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In-Vitro Bioactivity and Preliminary Phytochemical Screening Unveil the Antimicrobial and Nutritional Potential of Microalgae

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Abstract

Microalgae represent a diverse group of photosynthetic organisms known for their capacity to biosynthesize a wide spectrum of bioactive compounds with significant pharmaceutical potential. In the present study, twelve microalgal isolates were collected from various ecological niches across Meghalaya, India. The isolates were taxonomically identified through morphological characterization and 18S rDNA gene sequencing. Phytochemical profiling confirmed the presence of diverse bioactive constituents, including alkaloids, tannins, saponins, flavonoids, terpenoids, steroids, phenolics, and glycosides. Antioxidant potential was assessed using ABTS, FRAP, and DPPH radical scavenging assays, with *Parachlorella kessleri* exhibiting the highest antioxidant activity. Quantitative analysis further revealed that *Parachlorella kessleri* and *Chlamydomonas reinhardtii* possessed the highest total phenolic and flavonoid contents, respectively, whereas *Desmodesmus abundans* exhibited the highest carotenoid content. The in vitro antimicrobial screening against three bacterial pathogens demonstrated that *Chlorococcum chlorococcoides* is very effective in suppressing the growth of *Escherichia coli* and *Klebsiella pneumoniae*, while *Asterarcys quadricellulare* showed the highest zone of inhibition against *Staphylococcus aureus*. Furthermore, fatty acid methyl ester (FAME) analysis revealed a diverse composition of saturated, monounsaturated, and polyunsaturated fatty acids, such as myristic acid, myristoleic acid, erucic acid, 11,14-octadecadienoic acid, and heneicosapentaenoic acid. Overall, these findings underscore the biotechnological potential of microalgae from specific places of Meghalaya as a sustainable source of bioactive compounds for applications in the pharmaceutical, nutraceutical, and related industries.

Keywords: Phytochemicals; Antioxidants activity; Antimicrobial activity; Fatty acid methyl esters

Introduction

The escalating global health crisis, characterized by the emergence of multidrug-resistant pathogens, necessitates the discovery of novel antimicrobial agents. Infectious diseases prevalent nowadays have developed tolerance and resistance to most commercially available drugs resulting in substantial increases in healthcare costs (Da Silva et al., 2017). Alarmingly, drug discovery has slowed dramatically over the past few years and most clinical isolates of *Staphylococcus aureus*, *Streptococcus pyogenes* and *Mycobacterium tuberculosis* are considered highly resistant to most known antibiotics (Duin et al., 2016). Most of the antibiotics that we use today were discovered during the period spanning the 1940s to 1950s and natural products are the main source accounting for 70% of the total antibiotics (Demain, 2009). While traditional sources of antibiotics, such as

bacteria, fungi, and plants, have been extensively explored, microalgae represent a promising alternative (Ravindran et al., 2016). These photosynthetic microorganisms have evolved diverse mechanisms to survive in various environments, often exposed to pathogenic microbes. To survive, they had to develop tolerance or defense strategies that modulate metabolic pathways in response to environmental conditions resulting in a high diversity of compounds (Falaise et al., 2016). As a result, they produce a wide range of bioactive compounds with antimicrobial properties (Markou and Nerantzis, 2013). Several studies have highlighted the antimicrobial potential of microalgae-derived compounds. For instance, Michelon et al. (2021) demonstrated the antimicrobial activity of *Chlorella* sp. extract against Gram+ve and multidrug-resistant bacteria, attributing this effect to fatty acids in their LCMS profile. Similarly, eicosapentaenoic acid from *Phaeodactylum tricornutum* and linolenic acid from *Chlorococcum* sp. have been shown to exhibit antimicrobial properties against MRSA (Desbois et al., 2009 and Ohta et al., 1994). In addition, antimicrobial activities of hexadecanoic acid and 9,12-octadecadienoic acid from *Chlorella* sp. against Gram +ve and Gram – ve bacteria were also reported (Shaima et al., 2022). The antagonistic behavior of microalgal compounds is not limited to fatty acids alone. Compounds from various chemical classes, including diterpenes, terpenoids, and alkanes, have also been reported to exhibit antimicrobial activity (Ismail and Elkomy, 2022). For instance, phytol, a diterpene, demonstrates antimicrobial, anticancer, and antioxidant properties (Islam, 2018). Eicosane, an alkane, has shown anticancer, antifungal, antibacterial, and cytotoxic effects (Hussein et al., 2020). Additionally, isolated amino acids and steroid compounds extracted from Ulva sp. have been reported to possess antimicrobial activity against Gram +Ve and Gram -Ve bacteria (Yohannan et al., 2023).

Microalgae derived fatty acids also offer numerous health and nutrition benefits. They are rich in fatty acids like eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and other types of polyunsaturated fatty acids (PUFA). These fatty acids are therapeutic and have recently drawn attention in clinical and epidemiological investigations. PUFA are known for their clinical effect against cardiovascular diseases, blood coagulation, nervous system functions, and hypertension reduction (Maltsev et al., 2021). As well, microalgae are emerging as a sustainable source of biofuels. Their ability to accumulate high levels of lipids makes them attractive for biodiesel production (Zhang et al., 2022).

In recent years, there has been growing interest in natural antioxidants due to concerns about the potential health risks associated with synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxyltoluene (BHT). Microalgae are a rich source of natural antioxidants, including carotenoids, polyphenols, and polysaccharides. Catechin, epicatechin, gallic acid, and vanillic acid are natural antioxidants isolated from microalgae with better radical scavenging activity than BHA and BHT (Santiago-Díaz et al., 2021). These compounds have been shown to possess potent antioxidant properties, protecting cells from oxidative damage and reducing the risk of various diseases (Wei et al., 2013). A study conducted by Tannin-Spitz et al. (2005) shows that Water-soluble antioxidants sulphated polysaccharides discovered from microalgae *Porphyridium* can reduce the auto-oxidation of linoleic acid and shield 373 T lymphocytes from oxidative damage. In neuroblastoma SH-SY5Y cells, Bermejo-Bescos investigated the impact of spirulina extract on iron-induced oxidative stress (Bermejo-Bescos et al., 2011). Nacer et al. (2019) showed that *Nannochloropsis gaditana* has anti-diabetic effects by decreasing lipid peroxidation and raising catalase, GSH, and SOD activity. Different classes of vitamins, polyphenols, carotenoids, fatty acids, polysaccharides, phycobiliproteins, and peptides were also reported from these microorganisms with antioxidant activity (Ampofo, 2022).

Given their diverse bioactive properties and potential for sustainable production, microalgae represent a promising avenue for the development of novel therapeutic agents and functional foods cost-efficiently. Meghalaya, with its diverse ecosystems, offers a rich source of microalgal biodiversity. This study aims to explore the potential of Meghalaya's microalgae by identifying and characterizing isolates with antimicrobial, antioxidant, and nutraceutical properties.

Materials and methods

Isolation and culture conditions

Water samples were collected from the Jaintia, Ri-Bhoi, West Khasi Hills, and East Khasi Hills districts of Meghalaya. These samples were enriched using BG11 medium following the methodology described by Mandal et al. (2018). After visible growth, serial dilutions were performed and plated on 0.8% sterile agar plates to obtain isolated colonies. Single colonies were further purified through the streak plate method and transferred to 100 mL Erlenmeyer flasks containing fresh culture medium. Cultures were incubated at 25 ± 1°C under a 16:8-hour light-dark

cycle with a light intensity of 50 µmol photons $m^{-2}s^{-1}$. To ensure uniform cell distribution, cultures were gently shaken 3-4 times daily. Regular microscopic examination was conducted to maintain the purity of the cultures.

PCR amplification and phylogenetic analysis

Microalgae and cyanobacteria were harvested during the exponential growth phase (10-14 days) and subjected to DNA extraction using a plant genomic DNA extraction kit (Himedia) following the manufacturer's protocol. DNA concentration was determined spectrophotometrically by measuring absorbance at 260 nm.

Polymerase Chain Reaction (PCR) was employed for the amplification of the 18S rDNA regions. For the amplification, the universal primers EU328f-5'-ACCTGGTTGATCCTGCCAG- 3' and Chlo02r-5'-CTTCGAGCCCCCAACTTTC-3' were used (Mandal et al. 2018). The PCR conditions involved an initial denaturation step at 95°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 52°C for 2 minutes, and extension at 72°C for 3 minutes. A final extension step at 72°C for 10 minutes was included.

The PCR amplicons were separated on a 1.2% agarose gel and purified using a commercial PCR purification kit (Himedia). Sanger sequencing was performed by Eurofins Genomic India Pvt. Ltd., and the resulting sequences were edited using BioEdit 7.2 software. Species identification was carried out through BLAST analysis by comparing the sample sequences against the GenBank database. The best matches were selected based on alignment score, query cover, and identity percentage. A phylogenetic tree was constructed using MEGA v7 software, employing the neighbor-joining method to visualize the evolutionary relationships among the identified sequences (Schuelter et al., 2019).

Extract preparation

The microalgal biomass was extracted using a modified version of the method described by Nainangu et al. (2020). Briefly, the freeze-dried biomass was repeatedly homogenized with a methanol solvent mixture using a mortar and pestle. The homogenate was incubated overnight at room temperature with shaking. Subsequently, the mixture was centrifuged at 10,000 rpm for 15 minutes. This extraction process was repeated until the supernatant became colorless. The pooled supernatants were then dried using a rotary evaporator. The final crude extract was dissolved in DMSO for further bioactivity assays.

Phytochemical screening

Preliminary phytochemical screening was carried out according to the method described by Santhi et al. (2016) and Abdel-Karim et al. (2019).

Test for alkaloids Two drops of wagner's reagent were added to 3mL of extract mixed with 3 ml of 1% HCl. The formation of a brown/reddish brown precipitate indicates the presence of alkaloids.

Test for tannins Equal volume of extract was mixed with water and heated on a water bath. The mixture was filtered and few drops of ferric chloride were added to the filtrate. Formation of dark green colour indicates the presence of tannins.

Test for saponins In a test tube, 1 mL of distilled water is combined with 1 mL of extract and shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for flavonoids 1 mL of 2N NaOH solution was added to 1 mL of extract. The formation of a yellow precipitate was taken as a positive test for flavonoids.

Test for terpenoids 2 mL of extract were dissolved in 2 mL of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. An appearance of reddish brown colour in the inner face indicates that the presence of terpenoids.

Test for steroids 2 mL of extract were dissolved in 2 mL of chloroform and 2 mL concentrated sulphuric acid were added. The presence of steroids was determined by the formation of red colour in the lower chloroform layer.

Test for phenols To 1mL extract, 3-4 drops of 10% ferric chloride solution were added. Formation of dark green colour indicates the presence of phenols.

Test for glycosides using Keller-Kiliani test In a test tube, 2 mL of extract were dissolved in 2 mL of glacial acetic acid containing one drop of $FeCl_3$ solution. The mixture was then poured into a test tube containing 1 mL of concentrated H_2SO_4. A brown ring at the interphase indicates the presence of glycoside.

Proximate composition

Carbohydrate estimation

The estimation of carbohydrates was carried out according to the method described by Chanu (2020). In a test tube, 1.25 mL double distilled water was mixed with 1mg sample. To 1.25 ml blank/standard/sample solution, 4.0 mL of anthrone reagent was added and placed in a boiling water bath for 10 min. The absorbance of the supernatant was taken at 620 nm and glucose was used as the standard for calibration.

Lipid estimation

The estimation of Lipids was done based on the method described by Gwak et al. (2014). In a mortar and pestle, 100mg of dried cell pellet was grinded and mixed with a solution of chloroform: methanol: water (2:2:1, v/v) and vigorously agitated. Through centrifugation at 3000rpm for 5 mins, phase separation was achieved and the lower organic phase was collected and evaporated. The dry weights were measured as total lipid. Lipid content was expressed as % DCW (Dry Cell weight).

Protein estimation

Protein extraction was done using the alkali method by Rausch (1981). The sample was collected in a 2 mL eppendorf tube and centrifuged. The pellet was mixed with 1 mL of 0.5 N NaOH and extracted for 10 minutes at 80 °C while being stirred periodically. After cooling and centrifugation, the supernatant was transferred to new tubes. The alkali extraction was done three times. The final repeat was heated at 100 °C for 10 min for complete extraction of residual proteins. All three extractions were pooled and mixed well before analysis. The protein content was determined by the method described by Bradford using Bradford reagent (Barbarino, 2005). Bovine serum albumin (10 mg L^{-1}) was used as the standard for calibration.

Pigment composition

The estimation of carotenoid content was carried out according to the method described by Singh et al. (2022). Briefly, 2mL Samples were collected and centrifuged at 10000 rpm for 3min, the pellet was mixed vigorously with 2ml 99.9% methanol and incubated overnight in the dark at 4°C. The mixture was centrifuged (10000rpm, 5 mins) and the absorbance of the supernatant was taken at 470, 652.4, and 665.2 nm. The concentrations of different pigments were determined using the following formula (Lichtenthaler, 1987)

$$C_{Chlorophyll\ a} = 16.72\ x\ A_{665} - 9.16\ x\ A_{652}$$

$$C_{chlorophyll\ b} = 34.09\ x\ A_{652} - 15.28\ x\ A_{665}$$

$$C_{carotenoid} = (1000\ A_{470} - 1.63\ C_{\text{Chlorophyll a}} - 104.9\ \text{x}\ C_{\text{Chlorophyll b}})\ /\ 221$$

Total phenol content

Total phenolic content was determined by Folin-ciocalteu procedure following the method described by Abd El-Aty et al. (2014). In brief, 100 µL of the extract (1 mg/mL) was added to 400 µL of distilled water and mixed. To this solution, 150µL of Folin-ciocalteu reagent is added and incubated for 5 min. After incubation 500µL of sodium carbonate is added and kept at room temperature in the dark for 1 hr. Following incubation absorbance is then recorded at 650nm. Gallic acid was used as the standard to construct the calibration curve and total phenolic content was expressed as gallic acid equivalent (GAE) per gram of microalgae extract.

Total flavonoid content

Total flavonoid content was determined according to the method described by Safafar et al. (2015) with minor modifications. To 0.5mL extract (1 mg ML^{-1}), 500µL of methanol was added and mixed. Also, 1.4mL of distilled water is added followed by 100µL of 1M potassium acetate. The solution was kept for 5 min and thereafter, 100µL of 10% aluminium chloride was added and allowed to stand at room temperature for 30min. After which the absorbance was measured at 415nm. Total flavonoid content was calculated and expressed as quercetin equivalent (QE) per gram of microalgae extract.

DPPH Radical Scavenging Activity

The radical scavenging activity of microalgae extract was measured according to the method described by Blois (1958). In brief, different concentrations (0.4, 0.6, 0.8, 1, 1.2, 1.4 mg mL^{-1}) of the extract were prepared in methanol and then 100µLof each extract was added to 1mL of 0.1 mM DPPH in methanol. The solution is mixed properly and kept in the dark at room temperature for

30min. Absorbance is then measured at 517nm. BHT is used as the reference standard and the radical scavenging activity was measured using the formula.

$$\text{DPPH Radical Scavenging \%} = \left[\frac{\text{Abs DPPH} - \text{Abs Sample}}{\text{Abs DPPH}}\right] \text{X } 100$$

ABTS [2,2'-Azinobis (3-Ethylbenzothiazoline-6-Sulphonic Acid)] Free Radical Scavenging Activity Assay:

This experiment was performed according to Re et al. (1999) modified methodology. The first step entails the preparation of ABTS stock solution by reacting an equal volume of ABTS aqueous solution (7 mM) with 2.45 mM aqueous solution of potassium persulfate. The mixture was allowed to stand in the dark at room temperature for 16 hr before use. The $ABTS^{\cdot+}$stock solution was diluted with methanol to give an absorbance of 0.70 ± 0.02 at 734 nm. Then, 2.0 mL of $ABTS^{\cdot+}$working solution was mixed with 1 mL of the extracts at different concentrations ranges (50-300 µg/mL). The mixture was then incubated for 10 min at room temperature in the dark and the absorbance was immediately recorded at 734nm. The control was prepared by mixing 2.0 mL of $ABTS^{\cdot+}$ solution with 1 mL of double distilled water. BHT was used as reference standard. Samples were prepared and measured in triplicates. The results were expressed as the percentage of $ABTS^{\cdot+}$scavenging activity following the equation below, where A_0 is the absorbance of the control and A_s is the absorbance of the sample.

$$I\% = \left[\frac{A_0 - A_o}{A_o}\right] x\ 100$$

Determination of Ferric Reducing Antioxidant Power (FRAP):

The FRAP assay was done according to the method described by Andriopoulos (2022). The method is based on the reduction of ferric-tripyridyl triazine complex to its ferrous-blue-colored form in the presence of antioxidants. The FRAP reagent was freshly prepared by mixing 5ml of 10 mM TPTZ (2,4,6-tripyridyl-S-triazine) solution in 40 mM HCl and 5mL of 20 mM $FeCl_3$ solution. The mixture is then diluted five times by adding 50mL of 0.3 M acetate buffer (pH 3.6). In a test tube, 2.3mL of the FRAP reagent was mixed with 0.7 mL of the sample extracts at different concentrations (0.5-3.0 mg mL^{-1}). The mixture was then incubated in the dark at 37°C for 30 min. The absorbance was taken at 593 nm against a blank having all the reagents excluding the sample. All measurements were taken in triplicates with increasing absorbance of the reaction mixture indicate an increase of reduction capability. Ascorbic acid was used as the standard and the results were expressed in mg of ascorbic acid equivalents (AAE) mL^{-1} of extract.

Disc diffusion assay

The antimicrobial activity of the crude extract was tested against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae* using Kirby-Bauer disk diffusion assay following the method described by Abd El-Aty et al. (2014). Fresh bacterial cultures procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India were grown overnight in nutrient broth at 37°C. The bacterial cultures OD was then adjusted to 0.5 mcfarland standards and swapped over the Mueller Hinton agar plates (MHA). Sterile filter paper discs impregnated with 50µL crude extract (5mg mL^{-1}) were placed over the microbial lawn cultures and kept at room temperature to allow the extract to diffuse. The plates were then incubated at 37°C for 24hrs. Chloramphenicol was used as a positive control antibiotic and the inhibitory activity was determined by measuring the diameter of the zone of inhibition around each paper disc.

Minimum inhibitory concentration assay (MIC)

The MIC of the algal extract was determined by microdilution assay following the method described by Nainangu et al. 2020. The extract MIC was evaluated against the test bacterial strains (*Escherichia coli* MTCC M723, *Klebsiella pneumoniae* M109, and *Staphylococcus aureus* M96) at concentrations ranging from 5 mg mL^{-1} to 0.3125 mg mL^{-1} in 5 series of two-fold serial dilution. The inoculum of test organisms grown in Mueller Hinton broth was adjusted to 0.5 MacFarland standards (0.1 OD equals to 10^8 cfu mL^{-1}). In a 1 mL sterile tube, 100 µL of the standardized inoculum was added to 100 uL of the crude extract dissolved in 2.5% DMSO. It was incubated at 37°C for 24 hours. Following the incubation period, 20 uL of 0.5% of 2,3,5-Triphenyl Tetrazolium Chloride (TTC) prepared in sterile distilled water was added to each tube and further incubated for 2 hrs. The tube was then checked for color change where the pink color indicated bacterial growth and the original color indicated the absence of growth. The minimum concentration of extract which showed no color change indicated the absence of bacterial growth and was recorded as the MIC value of the extract against the

respective bacterial strain. In this experiment, chloramphenicol was used as the positive control, 2.5% DMSO was used as the negative control and sterile broth was used for sterility check.

FAME analysis

Lipid trans-esterification was achieved using a slightly modified method described by Mandal et al. (2018). In a mortar and pestle, 100 mg of lyophilized microalgae biomass was grinded with 1.5mL of NaOH-CH_3OH solution (0.5M NaOH). The mixture was saponified in a water bath at 75℃ for 10 minutes followed by methylation with 2mL of Boron Trifluoride-Methanol (1:2, v/v) at 75℃ for 10 min. Then 0.3mL of 0.9% NaCl and 2mL of hexane were added and centrifuged to allow phase separation. The upper phase consisting of fatty acids methyl esters (FAME) is collected and subjected to gas chromatography coupled with mass spectrometry (GC-MS). Elite-5MS Capillary Column was used for gas chromatography (PerkinElmer Clarus680 GC/600C MS, USA). Helium is used as a carrier gas with a flow rate of 1 mL min^{-1} and flame ionization detector (FID) is used to detect the analyte. The identification of the FAMEs is done by comparing the mass spectra pattern with the NIST library mass spectral database (S/W Turbomass NIST 2008).

Statistical analysis

The experiment was carried out in triplicates and the results were expressed as mean ± standard error (Safafar et al., 2015).

Results and Discussion

Identification and Phylogenetic analysis

Twelve mono-colonies were successfully obtained following the streak plate technique. The morphological features of the isolates were analyzed under the light microscope at 100X magnification and all showed significant growth in BG11 medium. The morphological features observed under the light microscope include non-motile unicellular cells ranging from oval to coccoid shape and chloroplast was highly visible in some isolates. These observations indicate that the microalgae isolates belong to the division Chlorophyta and may be of the genus *Chlorococcum* , *Desmodesmus*, *Chlorella* and *Graesiella* (Shubert and Gartner, 2015). Because of their phenotypic flexibility, microalgae may only be classified at the species level by molecular identification, even though microscopic observations may aid in genus-level identification.

DNA samples were isolated from all twelve microalgae isolates followed by PCR amplification of the 18S rDNA region. The sequence homology-based search carried out against sequences of microalgae in NCBI database identified eleven isolates at the species level while one isolate was only recognized at the genus level (Table 1). The BLAST analysis results showed that eleven isolates showed 90% above percentage identity except for *Chlamydomonas raudensis* which has an identity percentage of 89%. This indicates that the query sequences and the aligned NCBI database sequence are closely related and share the same lineage. Based on BLAST analysis, sequences with high similarity are subjected to phylogenetic analysis (Fig 1). A neighbour-joining phylogenetic tree was constructed using MEGA V11 software to further classify the isolates on the basis of their evolutionary relationship. From the phylogenetic tree, it was observed that all species that are closely related are represented under the same clade indicating that they are from the same ancestral lineage. The bootstrap method was used to determine the confidence level of the phylogenetic analysis which is represented by a number next to each clade. All clade that depicts the evolutionary relationship of the isolates at the species level has a bootstrap value above 90 % and these values reflect the reliability of the assessment. Overall based on the BLAST and phylogenetic analyses, the genus *Chlorococcum* is more dominant and is observed in 4 isolates, *Chlorococcum infusionum*, *Chlorococcum chlorococcoids*, *Chlorococcum oleofaciens* and *Oophila amblystomatis* or *Chlorococcum amblystomatis*. This genus is mostly distributed in the Jaintia hill district and is characterized by solitary unicellular cells, spherical or ellipsoidal with smooth cell walls (Bhagavathy et al., 2011). Often species from this genus have been explored for their excellent lipid productivity (Harwati et al. 2012). Other genus that was identified incudes *Chlamydomonas, Chlamydopodium, Oophila, Desmodesmus, Graesiella, Asterarcys* and *Parachlorella.*

Phytochemical Studies

Numerous species of microalgae are reported to be rich in lipids, proteins, carbohydrates and other phytochemical compounds. Microalgae like *Spirulina platensis* and *Chlorella* are directly consumed as food in many countries like China and Japan and have a significant impact on human health and nutrition (Koyande et al., 2019). In this study, twelve microalgae isolates were evaluated for the presence of eight phytochemicals such as alkaloids, tannins, saponins, flavonoids, terpenoids, steroids, phenolics and glycosides (Table 2). The phytochemical constituents were extracted from

their biomass using methanol as a solvent and are presented in Table 1. Alkaloids are the most abundant phytochemical and are detected in all twelve isolates. This phytochemical is known for its medicinal properties and is widely used in medication. For instance, drugs like quinine (anti-malaria), morphine (painkiller), codeine and aspirin (blood pressure regulator) all have alkaloidal structures (Hadizadeh et al., 2019). Some alkaloids have been reported as powerful poison and many have shown biological activities like antimicrobial, anti-inflammatory, and antimalarial (Kaur and Arora, 2015).

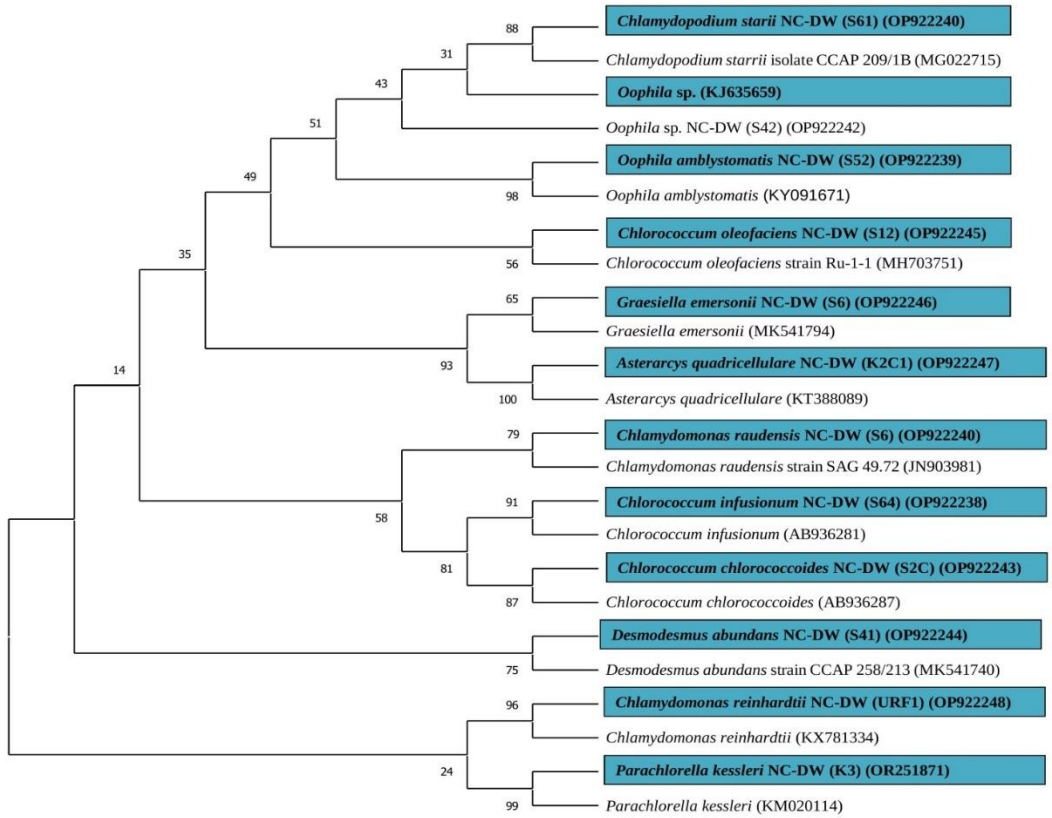

Fig 1. Phylogenetic tree depicting the evolutionary relationship of 18S rRNA sequence of 12 microalgae isolates. The tree was constructed by neighbor joining method with bootstrap values assigned at each branch.

Table 1. Molecular Identification of isolates as found using nucleotide BLAST along with their identity percentage, accession no. and their location.

Isolate	Abbreviations	Identity Per.	Accession No.	Location
Chlorococcum infusionum NC-DW (S64)	CI	91.74%	OP922238	Jaintia Hills, Khliehriat
Oophila amblystomatis NC-DW (S52)	QA	99.20%	OP922239	Jaintia Hills, Khliehriat
Chlamydopodium starii NC-DW (S61)	CS	98.53%	OP922240	Jaintia Hills, Khliehriat
Chlamydomonas raudensis NC-DW (S6)	CRD	89.30 %	OP922241	Jaintia Hills, Khliehriat
Oophila sp. NC-DW (S42)	O	97.45%	OP922242	Jaintia Hills, Khliehriat
Chlorococcum chlorococcoids NC-DW (S2C)	CC	97.91%	OP922243	Jaintia Hills, Khliehriat
Desmodesmus abundans NC-DW (S41)	DA	95.61%	OP922244	Ri-bhoi, rice field
Chlorococcum oleofaciens NC-DW (S12)	CO	92.73%	OP922245	Jaintia Hills, Khliehriat
Graesiella emersonii NC-DW (S6)	GE	91.93%	OP922246	Jaintia Hills, Khliehriat
Asterarcys quadricellulare NC-DW (K2C1)	AQ	92.41%	OP922247	East Khasi Hills, Kharmih
Chlamydomonas reinhardtii NC-DW (URF1)	CRH	97.63%	OP922248	Jaintia Hills, Khliehriat
Parachlorella kessleri NC-DW (K3)	PK	99.00%	OR251871	West Khasi Hills, River

Tannins, flavonoids, phenolics, and steroids are also reported in maximum number of isolates. All these phytochemicals are well known for their pharmaceutical properties and are widely used in medicine. Most widely known phytochemicals with antioxidant properties are phenolics, flavonoids, and tannins, these compounds can counteract the body's reactions to allergens, viruses, and carcinogens (Mutha et al., 202). Tannins for instance, are usually produced by plants in response to stress, and they have defensive properties that include photoprotection against free radicals and UV radiation. This property is attributed to the presence of phenolic rings allowing them to bind to a wide range of molecules and act as electron scavengers (Fraga-Corral et al., 2021). In contrast to

other phytochemicals, saponins and terpenoids are detected in only a few isolates. Saponins are detected in *Chlamydomonas raudensis, Desmodesmus abundans* and *Graesiella emersonii* and terpenoids are only found in *Desmodesmus abundans*. Saponins are compounds that consist of an isoprenoidal-derived aglycone, designated genin or sapogenin, covalently linked to one or more sugar moieties. Traditionally saponins were used for the treatment of coronary heart disease, hypertension, gastric ulcer, etc. (Akbarizare et al., 2019). Other phytochemicals compounds like steroids and glycosides were also reported from several isolates suggesting that most of the isolated strains are valuable reservoirs for bioactive compounds.

Table 2. Qualitative phytochemical screening of the methanolic microalgae extract

Isolate	Alka-loids	Tannins	Saponins	Flavonoids	Terpanoids	Steroids	Phenols	Glycosides
CI	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve
OA	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve
CS	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve
CRD	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
O	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve
CC	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve
DA	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve
CO	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve
GE	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
AQ	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve
CRH	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve
PK	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve

Proximate composition

Understanding the composition of carbohydrates, lipids, and protein contents is very crucial if microalgae are to be considered for their nutritional prospects. The analysis of dried microalgae biomass revealed differences in the proximate composition among all the isolates. From Table 3, it was observed that the proximate composition of *Asterarcys quadricellulare* had the highest protein content and carbohydrate content among all the twelve isolates with 220.68 ± 1.04 µg mL^{-1} and 87.97 ± 1.62 µg mL^{-1} respectively. Kang et al. (2024) also reported *Asterarcys quadricellulare* for its high protein content constituting up to 44.34% of the microalgae biomass, while carbohydrate and lipid content make up to 21.75% and 24.74% respectively. In terms of lipid content, *Oophila* sp shows the highest with 36.9% of dry cell weight followed by *Chlamydomonas raudensis* at 35.2%. Both these organisms were previously reported to be viable sources of polyunsaturated fatty acids like ω-3 α-linolenic acid and palmitic acid (Conde et al. 2022 and Kang et al. 2024). Furthermore, the high lipid level of *Oophila* sp makes it a desirable candidate for biodiesel production.

Numerous pigments that are extracted from microalgae have garnered significant interest in the fields of pharmacology, cosmetics and food. Two major groups of pigments that are found in microalgae include chlorophylls and carotenoids (Matos, 2017). *Desmodesmus abundans* has the highest chlorophyll a and b content with 18.4 ± 0.09 µgmL^{-1} and 7.73 ± 0.095µg mL^{-1} (Table 2.3). Similar range for chlorophyll a and b content was also observed by Salah et al. (2023) in *Desmodesmus* sp. with chlorophyll a and b values at 18.14 µg mL^{-1} and 7.72 µg mL^{-1} respectively. Chlorophyll a is the major pigment light harvesting pigment converting light energy to chemical energy while chlorophyll b is an accessory pigment. Therefore, the values of chlorophyll a in microalgae will always be higher than chlorophyll b.

Carotenoids are natural pigments widely present in both plants and algae. They play a major role in scavenging reactive oxygen species (ROS) generated during photosynthesis. Total carotenoid content was determined in all 12 isolates as depicted in Table 3. The highest carotenoid content was observed in *Desmodesmus abundans* with 7.69 ± 0.02 mg L^{-1} and the lowest carotenoid content was observed in *Graesiella emersonii* with 1.36 ± 0.19 mg L^{-1}. The range of carotenoid content observed in the studied isolates is very similar to the ones reported by Safafar et al. (2015), however when comparing data with other studies one should be careful to consider the type of medium used, the culture conditions and light intensity.

Total phenolic and total flavonoid content

Phenolic and flavonoid compounds are very important antioxidant molecules; they stabilize free radicals either by the transfer of hydrogen atoms or the addition of free electrons. The total phenolic

content for all samples was determined by Folin-ciocalteu procedure and the results were displayed in Table 4. The highest phenolic content in microalgae was observed in *Parachlorella kessleri* with 2.22 ± 0.25 mg GAE g^{-1} followed by *Chlorococcum infusionum* with 1.8 ± 0.07mg GAE g^{-1}, whereas *Oophila* sp. has the lowest phenolic content with 0.62±0.03 mg GAE g^{-1}. For flavonoids, the highest flavonoid content was observed in microalgae *by Chlamydomonas reinhardtii* with 10.03 ± 0.34 mg QE g^{-1} followed by *Chlorococcum chlorococcoids* with 8.84 ± 1.96 mg QE g^{-1}, whereas the lowest flavonoid content was observed in *Chlamydomonas raudensis* with 3.98 ± 0.97 mg QE g^{-1}.

Table 3. Proximate composition and pigment content of microalgae biomass

Isolate	Protein (µg/ml)	Carbohy-drate (µg/ml)	Lipid (%DCW)	Chloro-phyll a (µg/ml)	Chloro-phyll b (µg/ml)	Carotenoid (mg/L)
CI	185.06 ± 1.73	26.48 ± 1.52	28.9	9.24 ± 0.05	3.23 ± 0.12	3.38 ± 0.19
OA	136.62 ± 1.25	26.74 0.57	31.7	13.5 ± 0.18	0.57 ± 0.052	4.37 ± 0.20
CS	193.81 ± 0.75	26.16 ± 0.43	30.8	9.76 ± 0.05	2.68 ± 0.052	7.16 ± 0.036
CRD	190.5 ± 1.23	64.22 ± 0.78	35.2	4.92 ± 0.27	1.49 ± 0.07	2.73 ± 0.16
O	204.38 ± 0.78	72.24 ± 1.68	36.9	5.75 ± 0.09	7.66 ± 0.055	1.51 ± 0.03
CC	209.13 ± 0.35	80.33 ± 0.67	34.1	6.24 ± 0.17	2.62 ± 0.043	3.30 ± 0.07
DA	175.69 ± 0.67	64.09 ± 2,34	32.2	18.4 ± 0.09	7.73 ± 0.095	7.69 ± 0.02
CO	177.5 ± 0.56	60.65 ± 1.23	30.9	5.42 ± 0.12	1.69 ± 0.04	3.61 ± 0.08
GE	165.69 ± 0.67	78.89 ± 2,34	26	1.53 ± 0.03	1.62± 0.03	1.36 ± 0.19
AQ	220.68 ± 1.04	87.97 ± 1.62	34.7	6.48 ± 0.09	2.45± 0.05	3.45 ± 0.005
CRH	212.56 ± 2.4	28.22 ± 1.98	29.9	8.30 ± 0.15	4.78± 0.06	4.3 ± 0.19
PK	206.31 ± 2.09	54.47 ± 1.48	27.9	6.78 ± 0.06	2.31± 0.09	5.37 ± 0.03

There is a lot of variation when it comes to the phenolic and flavonoid content among the isolates and this could be because of the differences in their genetic factors (Papoulias et al., 2009). Also, the synthesis of bioactive compounds is deeply influenced by environmental conditions, thus the same species may show variations in their phytochemical content under different conditions. For example, the phenolic content of *Phaeodactylum tricornutum* (3.75 ± 0.46 mg GAE mg^{-1}) observed by Goiris et al. (2012) is much lower in comparison to the phenolic content observed by Maadane et al. (2015) at 16.8 ± 0.33 mg GAE g^{-1}, even though in both experiments ethanol extract was used. Such variation in phenolic content was also seen in *Synechococcus* sp. reported by Li et al. (2007). However, the variations observed by Li et al. (2007) are mainly because different solvents were used to extract phenol and the highest content was observed in ethyl acetate fraction. Similar observation by Abd El-Aty et al. (2014) in *Oscillatoria agardhii*, the methanol extract shows much higher phenolic content at 20.91 ± 0.21 mg GAE g^{-1} in comparison to the acetone and water extract which is at 16.23 ± 0.03 mg GAE g^{-1} and 8.04 ± 0.02 mg GAE g^{-1} respectively. Thus, the type of solvent used strongly influences the extraction of phytochemicals because polar compounds are more soluble in polar solvents and non-polar compounds are more soluble in non-polar solvents. Since both phenols and flavonoids are both polar compounds, methanol solvent is mainly recommended for the analysis of these compounds (Gallego et al., 2018).

Table 4. Summarized results of the total phenolic, flavonoids and carotenoids content. Values are represented as mean ± standard error.

Isolate	Total phenolic content (mg GAE g^{-1})	Total flavonoid content (mg QE g^{-1})
Chlamydomonas reinhardtii	1.4 ± 0.34	10.03 ± 0.34
Chlorococcum chlorococcoids	1.17 ± 0.25	8.84 ± 1.96
Graesiella emersonii	1.17± 0.39	7.28 ± 1.11
Asterarcys quadricellulare	1.2 ± 0.65	6.11 ± 1.94
Oophila amblystomatis	1.22 ± 0.53	5.87 ± 0.56
Parachlorella kessleri	2.22 ± 0.25	7.22 ± 1.79
Chlorococcum infusionum	1.8 ± 0.07	8.74 ± 1.78
Chlamydomonas raudensis	0.64 ± 0.23	3.98 ± 0.97
Chlamydopodium starii	1.22 ± 0.13	6.14 ± 1.73
Desmodesmus abundans	0.8 ± 0.03	5.02 ± 0.97
Chlorococcum oleofaciens	0.93±0.07	5.87±1.96
Oophila sp.	0.62±0.03	5.96±0.45

Antioxidant activities

Antioxidant activities of the methanol extracts derived from biomass of microalgae were investigated using ABTS, DPPH and FRAP assays and the results were depicted in Table 5. DPPH is

a stable free radical and is widely used to measure the antioxidant activity of the crude extract. It is characterized by its deep-violet colour with an absorption maximum at 515 nm. The reduction of this stable free radical by antioxidants will result in the formation of pale-yellow colour which could be easily monitored with a spectrophotometer. Extracts with lower IC50 values have more radical scavenging activity than those with higher IC50 values and all 12 isolates exhibit antioxidant activity. As depicted in Table 5, *Parachlorella kessleri* has the strongest DPPH scavenging activity with the lowest IC50 value (470.23±1.165 µg mL^{-1}) while the highest IC50 value was observed in *Oophila* sp. (1659.23±17.95 µg mL^{-1}) which means it has the lowest antioxidant activity. Sharma et al. (2019) reported a much lesser DPPH IC50 value for *Parachlorella kessleri* around 150± 0.004 µg mL^{-1} but according to their analysis, culture condition may results in variation of antioxidant activity and biochemical composition. ABTS assay also measures the antioxidant activity of the microalgae extract by inhibiting the absorbance of ABTS free radicals, which have a distinctive blue colour and show maximum absorption at 734 nm. The highest ABTS radical scavenging activity was shown by *Parachlorella kessleri* with the lowest IC50 value of 194.52±0.69 µg mL^{-1} followed by *Chlamydomonas reinhardtii* and *Oophila amblystomatis* with an IC50 value of 215.25±2.45µg mL^{-1} and 217.00±1.07µg mL^{-1} respectively. In both assays, the antioxidant activity is concentration dependent with maximum activity recorded as concentration goes higher. All 12 isolates had varying IC50 values in the DPPH and ABTS assays, ranging from 0.217 to 1.64 mg mL-1, indicating a wide range of antioxidant activity (Table 5).

In FRAP assay, *Parachlorella kessleri* showed the highest reducing potential in comparison to the other isolates with 11.22±0.17 mg AAE mL^{-1}followed by *Chlamydomonas reinhardtii* and *Chlorococcum chlorococcoids* with 9.28±0.05 mg AAE $mL^{-1}$and 8.53±0.07 mg AAE ML^{-1}respectively (Table 5). The FRAP reducing agent falls within the range of 3.30-11.22mg AAE $mL^{-1}$with *Chlamydopodium starii* exhibiting the lowest reducing power with 0.50± 0.05 mg AAE mL^{-1}. All three assays ABTS, FRAP, and DPPH show diverse radical scavenging activity among the microalgae species, this could be because of their differences in genus or the place from where they are isolated. However, the three antioxidant tests clearly indicate that *Parachlorella kessleri* has the highest radical scavenging activity among all the studied isolates. Also, at concentration 1mg mL^{-1} *Parachlorella kessleri* inhibition percentage is 103.83% for DPPH assay and 207.89% for ABTS assay. Similar range of antioxidant potential was also observed by Raikar et al. (2018) in *Chlorella vulgaris* and *Chlorella pyrenoidosa* which showed high free radical scavenging activity of 105.56% at a concentration of 1mg mL^{-1}, with an IC50 value of 412.51 µg mL^{-1}. Similarly, at 1mg mL^{-1}, the methanol extract of *C. pyrenoidosa* showed 88.6% free radical scavenging with IC50 value of 443.34 µg mL^{-1} (Raikar et al., 2018). It was observed that ABTS scavenging activity was higher than DPPH scavenging activity. This observation is because pigments absorb light at lower wavelength and may interfere with the overall reading of the result (Monteiro et al., 2020). A number of studies have used more than one type of solvent to evaluate antioxidant potential—particularly when targeting compounds such as phenols and flavonoids, for which polar solvents are generally recommended. For instance, Abd El-Aty et al. (2014) used three different solvents methanol, acetone and water to test the antioxidant potential of *Oscillatoria agardhii* and *Anabaena sphaerica*. It was found that methanol is the best among the three solvents. Similarly, Uma et al. (2011) observed that the methanolic extract show much better radical scavenging activity than the acetone and ethanolic extract of *Desmococcus olivaceous* and *Chlorococcum humicola*. It is universally known that the extraction of phytochemicals is deeply influenced by medium composition, temperature, culture condition and the type of sample used. Therefore, it is difficult to get consistent readings when determining the antioxidant capacity of microalgae. Our results indicate that all 12 microalgal isolates contain molecules that act as electron donors stabilizing radical species and counteracting their harmful effect, thus preventing damage to DNA, lipids, proteins, and other biomolecules.

Fatty acids

Microalgae are known to be rich sources of various fatty acids, including saturated, monounsaturated and polyunsaturated fatty acids. In this study, a diverse array of fatty acids was identified in microalgae samples, including erucic acid, eicosanoic acid, heptacosanoic acid, hexadecatetraenoate, eicosatrienoic acid, and eicosatetraenoate, among others. Tetradecanoic acid, also known as myristic acid, is a common saturated fatty acid found in microalgae (Table 6). Studies by Rasoul-Amini et al. (2011) have also reported the presence of myristic acid in microalgae species such as *Chlorella*. The nutritional value of microalgae is mostly determined by their fatty acid composition, and this differs considerably between microalgae species. The major fatty acids found in all twelve isolates are 10, 13-Dimethyltetradecanoic acid, cis-13-Octadecenoic acid, 2-hydroxy eicosanoic acid, and 11,14,17-eicosatrienoic acid. Microalgae lipids are composed of

saturated, monosaturated, and polyunsaturated fatty acids with chain lengths ranging from 14-25 carbon atoms and i-Propyl 7,10,13,16,19-docosapentaenoate has the highest chain length with up to 25 carbon atoms. 10, 13-Dimethyltetradecanoic acid (C16:0) saturated fatty acids ranging from 4% to 100% is the most commonly found fatty acid in all 12 isolates. In the case of unsaturated fatty acids, cis-13-Octadecenoic acid (C18:1) and 11,14,17-Eicosatrienoic acid (C20:3n-3) account for 2-43% of total fatty acid content and is present in almost all isolate.

Table 5. Summarized results of the radical scavenging activity of all the 12 isolates depicted through ABTS, DPPH and FRAP assay. Values are represented as mean ± standard error

Sample	DPPH IC50 (µg ML^{-1})	ABTS IC50 (µg ML^{-1})	FRAP [mg (AAE) ML^{-1}]
Chlamydomonas reinhardtii	619.27 ± 3.96	215.22 ± 2.45	9.28 ± 0.053
Desmodesmus abundans	1432.95 ± 11.27	628.91 ± 80.43	3.30 ± 0.04
Chlamydopodium starii	900.38 ± 3.79	277.56 ± 1.2	0.50± 0.05
Chlorococcum infusionum	856.02 ± 25.56	343.16 ± 1.35	4.59± 0.08
Asterarcys quadricellulare	579.4 ± 2.68	349.76 ± 2.45	6.54± 0.10
Oophila amblystomatis	570.075 ± 7.98	217 ± 1.07	4.69± 0.04
Chlamydomonas raudensis	771.40 ± 0.22	311.79 ± 0.96	6.94± 0.08
Chlorococcum chlorococcoids	706.79 ± 3.02	276.6 ± 38.87	8.53± 0.07
Oophila sp.	1659.29 ± 21.65	810.19 ± 0.57	3.39± 0.03
Chlorococcum oleofaciens	932.76 ± 25.56	247.53 ± 0.69	4.09± 0.05
Parachlorella kessleri	470.23 ± 1.16	194.52 ± 0.11	11.22± 0.17
Graesiella emersonii	1649.57	1260.68 ± 4.69	3.80 ± 0.18

The FAME profile result of this study was clearly different from the FAME profiles observed in previous studies, where linoleic acid, palmitic acid, oleic acid and linolenic acid were the most dominant fatty acid produced by *Chlorella, Demodesmus, Chlorococcum* and *Asterarcys* (Kaur et al., 2012 and Kang et al., 2024). These differences observed in the fame composition might be attributed to variations in microalgal species and culture conditions. Among the isolates *Chlamydomonas raudensis* has the highest composition of polyunsaturated fatty acids (PUFAs) with 99.78% and 0.22% saturated fatty acid (SFA). The most significant fatty acids identified from this isolate are the omega 3 and omega 6 fatty acids, including 11,14,17-eicosatrienoic acid (C20:3n-3), 8,11,14-eicosatrienoic acid (C20:3n-6), 8,11,14,17-eicosatetraenoate (C20:4n-3) and 10,12-Octadecadienoic acid (C18:2n-6). Similar composition was also reported by Dolhi et al. (2013) in *Chlamydomonas raudensis* UWO241 and *Chlamydomonas* sp. ICE-L with both organisms accumulating up to 75% polyunsaturated fatty acids. According to their reports, the high amount of PUFA in *Chlamydomonas raudensis* UWO241 membrane lipid was an adaptive strategy for survival at low temperatures, which included increasing the degree of FA unsaturation to maintain membrane fluidity. Other major polyunsaturated fatty acid group include methyl hexadeca-4,7,10,13-tetraenoate, methyl 6,9,12,15-hexadecatetraenoate and 11,14-octadecadienoic acid. The high level of unsaturation fatty acid of *Chlamydomonas raudensis* makes this strain suitable for edible oil production. Especially since the unsaturated Omega-3 and Omega-6 fatty acids are of nutritional interest within this group.

Antibacterial activity studies

The antibacterial property of all twelve isolates was tested against 3 bacterial strains *Escherichia coli*, *Staphylococcus aureus,* and *Klebsiella pneumoniae*. For this evaluation, disk diffusion assay and minimum inhibitory concentration were used to test the inhibitory potential, and all twelve isolates showed a certain level of antimicrobial activity against the tested bacterial strains (Table 7). In disk diffusion assay the three bacterial strains were exposed to 5 mg mL^{-1} of the crude extract and *Chlorococcum chlorococcoid* showed the maximum zone of inhibition against *E. coli* with 14 ± 0.5 mm, while *Asterarcys quadricellulare* showed the maximum zone of inhibition against *Staphylococcus aureus* with 16mm ± 0, followed by *Chlorococcum chlorococcoid* with 12 ± 0.5 mm. For *Klebsiella pneumoniae*, the maximum zone of inhibition was observed in *Chlorococcum chlorococcoids* with 14mm ± 0 (Fig 2). Although reports on *Chlorococcum chlorococcoid* antimicrobial properties are very limited, *Chlorococcum* species have been known to produce broad-spectrum antimicrobial compounds such as fatty acids, phenolics, and terpenoids (Stirk et al. 2022). These findings are supported by the work of Bhagavathy et al. (2011), who demonstrated that *Chlorococcum humicola* possesses notable antimicrobial properties against a wide range of pathogenic microorganisms.

In their study, various organic solvent extracts particularly benzene and ethyl acetate exhibited substantial inhibitory effects, with microbial growth inhibition rates reaching up to 80%. The

methanol extract also showed considerable activity against *E. coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*, which is very similar to the inhibitory effects observed in our study. Among many compounds with antibiotic effect, fatty acids such as linolenic acid, have been shown to significantly inhibit methicillin-resistant *Staphylococcus aureus* (MRSA) (Ohta et al., 1995). *Asterarcys quadricellulare* showed the highest activity against *Staphylococcus aureus*, possibly owing to the different types of fatty acids identified from this isolate. The observed zones of inhibition (10–16 mm) are consistent with earlier studies, further supporting the effectiveness of microalgae-derived compounds in combating bacterial pathogens (Elshobary et al., 2020).

Table 6: Fatty acid composition of microalgal lipids

Fatty acids	O	CRH	CS	GE	CRD	OA	PK	DA	CI	AQ	CC	CO
Saturated fatty acids												
Myristic acids (C14:0)	0.56	-	-	-	-	-	-	-	-	-	-	-
10,13-Dimethyltetradecanoic acid (C16:0)	4.15	23.95	50.58	40.99	-	26.44	16.94	28.86	39.77	25.06	100	100
Eicosanoic acid, 2-hydroxy (C20:0)	-	0.42	6.69	-	0.22	7.69	-	5.88	-	-	-	-
Monounsaturated fatty acids												
Myristoleic acid (C14:1)	-	0.31	-	-	-	-	-	-	-	-	-	-
13-Octadecenoic acid (C18:1)	41.10	18.51	-	28.66	-	22.31	-	31.94	26.68	17.57	-	-
Erucic Acid (C22:1)	-	-	1.43	-	-	-	-	-	-	-	-	-
Polysunsaturated fatty acids												
Methyl hexadeca-4,7,10,13-tetraenoate(C17:4n-3)	-	-	-	3.24	10.47	5.94	-	-	3.85	-	-	-
Methyl 6,9,12,15-Hexadecatetraenoate(C17:4n-3)	-	-	13.85	-	-	-	2.62	-	-	-	-	-
10,12-Octadecadienoic acid(C18:2n-6)	-	10.24	20.19	-	64.09	-	-	-	-	9.66	-	-
11,14-Octadecadienoic acid (C18:2n-4)	-	-	-	6.75	-	-	-	-	8.83	-	-	-
11,14,17-Eicosatrienoic acid (C20:3n-3)	33.37	42.37	-	20.36	2.38	37.62	-	33.32	20.87	38.04	-	-
11,14-Eicosadienoic acid (C20:2n-6)	-	0.15	-	-	-	-	-	-	-	-	-	-
8,11,14,17-eicosatetraenoate (C20:4n-3)	-	-	7.26	-	17.35	-	-	-	-	-	-	-
8,11,14-eicosatrienoic acid (C20:3n-6)	-	-	-	-	5.49	-	-	-	-	-	-	-
8(9)-epoxy-5Z,11Z,14Z-eicosatrienoic acid(C20:3n-6)	-	-	-	-	-	-	80.44	-	-	-	-	-
Heneicosapentaenoic Acid (C21:5n-3)	20.82	-	-	-	-	-	-	-	-	-	-	-
Methyl 6,9,12,15,18-heneicosapentaenoate(C21:5n-3)	-	-	-	-	-	-	-	-	-	5.98	-	-
i-Propyl 7,10,13,16,19-docosapentaenoate (C25:5n-3)	-	4.05	-	-	-	-	-	-	-	3.69	-	-
SFA	**4.71**	**24.37**	**57.27**	**40.99**	**0.22**	**34.13**	**16.94**	**34.74**	**39.77**	**25.06**	**100**	**100**
MUFA	**41.10**	**18.82**	**1.43**	**28.66**	**-**	**22.31**	**-**	**31.94**	**26.68**	**17.57**	**-**	**-**
PUFA	**54.19**	**56.81**	**41.30**	**30.35**	**99.78**	**43.56**	**83.06**	**33.32**	**33.55**	**57.37**	**-**	**-**

The minimum inhibitory concentration for all samples was examined at 5 different concentrations of the sample extract ranging from 5mg mL^{-1} to 0.3125 mg mL^{-1}. The highest dilution showing no microbial growth is then recorded as the MIC value. Results depicted in Table 7 indicate that all isolates exhibit antibacterial activity at high concentrations (5mg mL^{-1}), as we decrease the concentration the antibacterial activity also decreases. The lowest MIC value recorded among the isolates was 1.25mg mL^{-1} below this value no sign of growth inhibition was observed. *Chlorococcum chlorococcoid* has a MIC value of 1.25mg mL^{-1} for *E. coli* and *Staphylococcus aureus* whereas 2.5mg mL^{-1} for *Staphylococcus aureus*. A similar MIC value was also reported by Shaima et al. (2021) when they tested the antimicrobial activity of the methanolic extract from *Chlorella sp.* (UKM8). In their findings, the lowest MIC value for *E.coli, Staphylococcus aureus, and Klebsiella pneumoniae* was reported at 2.5mg mL^{-1}, 1.25mg mL^{-1} and 5mg mL^{-1} respectively.

Senhorinho et al. (2018) also reported a similar MIC value for *Chlamydomonas* sp at 1.24 mg mL^{-1} when tested against *Staphylococcus aureus*. There are also reports with much lower MIC values; for

example, Nainangu et al. (2020) tested the antimicrobial potential of *Oscillatoria* sp. SSCM01 and *Phormidium* sp. In their experiment, it was reported that the *Oscillatoria* sp. SSCM01 extract exhibits a MIC value of 31.2 µg mL^{-1}, 500µg mL^{-1} and 250 µg mL^{-1} against *Staphylococcus aureus*, *Klebsiella pneumoniae and E.coli* respectively. Such variation observed within the MIC value of microalgae and cyanobacteria is because the synthesis of bioactive compounds is heavily influenced by the culture condition which can alter the bioactivity of the particular species. And this has been proven by Mc Gee et al. (2020), in their experiment they observed an increase in the inhibitory property of polar extract against *Staphylococcus aureus* from 875 µg mL^{-1} to 438 µg mL^{-1} MIC when *Stauroneis* sp. is cultivated under white + red LEDs as compared to the monochromatic red LED-based cultivation. Several isolates show no zone of inhibition in the disk diffusion assay but exhibits inhibitory properties in the MIC assay. For example, *Desmodesmus abundans* has a MIC value of 2.5mg mL^{-1} for *Staphylococcus aureus* but exhibit no zone of inhibition in the disk diffusion assay. Such findings may be because the active component of the extract may have a very low diffusion rate (Alsenani et al., 2020).

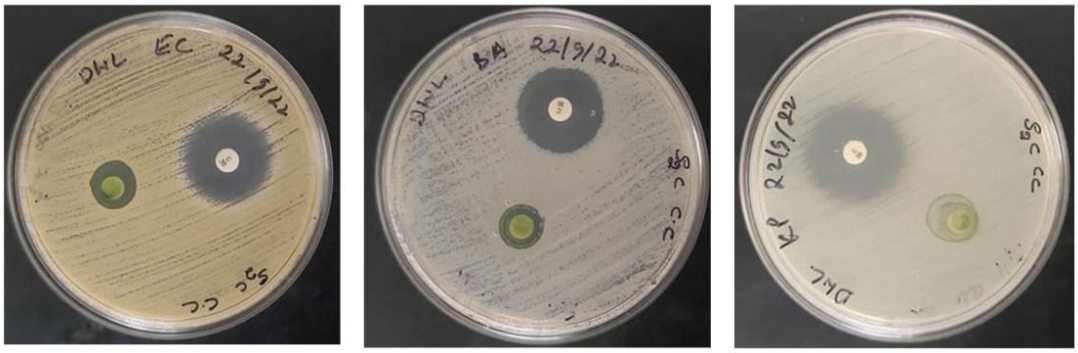

Fig 2. Zone of inhibition of *Chlorococcum chlorococcoid* methanol extract tested against *Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumoniae.* Chloramphenicol is used as a control.

Table 7. Summarized results of all 12 isolates antimicrobial properties. ZOI represent the zone of inhibition (ZOI) of the methanol extract (5mg mL^{-1}) in the disk diffusion assay and MIC represents the minimum inhibitory concentration value. Chloramphenicol is used as positive control and values are represented as Mean ± Standard error

Sample	*Escherichia coli*		*Staphylococcus aureus*		*Klebsiella pneumoniae*		*E. coli*	*S.aureus*	*K.pneumoniae*
	ZOI Control (mm)	ZOI Extract (mm)	ZOI Control (mm)	ZOI Extract (mm)	ZOI Control (mm)	ZOI Extract (mm)	MIC (mg/ml)	MIC (mg/ml)	MIC (mg/ml)
CRH	23 ± 0.5	11 ± 0	27 ± 1.1	12 ± 0.5	24 ± 0.5	-	2.5	1.25	1.25
CC	24 ± 0.5	14 ± 0.5	23 ± 1.1	12 ± 0.5	25 ± 0.5	14 ± 0	2.5	1.25	1.25
GE	23 ± 0.5	11 ± 0.5	28 ± 0.5	-	24 ± 0.5	10 ± 0	2.5	1.25	2.5
AQ	27 ± 0.5	11 ± 1.1	31 ± 0.5	16 ± 0	27 ± 0.5	-	1.25	2.5	2.5
OA	23 ± 0	11 ± 1.1	31 ± 0	11 ± 0	23 ± 0.5	11 ± 1.1	2.5	2.5	2.5
PK	19 ± 1.1	12 ± 0.5	21 ± 0	11 ± 1.1	23 ± 0	10 ± 1.1	.2.5	2.5	2.5
CI	23 ± 0	13 ± 0	32 ± 1.1	10 ± 0.5	23 ± 0	11 ± 0.5	1.25	1.25	2.5
CRD	21 ± 1.1	12 ± 1.1	19 ± 0.5	11 ± 0.5	23 ± 1.1	11 ± 0.5	5	2.5	2.5
CS	23 ± 0	>10±0.5	27 ± 1.1	11 ± 0.5	24 ± 1.1	10 ± 1.1	5	2.5	2.5
DA	27 ± 0.5	11 ± 0.5	28 ± 0	-	18 ± 1.1	-	2.5	2.5	2.5
CO	24 ± 1.1	13 ± 0.5	26 ± 0.5	11 ± 1.1	25 ± 0	10 ± 0.5	2.5	2.5	2.5
O	23 ± 0.5	10 ± 1.1	31 ± 1.1	11 ± 1.1	22 ± 0	11 ± 0.5	2.5	2.5	2.5

Conclusion

This study highlights the potential of microalgae isolated from Meghalaya as a valuable source for bioactive compounds. All twelve isolates exhibited varying degrees of antioxidant activity, as assessed by ABTS, FRAP, and DPPH assays. *Parachlorella kessleri* emerged as a promising candidate with the highest antioxidant activity, likely due to its high phenolic content. Additionally, all isolates demonstrated antimicrobial activity against pathogenic bacteria, with *Chlorococcum chlorococcoides* showing the strongest inhibitory effect against *Escherichia coli* and *Klebsiella pneumoniae*, while *Asterarcys quadricellulare* showed the maximum zone of inhibition against *Staphylococcus aureus*. GC-MS analysis revealed the presence of diverse fatty acids, including saturated, monounsaturated, and polyunsaturated fatty acids. Among the isolates *Chlamydomonas raudensis* is particularly rich in omega-3 and omega-6 fatty acids, making it a potential candidate for nutritional applications. These findings underscore the potential of microalgae from specific places of Meghalaya as a sustainable source of natural products with applications in various fields,

including pharmaceuticals and nutraceuticals. Further research is warranted to fully explore the bioactive potential of these promising microorganisms.

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Author Contributions

DWL: Conceptualization, Investigation, Manuscript preparation, Interpretation of results; BP: Investigation; SRJ: Investigation, manuscript review and editing and NC: Conceptualization, Investigation, Interpretation of results, Manuscript preparation, Manuscript review and editing; all authors approved the manuscript.

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Competing interest

The authors declare no competing interests.

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