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Unlocking antimicrobial potential of microalgae on food-borne bacteria: A standardized framework and future directions

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ABSTRACT

Foodborne infections are a global challenge, costing billions annually through food losses, trade restrictions, and healthcare expenses. Growing concerns over chemical antimicrobials such as antibiotics, sanitizers, and disinfectants, have driven interest in sustainable bio-control strategies for food systems. Microalgae, which produce a plethora of biomolecules including carbohydrates, lipids, proteins, and various secondary metabolites, represent a promising source of antimicrobial compounds. Despite numerous reports demonstrating antimicrobial activity in microalgal extracts, no microalgae-derived antimicrobials have yet reached commercialization.

This review focuses on some microalgal species already produced at commercial scale, including those with GRAS status (e.g., *Chlorella vulgaris* and *Chlamydomonas reinhardtii*). As for other microalgae-based products (e.g., biofuel oil), successful antimicrobial production depends on identifying key species and strains, optimizing growth conditions, and refining harvesting, cell disruption, and extraction protocols. Although research in this area is expanding, further studies are needed to improve our understanding of antimicrobials synthesis and to assess how these factors influence antimicrobial activity. Commonly used antibacterial assays such as disc diffusion and microdilution have limitations that must be considered when evaluating the antimicrobial activity of microalgal extracts. Overall, inconsistencies in testing and reporting have hindered the clear identification of microalgae as sources of effective antimicrobials. This review proposes a framework for future extract preparation and antimicrobial assessment and discusses future prospects to enhance the discovery and yield of microalgal antimicrobials.

Introduction

Food-borne infections result in 33 million disability-adjusted life years (DALYs) globally (WHO, 2016), and to an annual loss in productivity and medical expenses estimated at US $110 billion in low and middle-income economies (Jaffee et al., 2019). Among the most frequent causes of gastrointestinal illnesses are the Gram-negative bacteria *Salmonella* sp. and *Campylobacter* sp., which may lead to long-term health complications like reactive arthritis and irritable bowel syndrome, and pathogenic *Escherichia coli* infections, which can cause haemolytic uremic syndrome and acute kidney failure (WHO, 2018; WHO, 2020; Spinale et al., 2013). *Listeria monocytogenes*, although less common cause of illness, is among the deadliest food-borne pathogens, particularly for pregnant women, newborns, and immunocompromised individuals (Pogreba-Brown et al., 2022). Other identified food biological hazards are the Gram-positive bacteria *Bacillus cereus*; *Staphylococcus aureus*, *Clostridium botulinum* and *Clostridium perfringens*, as well as fungi such as *Aspergillus*, *Candida and Fusarium,* which cause food-borne illnesses through the production of entero- or mycotoxins (Benedict et al., 2016; Rajkovic et al., 2020). Controlling these pathogens is essential to mitigate the impact of food-borne infections on society.

In recent years, restrictions on the use of chemical antimicrobials, including antibiotics, sanitizers and disinfectants, in primary food production and food processing have been recommended or implemented in many countries (WHO, 2017; U.S. Department of Health and Human Services, 2021; European Parliament and the Council of the European Union, 2019) fueling the need for alternative bio-control strategies in

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food production systems.

Significant research has focused on antimicrobials from plants with more than 18,000 paper published in the last decade (Eloff, 2019). A Scopus search using the same keywords as Eloff (Eloff, 2019) but replacing "Plants" by "microalgae" (i.e. microalgae AND antimicrobial OR antibiotic OR antibacterial) evidenced a similar trend for microalgae with ~60 % of the total number of papers published in the last five years (Supplementary information S1). With an estimated 30,000 to 70,000 species, microalgae represent a significant natural reservoir of nutritional and bioactive elements, many of which exhibit health-beneficial and pharmacological properties (Borowitzka, 2013). A small number of microalgae species have been granted a Generally Recognized As Safe (GRAS) number or status. These microalgae effectively inhibit spoilage bacteria and extend the shelf life of minced meat, ice cream, milk and fermented products (Farag et al., 2025; Marrez et al., 2025; Beheshtipour et al., 2012; Csatlos et al., 2023). Microalgae-derived coatings can extend the shelf life of fruits and vegetables (de Oliveira et al., 2024), providing evidence for the potential of microalgae as effective biocontrols agents with a range of application systems. However, the studies that have compared a large number of microalgae strains or species have demonstrated significant variability in antimicrobial activity among and within species (see Table 1 for examples), with between 5 % and 91 % of the tested strains having antimicrobial activity against at least one pathogenic bacterial or fungal strain (Stirk and van Staden, 2022). Notably, there is a systematic lack of consistency in results among studies investigating the bioactivities of the same species (Aremu et al., 2014). Altogether, these illustrate the challenges in identifying key strains, growth conditions, and extraction protocols for broadening the obtention and use of microalgae bioactives with antimicrobial properties in the food industries.

The review focused specifically on the use of GRAS microalgae species already produced at commercial scale (e.g. *Chlorella vulgaris* and *Chlamydomonas reinhardtii*). In order to provide a theoretical and technical guidance for optimizing the yield and discovery of antimicrobial bioactives from microalgae, the published studies where antimicrobial activities were tested and reported were critically analyzed. In addition, this review outlines a set of recommendations applicable to all microalgae taxa for future research exploiting microalgae as sources of antimicrobial compounds.

Antimicrobial potential and testing strategies of microalgal extracts

General considerations on testing and effectiveness of antimicrobial activities from microalgae

There are many techniques for testing antimicrobial susceptibility (see (Balouiri et al., 2016) and these have been widely applied to test the antimicrobial activity of plants or microalgal extracts. The activities of microalgae against important microbial pathogens have generally been assessed from crude extracts or metabolite using agar diffusion susceptibility tests and macro- and microdilution tests (Box 1). "Crude extract" (also referred as "extract") is defined as the unrefined mixture of compounds obtained from the initial extraction process of algal biomass, while metabolites refer to the specific chemicals obtained through additional purification steps and identified by chromatography.

Using these methods, microalgae extracts demonstrated an efficacy generally lower than those of antibiotics currently commercialized for human therapeutic treatments (Table 1). However, microalgae crude extracts have been shown to enhance the antibacterial effects of these antibiotics by a factor of two to four-fold, even when added in small amount (Zaharieva et al., 2022).

Interestingly, the minimal bactericidal concentration (MBC), which is the minimum concentration of extract that kills 99 % of the bacteria, and can be used to assess whether the effect of the extract is bacteriostatic or bactericidal, has seldom been reported (Dantas et al., 2015; Plaza et al., 2010; Santhakumaran et al., 2020).

Applicability of current antibacterial activity assays to test microalgal crude extracts

Eloff (Eloff, 2019) provides an excellent background on the principles and pitfalls of existing antimicrobial activity tests when assessing plant extracts activities. These limitations apply to the testing of microalgae extracts and include the concentration of bioactives in the extracts, diffusion, loss, or precipitation of extract components during the assays, and the possibility of synergistic or antagonistic effects when testing crude extracts.

Najdenski (Najdenski et al., 2013) tested the same extracts by using the disc diffusion assays and the microdilution broth. They found that most of the tested extracts had a high activity when testing in liquid media, with MIC values as low as 0.125 $mg.mL^{-1}$, while they did not show zones of inhibition or gave small inhibitory zones in the agar diffusion assays, even when tested at high concentrations. Possible reasons for the difference between the two assays include low diffusion potential of non-polar active compounds (lipophilic compounds) through the aqueous agar matrix compared to polar compounds, or the degradation or loss by evaporation of volatile compounds during the disc diffusion assay (Stirk and van Staden, 2022; Shaima et al., 2022). Shaima (Shaima et al., 2022) indicated that the disc-diffusion assay, where most of the microalgae extracts diffuse from the surface of the agar, offers better inhibition ability compared to the well diffusion method, confirming the importance of the diffusion of the bioactives into the agar to detect activities. Other studies investigated the effect of extract concentration of the formation of inhibition halos in disc diffusion assays (Schuelter et al., 2019; Tavakoli et al., 2021). They reported that antimicrobial activities increased with increase in extract concentration or mass, although this increase in activity was not linear and tended to stabilize beyond an upper limit for extract concentration (Schuelter et al., 2019).

When using the broth dilution methods, extracts with MIC < 1 $mg.mL^{-1}$ have been considered to be of interest by many authors (Stirk and van Staden, 2022). However, most of microalgal extracts or compounds MIC are between 1 – 8 $mg.mL^{-1}$. Using MIC < 1 $mg.mL^{-1}$ as cut-off point for extracts containing multiple compounds with potential synergistic and antagonistic effects may result in biased and misleading results, risking targeting extracts with large quantities of moderately active compounds or discarding extract that showed poor results due to either the presence in low concentration of the active compound, or due to the presence of antagonists. Caesar (Caesar et al., 2018) have shown that the presence of antagonistic compounds in a plant extract could decrease the potency of a known spiked antimicrobial against *Staphylococcus aureus* by 70 %. Consequently, synergistic and antagonistic effects of bioactives compounds cannot be understated. The authors recommend the excellent discussion of (Caesar and Cech, 2019) on synergy and antagonism in natural products extracts.

Critically, it is essential to consider the limitations of each antimicrobial assay when testing microalgal extracts. Equally important is addressing the lack of consistency in reported data across studies, for example, reporting data on the influence of negative controls on bacterial inhibition and details on the origin of microalgal and bacterial isolates (Table 1). Without comprehensive and transparent reporting, repeatability is compromised, reducing the likelihood of successfully identifying and isolating microalgae-derived antimicrobials. Therefore, rigorous reporting of all experimental conditions and results is imperative to advance discovery efforts (further discussed in Section "Future prospects").

Table 1
Studies reporting antimicrobial activity of crude extracts from relevant microalgae. This table highlights a selection of published work to illustrate the diversity of approaches and data currently available.

Microalgae species	Microbial target	Activity of microalgae extract			Reference
		Zone of Inhibition (ZOI, in mm), obtained from disc or well diffusion assays	Minimum Inhibitory Concentration (MIC, in mg/mL), determined by macro- and micro-broth dilution methods	Negative control	
Chlorella vulgaris	*Bacillus Subtilis* ATCC 6633	25.6–27.2 mm (50 μL/well); smaller than ampicillin (40 μL/well: 30.0 mm)	1.0 mg/mL, MIC Ampicillin was not reported	No zone of inhibition reported for acetone, methanol and diethyl ether used to resuspend the extracts. Notreported for the broth dilution assays	(Alwathnani and Perveen, 2017)
	Escherichia coli ATCC 25922	28.5 – 28.6 mm (50 μL/well); smaller than ampicillin (40 μL/well: 31.8 mm)	0.6 mg/mL; MIC Ampicillin was not reported		
	Pseudomonas aeruginosa ATCC 27853	No inhibition, while for ampicillin (40 μL/well: 22–30 mm)	Not tested		
	Staphylococcus aureus ATCC 25928				
	Staphylococcus pyogenes grp A	17.4 – 19.1 mm (50 μL/well); smaller than for ampicillin (40 μL/well: 22.3 mm)	0.6 mg/mL; MIC Ampicillin was not reported		
Chlorella sorokiniana (UKM2), *Chlorella sp.*(UKM8) and *Scenedesmus* sp. (UKM9) local isolates	*Bacillus subtilis* ATCC 11774	8.0–13.8 mm (200 mg); smaller than Vancomycin (30 μg) or Gentamycin (10 μg): 14.0–21.0 mm	0.312 to 6.25 mg/mL. No result reported for the control Gentamicin (10 mg)	6 mm inhibition by 5 % Tween20 and 10 % DMSO in the disc diffusion assay	(Shaima et al., 2022)
	Enterobacter faecalis ATCC 14506				
	Escherichia coli ATCC 10536				
	Methicillin resistant Staphylococcus aureus ATCC 43300				
	Pseudomonas. Aeruginosa ATCC 10145				
	Serratia marcescens UKMCC0014				
Scenedesmus obliquus PACC 8610	*Escherichia coli* ATCC 35218	ND	12.5 mg/L; less effective than ciprofloxacin (0.0125 mg/L) or Enrofloxacin (0.0125 mg/L)	EtOH but no results reported	(Zaharieva et al., 2022)
	Salmonella typhimurium 123 (Collection of the Stephan Angeloff Institute of Microbiology)	ND	>12.5 mg/L; less effective than Ciprofloxacin (0.05 mg/L) or Enrofloxacin (0.05 mg/L)		
	Staphylococcus aureus ATCC 29213	ND	12.5 mg/L; less effective than Penicillin (0.125 mg/L), Ciprofloxacin (0.25 mg/L), or Enrofloxacin (0.05 mg/L)		
Scenedesmus obliquus GUBI-OTJT116	*Bacillus subtillis*	12–25 mm; smaller than the control (piperacillin + tazobactam: 35 mm) ZOI was similar to the controls when using extracts in concentration 6 to 100 times greater than the controls	MIC of the crude microalgae extracts were 3 to 25-fold greater than the controls (piperacillin + tazobactam) at the same concentration	No reported	(Mukherjee et al., 2024)
	Enterobacter aerogenes	10–20 mm; smaller than the control (piperacillin + tazobactam: 30 mm)			
	Escherichia coli	11–25 mm; smaller than the control (piperacillin + tazobactam: 25 mm)			
	Klebsiella pneumoniae	10–29 mm; typically smaller than the control (piperacillin + tazobactam: 30 mm)			
	Micrococcus luteus	15–23 mm; smaller than the control (piperacillin + tazobactam: 35 mm)			

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Table 1 (*continued*)

Microalgae species	Microbial target	Activity of microalgae extract			Reference
		Zone of Inhibition (ZOI, in mm), obtained from disc or well diffusion assays	Minimum Inhibitory Concentration (MIC, in mg/mL), determined by macro- and micro-broth dilution methods	Negative control	
	Pseudomonas aeruginosa	10–27 mm; smaller than the control (piperacillin + tazobactam: 30 mm)			
	Staphylococcus aureus	13–30 mm; smaller than the control (piperacillin + tazobactam: 40 mm)			
Scenedesmus sp. MACC-411	*Candida albicans* ATCC 10231	ND	0.156–2.50 mg/mL; less effective than Amphotericin B (0.005 mg/mL)	Water, 50 % DMSO and Mueller Hinton broth, but results not reported	(Aremu et al., 2014)
	Enterococcus Faecalis ATCC 19433	ND	0.078–0.625 mg/mL; more effective than Neomycin (0.625 mg/mL)		
	Escherochia Coli ATCC 11775	ND	0.156–1.250 mg/mL; less effective than Neomycin (0.004 mg/mL)		
	Pseudomonas aeruginosa ATCC 11775	ND	0.078–2.50 mg/mL; less effective than Neomycin (0.03 mg/mL)		
	Staphylococcus Aureus ATCC 12600	ND	0.208–2.5 mg/mL; less effective than Neomycin (0.002 mg/mL)		
Scenedesmus quadricauda (Turpin) Brébisson	*Aeromonas hydrophila* BIOTECH 10089	ND	No activity observed	Methanol, but results not reported	(Arguelles, 2018)
	Enterococcus aerogenes BIOTECH 1145		No activity observed		
	Escherichia coli BIOTECH 1825		No activity observed		
	Listeria monocytogenes BIOTECH 1958		1 mg/mL,control TET was not reported		
	Pseudomonas aeruginosa BIOTECH 1824		0.25 mg/mL,control TET was not reported		
	Salmonella. typhimurium BIOTECH 1826		No activity observed		
	Staphylococcus aureus BIOTECH 1823		0.25 mg/mL,control TET was not reported		
Chlamydomonas reinhardtii (isolated from paddy fields soil)	*Bacillus subtilis* PTCC 1023	8.0–11.0 mm (unspecified concentration); smaller than for Ampicillin (10 μg: 14.0–20.0 mm) or Gentamincin (10 μg: 10.0–16.0 mm)		Not reported	(Ghasemi et al., 2007)
	Escherichia coli PTCC 1114				
	Pseudomonas aeruginosa PTCC 1074				
	Staphylococcus aureus PTCC 1112				
	Staphylococcus epidermis PTCC 1114				
	Salmonella typhimurium PTCC 1609				
Chlamydomonas mexicana NIES-2218	*Mycobacterium tuberculosis* ATCC 27294		0.1 mg/mL; less effective than Thanisoniazid (<0.01 mg/mL)	Low inhibition with 1 % DMSO	(Ruiz-Güereca et al., 2019)
Chlamydomonas sp. isolates collected near abandoned mines (13 strains) and *C. reinhardtii* CPCC11 *Coccomyxa* sp. *Scenedesmus* sp. *S. dimorphus* UTEX1237	*Bacillus subtilis* ATCC 6051	5.0 – 15.0 mm (50 mg/mL); smaller than for Ampicillin (10 μg: 23.0 mm) and Streptomycin (10 μg: 17.0 mm).	ND	1 % DMSO but results not reported	(Senhorinho et al., 2018)
	Staphylococcus aureus ATCC 25923	0 – 9.3 mm (50 mg/mL); smaller than for Ampicillin (10 μg: 27 mm) and Streptomycin (10 μg: 18 mm).	0.02 – >1.024 mg/mL; less effective than Ampicillin (0.002 mg/mL)		

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Table 1 (*continued*)

Microalgae species	Microbial target	Activity of microalgae extract			Reference
		Zone of Inhibition (ZOI, in mm), obtained from disc or well diffusion assays	Minimum Inhibitory Concentration (MIC, in mg/mL), determined by macro- and micro-broth dilution methods	Negative control	
P. malhamensis	*Escherichia coli*	>14.0 mm (15 mg/mL); similar to Gentamicin (10 µg).	1.56 mg extract/mL	No zone of inhibition reported for water	(Schuelter et al., 2019)
	Salmonella typhimurium	10.9 mm; smaller to Gentamicin (10 µg)	6.25 mg/mL		
	Staphylococcus. aureus	5.4 mm; smaller to Gentamicin (10 µg)	12.5 mg/mL		
C. vulgaris and C. reinhardtii (Algal Culture Collection, Center for Advanced Studies in Botany, University of Madras)	*Bacillus subtilis* MTCC-441	11 – 12 mm (100 µg/mL); smaller than Streptomycin (100 µg/mL: 14.8 mm)		DMSO, but no results reported	(Jayshree et al., 2016)
	Escherichia coli MTCC-1687	14 – 15 mm (100 µg/mL); similar to Streptomycin (100 µg/mL: 14.8 mm)			
	Pseudomonas vulgaris MTCC-742	14.8 – 14.5 mm (100 µg/mL); similar to Streptomycin (100 µg/mL: 18.0 mm)			
	Pseudomonas aeruginosa MTCC-1688	17.0 – 18.0 mm (100 µg/mL); smaller than Streptomycin (100 µg/mL: 20.0 mm)			
	Staphylococcus aureus MTCC-96	0.0 – 13.0 mm, (100 µg/mL); smaller than Streptomycin (100 µg/mL: 14.0 mm)			
Chlamydomonas sp. YB-204	*Bacillus subtilis* SCTCC 100034	no activity (50 mg/mL)			(Sun et al., 2017)
	Escherichia coli SCTCC 100005	weak activity reported (50 mg/mL), no ZOI provided			
	Pseudomonas aeruginosa SCTCC 100200	16.4 mm (50 mg/mL)			
	Salmonella typhimurium SCTCC 100403	no activity (50 mg/mL)			
	Streptomyces albus SCTCC 100044	weak activity reported (50 mg/mL), no ZOI provided			
	Staphylococcus aureus SCTCC 100048	weak activity (50 mg/mL), no ZOI provided			
C. reinhardtii *(Ethanol extraction)*	*Bacillus subtilis* KCTC 1021	no activity (100 µL); Ampicillin and Kanamycin (concentration not specified)		DMSO, no activity reported	(Bashir et al., 2018)
	Escherichia coli KCTC 1116	no activity (100 µL)			
	Micrococcus luteus KCTC 1071	no activity (100 µL)			
	Pseudomonas aeruginosa KCTC 2004	15.0 mm (100 µL)			
	Salmonella enterica KCTC 2514	no activity (100 µL)			
	Staphylococcus aureus RN 4220	17.0 mm (100 µL)			
	Streptococcus iniae FP 5228	16.0 mm (100 µL)			
	Vibrio parahaemolyticus KCTC 2471	no activity or <13 mm (100 µL)			
C. reinhardtii CC-124	*Klebsiella pneumoniae*	21.5 mm (3.2 mg)	MIC50: 0.85 mg/mL	Not reported	(Vishwakarma et al., 2022)
	Serratia marcescens	19.2 mm (3.2 mg)	MIC50: 0.80 mg/mL		
Chlamydomonas sp. isolated from wells	*Escherichia coli* MTCC 1302	17.5 mm (concentration not specified); smaller than Chloramphenicol (concentration not specified: 21 mm)		DMSO, results not reported	(Guleria et al., 2024)
	Listeria monocytogenes MTCC 839	17.5 mm; smaller than Chloramphenicol (20 mm)			
	Salmonella typhimurium MTCC 1254	17.5 mm; smaller than Chloramphenicol (20 mm)			
	Shigella dysenteria MTCC 5151	17.5 mm; smaller than Chloramphenicol (20 mm)			

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Table 1 (*continued*)

Microalgae species	Microbial target	Activity of microalgae extract			Reference
		Zone of Inhibition (ZOI, in mm), obtained from disc or well diffusion assays	Minimum Inhibitory Concentration (MIC, in mg/mL), determined by macro- and micro-broth dilution methods	Negative control	
	Staphylococcus aureus MTCC 3160	17.5 mm; smaller than Chloramphenicol (21 mm)			
	Streptococcus lactis MTCC 5461	17.5 mm; smaller than Chloramphenicol (21 mm)			

ND: not done.

Box 1

Antimicrobial activity testing.

In the agar diffusion methods, microalgal extracts have been applied either on an absorbent filter paper disc (disc diffusion method) or dispensed into a hole punched into the agar (agar well method) to agar plates inoculated with the target microorganism. The antimicrobial activity is determined by measuring the diameter of the inhibition zone of bacterial growth around the extracts. Results can be easily compared to recognized international standards provided by EUCAST (EUropean Committee on Antimicrobial Susceptibility Testing, (European Committee on Antimicrobial Susceptibility Testing, 2025) or CLSI (Clinical & Laboratory Standards Institute) to evaluate their clinical relevance; Epidemiological Cutoff Value (ECOFF) can also be used to evaluate epidemiological relevance (Kronvall and Smith, 2016).

Another widely used method is the broth dilution method (referred to as macrodilution), which involves diluting microalgal extracts in a liquid growth medium containing the target microorganisms. This method allows for the determination of the Minimum Inhibitory Concentration (MIC), which is the lowest concentration of the extract that inhibits microbial growth in comparison with a known inhibitor, generally an antibiotic. A variation of the broth dilution method is the microdilution method, which is performed in microtiter plates, enabling high-throughput screening of multiple samples. The results of the microdilution method are often quantified using spectrophotometric measurements.

Production of antimicrobials by microalgae in cultivation

Influence of microalgal growth stage

Microalgal growth is characterized by a lag phase, an exponential growth phase, a stationary phase and a death phase. Each of these phases is characterised by the production of different metabolites, with the primary metabolites produced for microalgal cell development and growth first, and the secondary metabolites associated with cell survival, competition, or environmental defense (Sreenikethanam et al., 2022). The phytochemical contents of the microalgae crude extracts have been characterized by some authors, and the antimicrobial activities of green microalgae have been associated with the presence of several classes of metabolites, including fatty acids, plant-derived phenolics compounds, polysaccharides and carotenoids (Table 2).

Testing the inhibitory activities of freshwater microalgae against food-borne bacteria has been carried out using microalgae cultures in the exponential (Corona et al., 2017), the late exponential (Arguelles, 2018), or the stationary growth phase (Schuelter et al., 2019; Ördög et al., 2004; Cannell et al., 1988). For *Cyanobacteria* and *Chlorophyta* extracts, the best activities (defined as inhibitory activities observed with extract concentration < 1.0 mg/mL) against Gram-positive bacteria, Gram-negative bacteria, or fungus were obtained when the microalgae cultures were harvested between mid-exponential and early stationary phases, with differences reported among studies (Stirk and van Staden, 2022). Within the same microalgae species, some strains showed better growth inhibition of bacteria when harvested in the mid-exponential stage of growth, while other strains were more inhibitory in late exponential growth stage (Aremu et al., 2014). Variations in antimicrobial activities of freshwater microalgae were also observed during the stationary phase, which can last for at least 6 days (Schuelter et al., 2019). Meaningful comparison of activities between different growth stage could be confounded by biomass yield. Accurate reporting of the microalgae growth curve, growth rate over time and biomass is therefore critical to inform on optimal harvesting point associated with the greatest antimicrobial activities and yield. It would also facilitate cross-studies comparisons among species or within species grown under different conditions.

Influence of abiotic stresses

Manipulating cultivation conditions is a common strategy to promote the production of microalgal high-value bioactives. Abiotic stress conditions have been more widely examined than the response to biotic stresses (Rinaldi et al., 2024; Paliwal et al., 2017). Among abiotic stresses, salinity, temperature, pH variation, oxidation, light intensity and nutrient depletion, influence microalgal metabolism (Ördög et al., 2004; Cakmak et al., 2012; Mao et al., 2020; Plouviez et al., 2021). This section focused on light supplementation and nutritional stresses on the production of antimicrobial bioactives, as these conditions have typically been the most studied and shown to generate pronounced biochemical changes.

Light supplementation

The amount of light microalgal cells receive influences growth rate and biomass composition (Čermák et al., 2015; Vendruscolo et al., 2019). Light intensity and wavelength affect photosynthesis, modifying cellular metabolism and shifting metabolism from primary to secondary (Lehmuskero et al., 2018).

Studies that examined the effect of different light cultivation conditions on the production of bioactives against pathogenic bacteria have reported that green light appears to promote the production of antimicrobials. For example, Schuelter (Schuelter et al., 2019) tested different types of light sources to grow microalgae species isolated from continental freshwater. Compared to white, yellow, red or blue lights, the use of green light emitting diode bulbs resulted in the microalgal extracts

Table 2
Representative studies reporting metabolites attributed to antimicrobial activities in freshwater microalgae.

Class of metabolites	Identified Antimicrobial compounds	Separation and/or identification of antimicrobial compounds	Determination of antimicrobial activities	Reference
Saturated fatty acids (SFA)	Palmitic acid	FAME extract; SFA identified by Gas Chromatography (GC)	Microdilution Most active SFA inferred from its high proportion (>23 %) in the FAME extract	(Davoodbasha et al., 2018)
Saturated fatty acids (SFA)	Capric acid	Identification by GC	Extracts tested by Disc diffusion assays	(Ruffell et al., 2016)
	Erucic acid			
	Linolenic acid		Commercially acquired fatty acids individually tested to confirm their activity	
	Arachidonic acid			
	Palmitoleic acid			
Monosaturated fatty acids (MUFA)	Myristoleic acid			
	Palmitoleic acid			
	Erucic acid			
Polyunsaturated fatty acids (PUFA)	Linoleic acid			
	13–16 Docosadienoic acid			
	Arachidonic acid			
	Gamma-linolenic acid			
omega − 3 fatty acid	Docosahexaenoic acid			
SFA	Palmitic acid	nd	Microdilution	(Mukherjee et al., 2024)
MUFA	Oleic acid			
PUFA	Linoleic acid		Linear regressions of the antimicrobial activities in diluted extracts were used to test whether the most concentrated metabolites present in the extract confer the activity	
PUFA	Linolenic acids			
Aromatic alcohol	phenols	Concentration of total phenolic compounds in the extract determined by Folin-Ciocalieu reagents	Disc diffusion assays Largest zone of inhibitions observed for extracts containing higher levels of polyphenols	(Tavakoli et al., 2021)
Aromatic alcohol	Phenol	Identification by GC–MS	Well and agar diffusion	(Shaima et al., 2022)
SFA	Hexadecanoic acid			
diterpenoid alcohol	Phytol		Activity attributed to the most abundant compounds found in GC–MS peaks and previously described to possess antimicrobial activity	
bicyclic alkane	Bicyclo[3.1.1]heptane			
PUFA	Linoleic acid			
Sulphated polysaccharides	nd	Dried crude extract semi-purified using anion exchange column	Disc diffusion assays and microdilutions Fractions enriched with carbohydrate (70 %) and sulphate (30 %) tested for antimicrobial activities	(Vishwakarma and Vavilala, 2019)
SFA	Palmitic acid	Crude extracts fractionated using hexane, dichloromethane and methanol	Disc diffusion assays and microdilutions	(Alsenani et al., 2020)
SFA	Hexadecanoic acid			
SFA	Octadecanoic acid		Each fraction subjected to antimicrobial assays	
MUFA	Oleic acid	Each fraction analysed by UHPLC, Maldi TOF-MS and CG-MS		
PUFA	Linoleic acid			
PUFA	Linolenic acids			
	pheophytin *a*			
Peptides	Proteins Protein hydrolysates Peptide fraction < 5 and < 10 kDa	Enzymatic digestion of microalgae protein extract followed by ultrafiltration through 5 kDa and 10 kDa membranes	Disc diffusion Each fraction subjected to antimicrobial assays	(Tejano et al., 2019)
Peptides	Peptide fraction < 3 kDa, 3–10 kDa, and > 10 kDa molecular weight	Enzymatic digestion of microalgae protein extract followed by filtration through 3 kDa and 10 kDa membranes Identification of peptides by RPLC-mass spectrometry	Microdilution Each fraction subjected to antimicrobial assays	(Su et al., 2025)

(25 mg/mL) having the greatest level of antibacterial activity against *E. coli, P. aeruginosa, S. typhimurium* and *S. aureus*, with inhibition zones in disk diffusion tests comparable to those observed for gentamicin (10 ug). A positive association between the exposure to green light and antimicrobial activities of microalgae was also demonstrated by Zaharieva (Zaharieva et al., 2022), who used a combination of a continuous white light with either a green or a red light to grow *S. obliquus*. Only the extracts obtained from the cells cultivated under the green light enhanced the inhibitory effect of essential oregano oil on *S. aureus, E. coli* and *S. typhimurium*. In another study, Georgieva (Georgieva et al., 2024) investigated the influence of blue light on antimicrobial activities of *Coelastrella* sp. extracts. The extracts prepared from the cells cultivated under blue light or red/bue light were able to inhibit the growth of several bacteria, while the controls (cultivation under white light) were ineffective, confirming the importance of the light spectrum for microalgal metabolites bioactivity. As photoexposure to specific wavelengths can alter antimicrobial molecules and activate their inhibitory activities (Stojiljkovic et al., 2001), further research would be needed to determine if light increases the production of microalgal bioactives, or their activities.

Light photoperiod –e.g., cycles of illumination and dark perioddduring cultivation have been proposed to affect the antimicrobial activities in microalgae. However, the published data provides only indirect evidence, related to the bioaccumulation of metabolites with potential antimicrobial activities (Newsted, 2004; Sforza et al., 2012). Over illumination of microalgae cells can also lead to excessive formation of reactive oxygen species (Roach et al., 2015), which has negative effects on bacterial cellular functions and survival. León-Vaz (León-Vaz et al., 2023) showed that *Scenedesmus* sp. produced the most bioactive compounds, including carotenoids, when grown in high light (500 μmol.m^{-2}.s^{-1}) and cold (10 °C) stress conditions against the control grown at 100 μmol.m^{-2}.s^{-1} and 20 °C conditions. However, a careful analysis of light cultivation conditions applied before antimicrobial activity testing (Supplementary material Table S2) did not reveal any clear trend. Further research is needed to characterise metabolic adaptations triggered by light conditions, including concentration, type, and activities of antimicrobial bioactives.

Nutritional stresses

Nutrients, particularly nitrogen and phosphorus, are essential for microalgae growth. Depletion of N, P, or both can influence lipid synthesis and metabolite production. As observed for light supplementation, the large majority of the studies that investigated the effect of nutrient conditions on antimicrobial activities focused on the growth of microalgae culture and on the presence of extractable cellular compounds, including those with known antimicrobial properties. The findings from the few studies that reported direct evidence linking nutrients conditions and antimicrobial activities for freshwater microalgae suggest that the production of metabolites with antimicrobial activities may need to be balanced against the production of metabolites required for basic maintenance of cell functions. Hamouda and Abou-El-Souod (Hamouda and Abou-El-Souod, 2018) cultured *S. obliquus* in five different concentrations of phosphorous and reported a bell shape in the effectiveness of the microalgal extract to inhibit *E. coli*, with the greatest zone of inhibition observed when the phosphorus concentration was 0.007 g/L, and the smallest zone of inhibitions at higher or lower phosphorus concentration. Kilic (Kilic et al., 2022) tested the combined effect of nitrogen concentrations and temperatures on the antimicrobial activities of *C. vulgaris*. The largest zones of inhibition on disk diffusion assays were observed when *C. vulgaris* was cultivated in nitrogen concentration of 1.0 g/L and at 30 °C; they were reduced when the nitrogen concentration was decreased to 0.5 g/L or when the cultivation temperature was increased to 45 °C, but they increased at nitrogen concentration of 1.5 g/L when the temperature was 45 °C (Kilic et al., 2022). Similar complexities were observed for freshwater cyanobacteria (Noaman et al., 2004), further supporting the potential existence of fine regulatory mechanisms between nutrient availabilities and the production of antimicrobials by microalgae.

Unravelling the molecular mechanisms of antimicrobials production

Despite many studies reporting antimicrobial activities of microalgal extracts, the molecular mechanisms underlying the synthesis of these specific bioactives remain poorly understood (Yang et al., 2023). Investigations on other high-value bioactives produced by microalgae under environmental stress have however enabled the developmement of analytical "-omic" approaches that could be applied to the field of antimicrobials. For example, transcriptional expression of acetyl-CoA carboxylase and phosphoenolpyruvate carboxylase were correlated to the accumulation of lipids in *C. reinhardtii* under nitrogen-, sulfur-, and nitrogen and sulfur- deprivations (Gonzalez and Ynalvez, 2023). Transcriptomics analyses revealed that high salt stress stimulates the accumulation of astaxanthin in *Chromochloris zofingiensis* and *H. pluvialis* by suppressing competing pathway and enhancing carbon flux toward astaxanthin biosynthesis (Mao et al., 2020; Yang et al., 2023). These studies also identified key genes (*LCYb*; *LCYe, BKT1 and ZEP)* that could be potential engineering targets for redirecting precursors toward astaxanthin. Similarly, metabolomic analysis elucidated the differential metabolic pathways in high salinity and nitrogen-deficient conditions, and identified metabolic markers such as L-Proline, L-Aspartate, Uridine 5'-monophosphate (UMP), Succinate, L-2-Hydroxygluterate, L-Valine and Inosine 5'-monophosphate (IMP) associated to astaxanthin accumulation (Dou et al., 2025). ^{13}C tracer-based metabolic flux confirmed that optimal astaxanthin productivity could be achieved via balancing increasing glucose metabolism and inhibiting carotenoid synthesis (Wang et al., 2024). Applying −omics approches (alone or in combination) could accelerate the identification of key metabolic pathways and genes involved in the synthesis of bioactives by microalgae (see (Yang et al., 2023) for further details). A better understanding of these pathways and their regulation under environmental changes would optimise the production of antimicrobial bioactives by microalgae, either by optimizing cultivation conditions or by guiding genetic engineering.

Microalgae biomass harvesting and processing

Bioactive molecules can be synthesized within the algal cell matrix in a very small quantity, with reported yields as low as 5 mg/g dry weight (León-Vaz et al., 2023). Hence it is necessary to process the microalgae biomass to obtain an enriched and/or selective extract of the bioactive compounds. The biomass processing involves several steps, namely biomass harvesting, cell disruption, and purification of the extract or compound.

Microalgae harvesting

Separating the microalgae cells from the growth medium is critical to concentrate the biomass. Several methods have been applied, with centrifugating the cultures the most commonly used prior to antimicrobial activity tests (Supplementary material Table S1). The speed (1,200 *g* to 5,000 *g*) and duration (5 to 20 min) of centrifugation reported in the literature vary according to the types of microalgae (Najdenski et al., 2013; Corona et al., 2017; Mashhadinejad et al., 2016). These ranges reflect that microalgae pellets can be easily resuspended, and that in some instances longer centrifugation times are required to ensure the supernatant remains biomass-free. Alternatives to centrifugation consist of flocculation (using ferric chloride FeCl3 or NaOH) (Dantas et al., 2015; Mukherjee et al., 2024), or filtration (Alwathnani and Perveen, 2017). However, these approaches are limited by the need to remove or inactivate chemicals prior to antimicrobial testing and by the small size of the microalgae cells, which can limit recovery efficiency by gravity-based sedimentation or filtration (Muir et al., 2024). An approach that is gaining interest due to its potential to reduce harvest cost is algal bio-flocculation, which can be promoted by the presence of predators (e.g., zooplankton), bacteria or fungi (Muir et al., 2024). Bioflocculation approaches have yet to be explored for biomass harvesting prior to antimicrobial bioactive assays.

The antibacterial activities of the extracellular fraction (e.g., the liquid phase after biomass removal) compared to the crude extract (prepared from the pelleted cells) are inconsistent among studies (Ghasemi et al., 2007; Najdenski et al., 2013; Guedes et al., 2011) (Table 3). As expected, the concentration of active components in the algal extracellular fraction affects directly the inhibitory activities, with one study reporting increased growth of *B. subtillis* and *P. aeruginosa* when using algal extract with low concentration of exopolysaccharide, but inhibition of the same bacteria when using algal extract with 100-fold concentrated exopolysaccharides (Pointcheval et al., 2025). The need to process large volumes of the extracellular fraction to concentrate the bioactives and achieve bacterial inhibition would challenge cost-effective industrial upscaling, and this extracellular fraction has generally been discarded following the initial concentration step.

Due to its very high (>99 %) water content (Muir et al., 2025), the microalgal biomass is generally further concentrated post-harvest by removing any remaining liquid through lyophilisation, freeze-drying, or air-drying; the former being the optimal approach as it maintains the quality of the molecular components and the integrity of cell membrane while protecting heat- or light-sensitive compounds (Aljabri et al., 2023; Ren et al., 2021; Ryckebosch et al., 2012). The addition of a washing step with distilled water prior to drying the microalgal biomass has sometimes been used as it eliminates the components of the culture

Table 3
Antimicrobial activities of microalgae *exo*-metabolites (extracellular fraction).

Microalgae	Preparation of extracts and tests	Key findings	Reference
14 cyanobacterial and microalgal strains, including *S. obliquus, Chlorella* sp.	Supernatants from microalgae cultures in stationary phase freeze-dried, resuspended in double-distilled water. The extracellular polysaccharides (EPS) extracts then concentrated by precipitation in ethanol, heat drying, and resuspension in distilled water (2 mg mL^{-1}). Antimicrobial activity of each preparation against *S. aureus*, *B. cereus*, *E. coli*, *S. typhimurium*, and *Y. enterocolitica* tested by agar well diffusion test and the broth dilution method	No biological activity of EPS extracts from *S. obliquus or Chlorella* sp. detected against any of the tested organisms	(Najdenski et al., 2013)
60 microalgae strains, including *C. vulgaris* and *C. reinhardtii*	Supernatants from microalgae cultures collected and dried under pressure. Dried product resuspended in methanol. Antimicrobial activity against Bacillus subtilis, *Salmonella* sp., *P. aeruginosa*, *E. coli* and *S. aureus* determined by the disc method	The antimicrobial activities reported for the extracellular extract were consistently greater than the intracellular extracts[*]	(Ghasemi et al., 2007)
13 cyanobacteria strains and 12 microalgae strains, including *C. reinhardti,C. vulgaris S. obliquus, S. maximus,* and *D. pleiomorphus*	Supernatant of 30-day cultures lyophilized and resuspended in ethanol. Qualitative screening of the antimicrobial activities based on visual assessment of turbidity changes of a liquid media of *Salmonella* sp., *P. aeruginosa*, *E. coli* or *S. aureus)* supplemented with the extracts (extract: pathogen;1:5 (v/v) ratio.	The activities reported for the extracellular extract were consistently smaller than the intracellular extracts[*]	(Guedes et al., 2011)
2 cyanobacteria and 1 microalga (*C. reinhardtii CCAP 11/32B*)	EPS extracts obtained from ethanolic precipitation of cell-free supernatants. Following centrifugation pellets were freeze-dried and re-dissolved in sterile distilled water at a final concentration of 25 g · L^{-1}. Antimicrobial activity against *Pseudomonas aeruginosa* CIP A22 and *Bacillus subtilis* CIP 52.65 determined by the agar well-diffusion assay	*C. reinhardtii* EPS extract inhibited the growth rate of *B. subtilis* and *P. aeruginosa* by 87.1 and 86.2 % respectively. Extracts also had algicidal effect on *C. vulgaris.*	(Pointcheval et al., 2025)

[*] The comparison of the antimicrobial activities between cells fractions (internal vs external) should be treated with caution as the dry weight of each fraction is unknown.

medium potentially associated with the biomass (Schuelter et al., 2019; Mashhadinejad et al., 2016; Ren et al., 2021). However, rinsing with distilled water rather than with an isotonic solution needs to be considered carefully as it could induce cell lysis and loss of internal bioactives.

As discussed above, different harvesting techniques can be used to recover algal biomass. Conventional techniques such as centrifugation remain the technique of choice due to its efficiency, despite its costs. Significant research is focusing on microalgal harvesting and the development of emerging technologies (e.g. bio-floculation) to improve harvesting efficiency, production cost, and processing time. Staging and technology selection are essential for minimizing the costs and environmental impacts of harvesting. The selection of an appropriate harvesting method depends on numerous considerations, such as the characteristics of the microalgae cells, the media, the mode of operation and the final application considered. To the best of our knowledge, it is currently unknown if and how different methods may impact on the antibacterial activity of microalgae. Xu (Xu et al., 2015) showed that centrifugal stress (>5000 g) can lead to a 40 % loss of glycerol yields in *Dunaliella salina*. Further research should evaluate the influence of the harvesting methods on the antibacterial compounds yield and quality.

Microalgae cell disruption

Following the biomass harvest and concentration, disrupting or breaking the microalgae cell walls is required to release the intracellular components into the extraction liquid. Microalgae are composed of carbohydrates, lipids, proteins and many other compounds with potential bioactivity (Balasubramaniam et al., 2021). The methods used for cell disruption and extraction strongly affect the fraction found in the final extracts and consequently, the bioactivity of the extract. Currently, no universal or standardized method for processing of microalgal biomass exists. Highly influenced by the biochemical nature of each microalgae species tested, cell disruption and extraction methods can also be species and compounds specific and need to be carefully investigated. In addition, the development of efficient extraction and functionalization processes for antimicrobials deserves further investigation.

The cell disruption methods applied to the microalgal biomass prior to extraction of bioactives and testing for antimicrobial properties can be categorised into physical – also called mechanical – disruptions and chemical treatments. From the large variety of cell disruption methods available to extract biomolecules from microalgae (Nunes et al., 2024; Corrêa et al., 2021), the following section reports the methods applied for successful extraction of bioactives with antimicrobial properties.

Physical disruption techniques

The basic physical disruption methods have included the use of mortar and pestle (Davoodbasha et al., 2018), blender (Dineshkumar et al., 2017) or electric grinder (Das and Pradhan, 2010); these methods physically break down the cells walls and homogenise the microalgal biomass into small particle size. Another reported physical disruption method used successfully prior to antimicrobial testing is glass beads (Schuelter et al., 2019), which promote cell wall breakages by creating shear forces and collision between the cells and the beads.

More advanced approaches that have been used are sonication (Corona et al., 2017) and Ultrasound Assisted Extraction (UAE) (Zaharieva et al., 2022; Schuelter et al., 2019; Ördög et al., 2004). These cavitation-based extractions rely on the generation of sound waves within a liquid phase; the waves create alternating high pressure-low pressure cycles, leading to the formation of gas bubbles which expand and collapse, releasing large amounts of energy disrupting the microalgal cells. The recognized advantages of ultrasonic extractions include high extraction yields of bioactive compounds, minimal interference on the bioactives' integrity and low energy consumption (Corrêa et al., 2021; Jamshidi-Kia et al., 2024; Zhou et al., 2022).

The effectiveness of acoustic cavitation approaches in disrupting the cells varies according to wavelength frequency and microalgae species. Kurokawa, King, Wu, Joyce, Mason and Yamamoto (Kurokawa et al., 2016) assessed the damage of the diatom *Chaetoceros gracilis* subjected to different ultrasonication treatments. They found that an ultrasonication treatment at 2.2 MHz for 2 min disrupted 100 % of the cells, while treatments at a higher (4.3 MHz) or lower (0.4 MHz) frequency for the same duration were five times less effective. For *Nannochloropsis* sp., the number of disrupted cells increased with frequency, and 4.3 MHz was the most effective treatment (Kurokawa et al., 2016). Other authors compared the efficiency of 21 kHz sonication to disrupt three types of *Chlorophyceae,* and reported that sonication increased the proportion of the smallest particles for *C. variabilis and S. regularis*, but not for *A. gracilis* (Ma et al., 2015). Testing the extracts for their antimicrobial properties was out of the scope of these studies, but it would be valuable in future studies to test the degree of cell disruption required to achieve selective and effective extraction of antimicrobial compounds.

Pressurized Liquid Extraction (PLE) has also been applied to obtain functional compounds from *C. vulgaris* and other species (De Bhowmick et al., 2024; Plaza et al., 2012). PLE is based on the extraction of bioactives at temperature and pressure high enough to maintain the solvent in which cells were suspended in a liquid state during the whole extraction procedure. Results demonstrated that pressurized liquid extraction could achieve high extraction yields (in terms of biomass) and could produce extract with high content of carotenoids with low levels of chlorophyll. These extracts were as effective as the positive control against *E. coli*, *S. aureus* and *C. albicans* (Plaza et al., 2012).

Chemical disruption techniques

Chemical-based extractions involve the following steps: (1) addition of a large volume of a solvent into the microalgae biomass, (2) mixing for extended periods to ensure dissolution of the microalgae cell membrane phospholipids, and (3) evaporation or drying to concentrate the extracted bioactive compounds. From the existing literature, the initial amount of the microalgae biomass subjected to chemical treatment has ranged from 0.1 g to 40 g (reported as either fresh or dried weight), the ratio between microalgae biomass and solvent varied from 1:20 to 1:100 (w/v), and mixing duration was up to 3 days (Supplementary S2), illustrating the difficulty of developing a universal protocol. It is important to note that the selection of a solvent affects not only the disruption of microalgae membrane structure, but also the solubility and chemical nature of the bioactive compounds present in the crude extract (Santhakumaran et al., 2020; Mukherjee et al., 2024). Due to this dual effect of chemicals, chemical treatments have been reported in the literature either as cell disruption steps or as chemical extractions.

The chemicals used prior to antimicrobial testing against food-borne bacteria ranged from non (less)-polar solvents to polar solvents. Apolar solvents (hexane, chloroform, ethyl acetate, diethyl ether) have molecules with little to no difference in electronegativity between atoms, resulting in no significant charge separation. Extracts prepared with these solvents are usually rich in saturated and polyunsaturated fatty acids or carotenoids, with the type of solvents affecting the type of fatty acids detected in the extract (Stirk and van Staden, 2022; Mukherjee et al., 2024). Extracts prepared with polar solvents (such as acetone, ethanol, methanol, dichloromethane, dimethyl sulfoxide and water) are enriched in hydrophilic compounds like phenols, carbohydrates and polar pigments and proteins (Mukherjee et al., 2024; Georgieva et al., 2024).

Good antimicrobial activity against Gram-positive bacteria have been reported with microalgal extracts prepared with either apolar or polar solvents, while Gram-negative bacteria were more effectively inhibited by extracts prepared with polar solvents (Stirk and van Staden, 2022). Several studies compared the antimicrobial activities of freshwater microalgae, using extractions by either polar or apolar solvents (Supplementary Table S2); some reported that polar solvents yielded extracts more active than apolar solvents against Gram-negative bacteria (Dineshkumar et al., 2017; Hussein et al., 2020). Others obtained the best antimicrobial activities in extracts prepared with apolar solvents (Alwathnani and Perveen, 2017; Mukherjee et al., 2024).

Within the same class of solvent (e.g., polar versus apolar), difference in solvent polarity can affect the antimicrobial activities of the extracts. In the polar solvents category, ethanolic extracts of *Euglena viridis* and *C. vulgaris* demonstrated antibacterial activity greater than the corresponding methanolic extracts (Dineshkumar et al., 2017; Das and Pradhan, 2010). Similarly, the antimicrobial activities of *C. vulgaris* ethanolic extracts were found to be stronger than those obtained in water or acetone (Plaza et al., 2012).

Even small changes in solvent polarity could be leveraged to improve the resulting antimicrobial properties of extracts, as demonstrated by the findings of Tavakoli (Tavakoli et al., 2021). These authors engineered solvents with small differences in polarity by using different ratios of water:ethanol and examined the resulting antibacterial properties of *Spiriluna platensis* extracts; extracts prepared with ethanol 70 % generated slightly larger inhibition zones in disc assays compared to extracts prepared with pure ethanol (polarity index 5.2) or with distilled water (polarity index 9.0). It has been proposed that the presence of water may prevent the rearrangement of the membrane phospholipid bilayer partially dissolved by the solvent (Ren et al., 2021). Adding a water wash treatment to enhance the disruptive effect of an initial solvent could be another strategy to increase extractability of bioactives, although this strategy was effective in some but not all of the growth stage (Ren et al., 2021).

Overall, ethanol has emerged as a solvent suitable to confidently screen a large number of microalgae strains for their antimicrobial properties, and to yield extracts with reasonably strong antimicrobial activities (Dineshkumar et al., 2017; Das and Pradhan, 2010; Plaza et al., 2012). Compared to acetone, methanol, diethyl ether, chloroform, or hexane, another significant advantage of ethanol is its status as a greener solvent when considering health and safety risks, environmental protection, energy use and waste (Byrne et al., 2016). Beyond ethanol, natural deep eutectic solvents (NADES) are also emerging as sustainable solvents in algae-based extraction processes. NADES are composed of natural compounds like organic acids, alcohols, amino acids, sugars, and choline derivatives, and which have shown promise for extracting bioactives while also aligning with the Green Chemistry requirements (Vo et al., 2024).

Several strategies have been tested to improve metabolites extraction, and often chemical extraction is combined with physical treatment to increase extraction yields. Increasing the surface contact between microalgae cells and the solvent could positively contribute to extraction efficiency. Chemical disruptions have been performed on either fresh or dried microalgae biomass. Freeze-drying the microalgae biomass increases the surface area of the macroscopic structures (Bogolitsin et al., 2021) as well as improving the penetration of the solvent into the cells; as a consequence, it was found to enhance the disruptive action of solvent on the microalgae cell membrane structure and the release of bioactives (Ma et al., 2015).

The ratio between microalgal biomass and volume of extraction solvent can also affect the extraction yield of bioactives. Doubling the initial weight of lyophilized microalgae was found to significantly reduce the inhibitory activities of the algal extract against *E. coli* and *S. typhimurium*, suggesting that a smaller ratio between microalgae biomass and extraction buffer is more effective at disrupting cells (Schuelter et al., 2019). The action of solvents on the microalgae cell membrane were visualised by scanning electron microscopy (Ren et al., 2021). Treating freshwater microalgae *Phaeodactylum tricornutum* cells with an organic solvent resulted in the formation of holes in the microalgae cell walls, and in the diffusion of lipids from the intracellular compartment. The study also revealed that the cell membrane was not completely destabilized by the chemical treatment, explaining that 30–70 % of the lipids remained in the cells after a single solvent extraction. Combining the solvent extraction with a physical treatment (either vortex or sonication) improved the extraction efficiency of lipids by 8 to 27 % (Ren et al., 2021). In supercritical fluid extractions, the extraction performance of bioactives from microalgae can be promoted by the presence of co-solvent (ethanol), which decreases fluid viscosity and increases the movement of solvents into the algal matrix (Vo et al., 2024). Overall, these findings confirmed the importance of physical disruption of cell wall structure to ensure access and effectiveness of the solvents.

Heating solvents improves the dissolution of bioactives by increasing the mass transfer rate of soluble ingredients into the liquid phase (Mao et al., 2021). Extending the heating time from 5 min to 20 or 25 min improved the extraction yields from *Chlamydomonas* sp. and *Cladophora* sp. (Jamshidi-Kia et al., 2024). For *C. vulgaris,* prolonged heating at temperatures above 60 °C reduced extraction yield compared to extractions where heating was limited to 2 min (Jamshidi-Kia et al., 2024). Yield declined due to the denaturation of heat-sensitive compounds (Guleria et al., 2024). To establish the importance of treatment time on the extraction yield, Plaza (Plaza et al., 2012) tested extraction times sequentially (from 5 to 40 min in 5 min intervals) for ultrasound-assisted extraction in various solvents. They identified 25 min as optimal extraction duration for ethanol and hexane, and 30 min for acetone, but no reason was proposed to explain the decline for prolonged treatment. Overall, these findings have highlighted the importance of optimizing extraction conditions according to the strains and processes used.

Future prospects

A standardized framework for generating and testing extracts

The complexity of the microalgae physiology, the variability of biochemical and structural differences among species reported in the literature hinder the ability to accurately identify the best conditions associated with production and the extraction yield of bioactives. Some variations would be expected between species as well as within species grown under different cultivation conditions. The use of different extraction and activity assays could also influence the yield of bioactives and the efficacy of the extract (Table 1). Culture conditions for effective production of antimicrobial bioactives need to be optimised to reach a balance between optimal accumulation of bioactive and loss through cell lysis and deaths. Similarly, a balance between quantity and purity of bioactives in the extracts needs to be achieved for effective antimicrobial activity. By increasing co-extraction of large amounts of cell debris, the targeted molecules can be diluted or degraded, reducing the purity of the extract and ultimately leading to reduced antimicrobial activities (Guleria et al., 2024; Tavakoli et al., 2021).

Due to biochemical difference among microalgae species, there are currently no standard operating procedures to produce and extract microalgal antimicrobials. The antimicrobial compounds in higher plants have been extensively studied (Eloff, 2019), and much can be learnt from plant sciences to prevent the 're-allocation' of resources. Based on the knowledge presented in this review, the application of a standardized framework for microalgae antimicrobials, similar to that recommended for higher plants, would ensure higher success and traceability. This framework should include:

(i) Considering that each step involved in the generation of microalgal extracts can influence the composition and concentration of the bioactives, it is critical that each of those steps are clearly described in studies (i.e. microalgal growth, biomass concentration, sampling time, extraction etc).
(ii) In common with microalgae, bacterial isolates show differences in sensitivity to antimicrobials. Using well characterised bacterial isolates from culture collections is recommended to facilitate comparison between studies. This should be complemented by assays considering bacterial isolates physiologically more resistant to environmental stresses (e.g., by testing culture collection isolates preliminary subjected to stresses, or environmental isolates).
(iii) Testing extracts: The dose of the extract should always be disclosed and properly described (i.e. wet biomass vs dry biomass vs dry product after extraction). Importantly, the potential activity of the solvent used for extraction should always be assessed to prevent false positives. Negative and positive controls (e.g. standard antibiotics) should be included. The positive control is critical to provide evidence that the set-up was suitable and to assess the activity of the extract compared to the current clinical standard.
(iv) Considering the multitude of compounds with varying polarities found in extracts and parameters influencing its results, the agar diffusion assay should not be used alone as a screening tool for antimicrobial activity.
(v) For the disc assay, a positive control should be used in an amount relevant to the current clinical breakpoints, as these may vary according to the bacterial target. For example, the recommended amount of ampicillin is 2 ug when testing an antimicrobial activity against *Staphylococcus* spp. while it is 10 ug when testing against *Enterobacterales* (European Committee on Antimicrobial Susceptibility Testing, 2025).
(vi) With the development of high throughput techniques, bioactives isolation and identification should be further considered, while considering the drawback associated with loss of activity during extraction. Identified compounds should be tested *in vitro* and then *in vivo* for clinical relevance.

Advanced monitoring of microalgal cultures

The use of culture age to predict production of secondary metabolite levels needs to be used with caution as the relationship between culture age and metabolite levels is strain-specific and dependent on the types of phytochemicals involved (Aremu et al., 2014). Accurately determining the physiological and/or metabolic status of microalgae cells could be a better approach than age of culture to identify the optimal harvest time with maximized antibacterial activity.

Approaches to monitor the physiological status of microalgae cells during cultivation, and their advantages and disadvantages have been previously discussed (Bermejo et al., 2021). Cells in stationary phase are losing their homeostasis, hence release intracellular compounds in the media. Markers for cells lysis thus includes quantifying internal ATP, or appearance of DNA or free fatty acids or malondialdehyde as products of lipids degradation in the medium. The loss of cell viability can be monitored through counting the cells with chlorophyll (using specific dye); cell death can be indicated by change in RNA content in the culture medium, or by increase in Caspase-like activity. Alternatively, Vendruscolo (Vendruscolo et al., 2019) compared the intracellular amino acid content of *Scenedesmus obliquus* at different growth stage and identified glutamic acid or alanine as possible markers for cell stress and reduced growth. Advanced analytical methods such as spectroscopic techniques (e.g. hyperspectral), show potential for culture monitoring and microalgal phenotyping (Plouviez et al., 2024). By identifying specific trait, the use of these methods could improve monitoring of cultures for bioactives or the discrimination of species for specific antimicrobial applications based on their spectral signature.

Sampling sites/environment

Microalgae and cyanobacteria thriving in physiologically challenging environments have developed certain evolutionary adaptations and could be an interesting source of bioactives (Kochhar et al., 2022). Environments near abandoned mine sites seem to have a higher than average potential for harbouring antibacterial producers (Senhorinho et al., 2018). While marine microalgae are increasingly studied, extremophiles are also good candidates for the biodiscovery of antimicrobials (Rinaldi et al., 2024). The inclusion of metadata on sampling site (including conditions or historical use, as listed in Santhakumaran (Santhakumaran et al., 2020) could help with identifying potential new sampling sites.

Other, more widely accessible ecosystems, such as wastewater, could provide interesting sampling sites for antimicrobials-producing microalgae. Microalgae provide important functions in wastewater treatment, including removal of antibiotic and antibiotic residues (Ilieva et al., 2024; Xu et al., 2025). In these systems, symbiosis (mutualistic), communalistic and competitive interactions with other microbes occur. Because those interactions drive population dynamics and physiological plasticity, it may be assumed that the microalgae found in those ecosystems could be prone to synthesizing antimicrobial compounds against competitor or pathogens (Dineshkumar et al., 2017). In situ or co-cultivation studies in wastewater, combined with antimicrobial bioassays, are needed to understand those interactions. Identifying the bacteria triggering a microalgal antimicrobial response could be guided by metagenomic approaches characterizing the microbial community at taxonomic and functional levels (Durán et al., 2022; Kim et al., 2014; Krohn-Molt et al., 2017). Incubating the microalgae with the identified competitor and/or target bacteria in the laboratory could trigger defense responses, facilitating the study of the interactions involved and the identification of the antimicrobials produced (Borges Lopes et al., 2025). The use of droplet microfluidics could allow for mass screening analysis as done by Gopalakrishnappa (Gopalakrishnappa et al., 2024) who investigated over 100,000 cultures across more than 500 environmental conditions and determined how abiotic factors affect nutrient sharing in microalgal-bacterial communities.

Screening and transgene expression

Given the huge microalgal diversity, screening of more species is likely to result in the identification of new candidates for bioactives, including antimicrobials. From their thorough literature review, Stirk and van Staden (Stirk and van Staden, 2022) evidenced that fewer antimicrobial compounds have been identified in the *Chlorophyta* compared to *Cyanobacteria*. As *Chlorophyta* has a great commercial potential, future studies should focus on *Chlorophyta*.

Large screening of more species needs collaborative approaches and new technologies to prevent the use of resources for limited outcomes (Neergheen-Bhujun et al., 2017). The development of high throughput screening would accelerate the discovery of new compounds, but this is highly dependent on creating physical collections or virtual databases, which can be hindered by both ethical concerns and intellectual property rights (Mishra et al., 2025).

Microalgae, and specifically the model microalga *C. reinhardtii,* have been successfully engineered to produce target molecules by nuclear and chloroplast genome editing (Dubey et al., 2024; Liu et al., 2020; Sun et al., 2024; Zhuang et al., 2023). With new strains being developed for their compatibility with industrial production of transgene expression (Dementyeva et al., 2021), microalgae could be of great use in the production of specific antivirals and antibiotics from different microbial origins.

Identification of antimicrobials

Synergistic and antagonistic effects of microalgal compounds have not been thoroughly investigated to date. Further compound identification and characterization is therefore required. Identifying and characterizing the active compounds is also important to understand mode of actions, and to establish pharmacokinetics and pharmacodynamics that are critical in the development of antibacterial medicinal products.

Some antimicrobial bioactives from microalgae have been identified by chromatographic methods and/or mass spectrometry (Alsenani et al., 2020). Those techniques can be untargeted and do not require extensive fractionation. Nevertheless, activity-guided methods increase the chances of identifying molecules of interest because active compounds are first extracted based on their physicochemical properties and assessed for their biological activity in a step wise manner before identification by chromatographic techniques such as GC–MS (Kiani et al., 2022). High-throughput bioassay-guided fractionation techniques are being developed (Phillipson et al., 2002). Fractionation could be widely applied to microalgal extracts to increase the chances of identifying active compounds.

In addition, the potential of new approaches such as biochemometrics should be evaluated on microalgal extracts. Biochemometrics approaches rely on multivariate statistics to extract information from chemical and biological datasets. While not applied on microalgae yet, biochemometrics analyses have been successful in higher plant extracts to identify bioactives compounds present in low abundance or to differentiate between active and inactive compounds (Caesar et al., 2018; Kellogg et al., 2016; Ory et al., 2019; Pelzer et al., 2022; Wasilewicz et al., 2025). Consequently, such approaches could help with characterizing bioactives and enhance biodiscovery from microalgal extracts/biomass. Machine learning and artificial intelligence that already facilitate the analysis of omics datasets and have provided a leap forward in the identification of compounds, could play a big part in the applicability of biochemometrics (Wang et al., 2023; Lateef Junaid, 2025).

Bioinformatics and artificial intelligence

Bioinformatics tools are critical for processing and analysing big data such as the datasets generated in metabolomics (Deng et al., 2024). Bioinformatics tools are also being developed to determine the antimicrobial potential of molecules or design compounds with antimicrobial properties (Alexander et al., 2025; Wan et al., 2024; Gadiya et al., 2025; Lin et al., 2025; Wang et al., 2022). For example, Wong (Wong et al., 2024) used an artificial-intelligence graph neural network to identify chemical substructures with selective antibiotic activity that lead to the discovery of a new class of antibiotic potent against Gram-positive bacteria.

Conclusions

The demand for natural and sustainable antimicrobial agents continues to rise globally, particularly in the context of food safety and preservation, and microalgae are promising sources due to their rich and diverse bioactive profiles. In summarising the current status for extracting the bioactives with antimicrobial properties, this review highlighted the crucial importance of optimising each step leading to the extraction of bioactives, standardising the reporting of experimental conditions, and understanding the underlying mechanisms leading to bioactive expression. Recent years have seen how various −omics approaches could accelerate the screening and characterization of bioactive molecules or open new avenues for developing tailored microalgal strains with antimicrobial properties, ultimately unlocking the potential of microalgae for use in food producing systems.

CRediT authorship contribution statement

Delphine Rapp: Conceptualization, Data curation, Writing – review & editing. **Dieke Schelvis:** Writing – review & editing, Visualization. **Nigel P. French:** Writing – review & editing. **Maxence Plouviez:** Conceptualization, Writing – review & editing.

Declaration of competing interest

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

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Appendix A. Supplementary material

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