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REVIEW

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"Antimicrobial and antioxidant capacity of Dunaliella salina, Tetraselmis chuii and Isochrysis galbana and their potential use in food." a systematic review

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Abstract

The main compounds extracted from the biomass of marine microalgae have antioxidant, antimicrobial, antifungal, anti-inflammatory and antitumor effects, making the possibility of using these properties in the development of foods feasible. Despite the proven biological activity of microalgae, there are still challenges regarding the production and use of microalgal biomass or its derivatives in food industries that are related to high production costs, and there is little research regarding the evaluation of their safety and the search for their application in food development. Therefore, this research aimed to collect information regarding the biological activities of marine microalgae, which allows their use as a natural antimicrobial additive in food matrices and as an ingredient in the development of functional foods with antioxidant capacity. The search interval for the PRISMA (preferred reporting items for systematic reviews and meta-analyses) guidelines was five years. Different methods for the extraction of antioxidant and antimicrobial compounds from the marine microalgae *Dunaliella salina, Tetraselmis chuii* and *Isochrysis galbana* were compared and discussed, and the viability of their use and application in food matrices and in the food industry in general were analyzed. It was concluded that there are research gaps in the use of microalgae biomass as an extract, the identification of bioactive molecules for use in the food industry as antimicrobial agents and for the development of functional foods with antioxidant capacity.

Keywords Microalgae, Natural antimicrobial, Antioxidant capacity, Functional foods

Background

Marine microalgae are microscopic organisms that contain large amounts of chemical compounds that provide them with bioactive properties. One of the biological activities that Chlorophyta marine microalgae possess is antimicrobial capacity, *Chlorella vulgaris* and *Dunaliella salina* extracts, have demonstrated favorable results of inhibition against the growth of the pathogenic microorganism *Streptococcus mutans*. This capacity has also been observed in *Haptophyta* microalgae *Isochryris galbana, Isochryris litoralis* and *Isochryris maritima* extracts against *Escherichia coli*, *Pseudomonas aeruginosa*, among others (Jafari et al. 2018; Garcia & Del Rio, 2022). The antimicrobial capacity of Chlorophyta microalgae *Tetraselmis suecica* against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* Typhimurium, among others, can be attributed to the presence of peptides with bactericidal potential (Guzmán et al. 2019; Kokkali et al. 2020).

Another important biological activity demonstrated by microalgae is antioxidant capacity due to the presence

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of phenolic compounds and antioxidant peptides, which was verified by evaluating proteins extracted from *Dunaliella salina* subjected to simulated in vitro gastrointestinal digestion. High concentrations of fucoxanthin, a compound with high antioxidant capacity, have also been identified in *Isochryris galbana* (Mousavi & Hosseinzade, 2020).

Although preservative additives of synthetic origin are very important in the food industry, in recent decades, a growing consumer preference has been observed for the use of natural preservatives, such as those from marine microalgae (Gokoglu 2019; Aziz & Karboune 2018; Rathod et al. 2021), due to their possible toxicity, carcinogenicity, epithelial cell sensitization, and immune sensitization, which in turn can cause allergic contact dermatitis, urticaria, respiratory discomfort, mucosal symptoms, and even isolated inflammation of the tongue, in patients with a previous diagnosis of angioedema (Duus et al., 2020). These health conditions are associated with the most used synthetic preservatives in foods, such as isothiazolinones, parabens, methyldibromine glutaronitrile, sulfites, benzoic acid, ascorbic acid, and calcium acetate, among others.

Functional foods have a positive impact on consumer health, even more so when the bioactive compounds used in their preparation, such as antioxidants, come from natural sources such as marine microalgae extract (Nataraj et al. 2020; Chin et al., 2019). Bioactive compounds from marine microalgae can be isolated from aqueous solutions, from protein hydrolysates, extracts, fresh mass, among others (Admassu et al. 2018).

Despite the proven biological activity of microalgae, there are still challenges regarding the production and use of microalgal biomass or its derivatives in the food industry due to the high production costs, the limited research regarding the evaluation of toxicity and the search for the application of ideal concentrations when applying microalgae in foods (Tavakoli et al. 2022).

The antimicrobial and antioxidant capacities of the marine microalgae *Dunaliella salina, Tetraselmis chuii* and *Isochrysis galbana* have not been investigated in depth in countries of Latin America like Ecuador. Furthermore, research on antimicrobial and antioxidant capacities has been limited to confirming or denying these biological activities in microalgae, but their use as a raw material or as an ingredient for the development of functional foods with antioxidant capacity or their use as an antimicrobial in food processing has not been investigated, as well as the antimicrobial and antioxidant mechanisms themselves have not yet been investigated and explained.

Therefore, this review aims to collect information regarding the biological activity of marine microalgae, which, in the future, will allow its use as a natural antimicrobial additive in food matrices and as an ingredient in the development of functional foods with antioxidant capacity.

Methodology

A systematic review was carried out on the existing publications referring to the bioactive antimicrobial and antioxidant properties of the marine microalgae *Dunaliella salina, Tetraselmis chuii* and *Isochrysis galbana* and their possible application in the food industry as natural antimicrobial additives and for the development of functional foods with antioxidant capacity. The present research followed the assumptions described by the authors in the PRISMA technique (preferred reporting items for systematic reviews and meta-analyses) (Kitchenham 2004; Okoli & Schabram 2010). Figure 1 shows the flow chart of the PRISMA methodology applied for this research and its respective bibliometric results.

To comply with the first phase of the PRISMA method, the following research questions were used: What bioactive compounds with antimicrobial capacity do the algae *Dunaliella salina, Tetraselmis chuii* and *Isochrysis galbana*? What bioactive compounds with antioxidant capacity do the algae *Dunaliella salina, Tetraselmis chuii* and *Isochrysis galbana*? The antimicrobial capacity of the algae *Dunaliella salina, Tetraselmis chuii* and *Isochrysis galbana* allows their use as natural additives in food industry products? Can *Dunaliella salina, Tetraselmis chuii* and *Isochrysis galbana* be useful for the development of functional foods with antioxidant capacity? Which of the microalgae offers better antimicrobial capacity? Which of the microalgae offers better antioxidant capacity? These questions allowed adequate systematization of the information, thus facilitating its organization and comprehension.

The search interval was established in six years, which includes published research between January 2018 and March 2024, considering that the investigation of bioactive compounds from microscopic organisms is a current topic of interest in food science and technology.

To obtain relevant information on the topic, high-impact databases, including Springer, Science Direct, Refseek, Scopus, and Connected, and the searcher academic engine Google Scholar and Bielefeld Academic Search Engine were used. The searches were carried out in English, using bibliometric descriptors (Table 1) such as “antimicrobial capacity”, “antioxidant capacity”, and “bioactivity characteristics in microalgae". In addition, Boolean search engines were used to delimit the information regarding the microalgal species of interest: "antimicrobial capacity” and “antioxidant capacity”, “antimicrobial capacity” or “antioxidant capacity”, “bioactivity

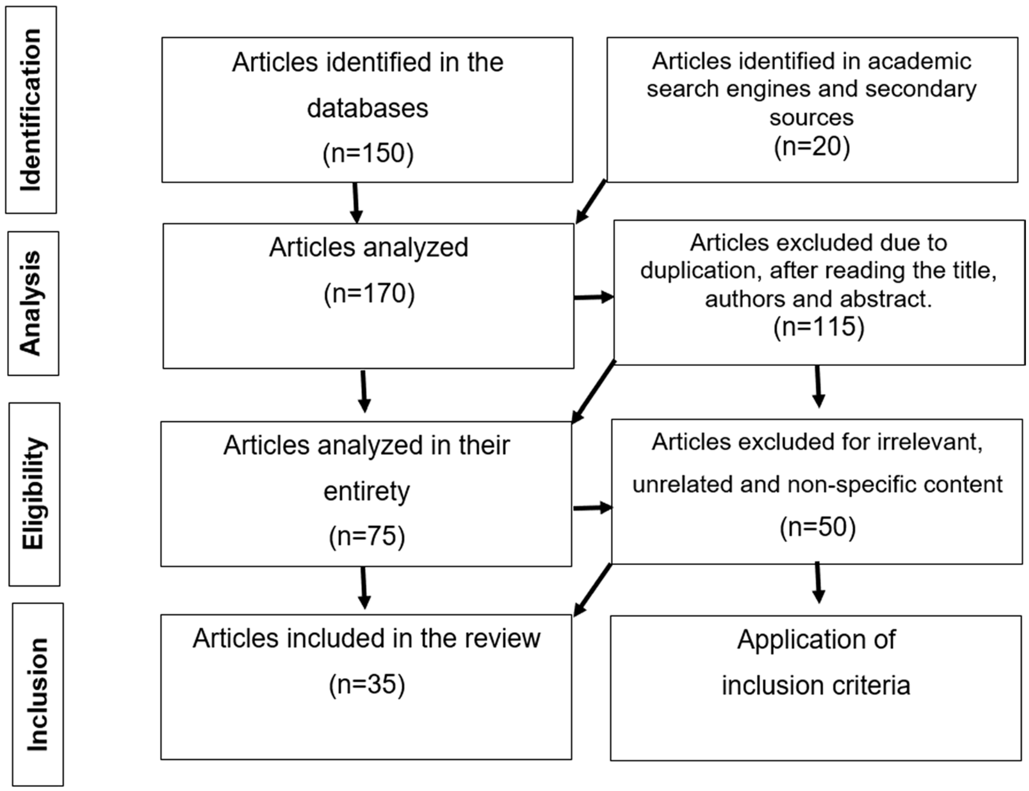

Fig. 1 PRISMA methodology flowchart

Table 1 Bibliometric results

Descriptor	Database					
	Springer	Science direct	Ref seek	Scopus	Connected papers	Google scholar
"Antimicrobial capacity of *Dunaliella salina*"	913	1640	232	311	400	2953
"Antioxidant capacity of *Dunaliella salina*	2953	16,300	1446	1883	844	7891
"Bioactive characteristics of *Dunaliella salina*"	5488	30	702	18,100	1159	1365
"Antimicrobial capacity of *Tetraselmis chuii*"	2303	2460	9402	7	2953	1280
"Bioactive characteristics of *Tetraselmis chuii*"	1	2951	472	85	160	199
"Antimicrobial capacity of *Isochryris galbana*"	2450	3681	3	2951	450	1993
"Antioxidant capacity of *Isochryris galbana*"	62	92	1560	1	1	1400
"Bioactive characteristics of *Isochryris galbana*"	4952	722	1762	238	2470	1290

characteristics in microalgae" + "Scientific Name of Microalgae".

Finally, the following descriptors were used: "antimicrobial capacity of *D.* salina", "antioxidant capacity of *D.* salina", "bioactive characteristics of *D.* salina", "antimicrobial capacity of *T. chuii*", "antioxidant capacity of *T. chuii*", "bioactive characteristics of *T. chuii*", "antimicrobial capacity of *I. galbana*", "antioxidant capacity of *I. galbana*", and "bioactive characteristics of *I. galbana*".

Inclusion criteria

This research was developed between 2018 and 2024 and included studies with quantitative, qualitative or mixed approaches and articles written in English or Spanish. The research objective, degree of relationship with the research questions, affinity of the study with the microalgal species of interest and affinity of the study with the bioactive characteristic of interest were considered.

Dunaliella salina

Generalities

Dunaliella salina is a unicellular marine phytoplankton belonging to the *Dunaliellaceae* family. Since the beginning of its study, it has been considered a fundamental part of the development and balance of primary marine life. Especially under hypersaline environmental conditions, so it can be used in research on the ability of algae and microalgae to adapt to salt. Furthermore, it has been proven that under optimal conditions for growth and development, plants can produce and accumulate large amounts of bioactive antioxidant compounds, especially β-carotene (El-Baz et al. 2020).

In addition to containing many antioxidants compounds, *Dunaliella salina* contains other bioactive molecules of great interest, such as lutein, zeaxanthin, chlorophyll and polyunsaturated fatty acids. Due to the large amount of β-carotene that this microalga contains, it is considered a powerful source of antioxidants, as well as a precursor and modulator of retinol, so it can even be used as a healthy food, nutritional supplement, and coloring additive either in food or cosmetics. There are also clinical reports showing that *Dunaliella salina* can have beneficial effects on health, reducing the risk of metabolic diseases such as obesity, diabetes mellitus, dyslipidemia and arterial hypertension (El-Baz et al. 2018).

Antioxidant capacity of *Dunaliella salina*

The way *Dunaliella salina* is grown and extracted influences the number of antioxidants that can be obtained from it. When a change in color goes from green to red is induced in the microalgae cells and NaCl and KNO_3 are added to the culture, lipophilic antioxidants, specifically carotenoids, are obtained. When the ultrasound-assisted extraction method is used, the production and yield of carotenoids are improved (Table 2), given that this extraction method is carried out at low temperatures and that ultrasonic waves allow for the effective release of carotenoids and chlorophyll into the solvent (Gallego et al., 2019; Nejadmansouri et al. 2021).

When the supercritical carbon dioxide extraction method is applied, it is important to establish the optimal extraction conditions, with the ideal conditions for *Dunaliella salina* being a pressure of 312.6 bar and a temperature of 45.0 °C, to guarantee the purity and high performance of the antioxidant (Bueno et al. 2020).

For the extraction of antioxidants to be selective, the extraction method assisted by supercritical carbon dioxide (SC-CO_2) can be used. For the selective extraction of β-carotene, the best solvent is ethanol, since it improves the solubility, increases the extraction yield and increases the mass fraction (Tirado & Calvo 2019).

The optimal extraction conditions in this case vary, and it is suggested to use 500 bar pressure, 70 °C and 10% by weight of ethanol as the cosolvent since this achieves a recovery of 90% of the antioxidant content and greater purity of β-carotene (Ludwig et al. 2020).

Antimicrobial capacity of *Dunaliella salina*

The antimicrobial capacity of *Dunaliella salina* microalga can be evaluated using extracts and suspensions, among others. By incorporating it into a suspension for the synthesis of gold nanoparticles (GNPs) and gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), an antimicrobial effect against Gram-negative and Gram-positive bacteria. The bacteria studied were *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Salmonella* Typhimurium, resulting in inhibition halos of significant sizes, as shown in Table 3 (Basiratnia et al. 2021). When the *Dunaliella salina* suspension is incorporated into silver nanoparticles (AgNPs), favorable results are obtained for antimicrobial activity against *Bacillus subtilis* and *Enterobacter tabaci* (Shantkriti et al. 2023).

On the other hand, microalgae extracts can be obtained with ethanol, hexane or another type of solvent; thus, they will have different antimicrobial activities depending on the solvent used, with the greatest effectiveness against *Pectobacterium carotovorum* subsp. *carotovorum* DSM30168, was achieved with the ethanol extract, while for *Pseudomonas syringae* pv.

Table 2 Antioxidant Capacity of *Dunaliella salina*

Method	Compound	Result	Author
Culture in J1 Medium, 4.0 M [NaCl-] and 0.50 mM [KNO_{3}]	Lipophilic Antioxidants Carotenoids	9.69 µg/mL	Gallego (2019)
Ultrasound-assisted extraction (10 min. Y < T°)	total carotenoids	2.62 µg/mg	Nejadmansouri et al. (2021)
	Chlorophyll *a*	1.83 µg/mg	
	Chlorophyll *b*	1.75 µg/mg	
Extraction with supercritical carbon dioxide (SC-CO_2) and ethanol	Total carotenoids	25 g/Kg microalgae	Stripped & Bald (2019)
Fluid extraction supercritical (CO_2 N_2 and Ethanol), 312 bar and 45 °C	Total carotenoids	60.8 mg/g of microalgae	Bueno et al. (2020)
Extraction with supercritical carbon dioxide (SC-CO_2) and Acetone	Beta carotenes	18 µg/mg	Ludwig et al. (2020)

Table 3 Antimicrobial Capacity of *Dunaliella salina*

Method	Inhibited microorganism	Inhibition Halo (mm)	Author
Synthesis of Gold Nanoparticles + *Dunaliella salina* Plate sowing	*Staphylococcus aureus*	15	Basiratnia et al. (2021)
	Bacillus cereus	14	
	Escherichia coli	11	
	Salmonella Paratyphi	12	

tomato EPS3, *Staphylococcus aureus* and *Escherichia coli* have better efficacy with hexane extract (Ambrico et al. 2020; Salim et al. 2018).

However, in in vivo studies in which young tomato plants and tomato and zucchini fruits were used as study subjects, *Dunaliella salina* extract obtained with hexane showed better antimicrobial efficacy (Table 4) and increased evasion and decreased severity of fruit disease due to pests, as well as a delay and decrease in fruit rot symptoms in tomato and zucchini (Ambrico et al. 2020).

When the sequential extraction of *Dunaliella salina* was carried out with hexane, ethyl acetate (AcOEt) and methanol, favorable results were obtained regarding the minimum antibiofilm inhibitory concentration (MBIC) against *Candida parapsilosis, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Staphylococcus epidermidis,* and *Pseudomonas aeruginosa*, demonstrating slightly greater efficacy when ethyl acetate was used as the main cosolvent (Iglesias et al., 2019).

Regarding the type of culture that allows better growth efficiency for *Dunaliella salina*, the findings suggest that the modified seawater medium is ideal (Salim et al. 2018).

Mechanism of the antimicrobial activity of *Dunaliella Salina*

The fresh mass extract of *Dunaliella salina* can be used for the biosynthesis of silver nanoparticles, these nanoparticles can connect to the microbial membrane and even pass through it (Fig. 2), once they have entered the microbial cell they interrupt cell division, alter the permeability of the cell membrane surface and respiratory functions, resulting in cell death (Shantkriti et al. 2023).

The direct antimicrobial mechanism of *Dunaliella salina* biomass has not been thoroughly evaluated so far, it has been hypothesized that the antimicrobial activity could be due to oxidized compounds, which react with sulfhydryl groups through additional non-specific interactions with proteins, the ability of terpenoids to disrupt the membrane by lipophilic compounds and intercalate

Table 4 Antimicrobial Capacity of *Dunaliella salina*

Method	Inhibited microorganism	Inhibition Halo (mm)/ Minimal inhibitory concentrations	Author
Extract With Solvents disk broadcast	*Pectobacterium carotovorum* subsp. *carotovorum,* extract	11	Ambrico, et al. (2020)
	Pseudomonas syringae pv. tomato, hexane extract	12	
Synthesis of Silver Nanoparticles + *Dunaliella salina* disk broadcast	*Escherichia coli*	4	Shantkriti et al. (2023)
	Bacillus subtilis	7	
	Enterobacter tabaci	7	
Extract with solvents Anti-biofilm	*Candida parapsilosis*	> 1.024 mg/L extract and methanol	Iglesias, et al. (2019)
	Escherichia coli		
	Klebsiella pneumoniae		
	Staphylococcus aureus		
	Staphylococcus epidermidis	512 mg/L, hexane extract and methanol	
	Pseudomonas aeruginosa		
	Candida albicans		
	Enterobacter cloacae		
Extract with solvents sowing in agar	*Staphylococcus aureus*	10	Salim et al. (2018)
	Escherichia coli	10.75	

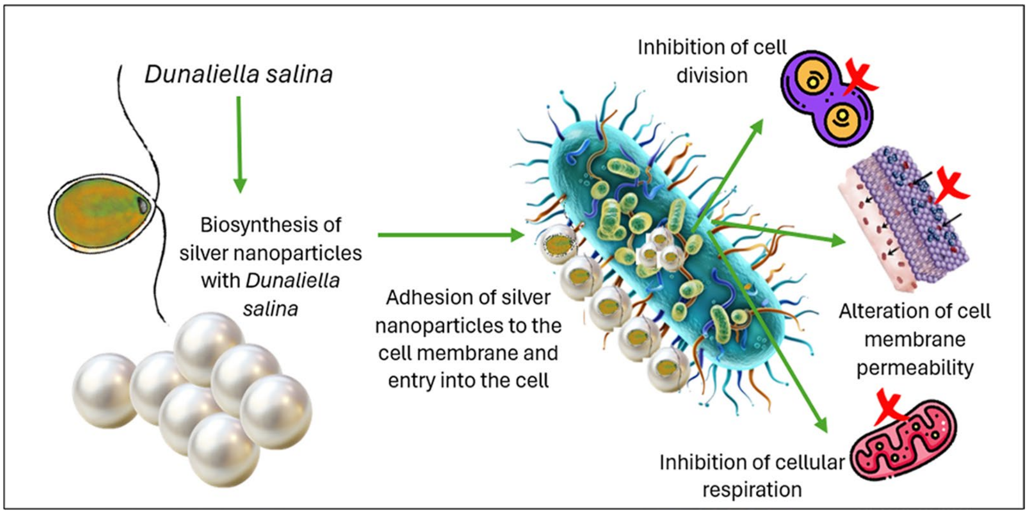

Fig. 2 Antimicrobial action mechanism of silver nanoparticles made from microalgae *Dunaliella salina*

with DNA, disruption of the electron transport chain and oxidative phosphorylation, destruction of nutrient uptake, degradation products of autoxidation and peroxidation production, direct lysis of bacterial cells or prohibition of enzymatic activity (Jafari et al. 2018).

Furthermore, it has been shown that the difference between the negative charge of a microorganism and the positive charge of a metallic nanoparticle acts as an absorbing electromagnet between the microbe and the nanoparticles. The binding of silver nanoparticles to the cell surface manages to perforate the wall, leading to bacterial death thanks to the interruption of several metabolic and reproductive pathways (Basiratnia et al. 2021).

Mechanism of the antioxidant activity of *Dunaliella Salina*

Microalgae cells in general, including *Dunaliella salina*, accumulate 30–40% of ascorbic acid that remains in the chloroplast. Ascorbic acid acts as a potent antioxidant due to its ability to donate electrons in enzymatic and non-enzymatic reactions, thus protecting cells by directly eliminating $O_2•-$, $HO_2•-$ and regenerating tocopherol from tocopheroxyl radicals (Fig. 3) (Roy et al., 2021).

Furthermore, the peptides isolated from *Dunaliella salina* contain basic amino acids such as Asn, Gln and Arg, which have a higher processing capacity to scavenge the free radical (2,2-Diphenyl-1-picrylhydrazyl) DPPH compared to acidic or neutral peptides. The

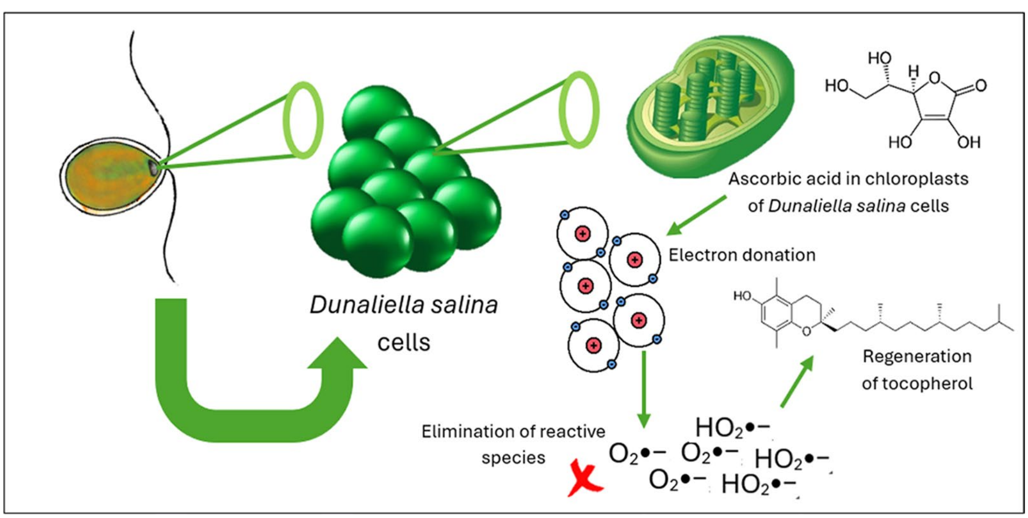

Fig. 3 Antioxidant action mechanism of the microalga *Dunaliella salina*

dominant peptide present in *Dunaliella salina* includes the hydrophobic amino acid residues Ile and Leu at the N-terminus, which might be mainly responsible for the antioxidant capacity of the entire digested fraction (Xia et al. 2019; Xu and Harvey 2019; Bahador et al. 2019).

Tetraselmis chuii

Generalities

Tetraselmis chuii is a unicellular and mobile marine microalga that is approximately 10 to 15 µm in size and has an ellipsoidal morphology, and its reproduction occurs due to the longitudinal fission process. The taxonomic classification of the microalga *Tetraselmis chuii* according to Butcher in 1959 is as follows: Kingdom Plantae, Phylum Chlorophyta; Class Chlorodendrophyceae, Order Chlorodendrales.

This microalgal strain was initially isolated and described in the 1950s on the coast of Great Britain and has subsequently been isolated in different parts of the world to study its functional and bioactive characteristics. It was identified 8/6 times in the Culture Collection of Algae and Protozoa (CCAP) of the United Kingdom (Barat et al., 2013).

Tetraselmis sp., is a widely known microalgae for its various applications as a live food used in the aquaculture industry, and its optimal growth conditions can vary according to the required culture conditions (Montoya & Acosta, 2021).

Furthermore, *Tetraselmis* sp., is a microalga with a wide variety of applications, especially in aquatic cultures. It can also be used to feed fish and shrimp, especially in specific stages of development of these animals, primarily for larval stages. They also serve as food of rotifers and crustaceans, due to their biochemical composition, which provides good nutritional characteristics, size and ease of cultivation, make these microalgae organisms of great research and industrial interest (Sacristán de Alva et al., 2018).

Antioxidant capacity of *Tetraselmis chuii*

The extraction of antioxidants and phenolic compounds can be optimized, and depending on the method used, the extraction assisted by pulsed electric fields (PEFs) combined with the aqueous solvent dimethyl sulfoxide (DMSO) allows for the effective recovery of pigments and polyphenols from microalgae (Kokkali et al. 2020).

The optimal conditions for the extraction of antioxidant compounds from *Tetraselmis chuii* are 24 h of maceration using DMSO as a solvent and a PEF at 1 kV/cm and 400 pulses since these conditions allow higher values of maximum antioxidant capacity (TAC) to be obtained, total phenolic compounds (TPC) and a greater recovery of carotenoids, as shown in Table 5 (Kokkali et al. 2020).

With the intention of preserving the integrity of the antioxidant compounds of the microalgae, microencapsulation of the fresh biomass can be carried out. The findings suggest that with microencapsulation, adequate conservation of beta-carotene and phenolic compounds is achieved when gelatin is used as a component of the wall and encapsulation is carried out at 110 °C in *Tetraselmis chuii* (Bonilla et al., 2018).

Light intensity and exposure time have an effect on the compounds that provide antioxidant activity; in this sense, it has been reported that chlorophyll *a*, chlorophyll *b*, carotenoids, α-tocopherol, the amount of chlorophyll *a*/*b* and the content of ascorbic acid from *Tetraselmis*

Table 5 Antioxidant Capacity of *Tetraselmis chuii*

Method	Compound/ Indicator	Result	Author
Pulsed electric fields (PEF) and aqueous solvent dimethyl sulfoxide (DMSO)	Maximum antioxidant capacity (TAC)	51.97 µM	Kkokali et al. (2020)
	Total phenolic compounds (TPC)	6.70 mg gallic acid equivalents (GAE)/g	
	Recovery of carotenoids	0.48 mg/g PS	
Cultivation in media with and without wastewater	Carotenoids Residual water	0.832 mg/L	Khatoon et al. (2018)
	Total Carotenoids in Conway + wastewater	0.681 mg/L	
	Conway Medium Carotenoids alone	0.649 mg/L	
Bread extract made from *Tetraselmis chuii*	Total phenols	0.11 mg/g GAE	Nunes et al. (2020)
	Antioxidant capacity	3.22 mg/g Vit. C	
Microencapsulation of biomass and spray drying	Total Carotenoids	76.5 µg/g	Bonilla et al. (2018)
	Carotenes	1 038.8 µg/g	
	Total phenolic compounds	46.2 mg/g GAE	
	Antioxidant capacity	7.5 mg/g Vit. C	
Culture in enriched seawater	Ascorbic acid	1.264 mg/g	Yusof et al. (2021)
	α-Tocopherol content	12.840 mg/g	

chuii increase with continuous exposure to light for 8 days when exposed to 12:12 and 24:0 h light/dark cycles at 24 ± 2 °C (Yusof et al. 2021).

The ideal culture medium also contributes significantly to a better antioxidant yield of the microalgae. As mentioned above, for *Tetraselmis chuii*, the best culture media in decreasing order are wastewater medium, Conway medium + wastewater and Conway medium alone, allowing better recovery of carotenoids (Khatoon et al. 2018).

The application of the antioxidant capacity of *Tetraselmis chuii* in foods is viable and can have a beneficial effect on human health. In this sense, fresh biomass of the microalgae was added to the bread formulation, resulting in a functional food with antioxidant capacity, and with changes in important technological measures such as the replacement of gluten, the addition of 4% fresh biomass allowed the bread to have considerable total antioxidant capacity and total phenolic content (Nunes et al. 2020).

Antimicrobial capacity of *Tetraselmis chuii*

The microalga *Tetraselmis chuii* has antimicrobial effects on *Listeria monocytogenes* CECT 935 and *Enterococcus faecalis* CECT 481 when an ethanolic extract of fresh biomass is used; however, its effectiveness against *Escherichia coli* and *Salmonella enterica*, has not been proven. Like other species of the *Tetraselmis* genus, *Tetraselmis* sp. Z3 and *Tetraselmis* sp. C6 has demonstrated antimicrobial efficacy against gram-negative pathogens (*Salmonella* Typhimurium, *Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-positive pathogens (*Bacillus subtilis, Staphylococcus aureus* and *Enterococcus faecalis)*, with important inhibition zone diameter values, such as those shown in Table 6, when the extract is ethanolic (Fajardo et al., 2020; Grubišić et al. 2022).

However, there was also a salvageable antimicrobial capacity for pathogens when the extract used for the study was methanolic. Additionally, the extract shows efficacy against *Pseudomonas fluorescens* and *Vibrio harveyi* (Salim et al. 2018).

As marine microalgae are food for rotifers, they can be used as subjects to study the antimicrobial capacity of microalgae. In this context, *Tetraselmis chuii* has been shown to have a bactericidal effect on the pathogen *Vibrio harvey* when it infects rotifers (Farisa et al. 2019).

Mechanism of the antimicrobial activity of *Tetraselmis chuii*

The ester groups, benzene, 9-hexadecenoic acid and fatty acids present in *Tetraselmis chuii*, provide the spectrum to inhibit the growth of bacteria, since they inhibit enzymes, interfere with the binding protein, form a complex with the cell wall, generate substrate loss and alter the permeability of the cell membrane; (Fig. 4) all this results in the cell death of pathogenic microorganisms (Maligan and Widayanti, 2013).

Mechanism of the antioxidant activity of *Tetraselmis* chuii and *Isochrysis* galbana

Alpha-tocopherol is a highly abundant antioxidant in the microalgae *Tetraselmis chuii* and *Isochrysis galbana*, this

Table 6 Antimicrobial Capacity of *Tetraselmis chuii*

Method	Inhibited microorganism	Inhibition Halo (mm) / Presence or Absence of inhibition zone	Author
Extract with Solvents Diffusion in Agar *Tetraselmis chuii*—fed rotifer extract Kirby -Bauer method	*Listeria monocytogenes* CECT 935	(+)	Fajardo et al. (2020)
	Enterococcus faecalis CECT 481	(+)	
	Escherichia coli	(−)	
	Salmonella enterica	(−)	
	Vibrio harveyi	7	Farisa et al. (2019)
Extract with Solvents Disk Broadcast	*Escherichia coli*	26.05	Grubišić et al. (2020)
	Salmonella typhi	20	
	Pseudomonas aeruginosa	17	
	Bacillus subtilis	10	
	Staphylococcus aureus	22	
	Enterococcus faecalis	18	
	Candida utilis	7	
Extract with Solvents Surface Sowing (In methanol extract)	*Staphylococcus aureus*	9	Salim et al. (2018)
	Escherichia coli	10.5	
Extract with Methanol Disk Broadcast	*Pseudomonas fluorescence*	4.2	Widowati et al. (2021)
	Vibrio harveyi	3	

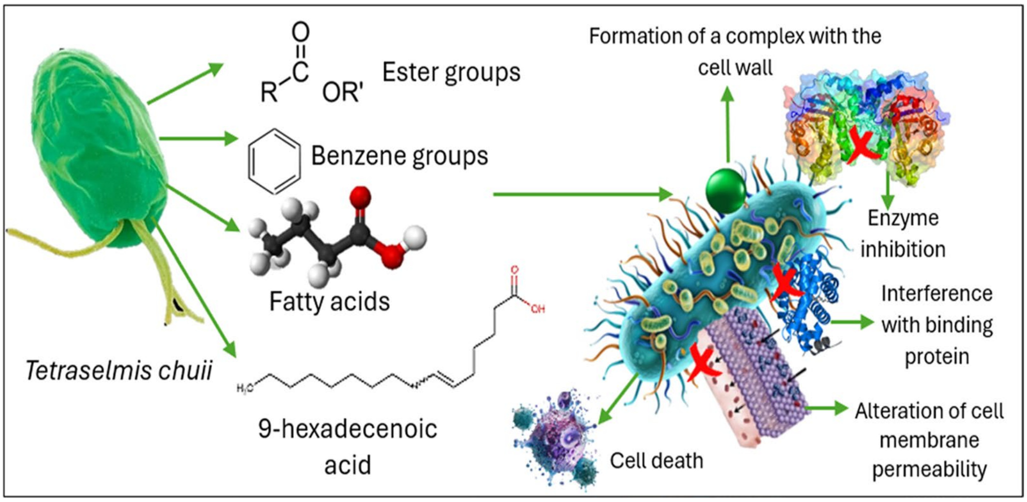

Fig. 4 Antimicrobial action mechanism of the microalgae *Tetraselmis chuii*

molecule is a chain-breaking molecule capable of suppressing $1O_2$, and $O_2 \bullet -$ and inhibiting lipid peroxidation by blocking the development of reactive oxygen species, which implies the inhibition of low-density lipoprotein oxidation (Fig. 5) (Yusof et al. 2021).

Isochrysis galbana

Generalities

Isochrysis galbana is a microalga that has ovoid morphology, with a size ranging between 5 and 7 µm. It also has two flagella, and it has been proven to be highly rich in docosahexaenoic acid (DHA), a bioactive component that is beneficial for the growth and development of marine fish larvae. It is considered an outstanding marine microalga because it contains a wide variety of active biomolecules of interest, such as lipids, antioxidants and DHA. Due to the presence of DHA, this alga can be used in aquaculture and as a fuel source (Medina et al., 2019).

Isochrysis galbana is a species of *Haptophyta* that has been widely used for years as food for bivalve larvae, which is currently an important part of the aquaculture industry. Furthermore, this microalga continues to be investigated due to its high content of bioactive substances, such as antioxidants, carotenoids and fucoxanthin, and it has a chloroplast whose genomic sequence was published in 2020 (Fang et al. 2020).

Antioxidant capacity of *Isochrysis* galbana

The sowing and harvesting method influence the number of antioxidants that can be recovered from marine

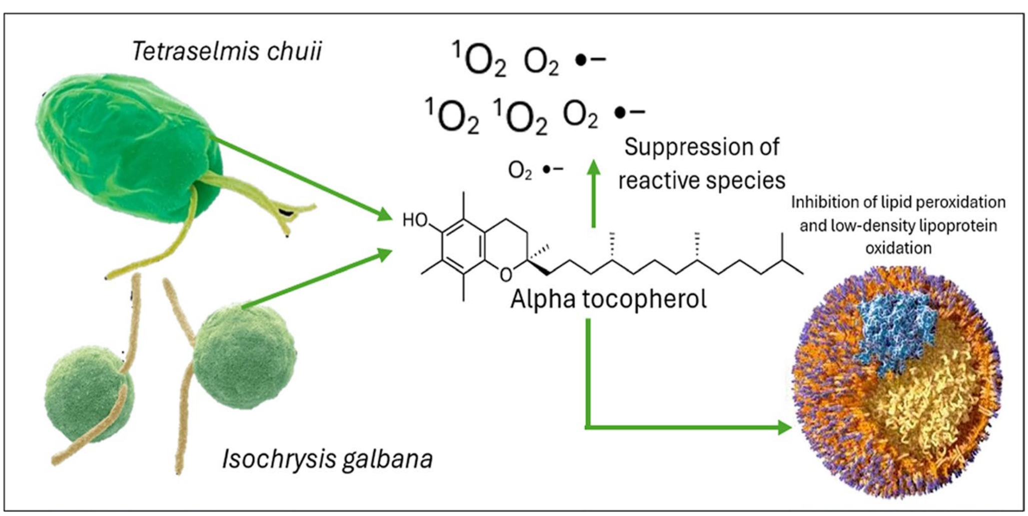

Fig. 5 Antioxidant action mechanism of the microalgae *Tetraselmis chuii* and *Isochrysis galbana*

microalgae. Electroflocculation decreased the antioxidant properties of methanolic extracts from the *Bacillariophyceae* marine microalgae *Isochrysis galbana,* and *Phaeodactylum tricornutum;* however, compared with the traditional centrifugation method, electroflocculation did not decrease the number of antioxidants in the algae biomass, while the centrifugation method did affect it (Ramos et al. 2020).

When supercritical fluids are used for extraction, favorable results are obtained (Table 7). In this case, the main challenge is to establish the optimal extraction conditions. However, when working with *Isochrysis galbana,* a concentration of ethanol is recommended 8% v/v of ethanol, a pressure of 40 MPa and a moderately high temperature of 50 °C (Ruiz et al., 2020).

Two of the most used methods are extraction in water and ethanol. Better results have been shown in the extraction process when the extraction is carried out with ethanol since the values of total polyphenols, laminarin content, coenzyme Q10, fucoxanthin and β-carotene increase in this extract (Matos et al. 2019).

The amount of antioxidants and the antioxidant capacity can be directly associated with the biological nature of the specimen, since when comparing the ethanolic and aqueous extracts of *Mychonastes homosphaera* (formerly *Chlorella minutissima*), *Dunaliella salina*, *Isochrysis galbana*, *Nannochloropsis oculate*, and *Tisochrysis lutea*. Under the same experimental conditions, it was observed that *Isochrysis galbana* presented a higher total phenolic content (TPC), and DPPH content, higher radical scavenging activity, higher iron chelating activity (ICA), and greater ferric reducing antioxidant power (FRAP) in the methanol extract (Andriopoulos et al. 2022).

Although research on the antioxidant properties of *Isochrysis galbana* in foods is scarce, its application in the formulation of canned fish burgers has provided acceptable values of chlorophyll and phycocyanin, resulting in a functional food with very effective antioxidant capacity compared to *Mychonastes homosphaera* (formerly *Chlorella minutissima*) and *Picochlorum* sp. (Chlorophyta), which demonstrated lower antioxidant values (Atitallah et al. 2019).

Antimicrobial capacity of *Isochrysis* galbana

Isochrysis galbana has demonstrated greater effectiveness against of the pathogenic microorganisms *Listeria monocytogenes, Staphylococcus aureus, Bacillus subtilis, Clavibacter michiganensis, Staphylococcus epidermidis, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Bacillus subtilis*

Table 7 Antioxidant capacity of *Isochrysis galbana*

Method	Compound/ indicator	Result	Author
Harvest by Electro-flocculation	Thin layer chromatography (TLC) Dimension of expressive reactive bands	24 and 26 mm	Ramos et al. (2020)
Ethanolic extract	Total phenolic contents	515 mg GAE/100 g dry weight	Matos et al. (2019)
	B-carotene	13.6 µg/g	
	Coenzyme Q10	2 µg/g	
	Fucoxanthin	246.3 µg/g	
Aqueous extract	Total phenolic contents	6.49 mg GAE/L	Andriopoulos et al. (2022)
	DPPH Radical scavenging activity (RSA DPPH)	0.98 mg Trolox equivalent per g dry matter	
Aqueous extract	Greater ferric reducing antioxidant power (FRAP)	19.04 mg Trolox equivalent per g dry matter	Andriopoulos et al. (2022)
Extraction with supercritical fluids (ethanol) and Behnken Design	Total carotenoids content	15.33 mg/g	Domínguez et al. (2020)
	Total carotenoids recovery	62.85% w/w	
	Total phenolic content	157.16 mg GAE/g	
	Antioxidant activity (TEAC method, trolox equivalents antioxidant capacity method)	0.31 mmol	
	Fatty acid identification and quantification, a standard fatty acid methyl ester (FAME)	7.19 mg/g	
Extract from canned fish burgers collaborated with *Isochrysis galbana*	Chlorophyll	23.28 mg/100 g	Atitallah et al. (2019)
	Phycocyanin	0.45 mg/100 g by dry matter	
	Carotenoids	12.5 mg/100 g by dry matter	
	Free radical scavenging activity	56%	
	Reducing power	0.75	

compared with *Scenedesmus microalgal* sp. NT8c, *Chlorella* sp. FN1 and *Nannochloropsis oculate* (Alsenani et al. 2020; Putra et al. 2022).

When *Isochrysis galbana* is compared with other specimens of the genus *Isochrysis*, such as *Isochrysis litoralis, Isochrysis maritima* and *Isochrysis galbana* demonstrates greater antimicrobial capacity against, *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans* and *Aspergillus niger* (Garcia & Del Rio, 2022).

The antimicrobial capacity of this alga is attributed to the presence of bioactive lipids, carotenoids, and phenolic compounds; such as, linoleic acid, oleic acid, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). Likewise, the antimicrobial capacity is also influenced by the type of extraction, since when the *Isochrysis galbana* extract is made in ethanol, better efficacy is obtained against the pathogen *Listeria monocytogenes* CECT 935, and for *Enterococcus faecalis* CECT 481 (Table 8) (Alsenani et al. 2020; Garcia & Del Rio, 2022).

Mechanism of the antimicrobial activity of *Isochrysis* galbana

The antimicrobial activity of *Isochrysis galbana* against pathogenic microorganisms depends on several factors, the composition of the bacterial membrane, the resistance capacity of yeasts, the structure of the polysaccharides, the degree of branching and the degree of sulfation. It is hypothesized that the polysaccharides present in *Isochrysis galbana* inhibit the growth of fungi by reacting with enzymes present in the hyphae and altering their main biological functions (Ben et al. 2017).

Future challenges

Applicability of antimicrobial bioactive compounds from microalgae to the food industry

As shown in the previous sections, microalgae extracts contain important bioactive compounds, some of which have the capacity to provide antimicrobial effects. Although the biological activities of each of the algae described is proven in isolation, they have a relatively limited antimicrobial effect. Therefore, a research topic of interest would be the study of the synergistic effect of the antimicrobial and antioxidant capacity when the extracts of the three are combined. Microalgae analyzed in this review, since bibliographic research shows that they have only been studied separately, which could enhance their effect and could be used as a natural antimicrobial additive in products developed in the food industry.

Table 8 Antimicrobial Capacity of *Isochrysis galbana*

Method	Inhibited microorganism	Inhibition Halo (mm)/ Minimum inhibitory concentration/ Absence or Presence of Inhibition Halo	Author
Extract with ethanol disk broadcast	*Listeria monocytogenes*	19.67	Alsenani et al. (2020)
	Staphylococcus aureus	20	
	Bacillus subtilis	19.33	
	Clavibacter michiganensis	16.33	
	Staphylococcus epidermidis	18.33	
	Enterococcus faecalis	18.33	
Extract By centrifugation diffusion on disco	*Escherichia coli*	9.99	Putra et al. (2022)
	Pseudomonas aeruginosa	9.09	
	Staphylococcus aureus	9.96	
	Bacillus subtilis	8.38	
Extract with ethanol minimum inhibitory concentration	*Escherichia coli*	mg extract per mL culture)7.26	Garcia & Del Rio (2022)
	Pseudomonas aeruginosa	> 10.00	
	Staphylococcus aureus	> 10.00	
	Candida albicans	6.10	
Extract with solvents diffusion in agar	*Listeria monocytogenes*	(+)	Fajardo et al. (2020)
	Enterococcus faecalis	(+)	
Extract with ethanol	*Listeria monocytogenes*	19.67	Alsenani et al., (2020)
	Staphylococcus aureus	20.00	
	Clavibacter michiganensis	16.33	
	Staphylococcus epidermidis	18.33	
	Enterococcus faecalis	19.33	

Furthermore, antimicrobial capacity studies of these microalgae have been carried out only on extracts obtained from fresh biomass, and their application in food products has not been investigated since they have not been used as an ingredient or additive in food for human consumption. This is reflected in the lack of information evaluating the antimicrobial capacity of microalgae in foods. Nor has the use of biomass been explored in another form of use; perhaps the most extensive is the use of extracts, but it does not refer to the possible use of pulverized, freeze-dried microalgae in tinctures, and other presentations which would allow greater versatility in its use.

Although the low use of bioactive compounds from microalgae could be associated with the lack of studies evaluating the toxicity of microalgae, they have been studied in vitro and in vivo in brine shrimp, fish, laboratory rats and tomato and pumpkin plants, suggesting that their consumption is safe.

Another possible cause that limits the use of microalgae as an antimicrobial additive is the fact that in research evaluating biological activity, identification, isolation and extraction studies of the molecules that provide antimicrobial effects have not been carried out.

Only one of the studies included in this review reports the identification of the molecules to which the antimicrobial capacity is attributed. In the study carried out by Jafari et al. (2018), linoleic acid, oleic acid, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are mentioned as the compounds responsible for the antimicrobial activity, and in turn, the analysis of these compounds in isolation would be required to guarantee their effectiveness and safety when used in food industry products.

Despite the limitations, the application of antimicrobial bioactive compounds from microalgae in the food industry is feasible; however, many more specific and in-depth studies are still required in foods for human consumption because of the current underutilization of the microbial bioactive potential of microalgae, at least in the food industry.

Potential use of antioxidant compounds from microalgae in the development of functional foods

The development of functional foods has been a growing topic in recent decades; however, research has focused only on fruits and vegetables as its main source, ignoring other matrices such as marine microalgae, which constitute a rich, innovative and natural source of antioxidants.

As evidenced in the compilation of research on the antioxidant capacity of *Dunaliella salina*, *Tetraselmis chuii* and *Isochrysis galbana*, they present a series of bioactive compounds that include carotenes, carotenoids, chlorophyll *a*, chlorophyll *b*, fucoxanthin, phycocyanin, α-tocopherol, coenzyme Q10, ascorbic acid, and substances that are widely used in the food industry and in the development of functional foods.

These compounds come from matrices that have already been widely studied, such as vegetables and fruits; however, the application of these molecules when they are extracted from marine microalgae is still limited in the food industry.

In addition, research and innovations in functional foods have focused especially on products from the dairy industry, such as yogurts, milk, and dairy drinks, when the antioxidant properties of microalgae are applicable in bakery products, where they not only improve the bioactive characteristics of the product but also improves the technological characteristics of the product. For example, in the study carried out by Nunes et al. (2020), the replacement of gluten in traditional bread was achieved by adding powdered *Isochrysis galbana*, which is used to develop a food that is nonallergenic, innovative and beneficial to human health. Likewise, it could be used in the meat industry since; according to the research, a fish burger was developed with the addition of *Tetraselmis chuii* powder, which has antioxidant properties.

Legislation for microalgae

World legislation

Regarding the legislation on the use of marine microalgae at the macro level, it is known that the incorporation of a species or its extracts in the development of products in the food industry at the European level is regulated by the Regulation on Novel Foods. This regulation applies to those species that have not previously been used as food or as an ingredient in a food product to a significant degree in the countries that are members of the European Union before May 15, 1997; this legislation includes marine microalgae (Lucakova et al. 2022).

When working with marine microalgae and intending to introduce them to the food market, algae must be subjected to authorization procedures so that their safety for human consumption can be guaranteed. Such authorization is required in the Regulation (EC) of Parliament. and European Council No 258/97 (Araújo et al. 2021).

The US Congress, in the Farm Bill, issued and redesigned in 2018, encourages researchers and experts in the area of cultivation and development of marine microalgae to include these microorganisms as agricultural products, meaning that currently, the Department of Agriculture (USDA) supports and allows the agricultural and laboratory cultivation of marine microalgae, in turn encouraging research programs and generating new markets for these species and the products made from them (CAESPA 2018).

The European Union, through Regulation (EU) 2017/2470, maintains an online list, which is updated automatically according to the respective approvals, where a catalog of new foods is displayed. This legislation also applies to microalgae intended to be used as food or as an ingredient in food and includes six microalgae: *Arthrospira platensis* (Cyanobacteria), *Jaagichlorella luteoviridis* (formerly *Chlorella luteoviridis*), *Auxenochlorella pyrenoidosa* (formerly *Chlorella pyrenoidosa*), *Chlorella vulgaris, Chlamydomonas reinhardtii* (Chlorophyta), and *Spirulina* sp. (Cyanobacteria) (Lucakova et al. 2022). As we can see, European legislation does not yet include the microalgae *Dunaliella salina*, *Tetraselmis chuii* and *Isochrysis galbana* on the list of permitted foods and ingredients, so without a doubt, much research is still required regarding their use of these organisms in the food industry, which guarantees and proves their safety for human consumption, relying on clinical, in vitro and in vivo studies.

In Australia and New Zealand, regulations on new food products or their ingredients, which include marine microalgae, are included in the category of foods and new food ingredients regulated in "Standard 1.5.1 Foods" of the Food Standards Code, from Australia and New Zealand. For example, in Japan, the Ministry of Health, Labor, and Welfare is the one that defines the specifications and standards for food additives, where the food additives approved under that context are reported. It is known that in Asian countries, species of the family of *Chlorella* microalgae are authorized for use as food additives: they provide color and flavor and enhance flavor, like the phycocyanins of *Arthrospira* sp., the carotene of *Dunaliella salina*, and the pulverized microalga *Haematococcus lacustris* (formerly *Haematococcus pluvialis*) (Chlorophyta), which is used as a dye (Matos et al. 2019).

Regional legislation

In the Second Territorial Report of the Technological Innovation Surveillance Unit of Argentina, the new bills and research promoted by the Honorable Chamber of Deputies of the Nation and Senators of the Nation were presented regarding the use of algae and microalgae in agricultural areas and with a special focus on their use to produce biodiesel (UVIT, 2016).

Continuing in the context of Argentina, the law regulating the exploitation of seaweed on beaches and the entire provincial territorial sea issued by the Provincial Legislative Power, Law 3273, which sets out regulations to comply with to be complied the extraction, exploitation and use of said specimens due to the 2012 Santa Cruz Provincial Legislative Branch.

In Peruvian Ministerial Resolution No. 211-2009, article 3 authorizes the collection and storage of stranded specimens of algae of the genus *Macrocystis* (sargassum, buoyant or balls*)* and *Lessonia* (aracanto or palo), if the collector abides by the measures. conservation of seaweed species, according to the regulations of the Peruvian Sea Institute of 2009.

Resolution 0942 of the Ministry of Environment and Sustainable Development of Colombia authorizes the use of marine algae and microalgae for research purposes upon request and analysis through an intermediary of an academic institution of higher education only for the period during which this research was carried out according to the Colombian Mining Association.

The Ministry of Economy, Development and Tourism of Chile, in Law 20925, Creates a Bonus for the Repopulation and Cultivation of Algae, with the objective of generating an increase in the existing biomass of algae and microalgae due to its ecological relevance and economy in the national territory for those individuals or associations that carry out activities that allow the recovery of said specimens in the intervention areas.

National legislation

In the Instructions for the General Regulations to Promote and Regulate Organic—Ecological—Biological Production in Ecuador, in the section on cultivation of wild algae, articles 62 to 66, the conditions that allow the cultivation, collection and use of algae and microalgae, in the agricultural field. However, no current regulations or legislation that specifically regulate the use and application of marine microalgae at the local level have been identified.

Conclusions

Microalgae extracts contain many bioactive compounds. Despite there being studies that have evaluated the antimicrobial and antioxidant capacities of the microalgae extracts cited in this review, these studies present research gaps in terms of their synergistic antimicrobial and antioxidant effects of more than one microalga, and they have been analyzed only in the form of extracts from fresh biomass, leaving aside other forms of use.

Additional studies to identify bioactive molecules have not been carried out. A more relevant aspect that lacks studies is the lack of application of both the bioactive properties of marine microalgae as a functional ingredient and as a natural additive in foods for human consumption and in the food industry in general.

Abbreviations

D. salina	Dunaliella salina
T. chui	Tetraselmis chui
I, galbana	Isochrysis galbana
PRISMA	Preferred Reporting Items for Systematic reviews and Meta- Analyses
I. litoralis	Isochrysis litoralis

I. maritima	Isochrysis maritima
S. aureus	Staphylococcus aureus
B. cereus	Bacillus cereus
E. coli	Escherichia coli
S.Typhimurium	Salmonella Typhimurium
GNP	Gold nanoparticles
P. carotovorum	Pectobacterium carotovorum
P. syringae	Pectobacterium syringae
C. parapsilopsis	Candida parapsilosis
K. pneumoniae	Klebsiella pneumoniae
S. epidermidis	Staphylococcus epidermidis
C. albicans	Candida albicans
E. cloacae	Enterobacter cloacae
CCAP	Culture Collection of Algae and Protozoa of the United Kingdom
PEF	Extraction assisted by pulsed electric fields
DMSO	Aqueous solvent dimethyl sulfoxide
TAC	Maximum antioxidant capacity
TPC	Total phenolic compounds
S. enterica	Salmonella enterica
B. subtilis	Bacillus subtilis
E. faecalis	Enterococcus faecalis
P. fluorescence	Pseudomonas fluorescens
V. harveyi	Vibrio harveyi
DHA	Docosahexaenoic Acid
S. epidermidis	Staphylococcus epidermidis
EPA	Eicosapentaenoic acid
L. monocytogenes	Listeria monocytogenes
C. michiganensis	Clavibacter michiganensis
H. lacustris	Hematococcus lacustris
H. pluvialis	Hematococcus pluvialis

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Author contributions

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The authors declare that they have no competing interests.

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References

Admassu H, Gasmalla MAA, Yang R, Zhao W. Bioactive peptides derived from seaweed protein and their health benefits: antihypertensive, antioxidant, and antidiabetic properties. J Food Sci. 2018. https://doi.org/10.1111/1750-3841.14011.

Alsenani F, Tupally KR, Chua ET, Eltanahy E, Alsufyani H, Parekh HS, Schenk PM. Evaluation of microalgae and cyanobacteria as potential sources of antimicrobial compounds. Saudi Pharmaceut J. 2020;28:1834–41. https://doi.org/10.1016/j.jsps.2020.11.010.

Ambrico A, Trupo M, Magarelli R, Balducchi R, Ferraro A, Hristoforou E, Marino T, Musmarra D, Casella P, Molino A. Effectiveness of *Dunaliella salina* extracts against *Bacillus subtilis* and bacterial plant pathogens. Pathogens. 2020;9:1–14. https://doi.org/10.3390/pathogens9080613.

Andriopoulos V, Gkioni MD, Koutra E, Mastropetros SG, Lamari FN, Hatziantoniou S, Kornaros M. Total phenolic content, biomass composition, and antioxidant activity of selected marine Microalgal species with potential as aquaculture feed. Antioxidants. 2022. https://doi.org/10.3390/antiox11071320.

Araújo R, Vázquez Calderón F, Sánchez López J, Azevedo IC, Bruhn A, Fluch S, Garcia Tasende M, Ghaderiardakani F, Ilmjärv T, Laurans M, Mac Monagail M, Mangini S, Peteiro C, Rebours C, Stefansson T, Ullmann J. Current status of the algae production industry in Europe: an emerging sector of the blue bioeconomy. Front Mar Sci. 2021. https://doi.org/10.3389/fmars.2020.626389.

Atitallah A, Hentati F, Dammak M, Hadrich B, Fendri I, Ayadi MA, Michaud P, Abdelkafi S, Barkallah M. Effect of microalgae incorporation on quality characteristics and functional and antioxidant capacities of ready-to-eat fish burgers made from common carp (*Cyprinus carpio*). Appl Sci. 2019. https://doi.org/10.3390/app9091830.

Aziz M, Karboune S. Natural antimicrobial/antioxidant agents in meat and poultry products as well as fruits and vegetables: a review. Crit Rev Food Sci Nutr. 2018;58:486–511. https://doi.org/10.1080/10408398.2016.1194256.

Bahador E, et al. Metabolic responses of the green microalga *Dunaliella salina* to silver nanoparticles-induced oxidative stress in the presence of salicylic acid treatment. Aquatic Toxicol. 2019. https://doi.org/10.1016/j.aquatox.2019.105356.

Barat Baviera M, Antonia Ferrús Pérez M, mina Font Pérez G, Hardisson de la Torre A, He rrera Marteache A, Lorente Toledano F, Marcos Sánchez A, Marti del Moral A, Rosario Martín de Santos M, Rosa Martínez Larrañaga M, Martínez Ló pez A, Martínez de Victoria Muñoz E, Nerín de la Puerta C, Pérez Martínez G, Picó Segura C, María Pintó Solé R, Pla Martínez A, Luis Ríos Ca ñavate J, Salas Salvadó J, Simal Gándara ----Secretario Técnico Vicente Calderón Pascual, J., 2013. Revista del comité científico de la aesan número dieciocho.

Basiratnia E, Einali A, Azizian-Shermeh O, Mollashahi E, Ghasemi A. Biological synthesis of gold nanoparticles from suspensions of green microalga *Dunaliella salina* and their antibacterial potential. Bionanoscience. 2021;11:977–88. https://doi.org/10.1007/s12668-021-00897-4.

Ben H, et al. Antimicrobial, antioxidant, cytotoxic and anticholinesterase activities of water-soluble polysaccharides extracted from microalgae *Isochrysis galbana* and *Nannochloropsis oculata*. J Serbian Chem Soc. 2017. https://doi.org/10.2298/JSC161016036B.

Bonilla-Ahumada FJ, Khandual S, Lugo-Cervantes EC. Microencapsulation of algal biomass (*Tetraselmis chuii*) by spray-drying using different encapsulation materials for better preservation of beta-carotene and antioxidant compounds. Algal Res. 2018;36:229–38. https://doi.org/10.1016/j.algal.2018.10.006.

Bueno M, Vitali C, Sánchez-Martínez JD, Mendiola JA, Cifuentes A, Ibáñez E, Herrero M. Compressed CO_2technologies for the recovery of carotenoid-enriched extracts from *Dunaliella salina* with potential neuroprotective activity. ACS Sustain Chem Eng. 2020;8:11413–23. https://doi.org/10.1021/acssuschemeng.0c03991.

CAESPA. Posibles impactos de la nueva Elaborada por CAESPA 1. 2018.

Chin Chew S, Tan C-H, Phing Pui L, Gunasekaran B. Encapsulation technologies: a tool for functional foods development discovery of dual inhibitor of pde1b and pde10a view project fats and oils chemistry and technology, bio-oil, nanoemulsion and microencapsulation technology view project. Int J Innov Technol Expl Eng. 2019;8(5):154.

Duus Johansen J, Mahler V, Lepoittevin J-P, Frosch PJ. Contact Dermatitis. Cham: Springer International Publishing; 2020.

El-Baz FK, Hussein RA, Mahmoud K, Abdo SM. Cytotoxic activity of carotenoid rich fractions from *Haematococcus pluvialis* and *Dunaliella salina* microalgae and the identification of the phytoconstituents using LC-DAD/ESI-MS. Phytother Res. 2018;32:298–304. https://doi.org/10.1002/ptr.5976.

El-Baz FK, Salama AAA, Hussein RA. *Dunaliella salina* microalgae oppose thioacetamide-induced hepatic fibrosis in rats. Toxicol Rep. 2020;7:36–45. https://doi.org/10.1016/j.toxrep.2019.10.017.

Fajardo P, Alonso M, Farabegoli F, Soula M. Evaluation of the antimicrobial activity of eight microalga species against aquaculture and food pathogens enhance microalgae. Foro Rec Mar Ac Rías Gal. 2020;405:412.

Fang J, Lin A, Yuan X, Chen Y, He W, Huang J, Zhang X, Lin G, Zhang J, Xue T. The complete chloroplast genome of *Isochrysis galbana* and comparison with related haptophyte species. Algal Res. 2020. https://doi.org/10.1016/j.algal.2020.101989.

Farisa MY, Namaskara KE, Yusuf MB. Antibacterial potention of extract of rotifers fed with different microalgae to control *Vibrio harveyi*. IOP Conf Series Earth Environ Sci. 2019. https://doi.org/10.1088/1755-1315/246/1/012058.

Gallego-Cartagena E, Castillo-Ramírez M, Martínez-Burgos W. Effect of stressful conditions on the carotenogenic activity of a Colombian strain of *Dunaliella salina*. Saudi J Biol Sci. 2019;26:1325–30. https://doi.org/10.1016/j.sjbs.2019.07.010.

Garcia-Mosaica C, Del Rio-Garati A. Antimicrobial activity of marine microalgae: *Isochrysis galbana* Isochrysis litoralis and Isochrysis maritima. J Anal Sci Appl Biotechnol. 2022. https://doi.org/10.48402/IMIST.PRSM/jasab-v4i2.39137.

Gokoglu N. Novel natural food preservatives and applications in seafood preservation: a review. J Sci Food Agric. 2019. https://doi.org/10.1002/jsfa.9416.

Grubišić M, Šantek B, Zorić Z, Čošić Z, Vrana I, Gašparović B, Čož-Rakovac R, Šantek MI. Bioprospecting of microalgae isolated from the Adriatic Sea: characterization of biomass, pigment, lipid and fatty acid composition, and antioxidant and antimicrobial activity. Molecules. 2022. https://doi.org/10.3390/molecules27041248.

Guzmán F, Wong G, Román T, Cárdenas C, Alvárez C, Schmitt P, Albericio F, Rojas V. Identification of antimicrobial peptides from the microalgae *Tetraselmis suecica* (Kylin) butcher and bactericidal activity improvement. Mar Drugs. 2019. https://doi.org/10.3390/md17080453.

Iglesias MJ, Soengas R, Probert I, Guilloud E, Gourvil P, Mehiri M, López Y, Cepas V, Gutiérrez-del-Río I, Redondo-Blanco S, Villar CJ, Lombó F, Soto S, Ortiz FL. NMR characterization and evaluation of antibacterial and antiobiofilm activity of organic extracts from stationary phase batch cultures of five marine microalgae (*Dunaliella sp D salina Chaetoceros calcitrans C gracilis* and *Tisochrysis lutea*). Phytochemistry. 2019. https://doi.org/10.1016/j.phytochem.2019.05.001.

Jafari S, Mobasher MA, Najafipour S, Ghasemi Y, Mohkam M, Ebrahimi MA, Mobasher N. Antibacterial potential of *Chlorella vulgaris* and *Dunaliella salina* extracts against Streptococcus mutans. Jundishapur J Nat Pharm Prod. 2018. https://doi.org/10.5812/jjnpp.13226.

Khatoon H, Haris H, Rahman NA, Zakaria MN, Begum H, Mian S. Growth, proximate composition and pigment production of *Tetraselmis chuii* cultured with aquaculture wastewater. J Ocean Univ China. 2018;17:641–6. https://doi.org/10.1007/s11802-018-3428-7.

Kitchenham, B. Procedures for performing systematic reviews. 2004.

Kokkali M, Martí-Quijal FJ, Taroncher M, Ruiz MJ, Kousoulaki K, Barba FJ. Improved extraction efficiency of antioxidant bioactive compounds from *Tetraselmis chuii* and *Phaedoactylum tricornutum* using pulsed electric fields. Molecules. 2020. https://doi.org/10.3390/molecules25173921.

Lucakova S, Branyikova I, Hayes M. Microalgal proteins and Bioactives for food, feed, and other applications. Appl Sci. 2022. https://doi.org/10.3390/app12094402.

Ludwig K, Rihko-Struckmann L, Brinitzer G, Unkelbach G, Sundmacher K. β-Carotene extraction from *Dunaliella salina* by supercritical CO_2. J Appl Phycol. 2020. https://doi.org/10.1007/s10811-021-02399-y/Published.

Maligan J, V Widayanti, Elok Zubaidah. "Production and identification of antimicrobial compounds from marine microalgae *Tetraselmis chuii* using ultrasound assisted extraction method." Proceedings of the 4th International Conference On Food Engineering And Biotechnology (ICFEB 2013), Copenhagen, Denmark. 2013.

Matos AP. Microalgae as a potential source of proteins in proteins sustainable source processing and applications. Amsterdam: Elsevier; 2019.

Matos J, Cardoso C, Gomes A, Campos AM, Falé P, Afonso C, Bandarra NM. Bioprospection of: *Isochrysis galbana* and its potential as a nutraceutical. Food Funct. 2019;10:7333–42. https://doi.org/10.1039/c9fo01364d.

Medina Pérez E, Ruiz Domínguez M, Morales-Espinoza J, Cerezal-Mezquita P. evaluación del perfil de ácidos grasos de *Isochrysis Galbana* Mediante El Uso De Métodos Ácidos Y Alcalinos De Transesterificación. Informador Técnico. 2019. https://doi.org/10.23850/225650351574.

Montoya Vallejo C, Acosta Cárdenas A. Crecimiento de Tetraselmis sp Empleando fertilizante como medio de cultivo. Revista. 2021. https://doi.org/10.18273/revion.v34n2-2021005.

Mousavi Nadushan R, Hosseinzade I. Optimization of production and antioxidant activity of fucoxanthin from marine haptophyte algae Isochrysis galbana. Iran J Fish Sci. 2020. https://doi.org/10.22092/ijfs.2020.122837.

Nataraj BH, Ali SA, Behare PV, Yadav H. Postbiotics-parabiotics: the new horizons in microbial biotherapy and functional foods. Microb Cell Fact. 2020. https://doi.org/10.1186/s12934-020-01426-w.

Nejadmansouri M, Golmakani M-T, Famouri M. Comparison of different methods for carotenoid extraction from *Dunaliella Salina* Carotenoid *Dunaliella salina* Extraction Microwave Ultrasound. Int J Nutr Sci. 2021. https://doi.org/10.30476/IJNS.2021.93230.1162.

Nunes MC, Fernandes I, Vasco I, Sousa I, Raymundo A. *Tetraselmis chuii* as a sustainable and healthy ingredient to produce gluten-free bread: impact on structure, colour and bioactivity. 2020. Foods. https://doi.org/10.3390/foods9050579.

Okoli C, Schabram K. Information systems a guide to conducting a systematic literature review of information systems research a guide to conducting a systematic literature review of information systems research. SSRN Elect J. 2010. https://doi.org/10.2139/ssrn.1954824.

Putra Y, Mustikasari I, Pangestuti R, Rahmadi P, Siahaan EA. Fatty acid profiles and biological activity of *Nannochloropsis oculata* and *Isochrysis galbana*, clone t-ISO. IOP Conf Series Earth Environ Sci. 2022. https://doi.org/10.1088/1755-1315/1083/1/012079.

Ramos LC, Batista R, Carneiro DC, Sousa LJ, Sales AN, Lima STC. Evaluation of electroflocculation harvesting on the antioxidant activity and toxicity of extracts from the microalgae *Isochrysis galbana* and *Phaeodactylum tricornutum*. J Appl Phycol. 2020;32:3853–9. https://doi.org/10.1007/s10811-020-02265-3.

Rathod NB, Ranveer RC, Benjakul S, Kim SK, Pagarkar AU, Patange S, Ozogul F. Recent developments of natural antimicrobials and antioxidants on fish and fishery food products. Compr Rev Food Sci Food Saf. 2021. https://doi.org/10.1111/1541-4337.12787.

Roy Uttam K, Nielsen BV, Milledge JJ. Antioxidant production in *Dunaliella*. Appl Sci. 2021. https://doi.org/10.3390/app11093959.

Ruiz-Domínguez MC, Cerezal P, Salinas F, Medina E, Renato-Castro G. Application of box-behnken design and desirability function for green prospection of bioactive compounds from *Isochrysis galbana*. Appl Sci. 2020. https://doi.org/10.3390/APP10082789.

Sacristán de Alva M, Luna Pabello VM, Orta Ledesma MT, Cruz Gómez MJ. Carbon, nitrogen, and phosphorus removal, and lipid production by three saline microalgae grown in synthetic wastewater irradiated with different photon fluxes. Algal Res. 2018. https://doi.org/10.1016/j.algal.2018.07.006.

Salim M, Dharma A, Wahyulya Putri A. Study and characterization growth of four microalgae species and test antimicrobial activity. Jurnal Zarah. 2018;6(2):53.

Shantkriti S, Pradeep M, Unish KK, Viji Das MS, Nidhin S, Gugan K, Murugan A. Bioynthesis of silver nanoparticles using *Dunaliella salina* and its antibacterial applications. Appl Surface Sci Adv. 2023. https://doi.org/10.1016/j.apsadv.2023.100377.

Tavakoli S, Regenstein JM, Daneshvar E, Bhatnagar A, Luo Y, Hong H. Recent advances in the application of microalgae and its derivatives for preservation, quality improvement, and shelf-life extension of seafood. Crit Rev Food Sci Nutr. 2022. https://doi.org/10.1080/10408398.2021.1895065.

Tirado DF, Calvo L. The Hansen theory to choose the best cosolvent for supercritical CO_2 extraction of Β-carotene from *Dunaliella salina*. J Supercrit Fluids. 2019;145:211–8. https://doi.org/10.1016/j.supflu.2018.12.013.

UVIT. Segundo Informe Territorial de la Unidad De Vigilancia de Innovaciones Tecnológicas de Argentina. 2016.

Xia E, et al. Optimization and identification of antioxidant peptide from underutilized *Dunaliella salina* protein: extraction, in vitro gastrointestinal

digestion, and fractionation. BioMed Res Int. 2019. https://doi.org/10.1155/2019/6424651.
Xu Y, Harvey PJ. Carotenoid production by *Dunaliella salina* under red light. Antioxidants. 2019;8(5):123. https://doi.org/10.3390/antiox8050123.
Yusof NS, Yeong YS, Zakeri HA, Wahid MEA, Ghafar SNA, Yusuf N. Photoperiod influenced the growth and antioxidative responses of *Chlorella vulgaris*, *Isochrysis galbana*, and *Tetraselmis chuii*. J Appl Pharm Sci. 2021;11:125–34. https://doi.org/10.7324/JAPS.2021.110415.

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