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PHYTOCHEMICAL CHARACTERIZATION AND ANTIBACTERIAL POTENTIAL OF SPIRULINA PLATENSIS

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ABSTRACT
Background: Algae are among the most diverse types of organisms on the planet. They have many advantages for humans. The most significant one is blue-green algae, which contain bioactive compounds, such as those found in *Spirulina platensis*. **Methodology:** The present study examines the antibacterial properties and phytochemical composition of *Spirulina platensis*, isolated from different freshwater bodies in the Chitradurga district, Karnataka, India. **Results & Discussions:** The anti-bacterial activity of the selected bacterial strains was investigated, and some appropriate results were found, which are discussed in the following sections. The methanolic extraction has revealed the maximum activity and the highest inhibition zones against *E. coli* and *P. aeruginosa*, followed by the acetone and the hexane extracts. The GC-MS profile examined has shown a range of bioactive compounds, viz., hexadecenoic acid, methyl esters, n-hexadecenoic acid, and glycerine. The occurrence of these compounds represents glycerine, ester compounds & lipid-derived metabolites, and the aromatic metabolites produced have the potential to exhibit antibacterial activity by *S. platensis*. It has the highest potential in the pharmaceuticals, nutraceuticals, and biotechnologies**. Conclusions**: The presence of these substances suggests that glycerine, esters, and aromatic compounds act in concert to produce potentially broad antibacterial activity by *S. platensis*. It can be inferred that the methanolic extract has shown high bioactivity.

INTRODUCTION

Algae, ranging from unicellular microalgae to multicellular macroalgae (seaweeds), are prolific producers of bioactive compounds. Due to their ecological adaptability and unique metabolic capabilities, many algal species synthesize metabolites with potent antimicrobial activity against bacteria, fungi, viruses & even protozoa [1]. Algal metabolites can inhibit microbial growth through various mechanisms, *viz*., Cell membrane disruption: Lipophilic compounds (e.g., fatty acids, halogenated metabolites) insert into microbial membranes, causing leakage. Enzyme inhibition: Phenolic compounds and terpenoids interfere with microbial enzymes. DNA replication interference: Some metabolites affect nucleic acid synthesis in bacteria. Quorum-sensing inhibition: Reduces bacterial virulence and biofilm formation [2, 3].

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The alarming rise of antimicrobial resistance (AMR) has intensified the global search for novel, effective, and sustainable antimicrobial agents. Algae, comprising diverse groups such as microalgae (e.g., *Chlorella*, *Spirulina*) and macroalgae or seaweeds (e.g., *Ulva*, *Sargassum*, *Gracilaria*), have emerged as promising bioresources due to their ability to synthesize a wide array of bioactive metabolites. These compounds exhibit potent antimicrobial activities against a broad spectrum of pathogenic microorganisms, including bacteria, fungi, and viruses [4, 5].

Unlike synthetic antibiotics, algal-derived antimicrobials are often biodegradable, eco-friendly, and less prone to inducing resistance. Algae produce unique secondary metabolites such as phlorotannins, halogenated terpenes, polyunsaturated fatty acids, alkaloids, and sulphated polysaccharides that have demonstrated significant inhibitory effects on microbial growth, quorum sensing, and biofilm formation [6]. The diversity and complexity of these compounds are influenced by species type, environmental conditions, and growth phase, making algae a rich and dynamic source of antimicrobial agents [7].

Recent research has focused on isolating, characterizing, and applying these bioactive molecules in pharmaceuticals, food preservation, aquaculture, and nanomedicine. Moreover, advances in metabolic and genome sequencing are facilitating the exploration of algal biosynthetic pathways, enabling sustainable production through biotechnology [8-10]. Applications of *Spirulina platensis* are wide-ranging in Biotechnology and Medicine. Some of the significant applications include Natural preservatives in food and cosmetics, Biomedical coatings for catheters and implants, and pharmaceutical agents, such as antiviral and antibacterial drugs [11, 12]. Agricultural biocontrol agents against plant pathogens [13, 14].

All these applications contributed to variability in concentrations and other extrinsic or intrinsic factors [15]. Variability in bioactivity based on environmental and seasonal factors. Extraction and purification processes are often complex and expensive [16]. Toxicity and biocompatibility need thorough evaluation before clinical application [17]. In the current study, antimicrobial activity against bacteria was evaluated. To study the antibacterial effect, the bacteria *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Streptococcus mutans* were used.

MATERIALS & METHODS

Sample Collection

Freshwater samples of *Spirulina spp.* were collected from Bharamasagara Lake in the Chitradurga district, Karnataka, India, during the early summer season (2nd March and 2nd April 2025). Samples were taken in 1-liter sterile containers, kept cool (~4°C), and transported to the laboratory within 6 hours of collection.

Isolation and Cultivation of *Spirulina platensis*.

The collected pond water was filtered using a muslin cloth to remove large debris. Centrifugation of the filtrate at 5,000 rpm for 10 minutes concentrated the algal biomass. The sediment was washed three times with sterile distilled water. The isolated *Spirulina* was inoculated into Zarrouk's medium, a commonly used alkaline medium for cyanobacterial culture, and incubated at 28 ± 2 °C under continuous light (2,000 lux) with gentle aeration (using an aquarium pump). Cultivation was continued for 10–15 days until the exponential growth phase.

Biomass Harvesting and Preparation of Extracts

After reaching optimal growth, the harvested *Spirulina* biomass was separated by centrifugation at 6,000 rpm for 15 minutes, and medium residues were removed by repeatedly washing with sterile distilled water. The biomass was then ground into a powder using a mortar and pestle after being shade-dried at room temperature.

Solvent Extraction

Approximately 10 g of dried biomass was subjected to solvent extraction using various solvents (methanol, acetone, and Hexane) in a Soxhlet apparatus for six hours. The extracts were filtered through Whatman No. 1 filter paper and then evaporated using a rotary evaporator to obtain the crude extracts. They were kept at 4°C for further analysis.

Test Microorganisms

The antimicrobial activity of *Spirulina* extracts was tested against the following bacterial strains:

Bacterial strains

- *Staphylococcus aureus* (Gram-positive)
- *Bacillus cereus* (Gram-positive)
- *Escherichia coli* (Gram-negative)
- *Pseudomonas aeruginosa* (Gram-negative)
- *Salmonella typhi* (Gram-negative)
- *Streptococcus mutans* (Gram-positive)

ANTIBACTERIAL ACTIVITY

Well diffusion method to check the Zone of inhibition

Samples extracted from various sources were assessed using the zone of inhibition against the organisms listed above.

Bacterial culture preparation

In four separate Erlenmeyer flasks, Luria-Bertani (LB) broth (Tryptone 10g, Sodium chloride 10g, Yeast extract 6g, Distilled water 1000mL) was prepared by adding Tryptone 0.3g, Sodium chloride 0.3g, Yeast extract 0.18g, and Distilled water 30mL. The flasks were then autoclaved for 15 minutes at 121°C. Subsequently, 30 millilitres of sterile LB broth flasks were infected with *E. coli* strain (MTCC 433), *P. aeruginosa* strain (MTCC 2453), *S. aureus* strain (MTCC 96), *S. mutans* strain (MTCC 497), *S. typhi*, and *B. cereus*, respectively, and incubated for 24 hours at 37˚ C. After centrifuging the cultured strains for 10 minutes at 6000 rpm, the pellets were dissolved in 1% (w/v) sodium chloride and adjusted to an absorbance of 1.000 at 600 nm using a UV spectrophotometer (Genesys 10S UV-VIS Spectrophotometer). The supernatant was disposed of.

Sample preparation

The sample (i.e., hexane, acetone, and methanol extracts of *Spirulina platensis*), 100 mg, was dissolved in 1 ml of Dimethyl sulfoxide (DMSO). The samples were prepared by pipetting 10 µL (1 mg), 20 µL (2 mg), 30 µL (3 mg), and 40 µL (4 mg) from the original 100 mg/mL stock solution, and DMSO was added to the final volume of each so it was up to 50 µL.

Standard/Control Antibiotic Compound Preparation

Tetracycline 10mg was dissolved in 1ml of DMSO. Different concentrations of Tetracycline were prepared by pipetting 10 µL (100 µg), 20 µL (200 µg), 30 µL (300 µg), 40 µL (400 µg), and 50 µL (500 µg), then making up to 50 µL with DMSO.

Plating to examine the zone of inhibition of organisms

The disinfected Petri dishes were filled with around 25 mL of the media (LB agar) and left to harden. A plate spreader was used to evenly distribute 200 µL of the produced inoculum onto agar plates. Using a borer, five 0.6 cm wells were drilled into each plate. 50 µL of the sample and the Control were added to the corresponding plate wells, while 50 µL of DMSO was added to the middle well as the negative control. The bacterial plates were incubated at 37 °C for 24 hours. The zone of inhibition was then measured in millimeters (mm).

Media preparation for antibacterial activity

In two Erlenmeyer flasks, Luria Bertani (LB) agar media (Tryptone 10g, Sodium chloride 10g, Yeast extract 6g, Agar 20g, Distilled water 1000mL) 500mL was made by adding Tryptone 5g, Sodium chloride 5g, Yeast extract 3g, Agar 10g, and Distilled water 500mL, respectively, and autoclaved for 15 minutes at 121°C.

RESULTS AND DISCUSSION

Spirulina isolated from the Chitradurga pond exhibited robust growth in Zarrouk's medium, reaching the exponential phase by the 10th day of incubation. The average dry biomass yield obtained after harvesting and drying was 2.6 ± 0.15 g/L.

Antimicrobial Activity of *Spirulina* Extracts

The zone of inhibition of the various solvent extracts was tested. The following sections detail the results obtained and the discussions. The methanolic extract exhibited the strongest antibacterial activity among the various solvent extracts, followed by the acetone and hexane extracts. The values in the tables represent the mean ± standard deviation across 3 replicates.

Table 1: Zone of Inhibition of methanol extract against bacteria (±SD)

Bacterial plates	Zone of Inhibition of methanol against bacteria (in Millimeter ±SD)			
	1mg	2mg	3mg	4mg
S. aureus	10± 0.6	11 ± 0.7	11 ± 0.7	13 ± 0.9
B. cereus	14 ± 0.7	15 ± 0.8	16 ± 0.9	17 ± 1.0
E. coli	28 ± 1.0	29 ± 1.1	29 ± 1.0	31 ± 1.2
P. aeruginosa	13 ± 0.9	15 ± 1.1	17 ± 1.3	21 ± 1.8
S. typhi	20 ± 0.9	21 ± 1.0	23 ± 1.2	24 ± 1.3
S. mutans	10 ± 0.7	12 ± 0.9	13 ± 1.0	14 ± 1.1

A table (1) represents the zone of inhibition of methanol extract against various organisms, viz., *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Salmonella typhi*, and *Streptococcus mutans*. In antimicrobial investigations, various concentrations of the extract were tested and represented in Table 1. Of the six bacterial sources, the methanol extract exhibits the following dose-dependent antibacterial activity: inhibitory activity increases with increasing dose. *Escherichia coli* was the most sensitive of all the bacteria studied, with inhibition areas ranging from 28 ± 1.0 mm to 31 ± 1.2 mm at 1 mg & 4 mg, respectively. This shows that the methanol extract has strong antibacterial

activity against Gram-negative intestinal infections. Additionally, there was a notable suppression of *Salmonella typhi.*

Salmonella typhi was likewise significantly inhibited in the same concentration range, ranging from 20 ± 0.9 mm to 24 ± 1.3 mm. *Pseudomonas aeruginosa* had moderate sensitivity and a significantly larger zone of inhibition, ranging from 13 ± 0.9 mm to 21 ± 1.8 mm, suggesting that the extract would be equally effective against this typically resistant microorganism. *Bacillus cereus* and *Staphylococcus aureus*, two gram-positive bacteria, showed only mild inhibition, with zones growing from 14 ± 0.7 to 17 ± 1.0 mm and 10 ± 0.6 to 13 ± 0.9 mm, respectively. For *Streptococcus mutans*, which may also be associated with tooth infections, this increment increased from 10 ± 0.7 mm to 14 ± 1.1 mm, with a steady but less pronounced effect. The permeability and reactivity of methanol-extracted phytochemicals, as well as variations in the cell wall structure of Gram-positive and Gram-negative bacteria, may be linked to this variability in sensitivity. Overall, this extract has broad-spectrum antibacterial capability and is more effective against Gram-negative pathogens than Gram-positive strains. Its good performance suggests that it could be used in plant-based antimicrobial treatments.

Standard deviation values indicate low to moderate variability in the data for zone of inhibition measurements, illustrating good reproducibility of the antibacterial assay. The lower average SD values at lower concentrations indicate more consistent inhibition. In contrast, the higher average SD values at higher concentrations, especially for *Pseudomonas aeruginosa*, indicate enhanced diffusion and more potent dose-dependent antibacterial activity. Overall, the SD values fall within acceptable limits for agar diffusion assays, confirming the reliability and consistency of the experimental results.

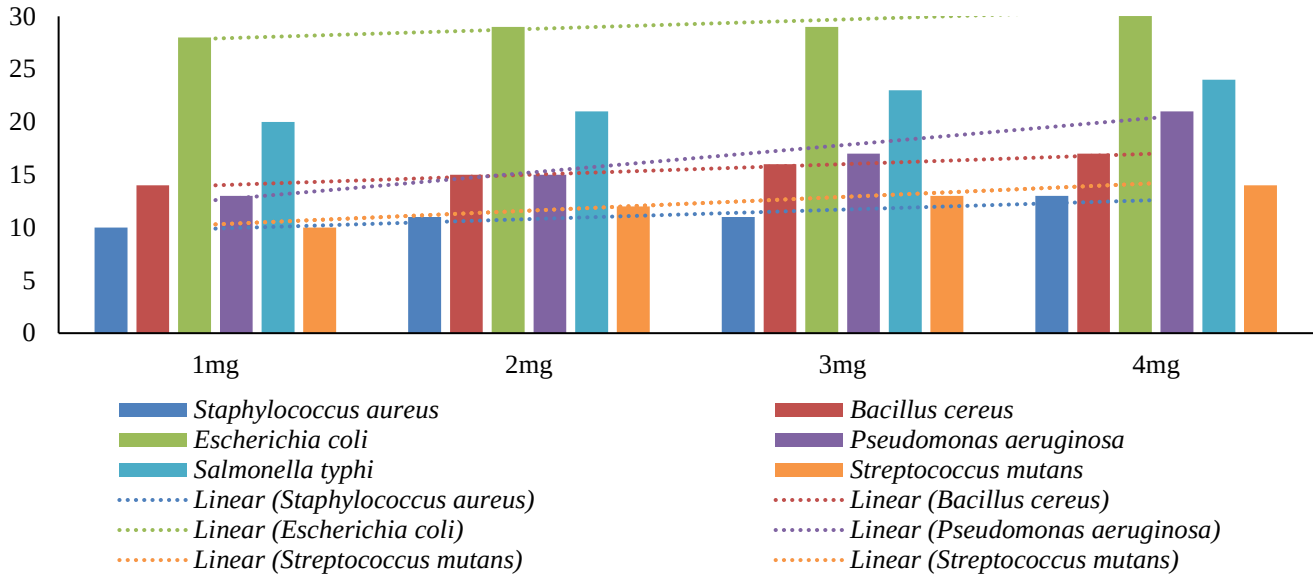


Figure 1: Linear regression plot for the Zone of Inhibition methanol extract tested against different bacteria

This graph shows a linear regression of zone-of-inhibition values for the methanol extract tested in vitro against various bacterial strains. A linear regression line indicates the trend of antimicrobial activity with respect to bacterial susceptibility (Figure 1). The negative slope indicates an inverse relationship: a higher extract concentration corresponds to a lower zone of inhibition, indicating better antibacterial activity. This is evidence that the methanol extract exhibits greater inhibitory activity at high doses. If the R^2 is around 1, it indicates a strong linear relationship between methanol extract concentration and inhibition of the bacterial environment. The bacterial sites along the linear regression line indicate each site's sensitivity. For instance, *S. aureus*, with a smaller zone of inhibition than *E. coli*, would be plotted near the origin, indicating higher sensitivity. The plot highlights the broad-spectrum antibacterial activity and consistent activity of the methanol extract. As the regression plot indicates, higher methanol concentration correlates with greater antibacterial activity across all tested bacteria. This shows a positive association between methanol extract and growth inhibition, as the area of inhibition increases with concentration.

Bacteria such as *S. aureus* and *P. aeruginosa* also show a steeper slope, indicating sensitivity to the extract. On the other hand, *the S. typhi and S. mutans* slopes show a flatter response, with a smaller slope. The goodness of fit (R^2 value), if the value is given, would indicate the strength of this correlation. The concentration-dependent sensitivity of methanol demonstrates its effectiveness as an antimicrobial agent, supporting its potential use in antibacterial formulations.

Table 2: Zone of Inhibition hexane extract against bacteria (±SD)

Bacterial Plates	Zone of Inhibition of hexane extract against bacteria (in Millimeter ±SD)			
	1mg	2mg	3mg	4mg
S. aureus	6 ± 0.5	7 ± 0.6	8 ± 0.7	9 ± 0.8
B. cereus	10 ± 0.7	11 ± 0.8	11 ± 0.8	12 ± 0.9
E. coli	25 ± 1.0	26 ± 1.1	27 ± 1.2	28 ± 1.3
P. aeruginosa	11 ± 0.8	12 ± 0.9	15 ± 1.2	18 ± 1.6
S. typhi	17 ± 0.9	18 ± 1.0	19 ± 1.1	20 ± 1.2
S. mutans	8 ± 0.6	10 ± 0.8	11 ± 0.9	12 ± 1.0

The hexane extract exhibits antibacterial activity, as the inhibitory zones for the test bacteria are consistent across all tested isolates. However, compared to the methanol extract, it is usually limited. *Escherichia coli* once again showed the highest sensitivity among the tested isolates, with inhibition zones ranging from 25 ± 1.0 mm at 1 mg to 28 ± 1.3 mm at 4 mg, suggesting the compounds isolated from hexane were highly sensitive. *Salmonella typhi* also exhibited a high response, with growth zones increasing from 17 ± 0.9 mm to 20 ± 1.2 mm, indicating the extract's potential activity against enteric Gram-negative pathogens. *Pseudomonas aeruginosa* was generally resistant to most antibiotics and showed a significant increase in inhibition from 11 ± 0.8 mm to 18 ± 1.6 mm, indicating that hexane-based phytochemicals at high concentrations exerted a significant influence. *Bacillus cereus* showed moderate sensitivity (10 ± 0.7 mm to 12 ± 0.9 mm) from Gram-positive bacteria. in relation to *S. aureus* and *Streptococcus mutans* (6 ± 0.5mm to 9 ± 0.8mm and 8 ± 0.6 mm to 12 ± 1.0mm). These small inhibition patterns could reflect lower solubility or the lower availability of polar antibacterial molecules in non-polar hexane solvent. Hexane extract showed antibacterial activity, mostly against Gram-negative strains, but its efficacy was lower than that of the methanol extract (Table 2), suggesting that the active compounds were more polar. Standard deviation values indicate the variation observed in the antibacterial activity of the hexane extract between experiments. Smaller SDs at inhibition zones indicate uniform and reproducible data, while slightly higher SDs at higher concentrations, especially in *P. aeruginosa*, indicate increased diffusion and a stronger dose-dependent response. In general, SD values are within the acceptable range for agar diffusion test procedures, confirming the reliability and repeatability of the antibacterial effects across the tested bacterial strains.

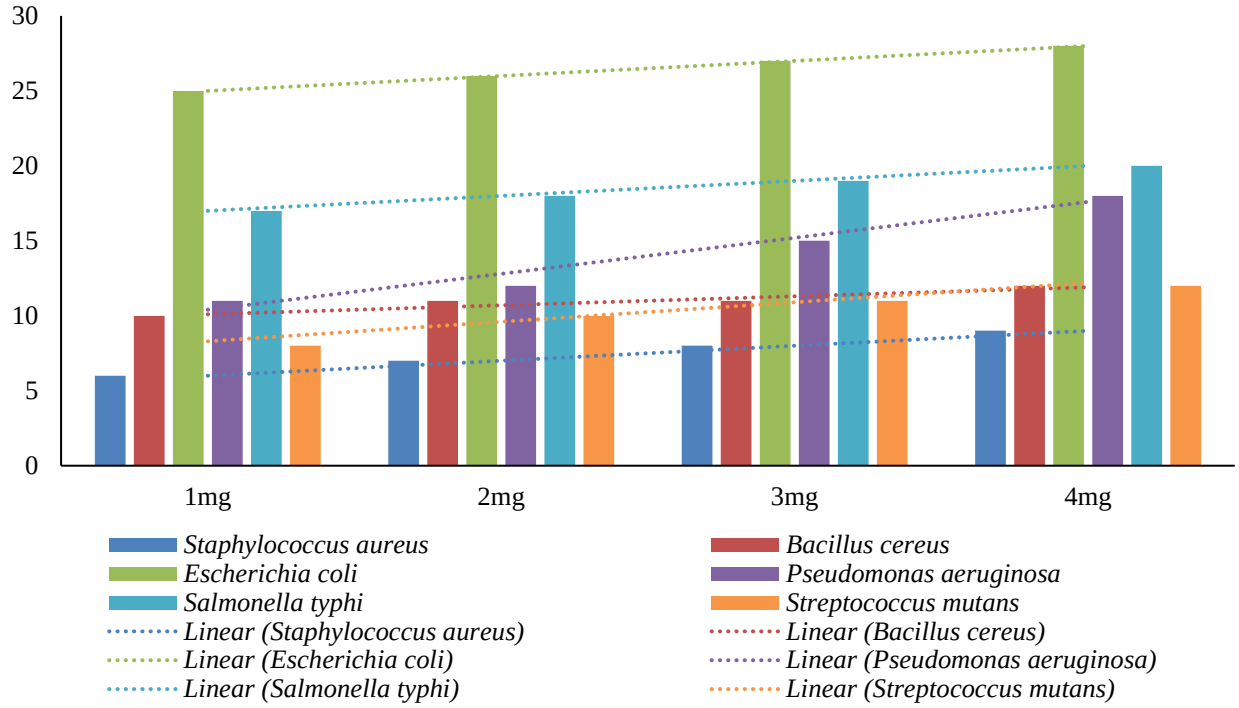


Figure 2: Linear regression plot for the Zone of Inhibition of the hexane extract tested against different bacteria.

Figure 2 shows the zone of inhibition response for the hexane extract. The hexane extract presumably exhibits, compared to methanol, flatter regression lines and possibly lower R^2 values, which could suggest a less effective antibacterial response or greater variability across bacterial strains. Hexane extracts are mainly composed of lipophilic substances that may not interact effectively with the bacterial cell walls of Gram-negative strains. The regression may show outliers (e.g., *P. aeruginosa*) with intrinsic resistance. A linear plot of zone-of-inhibition values for a hexane extract of the same bacterial species is shown in Figure 2. As with the prior case, the x-axis shows extract concentrations, and the y-axis shows inhibition zone diameters. The regression plots show how bacterial response changes with increasing extract concentration. Hexane, while causing a slightly more mixed response in bacteria than methanol extract, seems to provoke greater variation among them. Some bacteria show stronger inhibition under different concentrations; some also respond slowly. The slope of the line for *P. aeruginosa* or *B. cereus*, for example, could be steeper, indicating improved susceptibility. In contrast, for *S. mutans* and *S. typhi*, it may be only a shallow slope, which translates to reduced susceptibility. The magnitude of the regression (R^2) assesses the degree of predictable inhibition as concentration increases. Ultimately, this figure supports the idea that hexane compounds are selective, providing potent inhibition for some bacteria but not others. Some of this variability is likely due to the different cell wall structure & resistance mechanisms of Gram(+) & Gram(-) bacteria. Thus, while there may be some antibacterial activity, the trend line emphasizes its limited predictability and necessitates further compound isolation to identify potent constituents.

Table 3: Zone of Inhibition of Acetone extract against bacteria (±SD)

Bacterial Plates	Zone of Inhibition of acetone extract against bacteria (in Millimeter±SD)			
	1mg	2mg	3mg	4mg
S. aureus	6 ± 0.5	8 ± 0.7	9 ± 0.8	10 ± 0.9
B. cereus	10 ± 0.7	10 ± 0.7	11 ± 0.8	12 ± 0.9
E. coli	26 ± 1.1	27 ± 1.2	28 ± 1.3	29 ± 1.4
P. aeruginosa	10 ± 0.8	12 ± 1.0	15 ± 1.3	17 ± 1.5
S. typhi	18 ± 0.9	19 ± 1.0	20 ± 1.1	21 ± 1.2
S. mutans	7 ± 0.6	10 ± 0.9	12 ± 1.1	14 ± 1.3

The acetone extract was moderately potent against the bacterial strains under investigation, and the increase in inhibition zones with dose was dose-dependent. *E. coli* was the most sensitive, and there was a significant, positive zone of inhibition in the range of 26 ± 1.1 mm at 1 mg to 29 ± 1.4 mm at 4 mg, displaying the potent activity of soluble acetone compounds in this Gram-negative bacterium. The responses for *S. typhi* and *P. aeruginosa* were also positive, with zones of inhibition reaching up to 18 ± 0.9 mm to 21 ± 1.2mm and 10 ± 0.8 to 17 ± 1.5 mm, respectively, at the highest concentration. For Gram-positive bacterium *B. cereus*, the inhibition zone was medium (10 ± 0.7 to 12 ± 0.9 mm) & *S. aureus* & *S. mutans* exhibited lower but gradually increasing inhibition zones (6 ± 0.5 to 10 ± 0.9 mm and 7 ± 0.6 to 14 ± 1.3 mm, respectively). The findings highlight that although this acetone extract contains bioactive compounds that are effective against Gram (-) & Gram (+) bacteria, it is more potent against the former. The results affirm that acetone extractions can be used in practice to isolate antimicrobial agents from natural materials (Table 3).

The standard deviation values indicate low to moderate variability in the antibacterial activity of the acetone extract, indicating good experimental consistency. At lower concentrations, small SDs indicate uniformly distributed inhibition zones, whereas larger SDs at higher concentrations indicate enhanced diffusion and stronger dose-dependent effects. The increased variability in *Pseudomonas aeruginosa* and S*treptococcus mutans* was slight, indicating differential sensitivity among the bacteria. Overall, the SD values for agar diffusion assays were within acceptable limits, confirming the reliability of the results. The graph shows the trend in zone of inhibition for the acetone extract (Figure 3). The regression line here might be steeper than that of the hexane extract, but not as pronounced as that of methanol, reflecting moderate efficacy. Acetone extracts a mixed-polarity profile of phytochemicals that can interact with a broader range of microbial targets.

The plot helps visualize which bacterial species are more sensitive and predict a uniform zone-of-inhibition trend among strains. If the relationship with the regression line is very well fitted, with a high R^2, it could be concluded that the acetone extract is promising for broad applications on the antimicrobial front if additional preparation and research are conducted to confirm its properties. In Figure 3, we plot the antibacterial activity of the acetone extracts at specific concentrations against selected bacterial species using linear regression. Like in any prior figures, the x-axis corresponds to acetone extract concentrations, and the y-axis corresponds to inhibition zone measurements. There is a general positive trend in the regression lines, indicating that higher acetone extract concentrations are

associated with greater bacterial inhibition. Steeper regression lines for *Escherichia coli* and *Salmonella typhi*, which show greater sensitivity to the extract than a smaller sample size, reveal inhibition across different phases. *Salmonella aureus* and *Streptococcus mutans*, by contrast, have shown little increase in inhibition zones, reflecting greater susceptibility. The linear relationship shown indicates a dose-dependent response, although sensitivity varies by bacterial species. Based on the data, we infer that acetone extracts possess moderate to strong antibacterial activity depending on the target organism. More specifically, if the R^2 values are very high, it indicates that inhibition is increasing steadily with concentration. Overall, this figure confirms the suitability of acetone as an extraction solvent for antibacterial compounds and may guide solvent selection in natural product-based antimicrobial studies.

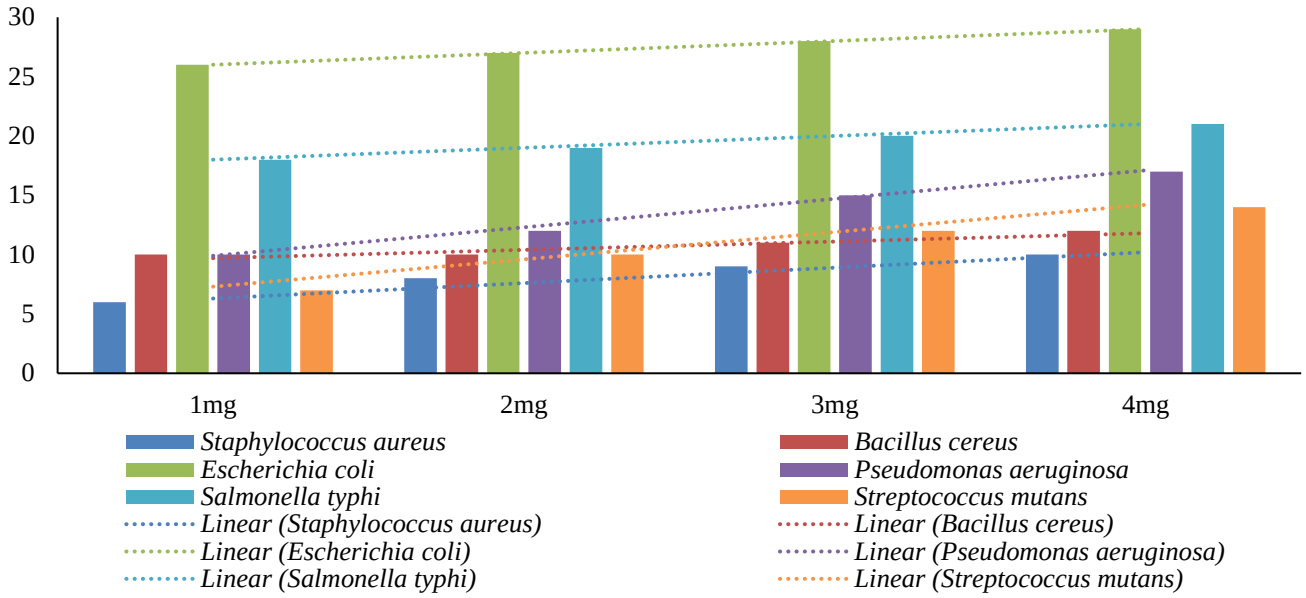


Figure 3: Linear regression plot for the Zone of Inhibition of the acetone extract tested against different bacteria

Table 4: Antibacterial activity of Tetracycline against *Spirulina platensis* extract (As a control experiment).

Organisms	100µg	200µg	300µg	400µg
S. aureus	22 ± 1.0	24 ± 1.1	26 ± 1.2	29 ± 1.4
B. cereus	14 ± 0.8	15 ± 0.9	17 ± 1.0	19 ± 1.1
E. coli	30 ± 1.3	32 ± 1.4	34 ± 1.5	36 ± 1.5
P. aeruginosa	25 ± 1.1	26 ± 1.2	27 ± 1.2	29 ± 1.3
S. typhi	22 ± 1.0	26 ± 1.3	28 ± 1.4	32 ± 1.6
S.mutans	22 ± 1.1	24 ± 1.1	26 ± 1.2	28 ± 1.3

Table 4 presents the antibacterial activity of a specific compound or extract tested at four concentrations (100 µg, 200 µg, 300 µg, 400 µg) against six bacterial species. Effectiveness is measured by the zone of inhibition (in millimetres), which indicates the extent to which bacterial growth is prevented around the compound's application site.

Table 4 shows the antimicrobial activity (probably expressed as zone of inhibition in mm) of the compound tested against six bacterial species at various concentrations (100 µg to 400 µg). The results reveal a dose-dependent increase in antimicrobial activity across all organisms under investigation. *E. coli* showed the highest sensitivity, with bands of inhibition increasing from 30 ± 1.3 mm at 100 µg to 36 ± 1.5 mm at 400 µg, indicating a reasonable level of protection against this Gram-negative organism. *Salmonella typhi* showed an even larger increase (from 22 ± 1.0 to 32 ± 1.6 mm), indicating a higher response. *Staphylococcus aureus* and *Streptococcus mutans* showed moderate inhibition, ranging from 22 ± 1.0 to 29 ± 1.4 mm and from 22 ± 1.1 to 28 ± 1.3 mm, respectively.

Pseudomonas aeruginosa displayed a consistent but somewhat low inhibition (from 25 ± 1.1 to 29 ± 1.3 mm), and *Bacillus cereus* displayed the lowest, with zones of 14 ± 0.8 to 19 ± 1.1 mm. These results further emphasize the compound's broad-spectrum antibacterial activity, particularly against *E. coli* and *S. typhi*. In summary, the data indicate that the tested compound is more effective in inhibiting bacterial growth, particularly against *Salmonella typhi* and *Pseudomonas aeruginosa*. The increasing trend across concentrations supports a dose-dependent antimicrobial activity. The variations in inhibition among

different bacteria likely reflect structural differences in cell walls, efflux mechanisms, or metabolic pathways. This table thus provides valuable insight into the compound's potential therapeutic use, warranting further investigation. In general, the standard deviation values indicate low to moderate variation in zone of inhibition across all concentrations tested. These comparatively small SDs indicate good experimental precision and reproducibility of the antibacterial assay. The gradual increase in SD with increasing concentration suggests enhanced diffusion and is therefore consistent with enhanced dose-dependent activity. The slightly higher variability in *S. typhi* and *E. coli* indicates differential bacterial sensitivity, although all values remain within the acceptable range for agar diffusion assays.

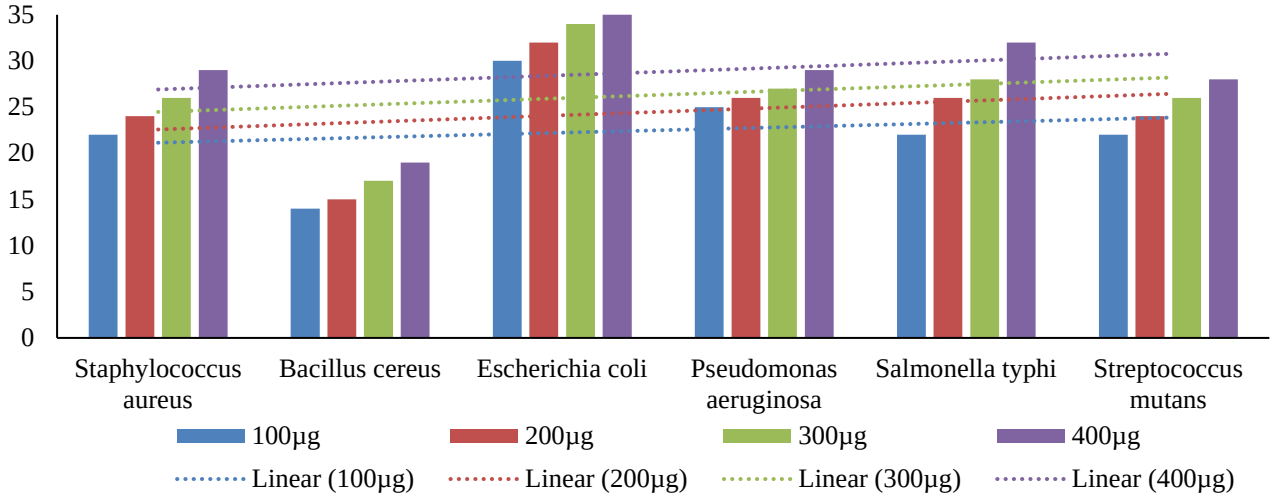


Figure 4: Antibacterial activity of *Spirulina platensis* on tetracycline extract against the selected bacteria

The antibacterial performance of tetracycline extract at various concentrations (100 µg, 200 µg, 300 µg, 400 µg) against six bacteria is reported in the graph. Higher concentration corresponds to greater activity. All bacteria exhibited an increase in antibacterial activity with concentration. Figure 4 demonstrates the antibacterial activity of *Spirulina platensis* with tetracycline extract against some bacterial species. The graph must show the zones of inhibition (in mm) for each bacterium to demonstrate the effectiveness of *Spirulina*-tetracycline in restricting bacterial growth. Bar or data points for different bacterial strains and their relative potency may be easily compared. These results suggest that the combination potentiates antibacterial activity, with larger zones of inhibition observed in the bacterial species tested.

The inhibition zones of bacteria such as *Pseudomonas aeruginosa* and *Salmonella typhi* could be much larger, which implies significantly greater sensitivity to the treatment. Some organisms, such as *Staphylococcus aureus* or *Streptococcus mutans*, may present instead smaller zones, indicating a modest to mild sensitivity. This figure highlights the potential for synergistic action between *Spirulina platensis* and tetracycline, whereby the bioactive compounds of *Spirulina* could enhance or augment the antibiotic's action. Figure 4 provides evidence that *Spirulina platensis* is a natural bio-enhancer that could enhance the activity of current antibiotics against a variety of bacterial pathogens.

Table 5: One-way ANOVA calculated among the various solvent extracts for their zone of inhibition.

Organism	F Statistic	P value	Conclusion(α=0.05)
S. aureus	7.662	0.0114	Significant
B. cereus	26.385	0.0002	Significant
E. coli	4.729	0.0395	Significant
P. aeruginosa	0.989	0.4089	Not Significant
S. typhi	5.850	0.0236	Significant
S. mutans	0.881	0.4471	Not Significant

Table 5 represents the one-way ANOVA analysis indicated that the solvent type (methanol, hexane and acetone) had a significant effect on the zone of inhibition for *Staphylococcus aureus* ($p = 0.0114$), *Bacillus cereus* ($p = 0.0002$), *Escherichia coli* ($p = 0.0395$) and *Salmonella typhi* ($p = 0.0236$), indicating that antimicrobial activity differed significantly among the three solvent extracts for these microorganisms. Of these, *Bacillus cereus* had the highest solvent-dependent change (i.e., it showed the greatest F-value). No significant differences were found for *P. aeruginosa* ($p = 0.4089$) & *S. mutans* ($p = 0.4471$), indicating that the antimicrobial activity of the extracts against these bacteria was comparable across all solvents.

Overall, these results may suggest that solvent selection strongly contributes to the antimicrobial efficacy of some bacterial species and is not significant for others.

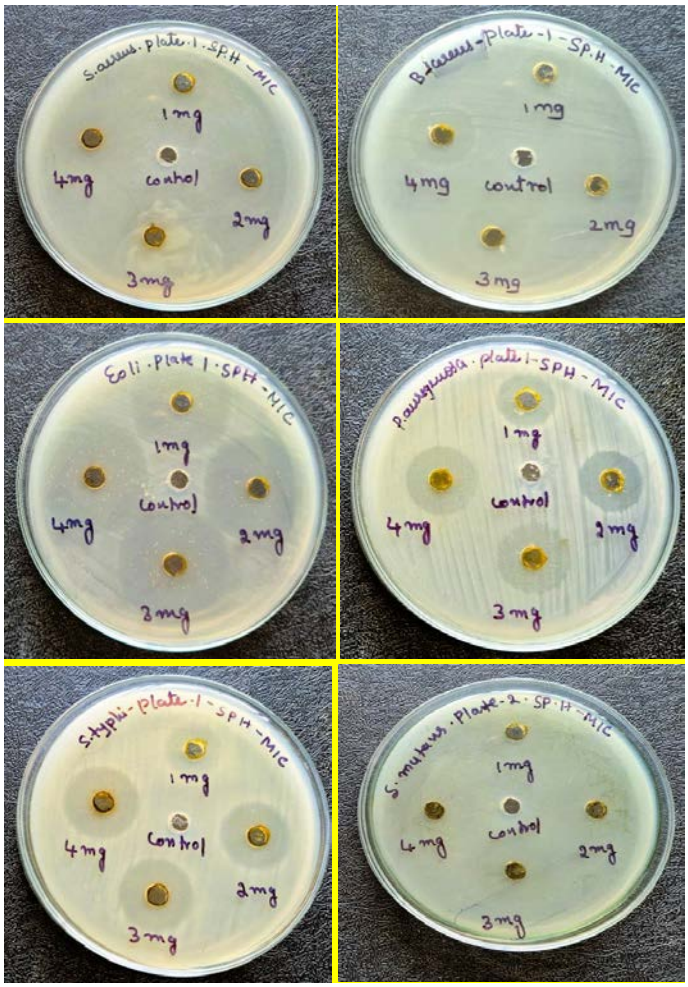


Figure 5: Antibacterial activity of hexane extracts of *Spirulina platensis.*

Antibacterial activity of hexane, acetone, and methanol extracts of *Spirulina platensis* against six pathogenic bacteria: *Pseudomonas aeruginosa, Streptococcus mutans, Escherichia coli, Staphylococcus aureus, Salmonella typhi,* and *Bacillus cereus*. An intermediate zone of inhibition was observed for the hexane extract (Figure 5). Thus, the phytochemical constituents of *Spirulina* were considered to exhibit antimicrobial properties due to their nonpolar nature. Semi-polar acetone extracts (Figure 6) exhibited elevated activity against *S. aureus* and *B. cereus*, demonstrating the presence of bioactive compounds such as flavonoids and fatty acids.

Methanol extracts (Figure 7) have been demonstrated to have the greatest inhibitory effect, indicating the importance of polar components, phenolics, peptides, and polysaccharides in *Spirulina* in the inhibition of bacterial growth. Methanolic extracts also exhibited broad-spectrum activity against *E. coli, S. typhi,* and *P. aeruginosa*.

Figure 6: Antibacterial activity of acetone extracts of *Spirulina platensis*

These findings prove that *Spirulina platensis* extracts possess powerful antibacterial activity, the efficiency of which is related to the polarity of the extraction solvent. Due to its high polarity, methanol yields more bioactive compounds than hexane or acetone, an essential material for the synthesis of antibacterial compounds from algal biomass.

The standard plates show qualitative evidence of the antimicrobial potential of *S. platensis*. The diameter of the zones of inhibition would be compared with those of standard antibiotics to assess potency.

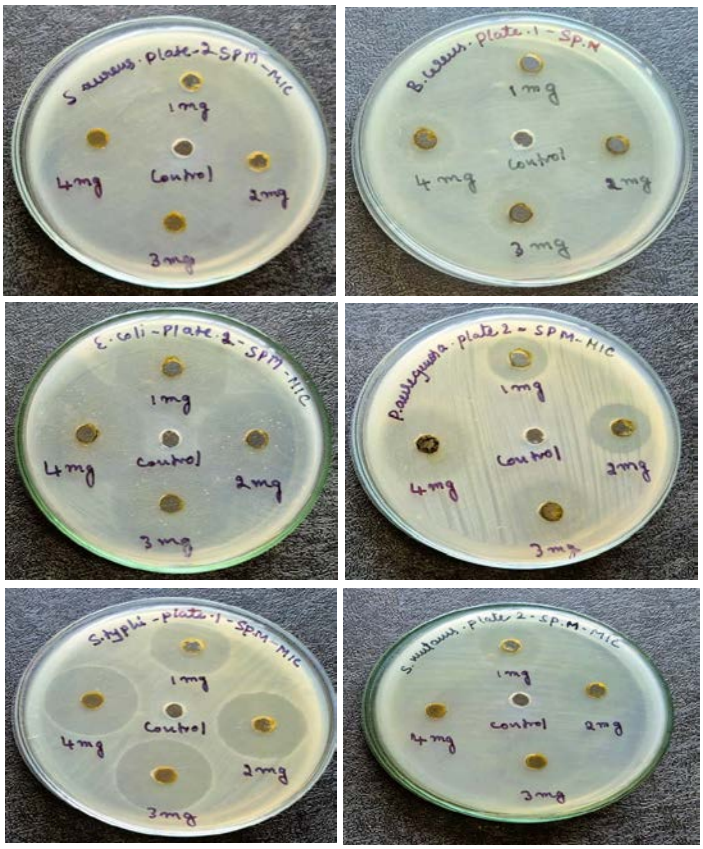


Figure 7: Antibacterial activity of methanol extracts of *Spirulina platensis*

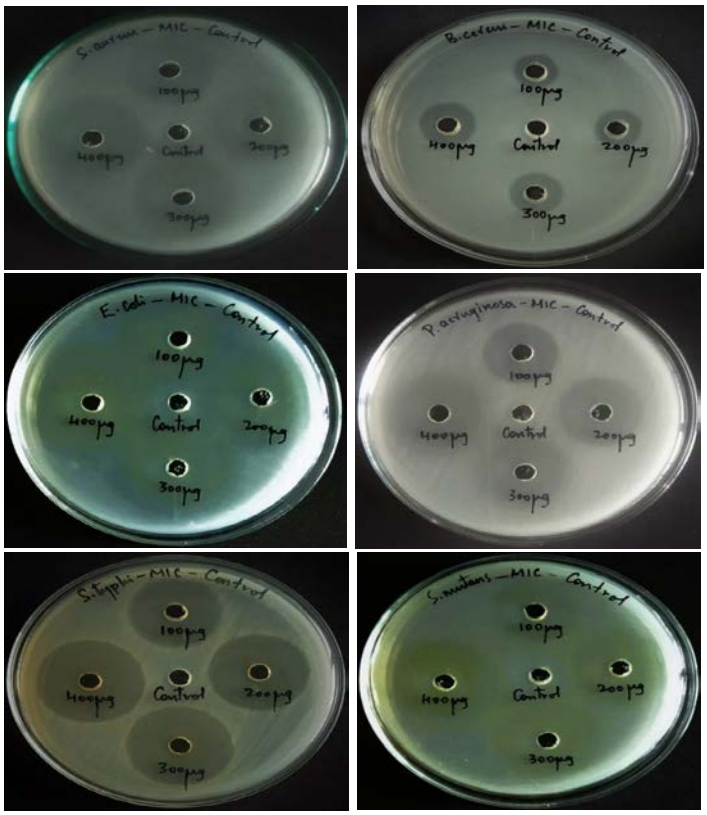


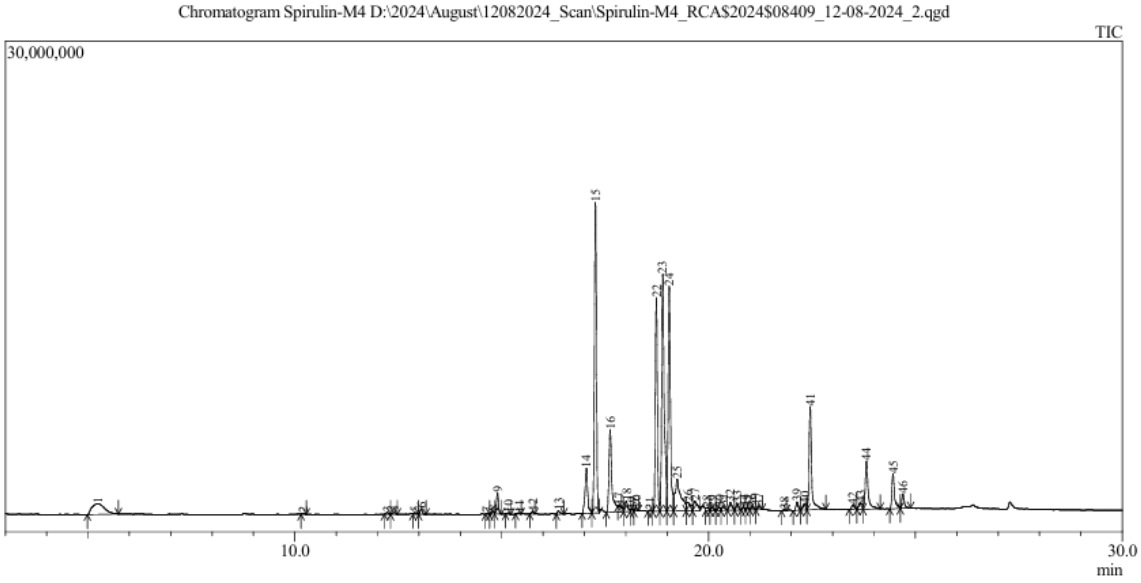
Figure 8: Antibacterial activity of tetracycline extracts of *Spirulina platensis*

The well of *P. aeruginosa* exhibited a clear zone of inhibition, indicating that the *Spirulina platensis* extract has strong antibacterial activity against this strain. The methanolic extract likely presented the largest zone of inhibition, consistent with your abstract. *S. mutans* is a Gram-positive bacterium associated with dental caries. If inhibition occurred, it would indicate the potential use of *S. platensis* in oral hygiene preparations. The size of the inhibition zone would indicate the sensitivity of this bacterium to the extract. *E. coli* is a Gram-negative enteric bacterium. The antibacterial action against *E. coli* is clinically important as it could suggest new therapeutic options against gastrointestinal infections. *S. aureus* is a Gram-positive pathogen responsible for various infections ranging from skin to systemic infections. The strong inhibitory activity indicates that this extract is highly potent and therefore has pharmaceutical applicability. The following section reveals the various phytochemicals eluted during the GCMS analysis and their details.

The Gas Chromatography–Mass Spectrometry (GC–MS) chromatogram of the bioactive compounds obtained from *Spirulina* is shown in Figure 9. The Total Ion Chromatogram (TIC) is shown as a chromatogram, where each peak corresponds to a specific chemical compound isolated by its volatility and detected via mass spectrometric ionization. The R.T, peak area, and identity of compounds together help to measure the chemical diversity and presence of metabolites in the extract with respect to their molecular and abundance characteristics. The chromatogram was performed over the time range from 0 to 30 minutes. It showed a relatively wide peak range, with durations of 15 to 22 minutes, indicating that the mid- to long-chain fatty acids, esters, and other hydrophobic bioactive compounds in *Spirulina* biomass eluted at high rates. Then the first peak (R.T 5.229 min) is for Glycerine, accounting for 3.49% of the peak area. Glycerine is a lipid formed metabolite, and it is present in extracts supplemented by hydro alcoholic or polar solvents. During 10–14 min, some low-to-medium-intensity peaks are associated with 2-Undecanol acetate, Bromoacetic acid dodecyl ester, Benzofuranone derivatives, and Heptadecanol. These compounds, however, appear quite rare (<1%), indicating aromatic intermediates and esterified fatty alcohols, underscoring their biochemical complexity. For example, there should be benzofuranone derivatives with antioxidant and probably antimicrobial actions. One remarkable feature of this chromatogram is that long-chain

fatty acids and their methyl esters dominate the mid-retention segment. One of them, Peak 15 at R.T 17.266 min, represents Hexadecenoic acid or palmitic acid, and represents 6.84% of the extract. It is a typical saturated fatty acid with antimicrobial, antioxidant, and anti-inflammatory activities, and is abundant in cyanobacteria. The same is true of peak 14 (R.T 17.050 min), 9-Hexadecenoic acid, methyl ester (Z-), with a wide band of 3.26 %, the concentration of monounsaturated fatty acids in *Spirulina.* At R.T 17.840 min, another important bioactive compound is γ-linolenic acid (GLA). Although at a rate of 0.05%, GLA is a beneficial omega-6 fatty acid, recognized as a component of the lipid content in *Spirulina* and a good source of anti-inflammatory, cardio-protective, and immune-modulatory effects.

The chromatogram also shows Eicosane at peak 9, a long-chain hydrocarbon that possesses antimicrobial and pesticidal properties. Other compounds of interest for study are Neophytadiene, Loliolide, and Dimethyl dioxane derivatives. Loliolide, a monoterpenoid lactone found in some algae, exhibits antioxidant & anti-aging behavior at 2 retention times, suggesting the presence of isomeric forms. In the presence of lipid-rich microalgae, these fatty acid esters and methyl esters are highly prevalent, indicating trans-esterification and natural esterification processes. These factors have reinforced the significance of *Spirulina* in its nutraceutical role and in scientific studies on biodiesel feedstock and therapeutic indications. These compositions therefore suggest that the *Spirulina* extract is a rich source of fatty acids (hexadecenoic acid and its esters and derivatives from linolenic acid), terpenoids, hydrocarbons, alcohols, aromatic compounds, and biological activity. This dominance of lipid derivatives aligns with the well-characterized biochemical architecture of *Spirulina* and its associated potential pharmacological, nutritional, and industrial applications.



Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	5.229	14206380	3.49	Glycerin
2	10.201	274018	0.07	2-Undecanol, acetate
3	12.229	503413	0.12	Bromoacetic acid, dodecyl ester
4	12.399	537077	0.13	3-Buten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-
5	12.901	462526	0.11	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-
6	13.053	1295427	0.32	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-
7	14.652	286697	0.07	n-Heptadecanol-1
8	14.790	738675	0.18	4,6,10,10-Tetramethyl-5-oxatricyclo[4.4.0.0(1,4)]dec-2-en-7-ol
9	14.898	4751913	1.17	Eicosane
10	15.151	273974	0.07	Methyl tetradecanoate
11	15.413	326141	0.08	Loliolide
12	15.744	535467	0.13	Loliolide
13	16.371	998868	0.25	Neophytadiene
14	17.050	13302637	3.26	9-Hexadecenoic acid, methyl ester, (Z)-
15	17.266	67993324	16.69	Hexadecanoic acid, methyl ester
16	17.623	27876561	6.84	n-Hexadecanoic acid
17	17.840	210564	0.05	Methyl .gamma.-linolenate
18	18.012	3270792	0.80	(Z)-Methyl heptadec-9-enoate
19	18.160	469389	0.12	5,5-Dimethyl-1,3-dioxane-2-ethanol, TBDMS derivative
20	18.240	677936	0.17	Hexadecanoic acid, 15-methyl-, methyl ester
21	18.597	293211	0.07	4-Methyl-Z-4-hexadecen-1-ol

Figure 9: GCMS results of *Spirulina* compound extracted.

Table 6: Represents the eluted compounds from *Spirulina platensis* and their biological activity.

Peak No	R Time	Area	Area %	Name of the compound	Biological activity
1	5.229	14206380	3.49	Glycerine	Inhibition of Enzymes Involved in Histidine Metabolism, Antimicrobial Activity, Anti-inflammatory Effects, and Inhibition of Aromatase and Estrogenic Activity, Antioxidant Activity, Potential as a Chelating Agent, Neuroprotective Activity.
2	10.201	274018	0.07	2-Undecanol, acetate	Membrane Interactions and Cell Signalling, Anti-inflammatory Effects, Neuroprotective Effects, Modulation of Endocannabinoid System.
3	12.229	503413	0.12	Bromoacetic acid, dodecyl ester	Anti-inflammatory Effects, Antioxidant Properties, Antimicrobial Activity, Skin Barrier Function and Moisturization, Neuroprotective Effects, Anti-cancer Potential, Lipid Metabolism Modulation.
4	12.399	537077	0.13	3-Buten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo [4.1.0] hept-1-yl)-	Antifungal, antimicrobial, skin and cosmetic applications, insecticidal activity, precursor for derivatives.
5	12.901	462526	0.11	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	Antioxidant activity, Anti-inflammatory Properties, Cytotoxic and Anticancer, antimicrobial, enzyme inhibition.
6	13.053	1295427	0.32	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	Structural Role in Membranes, myristylation, antimicrobial, energy source, pro-inflammatory, cosmetic and dermatological effects, lipid metabolism.
7	14.652	286697	0.07	n-Heptadecanol-1	Antioxidant Activity, Anti-inflammatory, antimicrobial, anticancer, neuroprotective, anxiolytic and sedative, Precursor to Vitamins and Chlorophyll, cosmetics, and skin care.
8	14.790	738675	0.18	4,6,10,10-Tetramethyl-5-oxatricyclo [4.4.0.0(1,4)] dec-2-en-7-ol	Antimicrobial, insecticidal and pesticidal, Anti-inflammatory, antioxidant, potential anticancer, fragrance, and cosmetics.
9	14.898	4751913	1.17	Eicosane	Antioxidant Activity, Anti-inflammatory, antimicrobial, anticancer, neuroprotective, anxiolytic and sedative, Precursor to Vitamins and Chlorophyll, cosmetics, and skin care.
10	15.151	273974	0.07	Methyl tetra decanoate	Antimicrobial, Potential Cytotoxicity, Enzyme Inhibition, Anti-inflammatory Properties, Insecticidal or Larvicidal Activity, Industrial and Biochemical Applications, Role in Membrane Studies.
11	15.413	326141	0.08	Loliolide	Antimicrobial, antioxidant, Anti-inflammatory, Potential in Cosmetic Applications, Industrial Applications.
12	15.744	535467	0.13	Loliolide	Antimicrobial, antioxidant, Anti-inflammatory, Potential in Cosmetic Applications, Industrial Applications.
13	16.371	998868	0.25	Neophytadiene	Cardiovascular Health, Anti-inflammatory Properties, Potential as an Essential Fatty Acid, Metabolic Regulation, Antibacterial Activity, Neuroprotective Effects.
14	17.050	13302637	3.26	9-Hexadecenoic acid, methyl ester, (Z)-	Anti-inflammatory Effects, Membrane Fluidity and Function, Membrane Fluidity and Function, Cardioprotective Potential, Neuroprotective Effects, Antioxidant Activity, Potential Antimicrobial Properties.

Peak No	R Time	Area	Area %	Name of the compound	Biological activity
15	17.266	67993324	16.69	Hexadecenoic acid, methyl ester	Antioxidant Activity, Anti-inflammatory Effects, Antimicrobial, Anticancer Activity, Hepatoprotective Effects, Anticonvulsant and Neuroprotective Effects, Immunomodulatory Effects, Anti-parasitic Activity, Wound Healing, and Skin Regeneration.
16	17.623	27876561	6.84	n-Hexadecenoic acid	Anti-inflammatory Effects, Cardiovascular Health, Anticancer Activity, Skin Health, Neurological Benefits, Anti-obesity and Metabolic Effects.
17	17.840	210564	0.05	Methyl .gamma.-linoleate	Membrane Fluidity and Function, Protein Modification (N-myristylation), Antimicrobial Activity, Immune System Modulation, Metabolic Effects, Skin and Cosmetic Benefits, Neurological and Cellular Signalling.
18	18.012	3270792	0.80	(Z)-Methyl heptadec-9-enoate	Potential as a Scaffold for Drug Design, Interaction with Enzymes, CNS Stimulatory or Depressive Effects, Cholinergic Activity.
19	18.160	469389	0.12	5,5-Dimethyl-1,3-dioxane-2-ethanol, TBDMS derivative	Antiviral Activity, Inhibition of DNA Polymerase, Antibacterial Properties.
20	18.240	677936	0.17	Hexadecenoic acid, 15-methyl-, methyl ester	Pesticidal Activity, Neuroprotective Potential, Potential as an Antioxidant, Lipid Metabolism Modulation, Anti-inflammatory Effects.
21	18.597	293211	0.07	4-Methyl-Z-4-hexadecen-1-ol	Antimicrobial Activity, Inhibition of Glycoside Hydrolases, Metabolic Effects, Neuroprotective Potential, Antioxidant Properties, Cryoprotective Activity.

Table 6 and Figure 9 represent the high-retention-time compounds eluted during the chromatographic analysis. Some compounds with lower RT have not been considered in this article. The GC–MS peak (Table 6) indicates the largest bioactive species extracted from *Spirulina*, with their retention time, peak area, and bioactivity. Alcohols, fatty acids, methyl esters, terpenoids, heterocyclic compounds, and hydrocarbons are also depicted in the spectrum, along with their nutraceutical and therapeutic properties. Glycerine (3.49%) is the most frequently reported compound, with diverse biological functions; it acts as an antimicrobial, anti-inflammatory, enzyme inhibitor, and antioxidant. The neuroprotective ability and chelating effect of *Spirulina* compounds further support its functional food constituent nature. Some medium-chain esters and aromatic derivatives are found in minute amounts (less than 1%) at 10-14 min. 2-Undecanol acetate has been found to exhibit anti-inflammatory and neuroprotective effects through membrane interaction and signalling pathways. Bromoacetic acid dodecyl ester and 3-Buten-2-one derivatives possess antifungal, antimicrobial, and antioxidant effects, and thus can promote *Spirulina's* antifungal protective effect. Benzofuranone derivatives (R.T. 12.9–13.0 min) exhibit antioxidant, antimicrobial, and cytotoxic/anticancer activities, indicating that they are promising candidates for pharmaceutical applications. This results in alcohols and terpenoid derivatives after 14–16 minutes. Although n-Heptadecanol-1 is not readily available, it exhibits anti-inflammatory, neuroprotective, anxiolytic, and skin-care effects. Neophytadiene (0.25%) is a diterpenoid hydrocarbon with antibacterial, cardioprotective, and metabolic-regulatory capacities, and is a significant bioactive molecule reported so far in a wide range of algae. There is an excellent development of fatty acid derivatives in 17 minutes, illustrating the lipid-rich profile of *Spirulina*. 9-Hexadecenoic acid, methyl ester (Z-) and Hexadecenoic acid, methyl ester, together form >20% of the compounds and are the most abundant compounds. These esters, comprising fatty acids, are potent sources of antioxidants, antibacterial compounds, anti-inflammatory compounds, neuroprotective compounds, hepatoprotective compounds, and wound-healing compounds. These ingredients are also extensively employed in nutraceutical products, biofuel precursors, and therapeutic strategies. Free hexadecenoic acid (palmitic acid) is likewise abundant (6.84%), indicating its cardiovascular benefits, anticancer effects, neurological effects, and metabolic control.

Methyl γ-linoleate is an omega-6 fatty acid valuable for immune regulation, membrane fluidity, and cell signalling, and skin healthy states, as well as for the maintenance of normal cell homeostasis, and is relatively rarely available. Its long-chain moieties, including (Z)-Methyl heptadec-9-enoate, Hexadecenoic acid, 15-methyl- ester, 4-Methyl-Z-4-hexadecen-1-ol, serve different roles in antimicrobial, neuroprotective, pesticidal, antioxidant, and antioxidant responses. It is concluded, in Table 6, that *Spirulina* is a mixture of bioactive metabolites (mainly fatty acids together with fatty esters) enriched by terpenoids, alcohols, and aromatic derivatives. It is these compounds that lead to the reported health benefits of *Spirulina*, such as anti-inflammatory, antioxidant, antimicrobial, anticancer, neuroprotective, and metabolic regulatory effects, which contribute to the high therapeutic value of the extract. The GC-MS results reveal a diverse range of bioactive compounds with numerous pharmaceutical and industrial uses. The high content of fatty acids, alcohols, esters, and aromatic compounds, most of which possess antioxidant, antimicrobial, anti-inflammatory, anticancer, and neuroprotective activities, suggests the extract's potential for therapeutic applications in inflammation-related diseases, skin care, or metabolic syndromes. In addition, further work is needed on isolation, structural elucidation, and in vitro/in vivo validation of these bioactivities and the compounds' synergistic effects. Aside from their pharmacological applications, the bioactive metabolites identified by GC–MS, namely fatty acids, fatty acid esters, terpenoids, alcohols, and aromatic derivatives, exert antimicrobial activity mainly by interacting with bacterial cell membranes. Fatty acids, being amphiphilic, can insert into the phospholipid bilayer, disrupting lipid packing and enhancing membrane fluidity and permeability. The leakage of intracellular components (ions, nucleotides), membrane depolarization, and eventual cell lysis result. Unsaturated fatty acids are particularly effective, as their double bonds introduce structural distortions that disrupt membrane integrity. Terpenoids and phenolic derivatives increase membrane damage by interacting with lipid bilayers and membrane proteins, thereby inhibiting respiration and nutrient transport. In Gram-negative bacteria, these compounds may destabilize the outer membrane by interacting with lipopolysaccharides and facilitating penetration of the cytoplasmic membrane. Direct disruption of the cytoplasmic membrane in Gram-positive bacteria causes loss of the proton motive force and inhibition of ATP synthesis. These metabolites facilitate membrane permeabilization and reduce the development of resistance, thereby enabling *Spirulina* extracts' broad-spectrum antimicrobial properties for therapeutic and industrial applications.

CONCLUSION

The compounds obtained from *Spirulina platensis* contain a wide range of fatty acid methyl esters, long-chain alcohols, and large amounts of cyclic ethers, which exhibit anti-inflammatory, antioxidant, antimicrobial, anticancer, and neuroprotective activities. Bioactive compounds reflect the characteristics of lake-grown *Spirulina* as an ideal natural food source for nutraceutical, pharmaceutical, and cosmetic applications, and they demand massive cultivation and biochemical use of the feeder. These agents are commonly employed for antimicrobial, anti-inflammatory, and antioxidant properties, all of which are fundamental components of *Spirulina's* therapeutic activity. A plethora of chemical molecules related to membrane stability and signalling mechanisms have been studied. The detection of *Spirulina* biomass indicates that it may be a valuable material for the formulation of nutraceuticals and bioactive food products for human wellness and immunity. Nevertheless, the discovery of isolates from natural freshwater lakes (Chitradurga district, Karnataka) for this genus of *Spirulina* shows that it has high regional significance and practical environmental applications. Lake-grown *Spirulina platensis* can be a low-resource, low-cost natural resource for several value products due to its effective bioactive profile. Fertile cultivation makes it not only suitable for local biotechnological projects, but it also reflects the international momentum towards green and bio-based technologies. Therefore, enabling production at scale of *Spirulina* material for industrial-scale agriculture and extraction may have wide-ranging implications for health, agriculture, and cosmetics, as well as for the economy and the environment. Most of the health benefits are antimicrobial, anti-inflammatory, anticancer, and cardiovascular, as well as dietary supplements. Hence, it can be a beneficial factor as mentioned above.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Arpitha M P contributed conceptualization, writing, software, methodology, and the first draft of the manuscript. Parameswara

Naik T Supervision and investigation of the whole work. He, along with Arpitha MP, contributed to the investigation and conducted the experimental work.

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