viable source of antimicrobial compounds and highlights the need for further research and development in this area. If successfully scaled up, the use of microalgae-derived antimicrobial compounds could potentially provide a sustainable and eco-friendly alternative to traditional antibiotics, which are facing increasing problems with resistance. In conclusion, the study suggests that microalgae have immense potential in producing novel antimicrobial compounds, which can effectively combat bacterial and fungal infections. Further research is needed to optimize the production and extraction processes and to evaluate the efficacy and safety of these compounds for clinical applications. The use of microalgae-derived antimicrobial compounds could have significant implications for the treatment of infectious diseases and the development of new antimicrobial agents.

References:


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