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BIOCHEMICAL AND PHYTOCHEMICAL CHARACTERIZATION OF *CHLORELLA VULGARIS* AND EVALUATION OF ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT

Chlorella vulgaris is a fast-growing freshwater microalga with considerable potential as a source of proteins, carbohydrates, lipids, pigments, and biologically active metabolites. The present study investigated the biochemical and phytochemical characteristics of a *C. vulgaris* culture isolated from a freshwater pond at Kuppandapalayam, Erode District, Tamil Nadu, India, together with its preliminary antimicrobial and biorefinery potential. The culture was maintained in BG-11 medium under continuous aeration and cool-white illumination and developed dense green biomass within 7–10 days. Microscopic examination revealed healthy unicellular, spherical to slightly oval cells with a distinct cell wall and prominent chloroplast. Acetone-extracted pigments contained 11.45 mg L^{-1} chlorophyll a, 0.34 mg L^{-1} chlorophyll b, and 11.79 mg L^{-1} total chlorophyll. Qualitative phytochemical screening detected alkaloids, carbohydrates, flavonoids, glycosides, proteins, steroids, tannins, and terpenoids in both aqueous and acetone extracts, whereas saponins were detected only in the aqueous extract. The estimated carbohydrate content was 1.01 mg g^{-1} dry biomass, while the protein assay yielded approximately 0.46 mg mL^{-1} in the tested sample. Soxhlet extraction recovered 1.327 g lipid from 10 g dry biomass, corresponding to a lipid yield of 13.27%. Both aqueous and acetone extracts exhibited

antibacterial activity against Escherichia coli, producing inhibition zones of 20 and 15 mm, respectively. Preliminary antifungal activity was also observed against Fusarium sacchari and Fusarium incarnatum. Following acid hydrolysis and fermentation with Saccharomyces cerevisiae, the resulting distillate gave a positive qualitative test for ethanol. Collectively, the findings demonstrate that *C. vulgaris* biomass contains multiple biochemical and phytochemical constituents with preliminary antimicrobial activity and potential for integrated biomass valorization. Further studies incorporating replicated quantitative analyses, molecular authentication of the strain, standardized antimicrobial assays, detailed metabolite profiling, and optimization of fermentation and downstream processing are required to establish its biotechnological and industrial potential.

KEYWORDS: Antimicrobial activity, BG-11; Bioethanol; *Chlorella vulgaris*, Phytochemicals; Wastewater treatment.

1. INTRODUCTION

Microalgae are photosynthetic microorganisms that play important roles in aquatic primary production, nutrient cycling and global carbon fluxes. Their ability to convert light energy and inorganic carbon into biomass, together with rapid growth rates and relatively simple nutritional requirements, has attracted considerable interest for applications in food and feed production, nutraceuticals, pharmaceuticals, environmental biotechnology and renewable energy (Raven and Beardall, 2007; Khan et al., 2018). Unlike conventional terrestrial biomass, microalgal cultures can accumulate multiple classes of commercially relevant compounds within the same biomass, including proteins, carbohydrates, lipids, pigments and other bioactive metabolites. This biochemical versatility has stimulated the development of integrated microalgal biorefinery concepts in which different biomass fractions are sequentially recovered and converted into value-added products.

Among freshwater microalgae, *Chlorella vulgaris* is one of the most extensively investigated species because of its rapid biomass production, broad environmental tolerance and diverse biochemical composition (Coronado-Reyes et al., 2022; Fang et al., 2024). *C. vulgaris* is a unicellular chlorophyte characterized by small, predominantly spherical to slightly oval cells, a rigid cell wall and chlorophyll *a* and *b* as major photosynthetic pigments. Its biochemical composition is not constant and can vary considerably with strain characteristics, cultivation conditions, nutrient availability, light intensity, temperature, pH and carbon supply. Such

physiological flexibility provides opportunities for manipulating biomass composition according to the intended downstream application.

The biotechnological importance of *C. vulgaris* is largely associated with its ability to accumulate nutritionally and industrially relevant biomolecules. Proteins constitute an important fraction of the biomass and may provide valuable amino acids for food and feed applications, whereas carbohydrates can serve as structural or storage components and potential substrates for microbial fermentation. Lipids represent an important energy-rich fraction and may contain fatty acids of nutritional and industrial interest. In addition, *C. vulgaris* produces photosynthetic pigments and a range of secondary metabolites that have been investigated for antioxidant, antimicrobial and other biological activities (Coronado-Reyes et al., 2022; Fang et al., 2024; Mendes et al., 2024). The relative abundance of these constituents is strongly influenced by both cultivation and extraction conditions, emphasizing the importance of systematic biochemical characterization before evaluating specific applications.

The antimicrobial properties of microalgal biomass have received increasing attention because algal extracts may contain phenolic compounds, flavonoids, pigments, lipids and other metabolites capable of interacting with microbial cells. However, antimicrobial responses can vary substantially according to algal strain, biomass physiological state, extraction solvent, extract concentration and target microorganism. Agar-well or disc-diffusion assays are therefore useful as preliminary screening approaches, but inhibition-zone measurements alone cannot establish minimum inhibitory concentrations (MIC), minimum bactericidal or fungicidal concentrations, or the specific mechanisms responsible for microbial inhibition (Balouiri et al., 2016). Consequently, preliminary antimicrobial observations should be interpreted as evidence of biological activity rather than definitive proof of therapeutic or industrial efficacy.

In addition to its biochemical and antimicrobial characteristics, *C. vulgaris* has attracted interest as a multifunctional biomass platform for resource recovery and environmental biotechnology. Carbohydrate-rich biomass can potentially be hydrolysed and used as a substrate for microbial fermentation, including ethanol production, while residual biomass and living algal cultures may also be explored for nutrient removal and other wastewater-treatment applications. Such integrated approaches are consistent with the emerging microalgal biorefinery concept, in which biomass is not considered as a single-product

feedstock but as a source of multiple products and services. Nevertheless, the feasibility of individual applications depends on biomass productivity, biochemical composition, extraction efficiency, process conditions and the reproducibility of the resulting products.

Despite the extensive literature on *C. vulgaris*, biochemical characteristics can differ substantially among strains originating from different freshwater environments. Therefore, characterization of locally isolated cultures remains relevant for identifying strains with desirable biochemical and functional properties. In particular, combining morphological characterization with pigment, protein, carbohydrate and lipid estimation, qualitative phytochemical profiling and preliminary antimicrobial screening can provide an initial assessment of the functional characteristics of an indigenous isolate.

Accordingly, the present study investigated a *C. vulgaris* culture isolated from a freshwater pond at Kuppandapalayam, Erode District, Tamil Nadu, India. The study integrated culture maintenance and morphological characterization with estimation of photosynthetic pigments and major biochemical constituents, qualitative phytochemical screening, and preliminary antibacterial and antifungal evaluation. Exploratory bioethanol production following biomass hydrolysis and fermentation was also examined to assess the potential of the carbohydrate fraction as a fermentation feedstock. In addition, the study investigated the applicability of the cultured biomass in an immobilized-cell wastewater-treatment experiment. The overall objective was to establish a preliminary biochemical and functional profile of the locally isolated *C. vulgaris* culture and to identify potential directions for subsequent quantitative optimization, strain authentication and application-specific process development.

2. MATERIALS AND METHODS

2.1 Collection of *Chlorella vulgaris* and sampling site

A freshwater microalgal sample was collected from a freshwater pond at Kuppandapalayam, Erode District, Tamil Nadu, India (11.407706° N, 77.699813° E) on 20 June. Approximately 5 mL of pond water containing naturally occurring microalgal populations was collected aseptically in sterile bottles at approximately 18: 15 IST and transported immediately to the laboratory for isolation and cultivation. The collected sample was processed under aseptic conditions to establish a laboratory culture of *Chlorella vulgaris*.

2.2 Culture conditions and biomass production

The microalgal culture was maintained in BG-11 medium. The basal medium contained $NaNO_3$ (1500 mg L^{-1}), K_2HPO_4 (40 mg L^{-1}), $MgSO_4 \cdot 7H_2O$ (75 mg L^{-1}), $CaCl_2 \cdot 2H_2O$ (36 mg L^{-1}), citric acid (6 mg L^{-1}), and 1 mL L^{-1} of trace-metal solution. The pH of the medium was adjusted to 7.0–7.2 before sterilization. The medium was autoclaved at 121 °C and 15 psi for 15–20 min and allowed to cool to room temperature prior to inoculation.

The initial culture was progressively scaled from approximately 5 mL to 15 mL and subsequently to 200 mL of BG-11 medium. For biomass production, the culture was maintained under continuous aeration using an aquarium air pump and illuminated with cool-white LED light at approximately 2500 lux. A 16: 8 h light: dark photoperiod was maintained throughout cultivation. The target cultivation temperature was 25 ± 2 °C. The culture was monitored daily for changes in colour, visible contamination and biomass development. Dense green biomass was generally obtained after 7–10 days of cultivation (Andersen, 2005; Ilavarasi et al., 2011).

2.3 Biomass harvesting and preparation

At the end of the cultivation period, algal biomass was harvested by centrifugation at 5000 rpm for 10 min at room temperature. The resulting biomass pellet was washed thoroughly with distilled water to remove residual culture medium and soluble salts. The washed biomass was dried to constant weight, finely powdered and stored in airtight containers at 4 °C until further analysis. Fresh biomass was used for microscopic observation and pigment-related analyses, whereas dried biomass was used for biochemical, phytochemical and antimicrobial analyses.

2.4 Growth monitoring and morphological characterization

Culture growth was monitored by visual assessment of culture colour and measurement of optical density at 680 nm using BG-11 medium as the blank. Cell density was additionally examined using a haemocytometer. Microscopic observations were performed using a digital microscope to assess cell morphology, including cell shape, cellular arrangement, pigmentation, cell-wall characteristics and chloroplast morphology. The culture was also examined periodically for evidence of bacterial, fungal or other algal contamination. The isolate was assigned to *C. vulgaris* based on its observed morphological characteristics; molecular confirmation was not performed in the present study.

2.5 Preparation of aqueous and acetone extracts

2.5.1 Aqueous extraction

For aqueous extraction, 10 g of dried and powdered *C. vulgaris* biomass was suspended in 100 mL of distilled water at a sample-to-solvent ratio of 1: 10 (w/v). The suspension was heated in a water bath for 1 h with intermittent agitation. Following extraction, the mixture was cooled to room temperature and filtered through Whatman No. 1 filter paper. The resulting filtrate was collected in amber-coloured bottles and stored at 4 °C until further analysis.

2.5.2 Acetone extraction

For acetone extraction, 10 g of dried powdered biomass was placed in a Soxhlet extraction thimble and extracted with 100 mL of acetone. Extraction was performed under controlled heating conditions for the specified extraction period. The resulting extract was filtered and concentrated under reduced pressure where required. The crude extract was transferred to amber bottles and stored at 4 °C until phytochemical and antimicrobial analyses. The extraction procedures were adapted from previously reported protocols for the recovery of bioactive constituents from microalgal biomass (Plaza et al., 2012; Agustiar et al., 2022; Georgiopoulou et al., 2022).

2.6 Qualitative phytochemical screening

The aqueous and acetone extracts were subjected to preliminary qualitative phytochemical screening to detect major classes of secondary metabolites. Tests were performed for alkaloids, carbohydrates, flavonoids, glycosides, proteins, saponins, steroids, tannins, phenolic compounds and terpenoids using standard qualitative reactions. Alkaloids were evaluated using Wagner's reagent, carbohydrates by the Molisch reaction, flavonoids using the alkaline-reagent test, glycosides by the Keller–Killiani reaction, proteins by the Biuret test, saponins by the foam test, steroids by the Liebermann–Burchard reaction, and phenolic compounds and tannins using ferric chloride-based reactions. Terpenoids were assessed using a Salkowski-type reaction. The results were recorded qualitatively based on the development of characteristic colour changes, precipitates or persistent foam (Gutiérrez et al., 2018).

2.7 Biochemical composition analysis

2.7.1 Total carbohydrate content

Total carbohydrate content was estimated using the orcinol–sulfuric acid method with D-glucose as the reference standard (Ashwell, 1957). Appropriate glucose standards were

prepared over the required concentration range, and the samples were treated with the colour-developing reagent followed by heating at 100 °C for 20 min. After cooling, absorbance was measured at 665 nm using a spectrophotometer. Carbohydrate concentration was calculated from the standard calibration curve and expressed as glucose equivalents per gram of dry biomass.

2.7.2 Total protein content

Total protein content was estimated using the Lowry method with bovine serum albumin as the standard (Lowry et al., 1951). Appropriate concentrations of BSA standards and biomass extracts were treated with alkaline copper reagent, followed by addition of Folin–Ciocalteu reagent. After colour development under dark conditions at room temperature, absorbance was measured at 660 nm. Protein concentration was calculated from the standard calibration curve and expressed according to the sample basis used for analysis.

2.7.3 Total lipid content

Total lipid was determined by Soxhlet extraction using dried *C. vulgaris* biomass. Approximately 10 g of dried biomass was subjected to solvent extraction, followed by removal of the solvent and gravimetric determination of the recovered lipid fraction. Lipid content was calculated using the following equation.

Lipid content (%) = (Mass of extracted lipid / Mass of dry biomass) × 100

The recovered lipid fraction was weighed after complete solvent removal and stored under appropriate conditions until further characterization.

2.8 Chlorophyll estimation

Photosynthetic pigments were extracted from dried *C. vulgaris* biomass using 90% acetone. Approximately 1 g of biomass was subjected to pigment extraction, and the final extract volume was adjusted to 10 mL. Absorbance was measured spectrophotometrically at 663 and 645 nm using 90% acetone as the blank. Chlorophyll *a*, chlorophyll *b* and total chlorophyll concentrations were calculated using the spectrophotometric equations described by Arnon (1949) and expressed on the basis of the biomass and extract volume used.

2.9 Antibacterial activity

The antibacterial activity of the aqueous and acetone extracts was evaluated against *Escherichia coli* using the agar-well diffusion method. A standardized bacterial suspension was prepared from an actively growing pure culture and uniformly inoculated onto the

surface of Mueller–Hinton agar plates. Sterile wells were prepared aseptically in the inoculated agar, and the respective *C. vulgaris* extracts were introduced into the wells at the designated test concentration.

A suitable antibacterial agent was included as the positive control, while the corresponding extract solvent was used as the negative control. The inoculated plates were incubated at 37 °C for 18–24 h. Following incubation, antibacterial activity was assessed by measuring the diameter of the clear inhibition zone surrounding each well in millimetres. The assay was conducted under identical conditions for the different extracts, and the inhibition-zone measurements were recorded for comparative evaluation (Perez, 1990; Balouiri et al., 2016).

3. RESULTS

3.1 Culture and growth

The freshwater microalgal inoculum was successfully established and maintained in BG-11 medium under the specified cultivation conditions. The culture exhibited a progressive change in colour from light green during the initial cultivation stage to green and subsequently dark green after approximately 7–10 days of incubation. The progressive intensification of green colour was consistent with increased algal biomass and pigment accumulation during culture development. The culture remained visually homogeneous throughout the cultivation period, and no apparent bacterial, fungal, or other algal contamination was observed. The development of a dense green culture after 7–10 days indicated successful establishment of the *C. vulgaris* culture and provided sufficient biomass for subsequent biochemical, phytochemical and antimicrobial analyses.

Table 1: Schematic workflow of the Culture and Growth.

Parameter	Observation
Initial culture appearance	Light green
Later culture appearance	Dark green
Time to dense biomass	7–10 days
Observed cultivation temperature	22–24 °C
Illumination	~2500 lux; 16: 8 h light: dark
Aeration	Continuous
Visible contamination	Not observed

3.2 Morphological characterization

Microscopic examination of the cultured microalga revealed healthy, green, unicellular cells that were predominantly spherical to slightly oval in shape. The cells exhibited a distinct cell

wall and a well-defined chloroplast. The absence of visible morphological abnormalities or contaminating microorganisms indicated good culture quality under the cultivation conditions employed. The observed cellular characteristics were consistent with the typical morphological features of *Chlorella vulgaris*, supporting the preliminary identification of the isolated culture as *C. vulgaris*.

Table 2: Morphological characterization.

Characteristic	Observation
Cell shape	Spherical to slightly oval
Cell colour	Bright green
Cell arrangement	Unicellular
Chloroplast	Single chloroplast
Cell wall	Distinct and smooth
Contamination	Not observed
Culture status	Healthy and actively growing

3.3 Chlorophyll Content

Microscopic examination of the established culture revealed predominantly healthy, green, unicellular cells with a spherical to slightly oval morphology. The cells exhibited a distinct cell wall and a well-defined chloroplast. No apparent morphological abnormalities or visible contamination were observed during microscopic examination. The observed cellular characteristics were consistent with the reported morphological features of *Chlorella vulgaris*, thereby supporting the preliminary morphological identification of the isolated culture as *C. vulgaris*.

Table 3: Chlorophyll content.

Parameter	Value
Absorbance at 663 nm	0.946
Absorbance at 645 nm	0.208
Chlorophyll a	11.45 mg L−1
Chlorophyll b	0.34 mg L−1
Total chlorophyll	11.79 mg L−1

3.4 Qualitative phytochemical profile

Preliminary phytochemical screening demonstrated the presence of several major classes of extractable constituents in the aqueous and acetone extracts of *Chlorella vulgaris* (Table 4). Alkaloids, carbohydrates, flavonoids, glycosides, proteins, steroids and tannins/phenolic compounds were detected in both extracts based on positive reactions in the respective qualitative assays. Saponins were detected exclusively in the aqueous extract, whereas

terpenoids were also detected in the aqueous extract but were not detected in the acetone extract. Overall, the aqueous extract exhibited positive reactions for all phytochemical groups examined, while the acetone extract showed positive reactions for all tested constituents except saponins and terpenoids. The observed differences indicate that solvent selection influenced the qualitative recovery of phytochemical constituents from *C. vulgaris* biomass. The results further demonstrate the presence of a chemically diverse range of detectable phytochemical classes in the investigated microalgal culture, providing a basis for subsequent quantitative and chromatographic characterization.

Table 4: Qualitative phytochemical profile.

Phytochemical	Test	Aqueous	Acetone
Alkaloids	Wagner	+	+
Carbohydrates	Molisch	+	+
Flavonoids	Alkaline reagent	+	+
Glycosides	Keller–Killiani	+	+
Proteins	Biuret	+	+
Saponins	Foam	+	–
Steroids	Liebermann–Burchard	+	+
Tannins/phenolics	Ferric chloride	+	+
Terpenoids	Salkowski	+	+

3.5 Total carbohydrate content

The total carbohydrate content of *Chlorella vulgaris* biomass was quantified using the glucose calibration method. The glucose standard and algal extract showed absorbance values of 0.753 and 0.757, respectively, at 665 nm. Based on the standard calibration curve, the carbohydrate content was estimated as 1.005 mg in the analyzed sample, corresponding to 1.01 mg g^{-1} dry weight (DW) (Table 5). The result confirms the presence of measurable carbohydrate reserves in the investigated *C. vulgaris* biomass. Expressing the carbohydrate concentration on a dry-weight basis provides a standardized measure for evaluating the biochemical composition of the biomass and facilitates comparison with other *C. vulgaris* strains and cultivation conditions.

Table 5: Estimation of total carbohydrate content of *Chlorella vulgaris* biomass.

Measurement	Value
Measurement wavelength	665 nm
Standard glucose absorbance	0.753
Sample absorbance	0.757
Carbohydrate in extract	1.005 mg
Reported total carbohydrate	1.01 mg g−1 DW

3.6 Total protein content

The protein content of *Chlorella vulgaris* biomass was quantified using the Lowry method with bovine serum albumin (BSA) as the reference standard. Absorbance was measured at 660 nm, and a concentration-dependent increase in absorbance was observed across the BSA standards. The absorbance values increased from 0.000 for the blank to 0.229 at 100 µg mL^{-1} BSA, while the standards containing 20, 40, 60 and 80 µg mL^{-1} BSA exhibited absorbance values of 0.002, 0.043, 0.152 and 0.200, respectively (Table 6). The progressive increase in absorbance with increasing BSA concentration confirmed the suitability of the calibration series for protein estimation.

The absorbance obtained for the *C. vulgaris* sample was subsequently compared with the BSA calibration relationship to determine its protein concentration. The result confirms the presence of measurable protein in the investigated algal biomass and supports the biochemical characterization of *C. vulgaris* as a protein-containing microalgal resource.

Table 6: BSA standard curve for estimation of total protein content.

BSA standard (µg mL−1)	OD at 660 nm
0	0.000
20	0.002
40	0.043
60	0.152
80	0.200
100	0.229

3.7 Total lipid content

Gravimetric determination following Soxhlet extraction showed that *Chlorella vulgaris* biomass contained a measurable lipid fraction. Extraction of 10 g of dried biomass yielded 1.327 g of recovered lipid, corresponding to a total lipid content of 13.27% (w/w). This value was calculated based on the mass of lipid recovered relative to the initial dry biomass. The result indicates that the investigated *C. vulgaris* culture accumulated a substantial proportion of extractable lipids, supporting its potential for downstream applications involving algal lipid recovery.

It should be noted that the source records contain a minor discrepancy, with the narrative reporting 1.323 g (13.23%), whereas the corresponding table reports 1.327 g (13.27%). The tabulated value of 1.327 g and 13.27% is retained here because it represents the value

provided in the results table. For final publication, this discrepancy should be verified against the original laboratory records.

Table 7: Total lipid content.

Parameter	Value
Initial dry biomass	10.000 g
Empty vial (W1)	13.851 g
Vial + extracted lipid (W2)	15.178 g
Extracted lipid (W2 − W1)	1.327 g
Lipid content	13.27% (w/w)

3.8 Antibacterial activity

Both aqueous and acetone extracts of *Chlorella vulgaris* exhibited measurable antibacterial activity against *Escherichia coli* (Table 7). The aqueous extract produced an inhibition zone of 20 mm, whereas the acetone extract produced a zone of 15 mm under the tested conditions. The corresponding positive-control values were 10 and 9 mm, respectively, while the negative control showed no detectable inhibition.

The comparatively greater inhibition observed with the aqueous extract indicates that the antibacterial activity was not confined to acetone-soluble constituents and may involve relatively polar, water-extractable metabolites. However, inhibition-zone diameter is influenced by several factors, including extract concentration, compound diffusion through the agar matrix, well size and physicochemical properties of the active constituents. Therefore, the larger inhibition zone observed for the algal extracts should not be interpreted as evidence of greater antibacterial potency than the positive control unless equivalent concentrations and standardized assay conditions are established. Further evaluation using MIC, MBC and concentration-dependent assays would provide more reliable evidence of the antibacterial efficacy of *C. vulgaris* extracts.

Table 8: Antibacterial activity.

Treatment	Zone of inhibition against *E. coli* (mm)
Aqueous extract	20
Positive control associated with aqueous assay	10
Acetone extract	15
Positive control associated with acetone assay	9
Negative control	0

4. DISCUSSION

The successful establishment of *Chlorella vulgaris* in BG-11 medium and the development of a dense green culture demonstrate that the investigated strain was capable of adapting to the laboratory cultivation conditions. The observed spherical to slightly oval, unicellular morphology, distinct cell wall and prominent chloroplast were consistent with the characteristic features generally associated with *Chlorella*. The progressive change in culture colour from light green to dark green during cultivation further suggested an increase in cellular biomass and photosynthetic pigment accumulation. The total chlorophyll concentration of 11.79 mg L^{-1}, with chlorophyll *a* representing the major pigment fraction, provides additional evidence of active photosynthetic biomass. Pigment biosynthesis in *C. vulgaris* is known to be strongly influenced by irradiance, nutrient availability, temperature, carbon supply and physiological growth stage; consequently, chlorophyll concentration should be interpreted in relation to the specific cultivation conditions rather than as a fixed characteristic of the species. Similar environmental dependence has been widely reported for microalgal pigment production and biomass composition.

Although the morphological characteristics supported the preliminary identification of the isolate as *C. vulgaris*, morphology alone may not provide sufficient resolution for reliable strain-level identification. Members of the genus *Chlorella* can exhibit considerable morphological similarity, while substantial differences in biochemical composition and bioactivity may occur among closely related strains. Molecular approaches, particularly 18S rRNA sequencing and, where appropriate, additional barcode markers, would therefore provide stronger taxonomic confirmation in future studies. Molecular authentication would also improve the reproducibility of subsequent biochemical and antimicrobial investigations.

The biochemical characterization demonstrated that the investigated biomass contained extractable lipids, carbohydrates and proteins, supporting the multifunctional biochemical nature of *C. vulgaris*. The lipid content of 13.27% (w/w) obtained by Soxhlet extraction falls within the broad range reported for *C. vulgaris*, although lipid accumulation can vary considerably depending on strain characteristics, cultivation conditions, nutrient availability and growth phase. Under nutrient limitation, particularly nitrogen stress, *C. vulgaris* can redirect cellular carbon metabolism towards storage lipid accumulation. Thus, the lipid value obtained in the present investigation represents the solvent-extractable fraction under the experimental conditions employed and should not be interpreted as a universal lipid content

for the species. Moreover, gravimetric Soxhlet extraction provides information on total recoverable lipid but does not distinguish neutral lipids, polar lipids or individual fatty acids. Fatty-acid profiling by GC–MS/GC-FID and comprehensive lipidomic analysis would therefore be necessary to establish the composition and potential application of the recovered lipid fraction.

The carbohydrate concentration was estimated as 1.01 mg g^{-1} dry weight, confirming the presence of measurable carbohydrate in the investigated biomass. However, this value appears relatively low when compared with carbohydrate concentrations commonly reported for *C. vulgaris*. The discrepancy may arise from differences in extraction procedure, analytical method, sample preparation or calculation. In the present dataset, the glucose standard and sample showed absorbance values of 0.753 and 0.757, respectively, at 665 nm. Because the sample response was very close to the standard response, the reported concentration should be carefully checked against the original calibration curve, dilution factor, extraction volume and biomass basis. This verification is important before using the value for quantitative comparison with published studies. Future work should employ a validated calibration curve with an appropriate concentration range and report analytical replicates together with regression statistics.

The protein estimation similarly requires careful consideration. The Lowry assay produced a concentration-dependent response for the BSA standards, confirming the suitability of the standard series for protein determination. However, the source data contain a discrepancy between the reported sample concentration of approximately 0.46 mg mL^{-1} and the value of 46 µg mL^{-1} presented elsewhere. These values differ by one order of magnitude and therefore should be reconciled using the original absorbance data, calibration equation, dilution factor and biomass basis. Such verification is essential because protein is one of the major commercially relevant components of *Chlorella* biomass and inaccurate reporting can substantially affect comparisons with other strains and cultivation systems. Future investigations should express protein concentration consistently on a dry-weight basis and include calibration-curve parameters, analytical replicates and appropriate uncertainty estimates.

The preliminary phytochemical screening further demonstrated that the aqueous and acetone extracts contained several detectable classes of compounds. Alkaloids, carbohydrates,

flavonoids, glycosides, proteins, steroids and tannins/phenolic compounds were detected in both extracts, whereas saponins and terpenoids were detected only in the aqueous extract. The occurrence of diverse phytochemical constituents in *C. vulgaris* is consistent with previous reports describing the presence of phenolic compounds, flavonoids, terpenoid-related metabolites and other biologically relevant constituents in microalgal biomass. The differences between the two extracts indicate that solvent selection influenced the qualitative recovery of detectable constituents. Water preferentially recovers highly polar and water-soluble components, whereas acetone can facilitate the extraction of a broader range of moderately polar metabolites and pigments. Therefore, the solvent-dependent profile observed in this study provides preliminary evidence that different extraction systems may selectively enrich different chemical fractions.

Nevertheless, the phytochemical screening should be interpreted cautiously. The colour reactions employed in qualitative screening are useful for preliminary assessment but are not compound-specific and cannot establish the identity, concentration or biological contribution of individual metabolites. A positive reaction for a particular phytochemical class should therefore not be regarded as confirmation of a specific compound. Chromatographic and spectrometric approaches such as HPLC-DAD, LC–MS/MS and GC–MS would provide more reliable chemical characterization. Quantification of individual phenolic and flavonoid compounds would also allow meaningful assessment of their relationship with the observed biological activities.

The antibacterial assay demonstrated that both *C. vulgaris* extracts inhibited the growth of *Escherichia coli*, with the aqueous extract producing a 20 mm inhibition zone and the acetone extract producing a 15 mm zone under the experimental conditions. The comparatively larger inhibition zone produced by the aqueous extract suggests that water-soluble constituents may contribute substantially to the observed antibacterial response. This observation is biologically plausible because microalgae contain diverse polar metabolites and other water-extractable compounds that may possess antimicrobial properties. Earlier studies have also demonstrated antibacterial activity associated with *Chlorella* species, supporting the present observation that *C. vulgaris* extracts can exhibit inhibitory effects against bacterial organisms.

However, the inhibition-zone measurements should not be interpreted as direct measures of intrinsic antibacterial potency. Agar diffusion is influenced not only by antimicrobial activity

but also by molecular size, polarity, solubility, diffusion coefficient, extract concentration, agar composition and inoculum density. Therefore, the 20-mm zone observed for the aqueous extract cannot, by itself, establish superior antibacterial potency relative to the acetone extract or the positive control. Standardized concentration-dependent assays, including minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determinations, would provide stronger quantitative evidence. Appropriate solvent controls and independent biological replicates should also be incorporated into subsequent experiments.

The preliminary antifungal observations against *Fusarium sacchari* and *Fusarium incarnatum* further indicate that the biological activity of the biomass may extend beyond antibacterial effects. However, because numerical inhibition-zone values and detailed replicate-level data were not available for these assays, the antifungal observations should be considered preliminary rather than quantitative evidence of antifungal potency. Future studies should determine MIC and MFC values, evaluate concentration-dependent responses and include appropriate reference antifungal agents. Such experiments would help distinguish genuine antifungal effects from differences in extract diffusion and assay conditions.

Taken together, the biochemical and biological findings indicate that the investigated *C. vulgaris* culture possesses characteristics relevant to a microalgal biorefinery approach. The simultaneous recovery of pigments, proteins, carbohydrates, lipids and bioactive extractable constituents suggests that the biomass should not necessarily be considered as a feedstock for a single end product. Instead, sequential or integrated processing could potentially recover multiple fractions, with lower-value residual biomass subsequently directed towards additional applications such as fermentation or environmental biotechnology. However, the present study provides a preliminary characterization rather than a complete biorefinery assessment. Process yields, mass balance, energy requirements, product purity and economic feasibility would need to be evaluated before any industrial-scale conclusions can be drawn.

Overall, the present findings establish a preliminary biochemical and biological profile of the investigated *C. vulgaris* culture. The combination of measurable pigments, lipid, carbohydrate and protein fractions, diverse qualitative phytochemical constituents and antibacterial activity supports further investigation of this microalga as a source of value-added biomolecules. Nevertheless, molecular strain authentication, validation of the carbohydrate and protein calculations, quantitative chemical profiling, replicated

antimicrobial assays and standardized potency measurements are necessary to strengthen the scientific basis of the findings. Such follow-up studies would enable clearer relationships to be established between cultivation conditions, biomass composition, extract chemistry and biological activity.

5. CONCLUSIONS

The present study established and characterized a laboratory culture of *Chlorella vulgaris* with respect to its photosynthetic pigments, biochemical composition, phytochemical profile and antibacterial activity. The cultivated biomass exhibited a total chlorophyll content of 11.79 mg L^{-1}, with chlorophyll *a* representing the predominant pigment. Biochemical analysis confirmed the presence of carbohydrates, proteins and lipids, with the lipid fraction accounting for 13.27% of the dry biomass. Qualitative phytochemical screening demonstrated the presence of multiple detectable metabolite classes, including alkaloids, carbohydrates, flavonoids, glycosides, proteins, steroids and tannins/phenolic compounds. Saponins and terpenoids were detected predominantly in the aqueous extract, indicating a solvent-dependent variation in the recovery of extractable constituents.

Both aqueous and acetone extracts exhibited antibacterial activity against *Escherichia coli*, producing inhibition zones of 20 and 15 mm, respectively, under the conditions employed. These findings provide preliminary evidence that *C. vulgaris* biomass contains biologically active constituents with antibacterial potential. However, the results should be considered an initial laboratory-level assessment, and the observed inhibition cannot be used alone to establish antimicrobial potency or identify the compounds responsible for the activity.

Overall, the study highlights the multifunctional biochemical and biotechnological potential of *C. vulgaris* as a source of pigments, lipids, proteins, carbohydrates and bioactive constituents. Further studies should focus on molecular authentication of the strain, validation of biochemical measurements, quantitative phytochemical profiling, compound-level characterization, MIC/MBC determination and statistically replicated antimicrobial assays. Such investigations will be essential for establishing reproducible relationships between *C. vulgaris* biomass composition, extract chemistry and biological activity and for determining its suitability for specific biotechnological applications.

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