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Review Paper

Exploration of secondary metabolites from green algae as antimicrobial agents: A comprehensive review

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ABSTRACT:
A number of advancements have been made in algal technology in different fields, such as medical, cosmetic, and pharmaceutical. Green algae (Chlorophyta) are a group of photosynthetic organisms which live in both aquatic and terrestrial environments. They are a potential source of bioactive compounds for the treatment and prevention of a wide range of infectious diseases caused by microorganisms. These compounds also possess anti-inflammatory, antioxidant, anticoagulant, antitumor, and immunomodulatory properties. A wide variety of bioactive compounds are produced, including polysaccharides, which exhibit antimicrobial properties capable of interfering with the cell walls, membranes, and nucleic acids of microorganisms. Membrane fluidity, permeability, or integrity can be affected by polyphenols and fatty acids which scavenge free radicals, chelate metal ions, or disrupt enzymes and membranes. Proteins and peptides form pores within the membranes which bind to specific receptors or inhibit enzymes. Adaptation to adverse environmental conditions, such as temperature extremes, photooxidation, salinity, or osmotic stress, results in the formation of bioactive compounds by altering the physiological and biochemical pathways of algae for the maintenance of cellular homeostasis. With changing consumer preferences and an increase in the number of resistant microorganisms, it is critical to seek novel antimicrobial compounds from green algae. The search for novel bioactive compounds with antimicrobial properties from green algae may serve as an alternative in the light of increased drug resistance in microorganisms. However, *in vitro* and *in vivo* evaluations of the safety, efficacy, and mechanism of action of the antimicrobial compounds from green algae require more research. Providing an overview of previous endeavours in this emerging field, this review provides perspectives and a summary of the bioactive compounds responsible for the antimicrobial properties of green algal extracts.

Keywords:
Chlorophyta, bioactive compounds, antibacterial, antifungal, antiviral, drug resistance.

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INTRODUCTION

It has long been known that natural products can be used as therapeutic agents. However, plant resources are often slow to grow and take years to reach their full potential. As a result of the restrictions imposed on a number of plant species, concerns about the illegal trading of natural resources, fear of depletion, and the risk of environmental impacts, researchers have begun to seek economically viable natural alternatives. As a result, green algal sources have been identified as potential therapeutics (Levy & Marshall 2004).

Infectious agents and emerging diseases pose a threat to modern medicine. The emergence of infectious diseases, such as whooping cough, bubonic plague, tuberculosis, etc., are interlinked with global climate change

and increasing mortality rates due to microbial infections (Baker *et al*. 2022). As a result of the overuse and inadequate dosing of drugs combined with the emergence of new resistant strains, conventional therapies for bacterial, fungal, and viral infections are becoming less effective. Hence, a combination of antimicrobial-antimicrobial, antimicrobial-adjuvant, and combination drug therapies have been used to combat drug resistance. Green biotechnology has thus emerged as a safer alternative to synthetic drugs, with algae gaining interest in this regard.

Algae, as an essential constituent of the aquatic ecosystem, play a significant role in productivity and carbon sequestration (Krause-Jensen & Duarte 2016). They are dynamic communities exhibiting spatial and temporal variations in species richness. Notably, Chlorophyta, along with marine red (Rhodophyta) and brown algae (Phaeophyceae), stands out as the most diverse group with various potential bioactive compounds (Shah *et al*. 2022). Environmental adaptations in algae result in the production of complex compounds. Algae inhabit numerous different environments, including freshwater, marine, and even terrestrial environments like soil and tree trunks. Such diverse habitats expose algae to varied environmental conditions, which in turn stimulate the production of a variety of compounds. The availability of nutrients, light, temperature, and salinity all influence the production of secondary metabolites in green algae. The genes involved in secondary metabolite biosynthesis can be affected by these factors, resulting in the production of a wide range of compounds with different biological functions. Several organic osmolytes are produced and accumulated to provide protection against environmental stressors, such as desiccation, high irradiation, and ultraviolet light. The osmolytes in algae aid in their adaptation to harsh environments. Defence and stress adaptation are also assisted by secondary metabolites, such as phenolics, flavonoids, and alkaloids, which often possess complex structures and exhibit a range of biological activities, including antimicrobial properties (Zhang *et al*. 2021).

Initially, species of *Chlorella* were used as model organisms for studying green algae metabolism because of their remarkable resistance to physical and chemical stress. The use of green algae as therapeutic agents has a long history (Dai *et al*. 2022). Early on, microalgal biomass was applied in tablets, powder, and water agents, and its pharmaceutical effects were assessed. In the past, compounds like calcium spirulan, and nostaflan from blue-green algae were found to have remarkable antiviral properties against HIV and HSV. As early as 1944, a growth-inhibiting substance called chlorellin was reported in cultures of *Chlorella vulgaris* and *C. pyrenoidosa* (Pratt *et al*. 1944). According to this study, the extracts exhibited antibiotic properties against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*, *Escherichia coli,* and *Pseudomonas pyocyanea*. The findings of this study paved the way for further studies into the potential of microalgae to provide antibiotics and other valuable renewable feedstock. The therapeutic potential of green algae is being explored even further today, driven by advancements in technology and research, particularly in the pharmaceutical and nutraceutical sectors. The bioactive molecules produced by green algae include carotenoids, polysaccharides, vitamins, unsaturated lactones, sulfur-containing compounds, cyanogenic glycosides, saponins, phytoalexins, terpenoids, steroids, phenols, amino acids, polyunsaturated fatty acids, and lipids (Afzal *et al*. 2023).

In addition to enzymatic inactivation and target site modification, bacteria which are resistant to antibiotics may reduce the concentration of the compounds or develop resistance through other mechanisms. The Public Health Action Plan to Combat Antimicrobial Resistance reports *Streptococcus pneumoniae*, *E. coli*, *S. aureus,* and species of the *Klebsiella* genus as being among the critical bacterial species resistant to antibiotics.

There are several advantages of using antimicrobial compounds from green algae over conventional medications: they are less likely to cause side effects, microorganisms have a lower risk of developing resistance, they are more environmentally friendly, and they may be used to develop new therapeutic agents in the future. This review presents the antimicrobial potential of extracts obtained from green algae, their specific bioactive compounds, and the novel green extraction technologies used to efficiently extract them.

Green algae as a source of natural antimicrobial agents

Studies have shown that the algae from polluted environments contain more metabolites with antimicrobial properties. For example, strains of *Dunaliella salina* collected from waters contaminated with sewage and industrial waste produced more compounds displaying antibiotic activity than strains from low-pollution areas (Little *et al*. 2021). Various types of organic pollutants can be absorbed and accumulated by algae. Secondary metabolites are produced in response to the absorbed pollutants, including dibenzofurans, depsidones, anthraquinones, xanthones, usnic acids, pulvinic acids, atranorins, and others which possess antimicrobial, antimutagenic, allelopathic, as well as anti-herbivorous properties (Shah *et al*. 2021). Figure 1 provides a schematic representation of the process of screening for metabolites from green algae. Developing algal-based antimicrobial products will present a number of challenges and opportunities, including safety, efficacy, stability, extraction, purification, and formulation. Several algal extracts demonstrate efficacy as antimicrobial compounds against different microorganisms. The secondary metabolites of green algae responsible for antimicrobial activity are given in Table 1.

Table 1. The secondary metabolites of green algae responsible for antimicrobial activity.

Algae	Extraction solvent	Bioactive compound	Sensitive microorganism	Ref. No.
Spirogyra varians	Hexane, chloroform, dichhloromethane, acetone, methanol	Proanthocyanidins, phenol, flavonoid, chlorophyll, carotenoid	*Bacillus cereus, Micrococcus luteus, Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, Pseudomonas aeruginosa , Klebsiella pneumoniae, Candida albicans*	10
Spirogyra sp.	Diethyl ether, acetone, ethyl acetate	Phenol, tannin, flavonoid	*Aeromonas hydrophila, Streptococcus agalacitace, Flavobacterium columnare*	72
Spirogyra nitida	Methanol	Fatty acids, phenols	*E. coli, C. albicans*	52
Spirogyra decimina, S. grantiana	Methanol, ethanol	Phenol, flavonoid, alkaloid	*S. aureus, Proteus vulgaris, P. mirabilis*	70
Spirogyra sp., *Oedogonium* sp.	Ethanol	Phenols	*Staphylococcus xylosus, S. aureus*	61
Oedogonium globosum, O. intermedium	Methanol	Terpenoids	*E. coli, S. aureus*	51
Pithophora varia	Ethanol	Alkaloids, phenols, flavonoids, steroids, quinones, amino acids, saponins, tannins	*Bacillus subtilis, E. coli, Trichoderma* sp., *Aspergillus niger*	3
Cladophora glomerata	Ethanol	Lipids, tannin, alkaloids, phenols, flavonoids	*E. coli, B. cereus, S. aureus, Salmonella typhimurium, L. monocytogenesis, P. aeruginosa, C. albicans, A. niger*	23; 26
	Methanol, chloroform, acetone	Indoles, terpenes, acetogenins, fattyacids, halogenated hydrocarbons	*B. subtilis, P. aeruginosa, Staphylococcus epidermidis, S. aureus, E. coli*	
Cladophora rupestris	Methanol, water	Polyphenols	*S. aureus, E. coli, C. albicans*	91
Tetraselmis sp.	Methanol, hexane, diethyl ether	Alkaloids, flavonoids, tannins, saponins, steroids	*E. coli, S. aureus, A. niger*, *C. albicans*	49
Tetraselmis sp., *Dunaliella* sp., *Chlorella* sp.	Methanol, hexane, diethyl ether	Benzoic acids, hexadecaonic acid, tetradecanoic acids	*E. coli*, *P. aeruginosa*, *Enterococcus* sp., *S. aureus, A. niger, C. albicans*	28
Ulva reticulata	Methanol, *n*-butanol, water	Steroids, fatty acids, phenol, (3,5-bis(1,1-dimethylethyl))	*C. albicans*, *S. aureus, E. coli*	25
Ulva lactuca	Ethyl ether	Acrylic acid, fatty acids, gallic acid	*E. coli, B. cereus, C. albicans, S. aureus*	18
Ulva fasciata	*n*-butanol	Phenols, flavonoids	*Penicillium digitatum, P. expansum, P. italicum*	31
Ulva fasciata, Codium elongatum	Methanol	Sulphated Polysaccharide	*S. aureus, C. albicans,* Herpes simples virus, Semliki Forest virus, Vaccinia virus	30
Ulva sp.	Water, ethyl acetate, ethanol	Alkaloids, carbohydrates, amino acids, flavonoids, sterols	*S. aureus, Streptococcus mutans, B. subtilis, S. typhimurium, Serratia marcescens, E. coli, Neisseria meningitis, C. albicans, Aspergillus fumigatus A. niger, Rhizopus oryzae, Mucor circinelloides*	74
Haematococcus pluvialis	Hexane, ethanol	Astaxanthin, propanoic acid, butanoic acid	*A. niger, C. albicans*	77
Codium adharens	Methanol, hexane, diethyl ether	Lipids, alkaloids, flavonoids, proteins, free amino acids	*E. coli, S. aureus, Vibrio cholerae*	83
Caulerpa brachypus, Caulerpa scapelliformis, Caulerpa okamurai, Chaetomorpha crassa, Chaetomorpha spiralis, Monostroma nitidum, Codium adhaerens	*n*-butanol	Sulphated Polysaccharide	*E. coli, S. aureus,* HSV-1	45
Caulerpa racemosa	Methanol	Sulphated Polysaccharide	HSV-2	59
	Water, ethanol	Polyphenol, tannin	*S. aureus, P. aeruginosa*	
Enteromorpha prolifera, Ulva reticulata	Ethyl acetate, petroleum ether, *n*-butanol	Lipophilic, phenolic compounds, sulfated polysaccharide	*S. aureus, K. pneumoniae, E. coli*	96
Chaetomorpha aerea	Water	Sulfated galactan	*S. aureus, E. coli*	100

Antibacterial activity

The antibacterial activity of algal extracts is dependent on the contents of quercetin (flavonoid), phycocyanin, carotene, phenolic compounds, and metabolically active polysaccharides (Gomez-Guzman *et al.* 2018). Due to their antibiofilm activity, bioactive compounds play a crucial role in combating infectious diseases. Prarthana & Maruthi (2017) reported the presence of

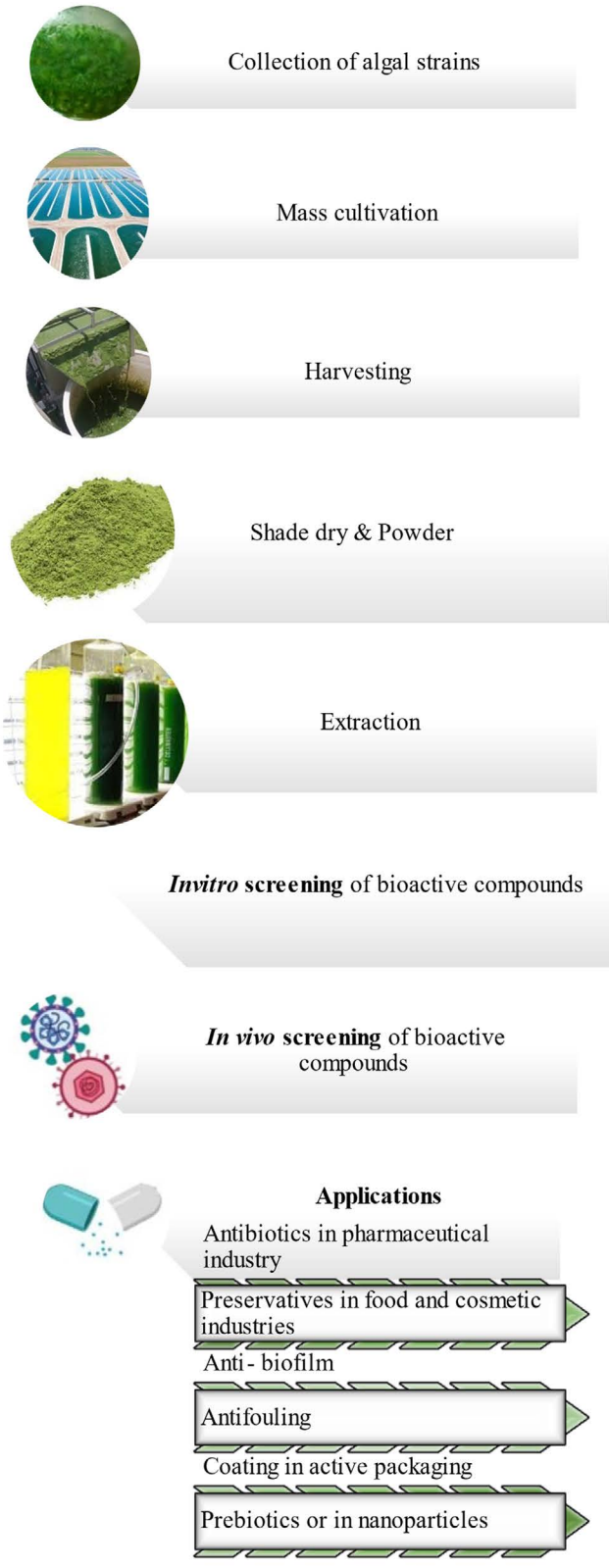

Fig. 1. A schematic representation of the processes involved in the screening of green algae for potential metabolites.

several antimicrobial compounds in the extracts of *Spirogyra* sp. including bis(2-ethylhexyl) phthalate, cholestanol-3-ol,2-methylene-(3β,5α), octadecanoic and pentadecanoic acids. Similarly, experiments conducted with the ethanolic and methanolic extracts of *S. grantiana* demonstrated activity against *E. coli* and *Salmonella typhi*, thus indicating the potential use of the active compounds present in extracts from this alga to treat urinary tract infections, diarrhoea, pyogenic infections, and septicemia. The hexane extract of *S. biformis* and *S. decimina* showed antibacterial activity against *S. aureus* and can potentially be used to treat septicemia, sinusitis, and tonsilitis infections. Furthermore, causative agents of urinary tract and wound infections, *Proteus vulgaris* and *P. mirabilis*, were susceptible to the ethanolic and methanolic extracts of *S. biformis* and *S. grantiana,* respectively (Prakash *et al*. 2011).

A number of natural compounds from green algae were shown to outperform synthetic compounds, including eicosapentaenoic acid, phthalate ester, and microcolin-A (Osman *et al*. 2013). Pharmaceutical biotechnology frequently relies on *Chlamydomonas reinhardtii*. Extracts of *C. reinhardtii* are effective against bacterial pathogens, including *P. mirabilis* (Alsenani *et al*. 2020). A chimeric peptide derived from *C. reinhardtii* containing Mytichitin CB and Hispidalin inhibits bacterial growth by disrupting the integrity of the membrane. This makes it an excellent candidate for the eventual replacement of conventional antibiotics (Wang *et al*. 2023). Due to the presence of flavonoids, steroids, polysaccharides, terpenoids, and fatty acids in the crude methanolic extracts of *Hydrodictyon reticulatum*, the growth of *Klebsiella pneumoniae* and *S. aureus* was inhibited (Munir *et al*. 2020). *Oscillatoria sancta* extracts were reported to be active against *P. mirabilis*, *S. pyogenes*, and *P. vulgaris* (Prakash *et al*. 2011). Similarly, the methanolic and chloroform extracts of *C. vulgaris* showed inhibitory activity against the pathogens *B. subtilis, K. pneumoniae, Aerobacter aerogenes*, and *E. coli*. Their antibacterial activity is primarily attributed to saturated and unsaturated fatty acids. Ciprofloxacin and norfloxacin work synergistically in methanolic extracts of *C. vulgaris* and exert enhanced antibacterial potency against *E. coli* (Pradhan *et al*. 2021). Ethanol extracts of *Desmococcus olivaceus* and *C. vulgaris* are recommended to treat *Clostridium botulinum, E. coli*, and *S. typhi* infections due to the presence of flavonoids, terpenes, and carbohydrates (Ramar *et al*. 2016). Likewise, the hexane, methanol, and ethanol extracts of *Oedogonium echinospermum* are effective antimicrobial agents against enteric fever, septicemia, and gastroenteritis caused by *S. typhi*. The ethanolic extracts of *Pithophora* sp. and *Oedogonium* sp. are found to be effective against species of *Salmonella* and *Staphylococcus* (Danyal *et al*. 2013).

Extracts obtained from *Haematococcus pluvialis*, a marine microalga, exhibited antibacterial activity against *S. aureus* and *E. coli* due to the presence of fatty

Table 2. A list of microorganisms resistant to antibiotics and mechanisms of resistance.

Antibiotic class	Resistant bacteria	Mode of action	Ref. No.
Aminoglycoside	*Pseudomonas aeruginosa*	Enzymatic modification	40
β-Lactams	*Staphylococcus aureus, S. pneumoniae*	Enzymatic degradation	
Glycopeptides	*Enterococcus faecium, E. faecalis*	Altered target	
Macrolides	*S. aureus, S. pneumoniae, S. pyrogenes*		
Oxazolidinones	*E. faecium, S. aureus*		
Quinolones	*S. aureus*		
Sulfa drugs	*Escherichia coli, S. aureus, S. pneumoniae*		
Chloramphenicol	*S. pneumoniae*	Antibiotic inactivation	

acids (Rodriguez-Meizoso *et al*. 2010). It has been reported that α-linolenic acid, a form of fatty acid in algae, can inhibit the growth of bacteria, including *S. aureus* and *E. coli*. Also identified in *Ulva rigida,* fatty acids such as oleic, linoleic, palmitic, and stearic acids, possess antibacterial effects primarily against *S. aureus*, *Enterococcus faecalis,* and *E. faecium* (Ismail *et al*. 2018). *Ulva intestinalis* is a commonly available species containing bioactive compounds, such as laxaphycins, isomalyngamide, and antitoxin, which have antibacterial activity against *B. cereus* and *S. aureus* (Tolpeznikaite *et al*. 2021). According to studies conducted by Osman *et al*. (2010), *E. compressa* was effective against *P. aeruginosa*, *S. aureus*, and *S. pneumoniae*. An evaluation of the potential antibacterial activity of *Caulerpa lentillifera* extracts and caulerpin against four common food pathogens, including *E. coli*, *Salmonella* sp., *Streptococcus* sp., and *S. aureus* found that the activity was due to the presence of bioactive compounds such as polyphenols (Nagappan & Vairappan 2014). Algae extracts are generally most effective on the following microbial species: *S. aureus*, *E. coli*, and *Bacillus cereus* (Danyal *et al*. 2013; Jang & Lee 2015).

Antifungal activity

In addition to antibacterial compounds, green algae are also a source of antimycotic compounds. Fewer studies have been carried out on the antifungal activity of green algae metabolites than on their antibacterial activity. According to Hamed *et al*. (2018), the presence of phenolic compounds, polyunsaturated fatty acids, and various terpenoids is responsible for the antifungal activity of green algae extracts. Ethanolic extracts of *Pithophora* sp. and *Oedogonium* sp. are found to be effective against *Penicillium viridicatum*, and *Fusarium solani* (Danyal *et al*. 2013). *Aspergillus niger* and *A. fumigatus* are both susceptible to methanolic extracts of *Chlamydomonas reinhardtii* (Alsenani *et al*. 2020).

Aspergillus niger, which causes respiratory infections and food spoilage, is inhibited by a lectin produced by *Caulerpa racemosa*. Compounds from marine algae *Codium decorticatum* and *Caulerpa scalpelliformis*, obtained using acetone, methanol, chloroform, diethyl ether, ethyl acetate, and hexane solvents, were evaluated for their antifungal activity against *Fusarium oxysporum*, *F. udum*, *F. solani*, *Rhizoctonia solani*, *Alternaria alternata*, *Botrytis cinerea*, *Candida albicans*, *C. krusei*, *A. niger* and *A. flavus* (Lavanya & Veerappan 2012). In recent studies, phenolic and flavonoid compounds in *Ulva fasciata* extracts have been found to possess antifungal activity against *Penicillium digitatum*, *P. expansum*, and *P. italicum* (Fayzi *et al*. 2022). In a study conducted by Mickymaray & Alturaiki (2018) involving chronic asthmatic patients with acute respiratory distress, *Ulva prolifera* and *Cladophoropsis* sp. ethanolic extracts exhibited the most potent antifungal activity against *C. albicans*. According to Indira *et al*. (2013), methanolic extracts of *Halimeda tuna* extracts were highly effective against *A. niger, A. flavus*, *A. alternaria*, *C. albicans*, and *Epidermophyton floccosum*. Bioactive compounds of *Enteromorpha linza* were reported to have a stronger antifungal effect than nystatin against *A. niger* and *C. albicans* in a study by Erturk & Tas (2011).

Antiviral activity

The emergence or (re)emergence of contagious diseases has resulted in a massive increase in efforts to develop antiviral drugs. Many different types of viruses have been shown to be susceptible to compounds obtained from green algae. Sulfated polysaccharides derived from seaweed have shown antiviral activity against a broad spectrum of viruses, including common DNA and RNA viruses. They can inhibit different stages of the viral infection inside the host cell, such as blocking the initial entry of the virus or inhibiting its transcription and translation (Wei *et al*. 2022).

Caulerpenyne, a sesquiterpene isolated from the algae *C. racemose,* has demonstrated inhibitory effects on the herpes simplex virus, the human immunodeficiency virus, and the hepatitis C virus. A variety of green algae, including *Ulva lactuca*, *E. compressa*, and *Monostroma nitidum*, contain sulfated polysaccharides, such as ulvan and rhamnan, which have shown antiviral activity against HSV-1, HSV-2, the respiratory syncytial virus, and the influenza A virus (Xu *et al*. 2023). Wang *et al*. (2012) reported effective antiviral activity against the

dengue virus induced by sulfated polysaccharide of *C. racemosa*.

Deethae *et al*. (2018) found that ethanolic extracts of *Spirogyra* sp. contain alkaloids, essential oils, and terpenoids which are effective against the Herpes simplex virus type 1 and type 2. A study conducted by Hayashi *et al*. (2019) investigated the effectiveness of monogalactosyl diacylglycerides derived from the green microalgae *Coccomyxa* sp. which disrupt the envelope of the human papillomavirus and prevent it from interacting with the host cell.

Mode of action of antimicrobial compounds

Comprehending bacterial resistance to antibiotics requires an understanding of both the mechanism of action and the nature of antibacterial agents. The variation in the levels of antimicrobial compounds in algae during different growth stages highlights the diversity of the bioactive compounds they possess. For example, compounds obtained from *Codium* sp. have the highest efficiency during spring (Parsaeimehr & Lutzu 2016). In a study conducted by Tolpeznikaite *et al*. (2021), the antimicrobial activity of *C. rupestris* compounds was found to be higher in samples collected in autumn compared to those collected in spring. Microorganisms resistant to antibiotics and mechanisms of resistance are listed in Table 2. Mechanisms of action of antimicrobial compounds are presented in Figure 2.

Antibacterial activity

Bioactive metabolites play a significant role in modulating the activity of antimicrobial agents. They can enhance their efficacy by altering interactions with host cells, potentially leading to more effective treatment outcomes (Perez *et al*. 2016). The antibacterial action of glycol compounds is due to their interaction with lipopolysaccharides or peptidoglycans in bacterial cell walls (Besednova *et al*. 2015). A number of cyclic esters, including furanones and lactones, are known to possess antibacterial properties. Synthetic sanitisers and antibiotics can also be substituted with algal furanones. The mechanism of action of furanones is often related to their ability to interfere with bacterial communication systems, referred to as quorum sensing. By disrupting these systems, furanones can prevent bacteria from coordinating certain behaviours, including the formation of biofilms, often involved in antibiotic resistance (Alizadeh *et al*. 2020). The antimicrobial activity of lactones is often associated with the presence of an α,β-unsaturated γ-lactone moiety. This structural feature can interact with microbial enzymes and proteins, disrupting their normal function, thus leading to the inhibition of microbial growth (Mazur & Maslowiec 2022).

Algal polysaccharide glycoprotein receptors bind to hyaluronic acid-inhibiting enzymes in the cytoplasm and inhibit DNA replication and colonisation. In *Tetraselmis suecica*, peptides cause cytoplasmic leakage in bacteria, eventually leading to their death. Ultimately, the antimicrobial proteins and peptides target the nuclear proteins of the bacteria creating pores, disrupting membranes, and causing death (Bhowmick *et al*. 2020). The peptide, SP-1, isolated from *Spirulina platensis* has been reported to possess antimicrobial properties against *E. coli* and *S. aureus*. This alga has been used to isolate the first antimicrobial peptide, SP-1. Generally, antimicrobial peptides (AMPs) exert effects via several mechanisms. They can disrupt the integrity of the microbial cell membrane, leading to cell lysis. They can also penetrate the microbial cells and interfere with vital intracellular processes. Some AMPs can even modulate the host's immune response (Sun *et al*. 2016).

Algal lipid metabolites play an indispensable role in antimicrobial activity. The fatty acid composition of marine algae is a crucial factor in antimicrobial effectiveness, with longer and more unsaturated chains being particularly potent. Marine algae-derived fatty acids target the fatty acid metabolism of bacterial cells, thereby inhibiting the electron transport chain and oxidative phosphorylation in cell membranes. This leads to the formation of peroxidation and auto-oxidation degradation products and cellular lysis (Guedes *et al*. 2011). Extracts from *Planktochlorella nurekis* contain long-chain fatty acids and are potent inhibitors of several bacteria, including *Campylobacter jejuni*, *E. coli*, *Salmonella enterica*, *Arcobacter butzleri*, and *Lactobacillus johnsonii* (Cermak *et al*. 2015).

Lysozyme enzymes digest bacterial cell walls, while polyphenols disrupt the cell membrane and inhibit enzymes, deprive substrates, and bind to adhesins. Tolpeznikaite *et al*. (2021) reported the efficacy of *Cladophora rupestris* extracts against *Staphylococcus hemolyticus*. According to Yuvaraj *et al*. (2011), *C. glomerata* has potential as a source of natural antimicrobial compounds, with a particular effect against *Vibrio* species, *E. coli*, and *B. cereus*. The study also hypothesised that long-chain hydrocarbons were potential bioactive substances which could be used in pharmaceutical preparations. Stabili *et al*. (2014) suggest that linolenic acid, which is abundant in *C. glomerata*, could be responsible for its antimicrobial activity against *Vibrio* species.

Antibiotics are reported to be more effective against gram-positive bacteria. Gram-negative bacteria, characterised by a thin layer of peptidoglycan with an outer membrane containing lipopolysaccharides, are more complex than gram-positive bacteria, possessing multilayered membranes which act as a barrier. The outer membrane, containing porins which allow passage for small molecules acts as a protective barrier for gram-negative bacteria against substances such as antibiotics, detergents, and enzymes (Bhowmick *et al*. 2020).

Ulvans, sulfated polysaccharides sourced from species of *Ulva*, exhibit antimicrobial, anticoagulant, anti-

oxidant, anticancer, and antihyperlipidemic properties. They typically exist as tightly linked covalent bonds within algal cell walls, where their structure is determined by their molecular weight and degree of sulfation. Higher sulfation and lower molecular weight ulvans display more activity against gram-positive bacteria than gram-negative bacteria (Besednova *et al*. 2020). Santoyo *et al*. (2009) demonstrated the antibacterial activity of *Haematococcus pluvialis* extracts against *S. aureus* (gram-positive), primarily attributed to membrane disruption caused by butanoic and methyl lactic acids. This disruption is caused by the acidic nature of the compounds, which disrupts the bacterial cell membrane. Further research is needed to identify and characterise novel compounds, as well as to understand their mode of action, toxicity, and pharmacokinetics.

Antifungal activity

A few plausible explanations regarding the mechanism underlying antimycotic compounds in green algae have been derived from their chemical structure and biological activity. The mechanisms through which green algal compounds operate differ depending on the type of compound, microorganism, and host cell involved. Phlorotannins are polyphenolic compounds which are present in some green algae such as species of *Ulva* and *Codium*. They possess the ability to inhibit the growth of fungal pathogens (*Candida*, *Aspergillus*, and *Fusarium* species) by disrupting cell wall synthesis and membrane integrity. Algae from the *Ulva* and *Enteromorpha* genera have been found to contain sulfated polysaccharides, which are complex carbohydrates possessing antifungal properties against various fungal pathogens. They operate by binding to fungal cell surface receptors, disrupting membrane permeability, and triggering apoptosis (Alam *et al*. 2021).

Fatty acids isolated from species of *Cladophora* inhibit the growth of fungal pathogens such as *Botrytis*, *Fusarium*, and *Colletotrichum* by altering the fluidity of the fungal membrane, interrupting electron transport, and disrupting oxidative phosphorylation. In addition to inhibiting germ tube formation, algal metabolites also reduce yeast attachment to host cells, thus reducing their pathogenicity (Nazzaro *et al*. 2019).

Antiviral activity

Compounds derived from green algae may prove to be promising candidates for the development of novel antiviral drugs since they are natural, structurally diverse, exhibit low toxicity, and are environmentally sustainable. In addition to interfering with viral attachment, entry, replication, and release, green algal compounds may also modulate host immune responses, inflammation, and oxidative stress, which are involved in viral infection. Viruses can change their genetic composition independently in response to interactions with treatment strategies, which in turn may lead to drug resistance.

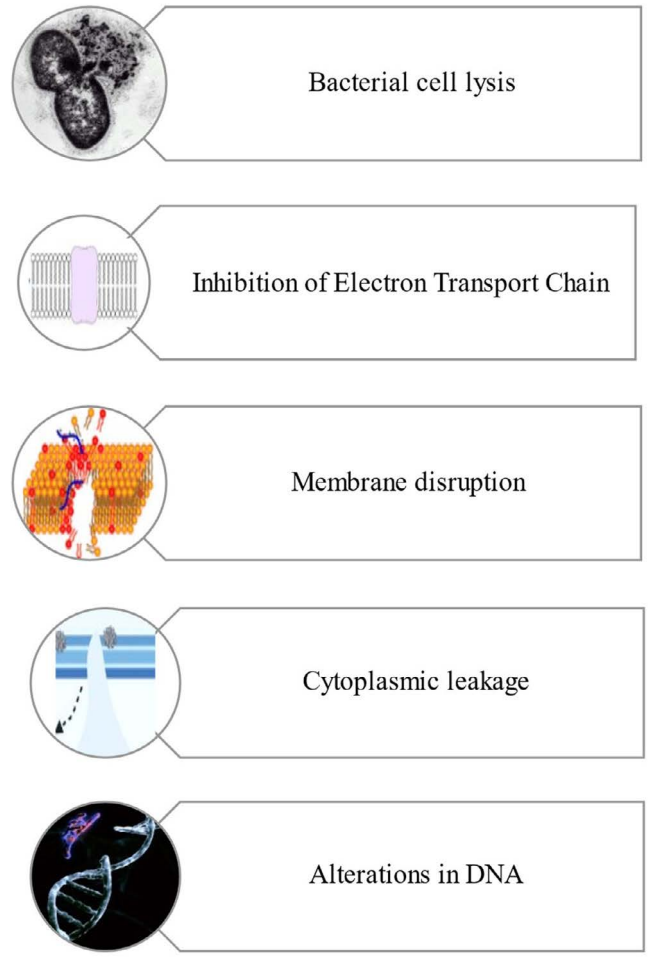

Fig. 2. Mechanisms of action of antimicrobial compounds.

Algae have been reported to contain antiviral compounds such as lectins and sulphated polysaccharides which can boost immunity. *Caulerpa cylindracea* develops immunity against *Vibrio* species, which has been integrated into therapeutics and is currently being evaluated for its use as an antiviral agent (Rizzo *et al*. 2016). In order to optimise extraction, purification, characterisation, formulation, delivery, and clinical evaluation, further detailed *in vivo* toxicological research is required. Sulfated polysaccharides produced by *Caulerpa* species interfere with the binding of the receptors on the cell surface and can inhibit the entry of the herpes simplex virus type 1 (HSV-1) and the human immunodeficiency virus type 1. In the case of HIV-1 replication, caulerpin, an extract from *C. racemosa*, inhibits reverse transcriptase, an enzyme essential for the replication of retroviruses. Similarly, Kahalalide F from *Bryopsis* spp. inhibits the production of hepatitis C virus infectious particles. By influencing the processing and trafficking of viral proteins, caulerpenyne from *Caulerpa taxifolia* is capable of inhibiting HIV-1 assembly and release. By

altering the composition of the lipids in the membranes of the host, halovirs from *Halimeda discoidea* are also able to inhibit the assembly and release of the dengue virus (Faisal *et al*. 2023).

Polysaccharides, polyphenols, pigments, peptides, and proteins produced by green algae have different antiviral mechanisms. Viral attachment and entry can be inhibited by polysaccharides, while viral replication and assembly can be restricted by polyphenols. Green algae have demonstrated antiviral activities against several viruses including the herpes simplex virus, the human immunodeficiency virus, the influenza virus, and the hepatitis C virus. Virus characteristics, such as the envelope, genome, and replication cycle, can affect antiviral efficacy (Afzal *et al*. 2023).

Recent reports indicate worldwide interest in the antiviral potential of green algal metabolites against Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2). Astaxanthin, a carotenoid with anti-inflammatory and immunomodulatory properties, could alleviate cytokine storm, preventing acute respiratory distress syndrome (ARDS), a major cause of death in COVID-19. Phycocyanin, a pigment from *Spirulina* sp., inhibits NADPH oxidase and has anti-inflammatory activity, making it an effective candidate for adjuvant therapy in COVID-19 patients. Metabolites from algae of the *Ulva* and *Caulerpa* genera are being studied for antiviral activity, with promising outcomes. Caulerpin demonstrated high binding energies towards SARS-CoV-2 protein receptors (Moo-Puc *et al*. 2009). Although this field of study is still in its infancy, there is confidence that in the future algal species will be used as immunity boosters to reduce viral activity in humans as well as in COVID-19 prevention.

Extraction techniques for obtaining antimicrobial metabolites from green algae

Extraction techniques play a crucial role in the antimicrobial activity of green algal metabolites. The effectiveness of metabolites can vary based on the species of algae, the strain of the bacterial pathogen, and the solvent used to extract the metabolites. Different extraction procedures extract different metabolites with antimicrobial activity (Esquivel-Hernandez *et al*. 2016). A homogeneous ternary system of solvents such as ethanol-hexane-water in a 77:17:6 ratio was recently used to enhance the yield percentage and compound purity (Parniakov *et al*. 2015). Different extraction methods also play a crucial role in the extraction of bioactive compounds. Soxhlet extraction is typically employed for the extraction of thermo-stable compounds, while the cold extraction method is generally used for lipids and hydrocarbons. Carullo *et al*. (2018) reported that lipids, pigments, and polyphenols were extracted within a short period of time using Pulsed Electric Fields (PEF). When compared to the methods previously discussed, this method yielded higher extraction yields than traditional extraction techniques. Similarly, high-pressure homogenisation (HEH) methods have recently been employed in the disruption of microalgal cells to extract bioactive compounds. PEF is advantageous over HEH in that it induces the permeabilisation of cell membranes without creating any debris, thus enabling the selective extraction of carbohydrates and low molecular weight proteins (Scherer *et al*. 2019). Supercritical fluid extraction and subcritical water extraction are regarded as the most advanced techniques for the extraction of high-value bioactive compounds from algae due to their high selectivity, reduced extraction time, and minimal use of toxic organic solvents (Santana *et al*. 2012). Another novel method is ultrasound-assisted extraction, which generates shear solid forces on algal cell walls for the efficient extraction of polysaccharides such as carrageenans and alginate, pigments including fucoxanthin, chlorophylls, or β-carotene, and phenolic compounds. The advantage of this technique is that it requires less solvent and energy and preserves the integrity of the compounds (Carreira-Casais *et al*. 2021). Although polymers such as algaenan and sporopollenin from the microalgal cell wall are difficult to extract, microwave-assisted extraction employed for lipid extraction is efficient with a higher yield and purity of compounds (Kapoor *et al*. 2018). The efficient extraction of lipids and the quantification of fatty acids from algal biomass can be achieved with accelerated solvent extraction (Tang *et al*. 2016). These green extraction technologies are economic and environmentally friendly alternatives which avoid the use of dangerous chemical solvents. The role of extraction techniques in the antimicrobial activity of green algae metabolites is significant as it influences both the effectiveness of the metabolites and the potential for their development.

The role of extraction solvents in determining the efficacy of antimicrobial agents

Bioactive compounds differ in their response due to diverse chemical structures, polarities, and solubilities (Afzal *et al*. 2023). Polar and non-polar solvent extracts of algae have shown potential against pathogenic bacteria. Polar solvents like methanol and water, and non-polar solvents like benzene and hexane, extract phenolic and flavonoid compounds, respectively. Fundamentally, the ability of solvent systems to extract bioactive compounds determines the antimicrobial potential of algae (Al-Saif *et al*. 2014).

As algal compounds have varying chemical properties, solvent selection depends on the specific compound of interest. The efficiency of using organic solvents like chloroform for extracting hydroquinones, phenols, sesterpinoids, etc. is evident from the study carried out by Vijaya Rani *et al*. (2018) where metabolites from the filamentous algae *Rhizoclonium* sp. and *Pithophora*

sp. showed greater antibacterial activity against *S. aureus* when extracted using chloroform. The chloroform extracts of *Spirogyra rhizopus* displayed promising inhibitory action against *Salmonella typhimurium, P. aeruginosa, S. aureus,* and *E. coli* (Daniel *et al*. 2019). Furthermore, studies emphasise that the chloroform extracts of *Spirogyra quinina* and *Zygnema stellinum* are proven to be more effective than other extracts and exhibit activity against *Aeromonas hydrophila, B. subtilis, P. vulgaris, E. coli*, and *Candida albicans* (Mugilan & Sivakami 2016).

Petroleum ether, chloroform, and methanol extracts of *Codium decorticatum* are effective against *S. pneumoniae* and *K. pneumoniae* due to the presence of alkaloids. The study demonstrated the effectiveness of extracts of *C. decorticatum* when compared to traditional drugs such as gentamicin and ampicillin (Anbu *et al*. 2009). A sulfoquinovosyl diacylglycerol isolated from an *n*-butanol fraction of *C. racemosa* showed antiviral effects against the Herpes simplex virus type 1 (Wang *et al*. 2007).

Both methanolic and ethanolic extracts of *Oedogonium capillare* showed maximum inhibitory effects on gram-positive and -negative bacteria such as *S. aureus, K. pneumonia, E. coli,* and *P. aeruginosa* and fungi *C. albicans*, and *A. niger*. It is inferred that methanol may serve as a better solvent for extracting potent antimicrobial compounds (Adegoke *et al*. 2018). For instance, the methanolic and ethanolic extracts of *Halimeda tuna* exhibited greater activity against *A. niger* than the aqueous extracts. *Halimeda tuna* methanolic extracts were effective against *A. niger, A. flavus*, *A. alternaria*, *C. albicans*, and *Epidermophyton floccosum* (Indira *et al*. 2013). Methanolic extracts of *Ulva fasciata* are efficient against *S. aureus, B. subtilis*, *P. aeruginosa*, and *E. coli* (Fayzi *et al*. 2020). Algal extracts from *U. fasciata*, *Chaetomorpha antennina*, *Acrosiphonia orientalis*, and *Padina tetrastromatica* were tested for their antimicrobial properties against *E. coli*, *S. aureus*, and *S. pyogenes*. In particular, extracts of *A. orientalis* showed activity against 70% of the tested organisms (Santhanam *et al*. 2008).

Methanol-toluene mixtures (3:1 v/v) were most efficient in extracting antimicrobial compounds from fresh biomass (Prarthana & Maruthi 2019). Bio-oil and lipids can be extracted from algae using ethanol and cyclopentyl methyl ether (Santoro *et al*. 2019). The "best" solvent will depend on the specific bioactive compound, the type of algae, and the extraction method. The extraction process must be optimised for each specific case.

The advantages of using green algae as a source of antimicrobial agents

Despite being considered a waste product, algal biomass has the potential to yield bioactive compounds at a reasonable cost. With minimal inputs, commercial cultivation can be conducted in photobioreactors. Due to its hypersaline environment, *D. salina* naturally produces significant quantities of beta-carotene. Bioprocessors utilising *D. salina* regulate salinity to increase carotene production, which can then be extracted for human use (Borowitzka 2015). Recent research has focused on developing efficient and green technologies for extracting algal polyphenols. Polyphenols can be extracted using deep eutectic solvents as an eco-friendly alternative to ionic liquids. Polyphenols can be selectively extracted using these solvents due to their ability to form hydrogen bonds. By using agricultural and other industrial waste the cost of extraction can be dramatically reduced. Algal compounds are less expensive to extract and isolate because of these factors (Lemes *et al*. 2022). It is important to note, however, that the actual cost may vary based on the compounds being extracted as well as the extraction method. Researchers constantly seek economically viable, environmentally safe natural resources for continual drug development, such as algae, which possess numerous advantages. Algae grow quickly and adapt to different environments. Temperature extremes, high or low salinity, and osmotic stress are all conditions in which they are able to thrive. As with land plants, algae grow by converting sunlight and carbon dioxide into new matter. However, unlike land plants, algae grow more efficiently because they do not require support structures. The production of algae on non-arable land using non-potable water (brackish or seawater) complements rather than competes with traditional agriculture. Thus, the cultivation of algae does not deplete other agricultural resources. Finally, algae can reduce the carbon footprint of their production by sequestering CO_2 (Diaz *et al*. 2023).

Challenges

From the standpoint of sustainability and renewables at a lower cost, microalgae can help to bridge the gap between global population growth and climate change. Accessibility, availability, sustainable methods for extracting and purifying algal metabolites, the preservation of functional structure during and after extraction, safety, sensory quality, and formulation are some of the obstacles to algal utilisation. Concerns about their endangered status of certain plants have restricted the use of active drugs isolated from them. Alternatives include the utilisation of algae so initiatives to bring algal products into practice are important.

Incorporating bioactive compounds into packaging films may serve to increase drug efficacy. Prior to application, allergenicity tests should be carried out *in vitro*, *in vivo*, and *in silico*. It may also be possible to use drug synergism to enhance drug therapy where the combined effect is greater than expected (Tallarida 2011). The prevention of livestock and poultry diseases ensures the safety of microbes in animal products. This can be achieved by replacing in-feed antibiotics with green microalgae. Analysing alternative bioassay models is nec-

essary to maintain product safety, efficacy, and quality.

The contribution of algae to human pharmaceutics has great potential, but their acceptance largely depends on safety. Limitations include insufficient data on the safety and efficacy of microalgae-based ingredients and potential human allergic reactions, thus necessitating further research. The high nucleic acid concentration in the algal biomass can lead to negative health outcomes such as gout and kidney stones upon metabolism (Nethravathy *et al*. 2019). Algal cultivation and processing can pose health risks if not done safely and require constant monitoring.

Like other medications, algae-derived drugs can have side effects, including allergic reactions. While hydroxybenzoic acid esters (paraben), commonly found in cosmetic products, mimic estrogen and can increase breast cancer risks, toxic elements like cadmium and fucotoxins, defence compounds against herbivores and pathogens, should be avoided in algae-based food products. In contrast, natural compounds from algae have fewer side effects due to cytotoxic, chemosensitising, and synergistic interactions (Thiyagarasaiyar *et al*. 2020).

The development of drugs using algae is challenging (Pereira & Valado 2023). Local wild-type strains often yield suboptimal quantities of bioactive compounds of interest, limiting their industrial applications. Using recently developed biotechnological applications such as transcriptomics, nutrition genomics, metabolomics, proteomics, and metagenomics will facilitate the study of algal genomes and their pharmacological interactions with bacteria (Kumar *et al*. 2016). However, genetic engineering tools remain underutilised for microalgae compared to other microorganisms despite the fact that genetic engineering can overcome strain improvement limitations (Paull 2013). Increasing the growth rate and product synthesis of microalgae is a challenging and crucial issue as there are costs and limitations concerning production scale. Biomass production challenges include dewatering algae cultures, pre-treating biomass, and managing genetically engineered algae. The induction of oxidative stress (elicitation) has been less explored through physical techniques (pulsed electrical fields and ultrasonication) and abiotic environmental factors (PH, temperature, and light). In order to provide bioactive-rich biomass to consumers, an all-encompassing approach should be proposed. For elicitation techniques to be effective, investigations into metabolic pathways are needed. Clinical *in vivo* studies are necessary to determine whether their bioactive compounds are accessible, bioavailable, and effective upon consumption. Algal biomass can be processed sustainably using fermentation, ultrasonication, pulsed electric field, microwave, and enzyme-assisted methods. Researchers and algae-based industries should also collaborate effectively to gain a better understanding of industrial loopholes and consumer requirements.

CONCLUSION

The antimicrobial potential of algal extracts is significantly influenced by the extraction techniques and solvents used, the extracted compounds, the concentration of the extracts, habitat, harvesting period, and seasonal variations like temperature and the cellular metabolism of the algae. Algae are gaining more interest as a valuable source of bioactive compounds which can sustain human health owing to their antibacterial, antifungal, and antiviral activities. As a result of defence strategies to survive in competitive environments, algae produce numerous metabolites. Their effective antimicrobial potential has been demonstrated against various pathogenic microorganisms. Detailed studies are required to identify the bioactive compounds and the toxicological effects of the same in the human body, thereby initiating research for the substitute of conventional antibiotics. The mechanisms underlying the health benefits should be evaluated to further analyse their nutritional properties, which are necessary to fully achieve the total commercial potential of green algal technology.

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REZIME

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Istraživanje sekundarnih metabolita iz zelenih algi kao antimikrobnih agenasa: sveobuhvatan pregled

Elsa Shibu Sruthy i Edathiruthi Kottukkal Chandran Baiju

Tehnologija algi je omogućila brojne napretke u različiti oblastima, kao što su medicina, kozmetika i farmacija. Zelene alge (Chlorophyta) su grupa fotosintetskih organizama koji žive u vodenoj i kopnenoj sredini. Potencijalni su izvor bioaktivnih jedinjenja za lečenje i prevenciju širokog spektra zaraznih bolesti uzrokovanih mikroorganizmima. Ova jedinjenja takođe poseduju protivupalna, antioksidativna, antikoagulantna, antitumorska i imunomodulatorna svojstva. Proizvodi se širok spektar bioaktivnih jedinjenja, uključujući polisaharide, koji imaju antimikrobna svojstva; sposobni su uticati na ćelijske zidove, membrane i nukleinske kiseline mikroorganizama. Fluidnost membrane, propustljivost ili njena celovitost mogu biti pod uticajem polifenola i masnih kiselina koji hvataju slobodne radikale, heliraju metalne jone ili ometaju enzime i membrane. Proteini stvaraju pore unutar membrana koje se vežu na specifične receptore ili inhibiraju enzime. Prilagođavanje nepovoljnim uslovima životne sredine, kao što su temperaturni ekstremi, fotooksidacija, salinitet ili osmotski stres, rezultira stvaranjem bioaktivnih jedinjenja promenom fizioloških i biohemijskih puteva algi za održavanje ćelijske homeostaze. Sa promenom preferencija potrošača i povećanjem broja otpornih mikroorganizama, ključno je tražiti nova antimikrobna jedinjenja iz zelenih algi. Potraga za novim bioaktivnim jedinjenjima sa antimikrobnim svojstvima iz zelenih algi mogla bi biti alternativa povećanoj otpornosti mikroorganizama na lekove. *In vitro* i *in vivo* procene bezbednosti, efikasnosti i mehanizma delovanja antimikrobnih jedinjenja iz zelenih algi zahtevaju više istraživanja. Pružajući pregled ranijih napora u ovoj oblasti u nastajanju, ova studija pruža perspektive i rezime bioaktivnih jedinjenja odgovornih za antimikrobna svojstva ekstrakata zelenih algi.

Ključne reči: Chlorophyta, bioaktivna jedinjenja, antibakterijska otpornost, antifungalna otpornost, antiviralna otpornost, otpornost na lekove.

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