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Research Article

Determination of the Antibacterial Activity of Microalgae Isolated from Giresun Streams

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ABSTRACT

The rapid emergence of antibiotic resistance has become a global crisis, threatening public health, food security, and agriculture. Particularly, the control of zoonotic diseases and the assurance of microbial safety in animal products necessitate the development of new and sustainable solutions. In this context, research on antimicrobial agents derived from natural sources has been gaining significant importance. Microalgae, with their ability to synthesize bioactive compounds, represent a promising natural resource in this regard. Studies on the antibacterial properties of freshwater microalgae in Türkiye remain limited. However, the rich biodiversity of these ecosystems provides valuable opportunities for the discovery of novel antimicrobial agents. This study investigates the antibacterial activity of *Chlorococcum hypnosporum*, *Stichococcus bacillaris*, *Chlorella vulgaris*, *Chlorolilaea pamvotia*, and *Desmodesmus opoliensis* isolated from the Aksu, Batlama, and Büyük Güre streams in Giresun, Türkiye. Bioactive compounds were extracted using acetone, ethanol, and methanol, and their antibacterial effects were tested against five bacterial strains via the agar well diffusion method. Notably, the acetone extract of *Chlorella vulgaris* exhibited significant activity against *Bacillus subtilis* at 40 µl/petri, and also showed high antibacterial activity against *Escherichia coli*. Additionally, the ethanol extract of *Chlorococcum hypnosporum* exhibited antibacterial activity against both *Salmonella Typhimurium* and *Staphylococcus aureus*. Other microalgae species also demonstrated significant antibacterial properties against the tested bacterial strains. These findings enhance our understanding of the antibacterial potential of Türkiye's freshwater microalgae and highlight their potential as sustainable antimicrobial agents for ensuring microbial safety in animal products. This study further emphasizes the importance of microalgae as natural and environmentally friendly alternatives in combating antibiotic resistance and preventing agricultural microbial contamination.

Keywords: Microalgae, freshwater, molecular characterisation, antibacterial activity

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INTRODUCTION

The discovery of antibiotics marked a revolutionary step in medical science, enabling the effective treatment of various infectious diseases. However, prolonged and widespread use of antibiotics has led to the development of resistance by microorganisms. This resistance, driven by the evolutionary survival mechanisms of bacteria, progressively reduces the effectiveness of antibiotics (Salam et al., 2023). Antibiotic resistance has emerged as one of the most critical issues on the global agenda in recent years, not only due to its significant impact on public health but also because of the economic burden it imposes. The rapidly increasing rates of antibiotic resistance worldwide have profound implications for health, sustainable development, the economy, trade, and the stability of nations. It is anticipated that this issue will have far-reaching consequences in the years to come (Demyanyuk et al., 2023; Vanegas-Múnera

and Jiménez-Quiceno, 2020; Frieri et al., 2017; Lee Ventola, 2015).

The increasing threat of antibiotic resistance underscores the importance of developing alternative therapeutic strategies and discovering new antibiotics (Wright, 2014). In this context, the investigation of biologically active compounds derived from microorganisms in natural sources has become a significant area of research. Algae produce secondary metabolites in response to various environmental stress factors, such as high UV radiation, salinity, biofilm formation, thallus damage, and competition with other aquatic organisms (Kolácková et al., 2023). These metabolites play a critical role in the algae's survival and adaptation to stress conditions, and exhibit biological activities such as antibacterial, antifungal, and antioxidant properties (Little et al., 2021; Ördög et al., 2004). Among these metabolites, long-chain unsaturated fatty acids are particularly associated with the antibacterial activity of algae. These fatty acids target bacterial cell membranes, causing membrane damage, disrupting cellular respiration, and allowing intracellular contents to leak out (Saritha et al., 2018; Amaro et al., 2011; Borowitzka, 1995). The antibacterial potential of algae has gained prominence in recent years as a promising alternative in the fight against antibiotic-resistant bacteria. Studies have demonstrated that secondary metabolites derived from algae exhibit potent antibacterial effects against pathogenic bacteria (Bhowmick et al., 2020; Besednova et al., 2020). In this context, the biotechnological applications of algae have generated considerable interest, not only as antibacterial agents but also as potential biological sources for the pharmaceutical and cosmetic industries (Adhithya et al., 2022). Studies on the antibacterial potential of algae have demonstrated these organisms as promising alternatives in the fight against antibiotic-resistant bacteria. In this context, the biochemical properties of various algae species, particularly their antibacterial activities, have gained significant attention. This study investigates the antibacterial effectiveness of microalgae species such as *Chlorococcum hypnosporum*, *Stichococcus bacillaris*, *Chlorella vulgaris*, *Chlorolilaea pamvotia*, and *Desmodesmus opoliensis*.

Chlorococcum hypnosporum is a unicellular green microalga belonging to the Chlorophyceae family. The cell size of this species ranges from 21 to 20 microns, and the cell wall thickness is less than 0.50 microns. Cultured cells are always green (Lee, 1970). It is known that *Chlorococcum* produces astaxanthin, adonixanthin, cantaxanthin, β-carotene, lutein, and ketocarotenoids (Yuan et al., 2002; Zhang and Lee, 1999). Astaxanthin is a natural carotenoid that possess antibacterial activity (Karpinski et al., 2021; Shanmugapriya et al., 2018). *Stichococcus bacillaris* is a green soil microalga characterized by rod-shaped or cube-shaped cells and containing more than 14 strains (Neustupa et al., 2007). A study by Harder and Opperman (1953) reported that *Stichococcus bacillaris* exhibits antibiotic activity due to the fatty acids it contains. Nucleic acids, vitamins, minerals, amino acids, essential fatty acids, carotenoids, and enzymes are powerful sources of bioactive compounds that can be produced by *C. vulgaris*, which has great potential in various biological applications related to human health (Sarkar et al., 2021; Maadane et al., 2017; Rao et al., 2010). *Chlorolilaea pamvotia*, first isolated in Türkiye by Altürk Karaca and Soylu (2025), is recognized for its chloroplasts, which vary from cup-like to reticulate shapes, and its ability to accumulate lipid droplets and starch. Lortou and Gkelis (2023) investigated the antibacterial properties of *Chlorolilaea pamvotia* in their study and reported that this species exhibits significant antibacterial activity. *Desmodesmus opoliensis* consists of tapered, fusiform cells arranged in groups of 2, 4, or 8. The cell dimensions are 15 x 6 micrometers (Médard et al., 2018). *Desmodesmus* has significant potential for producing algal biomass rich in carbohydrates, vitamins, proteins, as well as micro and macro elements (Hosseini et al., 2020). Previous studies have reported the antibacterial properties of species such as *Chlorella vulgaris* and *Stichococcus bacillaris*, and the secondary metabolites produced by *Chlorococcum* and *Desmodesmus* have also been shown to exhibit antibacterial effects in response to environmental stress (Rinaldi et al., 2024; Hussein et al., 2018; Sivasubramanian, 2011).

The streams in Giresun are vital ecosystems in the Black Sea Region, providing rich biodiversity and ideal conditions for microalgae growth. Their nutrient-rich waters make them excellent sources for isolating microalgae with potential antibacterial and biotechnological applications. Studying these streams not only enhances our understanding of regional biodiversity but also supports sustainable utilization of their biological resources.

This study aims to evaluate the antibacterial activities of *Desmodesmus opoliensis*, *Chlorella vulgaris*, *Chlorococcum hypnosporum*, *Stichococcus bacillaris*, and *Chlorolilaea pamvotia*, isolated from water samples collected from the Batlama, Aksu, and Büyük Güre Streams in Giresun, against pathogenic bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Salmonella Typhimurium*, and *Enterococcus faecalis*. By investigating the antimicrobial potential of these microalgal species, the study seeks to provide valuable insights into their biological activities and contribute to the development of alternative strategies for managing bacterial infections. Additionally, the research aims to address global health challenges such as the decreasing efficacy of antibiotics and the growing threat of antibiotic resistance by identifying new biologically active compounds. Exploring the antibacterial properties of these microalgae could play a significant role in the discovery of novel therapeutic agents targeting both bacterial infections and resistant strains. The findings may support the development of effective and sustainable treatment strategies, aligning with global efforts to reduce reliance on antibiotics.

MATERIALS AND METHODS

Isolation of Algal Species

Water samples were collected from the benthic and pelagic zones of the Aksu, Batlama, and Büyük Güre Streams within the central district of Giresun Province using 1 L plastic bottles and transported to the laboratory. The microalgae samples collected are provided in Table 1.One milliliter of each water sample was inoculated into BG11 and Allen media solidified with 1% agar (Allen, 1984; Allen and Stanier, 1968). The culture plates were incubated at 26°C in a SANYO MLR 351 incubator under a light intensity of approximately 155 µmol/m²/s with a 12:12 light (L) pho-

toperiod. After one month, all distinct colonies formed on the agar plates were transferred using an inoculating loop to fresh solid media. This process was repeated until single-species isolates were obtained (Demiriz, 2008). The isolated species were then transferred to liquid media and left to grow under controlled incubation conditions.

Table 1. Streams of Microalgae Sample Collection.

Sample	Stream	Coordinates
A1	Aksu	40.561389 38.216111
A2	Aksu	40.561389 38.216111
A3	Aksu	40.561389 38.216111
B3	Batlama	40.909003 38.355779
BG2	Büyük Güre	40.915570 38.334224

Morphological Identification and Molecular Characterization of Algal Species

Samples were taken aseptically from the cultures grown in liquid media. Light microscopy and inverted microscopy were employed for species identification, and measurements were conducted using a micrometric eyepiece. The identification process utilized references such as *Freshwater Algae of North America* and the AlgaeBase database (Wehr and Sheath, 2003; Guiry and Guiry, 2023).

Light microscopy, although widely used for the identification of microalgae, makes it difficult to distinguish morphologically similar species, particularly those that are small or challenging to identify. Due to these limitations, molecular methods provide a more accurate and rapid means of identifying algal species. Ribosomal RNA gene sequencing is an effective technique that facilitates the phylogenetic identification of microalgae, especially for species that cannot be cultured. This method is widely used to assess microbial population diversity and provides more precise results compared to microscopic identification and culturing techniques. In this study, ribosomal RNA gene sequencing was employed for the molecular characterization of microalgal species. The primary goal was to accurately identify species that are difficult to define with light microscopy and cannot be cultured. The 18S rRNA gene sequencing served as an important tool in determining the phylogenetic relationships of microalgae, leading to a more accurate classification of the species. Molecular methods enable the reliable identification of species that may be overlooked in traditional microscopic examinations (Fawley et al., 2004; Fawley et al., 2005; Lewis and Lewis, 2005; Hoshina, 2014). DNA isolation and molecular identification of the algal species were conducted by BM Software Consulting and Laboratory Limited Company. DNA isolation was performed using the EURx GeneMATRIX Isolation Kit (EURx, Gdańsk, Poland) following the manufacturer's protocol.After DNA isolation, the quantity and purity of the obtained DNA were assessed through spectrophotometric measurements using a Thermo Scientific Nanodrop 2000 (USA) device. In the PCR procedure, target gene regions were amplified using the universal primers EukA and EukB for species identification. The primer sequences and PCR conditions are as follows:

EUK A (5′-AACCTGGTTGATCCTGCCAGT-3′),

EUK B (5′-GATCCTTCTGCAGGTTCACCTAC-3′).

The PCR procedure was carried out.First, an initial denaturation was performed at 95°C for 5 minutes. Then, 30 cycles were conducted with the following conditions for each cycle: denaturation at 95°C for 45 seconds, annealing at 57°C for 45 seconds, and extension at 72°C for 90 seconds. After the cycles, a final extension was performed at 72°C for 5 minutes, and the reaction was completed by cooling to 4°C. The amplification results obtained by PCR (kyratec thermocycler) were run on a 1.5% agarose gel prepared with 1x TAE buffer at 100 volts for 90 minutes, and the gel was stained with ethidium bromide and visualized under UV light. A single-step PCR was performed to amplify the approximately 1800 base pair region. PCR reactions were carried out with Solis Biodyne (Estonia) FIREPol® DNA Polymerase Taq polymerase enzyme.Results were evaluated using the NCBI-BLAST program.

Preparation of Algal Extracts

The species *Desmodesmus opoliensis* (P.G. 16 Richter) E. Hegewald, *Chlorella vulgaris* Beijerinck, *Chlorococcum hypnosporum* R.C. Starr 1955, *Stichococcus bacillaris* Nägeli, and *Chlorolilaea pamvotia* (Lortou & Gkelis) Lortou & Gkelis were subjected to centrifugation at 8000 rpm for 5 minutes to concentrate the algal cells. The resulting algal pellets were then dried in an oven at 65°C for 24 hours to remove excess moisture.

One gram of dried algal biomass was extracted with 10 mL of ethanol, methanol, and acetone to evaluate their efficiency in extracting bioactive compounds. The extraction was carried out in a water bath at 50°C for 48 hours to ensure optimal conditions for the release of bioactive compounds (Vehapi et al., 2018; Kumar et al., 2023). Following extraction, the samples were filtered using Whatman No. 1 filter paper to remove any solid residues. Subsequently, the solvents were evaporated in an oven for 2 hours under dark conditions to isolate the extracted bioactive compounds, making the samples suitable for further analysis (Bennour et al., 2020; Foerster et al.,2023). This procedure ensured that the extracts were free of solvents and prepared for subsequent testing.

Determination of Antibacterial Activity of Algal Extracts

The bacterial strains used in this study included *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6051), *Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 29212), and *Salmonella enterica Serovar Typhimurium* (ATCC 14028). The antibacterial activity of the algal extracts was assessed using the agar well diffusion method (Perez et al., 1990). Bacteria were pre-incubated on Nutrient Agar (NA) at 37°C overnight. A single colony from each bacterial strain was inoculated into 5 mL of Nutrient Broth and incubated at 37°C for 18 hours. These bacterial cultures were spread onto NA plates using sterile cotton swabs. Wells with a diameter of approximately 6 mm were created on

the NA plates, and 20 µL or 40 µL of algal extracts were added to each well. Plates were left at +4°C overnight to allow diffusion. Negative controls included acetone, methanol, and ethanol. After 24 hours of incubation at 37°C, the inhibition zones around the wells were measured using a millimetric ruler. The study was conducted under aseptic conditions and repeated three times.

RESULTS AND DISCUSSION

Morphological and Molecular Characterization of Algae

In this study, the morphological and molecular characterization of five isolated algal species was conducted (Table 1). The molecular characterization of the isolated microalgae species revealed that *Chlorococcum hypnosporum* (A1) was identified with 100% similarity. Additionally, *Chlorella vulgaris* (A3) showed 99.90% similarity, *Chlorolilaea pamvotia* (B3) showed 97.39% similarity, and *Desmodesmus opoliensis* (BG2) showed 99.55% similarity based on 18S rRNA analysis. Owing to the unsuccessful PCR amplification, the characterization of *Stichococcus bacillaris* (A2) was performed only morphologically (Table 2).

Determination of Antibacterial Activity Using the Agar Well Diffusion Method

The antibacterial effects of *Chlorococcum hypnosporum* (A1), *Stichococcus bacillaris* (A2), *Chlorella vulgaris* (A3), *Chlorolilaea pamvotia* (B3), and *Desmodesmus opoliensis* (BG2) against *Bacillus subtilis*, *Enterococcus faecalis*, *Salmonella Typhimurium*, *Staphylococcus aureus*, and *Escherichia coli* were investigated. In this study, solvents based on acetone, ethanol, and methanol were used to prepare the algal extracts. The extracts were tested on NA medium at concentrations of 20 µL/petri and 40 µL/petri.

Table 3 presents the antibacterial activity of acetone, ethanol, and methanol extracts from *Chlorococcum hypnosporum* at concentrations of 20 µL/petri and 40 µL/petri against the test bacteria.In our study, the ethanol extract obtained from *Chlorococcum hypnosporum* at a dosage of 40 µL/petri dish exhibited the highest antibacterial activity against *Bacillus subtilis* ATCC 6051. Both ethanol and methanol extracts at 40 µL/petri dish demonstrated antibacterial activity against *Escherichia coli* ATCC 25922, while acetone, ethanol, and methanol extracts at 20 µL/petri dish did not show any antibacterial activity against the same bacterium. Additionally, Staphylococcus aureus ATCC 25923 exhibited resistance to the methanol extract, whereas it was inhibited by both the acetone and ethanol extracts. In the study conducted by Elshobary et al. (2020), which investigated the antibacterial activity of *Chlorococcum minutum*, it was found that the acetone extracts exhibited the highest antibacterial activity, inhibiting the most sensitive pathogens, while methanol and ethanol extracts showed lower activity. In contrast, in our study, the ethanol extract of *Chlorococcum hypnosporum* demonstrated high antibacterial activity against *Bacillus subtilis* ATCC 6051. While acetone extracts generally exhibited higher activity in the study by Elshobary and colleagues (2020), our study observed that the ethanol extract was more effective. These differences may be attributed to variations in the biochemical composition of the microalgae species used, the diversity in extraction methods. Elshobary et al. employed sequential extraction techniques, while our study utilized a single solvent extraction method. This could be a significant factor influencing antibacterial activity. Furthermore, the varying susceptibilities of bacterial strains and the polarity of solvents may also contribute to these differences.

Table 4 shows the antibacterial effects of 20 µL/petri and 40 µL/petri acetone, ethanol, and methanol extracts obtained from *Stichococcus bacillaris* against the test bacteria.It was determined that the *Salmonella Typhimurium* ATCC 14028 bacterial strain was resistant

Table 2. Sample Code Sequencing Data Results and BLAST Results.

Sample Code	Sequencing Data Results	BLAST Results
A1	CCATGCATGTCTAAGTATAGTCCCTTATACTGCGAAACTGCGAATGGCTCATTA- AACAGTTATAATTTATTTGATGGTACTTACTACTCGGATAACCGTAGTAATTCTA- GAGCTAATACGTGCGCAAATCCCGACTTCTGGAAGGGACGTATTTATTAGATA- AAAGGCCAGCCGGGCTTTGCCCGACCTGCGTTGATTCATAATAACCATAC- GAATCGCATGGCCTTGTGCCGGCGATGTTTCAAATAAATATCTGCCCTAT- CAACTTTCGATGGTAGGATAGAGGCCTACCATGGTGGTAACGGGTGACG- GAGGATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCA- CATCCAAGGAAGGCAGCAGGCGCGCAAATTGCCCAATCCCGATTCGGGGAG- GCAGTGACAATAAATAACAATACCGGGCATTCAATGTCTGGTAATTGGAAT- GAGTACAATCTAAATCTCTTAACGAGGATCCATTGGAGGGCAAGTCTGGT- GCCAGCAGCCGCGGTAATTCCAGCTCCAAAAGCGTATATTTAAGTTGTTG- CAGTTAAAAGGCTCGTAGTCGAAACTCGGGTTCGGTCCAGCGGTCCG- CCTCTGGTGTGCACTGCTGGTACTGTTCCTTTCTGTCGGGGACGGGCTCCT- GGGCTTCATTGTCTGGGACCCGGGCTCGGCGAGGTTACTTTGAGTAAATT- AGAGTGTTCAAAGCAGGCGATCGCCCTGAATACATTAGCATGGAATAGCAC- GATAGGACTCTGGCCTATCTTGCTGGTCTGTAGGACCGGAGTAATGATTAA- GAGGGACAGTCGGGGGCATTGGTATTTCCGGGTCAGAGGTGAAATTCTTG- GATTCCGGAAAGACCATCCACTGCGAAAGCATTTGCCAAGGATGTTTTCATT- GATCAAGAACGAAAGTTGGGGGCTCGAAGACGATTAGATACCGTCGTAGTCT- CAACCATAAACGATGCCGACCAGGGATTGGCGGGCGTTCTTTTGATGAC	*Chlorococcum hypnosporum* 100 %
A2	Cannot be evaluated according to the sequencing analysis	

Table 2. Continued.

Sample Code	Sequencing Data Results	BLAST Results
A3	GTCTAAGTATAAACTGCTTTATACTGTGAAACTGCGAATGGCTCATTAAAT- CAGTTATAGTTTATTTGATGGTACTTACTACTCGGATACCCGTAGTAAATCTA- GAGCTAATACGTGCGTAAATCCCGACTTCTGGAAGGGACGTATTTATTAGATA- AAAGGCCGACCGGGCTTCTGCCCGACTCGCGGTGAATCATGATAACTTCAC- GAATCGCATGGCCTTGTGCCGGCGATGTTTCATTCAAATTTCTGCCCTAT- CAACTTTTGATGGTAGGATAGAGGCCTACCATGGTGGTAACGGGTGACG- GAGGATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCA- CATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACAGG- GAGGTAGTGACAATAAATAACAATACTGGGCCTTTTCAGGTCTGGTAATTG- GAATGAGTACAATCTAAACCCCTTAACGAGGATCAATTGGAGGGCAAGTCT- GGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTTAAGTTGCT- GCAGTTAAAAAGCTCGTAGTTGGATTTCGGGTGGGATCTGCCGGTCCGC- CGTTTCGGTGTGCACTGGCAGGGCTCACCTTGTTGCCGGGGACGGGCTCCT- GGGCTTCACTGTCCGGGACTCGGAGTCGGCGCTGTTACTTTGAGTAAATT- AGAGTGTTCAAAGCAGGCCTACGCTCTGAATACATTAGCATGGAATAACAC- GATAGGACTCTGGCCTATCCTGTTGGTCTGTAGGACCGGAGTAATGATTA- AGAGGGACAGTCGGGGGCATTCGTATTTCATTGTCAGAGGTGAAATTCTTG- GATTTATGAAAGACGAACTACTGCGAAAGCATTTGCCAAGGATGTTTTCATTA- ATCAAGAACGAAAGTTGGGGGCTCGAAGACGATTAGATACCGTCCTAGTCT- CAACCATAAACGATGCCGACTAGGGATCGGCGGATGTTTCTTCGATGACTCC	*Chlorella vulgaris* 99.90 %
B3	TCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGG- CAGCAGGCGCGC AAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAATAACGATACTG- GGCATTTTTGTCTGGTAATCGGAATGAGCACAACGTAAATACCTTAACGAG- TATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTC- CAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTC- GGGCGGCGCGTCGCGGTCTGCCCTTTGGGTACGTACTGCGCCCGCGTC- GCCTTGCTGCCGGGGACGCGCTCCTGGGCTTCACTGTCCGGGACGCG- GAGTCGGCGAGGTGACCTTGAGCAAACAGGAGTGTTCATAGCAAGCGCAC- GCTCTGAACTATTTAGCATGGAATCACGCGATAGGACTCTGGCCTATCCCG- GTGGTCTGTAAGACCGGAGTAATGATTAAGAGGGACAGTCGGGGGCAT- TCGTATTTCATTGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGAACTTCT- GCGAAAGCATTTGCCAGGGATGTTCTCATTGATCAAGAACGAAAGTTGGG- GGCTCGAAGACGATTAGATACCGTCGTAGTCTCACCCATAAACGATGCCG- ACTAGGGATCGGCGGGTGTTCCATTGAT	*Chlorolilaea pamvotia* 97.39 %
BG2	TCTAAGTATAAACTGCTTATACTGTGAAACTGCGAATGGCTCATTAAATCAGT- TATAGTTTATTTGGTGGTACCTTCTTACTCGGAATAACCGTAAGAAAATTA- GAGCTAATACGTGCGTAAATCCCGACTTCTGGAAGGGACGTATATATTAGATA- AAAGGCCGACCGGGCTCTGCCCGACCCGCGGTGAATCATGATATCTTCAC- GAAGCGCATGGCCTTGTGCCGGCGCTGTTCCATTCAAATTTCTGCCCTAT- CAACTTTCGATGGTAGGATAGAGGCCTACCATGGTGGTAACGGGTGACG- GAGGATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCA- CATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGATACGGG- GAGGTAGTGACAATAAATAACAATACCGGGCATTTCATGTCTGGTAATTG- GAATGAGTACAATCTAAATCCCTTAACGAGGATCCATTGGAGGGCAAGTCTG- GTGAACCAAGCAACGCAATGCTGTTGACGCCAGAGATAGTAGGGCAGTTG- CACTCTAGTGTACCTGTTACGCCTGCTAGTCGAGCAATCATTCAATTGAACGG- GCTGATTGCCGGCAAGACGACCTGGTACGGGGAAGGCCTTCAAGATCCACT- GGCTAATCCCGTGGCGAGCTTGCATAGGGTGACCTTTGTAAAGCCGTCGTAAC- GCACGCAAAGGCGTCGGCTGACTCACTGAAGTTGGCTTAAGGGACGTGCTA- ACCCCATCCGATGATAAAGGATGCTTGAAGCAATAGCACCCGTTCTGCAAAG- GCTTCAAGGGGCAATAGTGTGCTGAGGAGATGCTTCACACTGCTCGGTAT- TCAAGCATTGGAAACTCAATCTGTGGCGACTAGGCAAGCGTGACGACAATTA- AGCTCGCAAATCGCGATTCTTGGAATTGACGTCGTCGCTGCCTCGTACAGATC- GAATCCAGTGCGAAAGGGGCCAGCAGCCGCGGTAATTCCAGCTCCAATAG	*Desmodesmus opoliensis* 99.55 %

to the 40 µL/petri acetone extract obtained from *Stichococcus bacillaris*. Additionally, *Staphylococcus aureus* ATCC 25923 was found to be resistant to the 40 µL/petri methanol extract of *Stichococcus bacillaris*. No antibacterial activity was observed for the 20 µL/petri and 40 µL/petri acetone, ethanol, and methanol extracts of *Stichococcus bacillaris* against *Bacillus subtilis* ATCC 6051, *Enterococcus faecalis* ATCC 29212, and *Escherichia coli* ATCC 25922. Sivakumar et al. (2014) reported that the *Stichococcus bacillaris* Sia2011 strain produced high levels of lipids, suggesting its potential use in the pharmaceutical industry. Bozkurt (2019), in their thesis study, reported that the ethanol and methanol extracts of *Stichococcus bacillaris* demonstrated significant antimicrobial activity against *S. aureus*, while the methanol extract exhibited weak antibacterial activity against *E. coli*. In contrast, the ethanol extract showed no antibacterial activity against *E. coli*. These findings align with our study, which revealed resistance of *S. aureus* to the methanol extract and the lack of activity of the ethanol extract against *E. coli* for the same species.

Table 5 exhibits the antibacterial effects of 20 µL/petri and 40 µL/petri acetone, ethanol, and methanol extracts obtained from *Chlorella vulgaris* against the test bacteria.It was determined that the 20 µL/petri and 40 µL/petri acetone extracts obtained from *Chlorella vulgaris* exhibited antibacterial activity against *Bacillus subtilis* ATCC 6051. Additionally, only the 40 µL/petri acetone, ethanol, and methanol extracts of *Chlorella vulgaris* demonstrated antibacterial activity against *Enterococcus faecalis* ATCC 29212, while the 20 µL/petri acetone and methanol extracts showed no activity against this strain. *Typhimurium* ATCC 14028 exhibited resistance to the 40 µL/petri acetone extract of *Chlorella vulgaris*, with an inhibition zone diameter of 8 mm. Moreover, *Staphylococcus aureus* ATCC 25923 was resistant to the 40 µL/petri ethanol extract, forming a 6 mm inhibition zone, while the 20 µL/petri methanol extract had no antibacterial effect on this microorganism. The 40 µL/petri acetone extract of *Chlorella vulgaris* showed sensitivity to *Escherichia coli* ATCC 25922, producing a 14 mm inhibition zone. Syed et al. (2015), in their study investigating the antibacterial effects of ethanol, methanol, and chloroform extracts of *Chlorella vulgaris* against *Klebsiella* sp., *E. coli*, and *Bacillus* sp., reported that the ethanol extract exhibited high antibacterial activity against *E. coli*, while the methanol extract produced a lower inhibition zone. Similarly, Sukhikh et al. (2022) noted that the fatty acid content of *Chlorella vulgaris* contributed to its antibacterial effect against *Bacillus subtilis*.

Table 3. Antibacterial activity of 20 µL/petri and 40 µL/petri acetone, ethanol, and methanol extracts of *Chlorococcum hypnosporum* microalgae against test bacteria.

Extract	Microalgae doses	*B. subtilis*	*E. faecalis*	*S.Typhimurium*	*S. aureus*	*E. coli*
Acetone	20 µL/petri	4 mm	4 mm	-	5 mm	-
	40 µL/petri	7 mm	5 mm	-	3 mm	-
Ethanol	20 µL/petri	-	4 mm	3 mm	4 mm	-
	40 µL/petri	8 mm	6 mm	10 mm	10 mm	8 mm
Methanol	20 µL/petri	2 mm	-	-	-	-
	40 µL/petri	3 mm	2 mm	-	-	7 mm

Table 4. Antibacterial activity of 20 µL/petri and 40 µL/petri acetone, ethanol, and methanol extracts of *Stichococcus bacillaris* microalgae against test bacteria.

Extract	Microalgae doses	*B. subtilis*	*E. faecalis*	*S. Typhimurium*	*S. aureus*	*E. coli*
Acetone	20 µL/petri	-	-	-	-	-
	40 µL/petri	-	-	6 mm	-	-
Ethanol	20 µL/petri	-	-	-	-	-
	40 µL/petri	-	-	-	-	-
Methanol	20 µL/petri	-	-	-	-	-
	40 µL/petri	-	-	-	4 mm	-

Table 5. Antibacterial activity of 20 µL/petri and 40 µL/petri acetone, ethanol, and methanol extracts of *Chlorella vulgaris* microalgae against test bacteria.

Extract	Microalgae doses	*B. subtilis*	*E. faecalis*	*S. Typhimurium*	*S. aureus*	*E. coli*
Acetone	20 µL/petri	2 mm	-	-	2 mm	2 mm
	40 µL/petri	12 mm	4 mm	8 mm	3 mm	14 mm
Ethanol	20 µL/petri	4 mm	-	2 mm	3 mm	-
	40 µL/petri	6 mm	4 mm	3 mm	6 mm	5 mm
Methanol	20 µL/petri	3 mm	-	-	-	-
	40 µL/petri	12 mm	2 mm	2 mm	2 mm	7 mm

Table 6 shows the antibacterial effects of 20 µL/petri and 40 µL/petri acetone, ethanol, and methanol extracts obtained from *Chlorolilaea pamvotia* against the test bacteria. It was determined that the 40 µL/petri ethanol and methanol extracts obtained from *Chlorolilaea pamvotia* exhibited antibacterial activity against *Bacillus subtilis* ATCC 6051. The 20 µL/petri and 40 µL/petri acetone extracts of *Chlorolilaea pamvotia* showed no antibacterial activity against *Enterococcus faecalis* ATCC 29212, while both the 20 µL/petri and 40 µL/petri ethanol extracts demonstrated antibacterial activity against this microorganism. Specifically, the 40 µL/petri ethanol extract produced a 9 mm inhibition zone against *E. faecalis* ATCC 29212, indicating sensitivity to this extract. On the other hand, no extracts of *Chlorolilaea pamvotia* displayed antibacterial activity against Salmonella *Typhimurium* ATCC 14028 and *Escherichia coli* ATCC 25922. In the study conducted by Lortou and Gkelis (2023), it was reported that *Chlorolilaea pamvotia* exhibited antibacterial activity against *S. Typhimurium*. However, in our study, no antibacterial activity of this species against the same microorganism was observed. This discrepancy could be attributed to methodological differences. Specifically, factors such as the extraction method, the type of solvent used, genetic or phenotypic variations in the microbial strains tested, and experimental conditions (e.g., incubation time, pH, or temperature) may have influenced the results.Moreover, variations in the chemical composition of extracts obtained from *C. pamvotia* could result in differences in the quantity and diversity of active compounds, thereby affecting antibacterial activity. This highlights the importance of standardizing methods in such studies and underscores the significant influence of environmental and experimental conditions on the biological activities of microalgae.

Table 7 illustrates the antibacterial activity of acetone, ethanol, and methanol extracts (20 µL/petri and 40 µL/petri) derived from *Desmodesmus opoliensis* against the test bacteria The 40 µL/petri acetone, ethanol, and methanol extracts of the microalgae *Desmodesmus opoliensis* exhibited antibacterial activity against *Bacillus subtilis* ATCC 6051 and *Enterococcus faecalis* ATCC 2921. The 40 µL/petri acetone extract demonstrated antibacterial activity against *Salmonella Typhimurium* ATCC 14028. Additionally, the 40 µL/petri ethanol extract exhibited antibacterial activity against *Staphylococcus aureus* ATCC 25923. In the 20 µL/petri ethanol extract, antibacterial activity was observed only against *Staphylococcus aureus*, whereas in the 40 µL/petri ethanol extract, activity was recorded against *Bacillus subtilis*, *Enterococcus faecalis*, *S. aureus*, and *E. coli*. Hosseini et al. (2020) demonstrated in their study that *Desmodesmus* microalgae exhibit notable antibacterial activity, particularly when cultured under stress conditions.

The antibacterial effects of the solvents themselves were evaluated, and no inhibition zones were observed with acetone, ethanol, or methanol against *Bacillus subtilis* ATCC 6051, *Enterococcus faecalis* ATCC 29212, *Salmonella Typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 25923, and *Escherichia coli* ATCC 25922. Similarly, in the study conducted by Demiriz (2008), the antibacterial effects of methanol, ethanol, n-butanol, acetone, hexane, and 0.5M Tris-HCl solvents on test bacteria were evaluated, and it was concluded that none of these solvents exhibited antibacterial activity against *Salmonella enteritidis* ATCC 13076, *Escherichia coli* O157:H7, *Staphylococcus aureus* ATCC 19213, and *Bacillus subtilis* ATCC 6633.Both studies highlight the ineffectiveness of solvents and algal extracts at low doses against the tested bacteria. The findings suggest the need for a more comprehensive investigation of antibacterial activity involving various solvent types and microalgal metabolites.

Consequently, the variation in the inhibitory activity of each isolated algal species against Gram-positive and Gram-negative bacteria is believed to be related to the antibacterial com-

Table 6. Antibacterial activity of 20 µL/petri and 40 µL/petri acetone, ethanol, and methanol extracts of *Chlorolilaea pamvotia* microalgae against test bacteria.

Extract	Microalgae doses	*B. subtilis*	*E. faecalis*	*S. Typhimurium*	*S. aureus*	*E. coli*
Acetone	20 µL/petri	-	-	-	-	-
	40 µL/petri	-	-	-	-	-
Ethanol	20 µL/petri	-	2 mm	-	-	-
	40 µL/petri	3 mm	9 mm	-	2 mm	-
Methanol	20 µL/petri	-	-	-	-	-
	40 µL/petri	2 mm	5 mm	-	2 mm	-

Table 7. Antibacterial activity of 20 µL/petri and 40 µL/petri acetone, ethanol, and methanol extracts of *Desmodesmus opoliensis* microalgae against test bacteria.

Extract	Microalgae doses	*B. subtilis*	*E. faecalis*	*S. Typhimurium*	*S. aureus*	*E. coli*
Acetone	20 µL/petri	2 mm	-	-	-	-
	40 µL/petri	3 mm	2 mm	2 mm	2 mm	-
Ethanol	20 µL/petri	-	-	-	2 mm	-
	40 µL/petri	4 mm	4 mm	-	2 mm	4 mm
Methanol	20 µL/petri	4 mm	-	-	-	-
	40 µL/petri	4 mm	2 mm	-	2 mm	-

pounds, specifically secondary metabolites, which are present in varying concentrations in each algal species and influence the mechanism of action against the bacteria. In this study, it was concluded that as the doses of the extracts prepared for the tested bacteria increased, the antimicrobial activity also increased. Vehapi (2016) reported that with the increase in concentration of all microalgae extracts, there was an observed increase in the inhibition rates against Gram positive; *Mycobacterium smegmatis* RUT and Gram negative; *Morganella morganii*, *Proteus mirabilis* BC 6624, *Aeromonas hydrophila* ATCC 7965.

CONCLUSION

This study highlights the antibacterial potential of green microalgae and emphasizes their biotechnological significance as natural alternatives to antibiotics. Specifically, the antibacterial activity exhibited by *Chlorococcum hypnosporum*, *Stichococcus bacillaris*, *Chlorella vulgaris*, *Chlorolilaea pamvotia*, and *Desmodesmus opoliensis* species against various pathogenic bacteria demonstrates their capacity to produce bioactive compounds with antibacterial properties. This finding reveals the potential of these microalgae as antimicrobial agents. For instance, the ethanol and methanol extracts of *Chlorococcum hypnosporum* and *Chlorella vulgaris* exhibit significant antibacterial activity against *Bacillus subtilis* and *Escherichia coli*, underscoring their potential as new therapeutic options in health-related applications.

Furthermore, this study points to the importance of microalgae as a biotechnological resource for combating antibiotic-resistant bacterial strains. In the face of rising concerns over antibiotic resistance, microalgae present a promising natural alternative. Species such as *Chlorococcum hypnosporum*, *Stichococcus bacillaris*, *Chlorella vulgaris*, *Chlorolilaea pamvotia*, and *Desmodesmus opoliensis* offer considerable promise for the development of alternative therapeutic agents and natural preservatives.

Species like *Chlorella vulgaris*, *Desmodesmus opoliensis*, and *Stichococcus bacillaris* are well-adapted to diverse environments, from freshwater to marine habitats, making them ideal for biotechnological applications. Their genetic diversity and environmental adaptability enhance their suitability for large-scale industrial production. For example, the antibacterial properties of *Chlorella vulgaris* and its ability to thrive in various conditions make it an excellent candidate for use in the pharmaceutical and food safety sectors. Similarly, the antibacterial activities and environmental resilience of *Desmodesmus opoliensis* and *Stichococcus bacillaris* support their potential in sustainable biotechnological processes.

In conclusion, this study demonstrates that green microalgae possess significant antibacterial potential and could be used as natural alternatives to antibiotics in preventing zoonotic diseases and ensuring the microbial safety of animal products. Given their ability to produce bioactive compounds, microalgae warrant further research for the discovery of new antimicrobial agents and expanded biotechnological applications. The results of this study strongly support the notion that microalgae represent a promising resource for the development of alternative antimicrobial solutions.

Conflict of Interest: The authors have no conflicts of interest to declare.

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