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Investigation of the Bioactive Compounds, Antimicrobial Activity, and Phyco-Pigment Contents of Some Microalgae Extracts

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ABSTRACT

This study investigated the bioactive compounds, antioxidant and antimicrobial properties of three microalgal species: *Spirulina platensis*, *Scenedesmus obliquus*, and *Chlorella vulgaris*, to evaluate their potential as natural bioactive agents. The antioxidant activity was assessed using free radical scavenging (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assays. At the same time, antimicrobial efficacy was tested against various pathogens, including Gram-positive and Gram-negative bacteria and fungi. The three algae were also screened for their phyco-pigment content including chlorophyll, carotenoids, phycocyanin, allophycocyanin, and phycoerythrin. The bioactive composition of the three microalgal species was determined by gas chromatography-mass analysis. The data revealed that *Spirulina platensis* exhibited the highest DPPH radical scavenging activity (70.35% inhibition at 200mg/ L), linked to its abundant phycocyanin, chlorophyll, and carotenoid content. *Scenedesmus obliquus* demonstrated the highest ferric reducing power (maximum absorbance 1.1239), likely due to its high concentration of chlorophyll b and fatty acids, which support electron donation and antioxidant activity. The GC-MS analysis identified the key bioactive compounds in each algal species, including polyunsaturated fatty acids, flavonoids, and terpenes, contributing to their antioxidant and antimicrobial effects. *Spirulina platensis* showed the strongest antimicrobial activity against *Bacillus subtilis* and *Candida albicans*, while *Scenedesmus obliquus* was most effective against *Staphylococcus aureus* and *Escherichia coli*. These findings highlight the potential of these microalgae as natural sources of antioxidants and antimicrobials, with promising applications in pharmaceuticals, food preservation, and managing antimicrobial resistance.

INTRODUCTION

Microalgae have gained increasing attention as a precious source of bioactive components with potential applications in pharmaceuticals, nutraceuticals, and functional foods. These microscopic organisms, existing in diverse aquatic environments, produce various metabolites, including proteins, lipids, pigments, and antioxidants, contributing to their health-promoting properties. Among the most studied microalgae are *Scenedesmus*

obliquus, *Chlorella vulgaris*, and *Spirulina platensis*, which are known for their rich biochemical composition and wide-ranging biological activities, such as antimicrobial, antioxidant, and anti-inflammatory effects **(Shevelyuhina *et al.*, 2022)**.

The growing interest in natural products derived from microalgae is driven by the need to find sustainable and effective alternatives to synthetic chemicals in various industries. Microalgal extracts, particularly those rich in bioactive lipids, pigments, and phenolic compounds, have shown promise as antimicrobial agents, combating pathogenic microorganisms that contribute to food spoilage and human diseases. Furthermore, the antioxidant properties of these microalgae, owing to their high levels of carotenoids, chlorophylls, and vitamins, offer protection against oxidative stress, a key factor in the development of chronic effects like cancer, cardiovascular disorders, and neurodegenerative disease **(Naik *et al.*, 2024)**.

Additionally, microalgae produce unique pigments, such as chlorophylls, carotenoids, and phycobiliproteins that have significant nutritional and therapeutic benefits. Phycobiliproteins, for instance, are water-soluble pigments found in cyanobacteria and red algae, which have potent antioxidant and anti-inflammatory properties. These pigments not only provide essential nutrients but also serve as natural colorants in food and cosmetics, making microalgae a highly valuable resource **(Aizpuru & González-Sánchez, 2024)**.

Despite the extensive body of research on microalgae, there remains a need to further explore the specific bioactive compounds that contribute to their antimicrobial and antioxidant activities. Furthermore, understanding the pigment content of different microalgal species can help identify the most promising strains for various industrial applications. In this study, we focused on investigating the bioactive compounds, antimicrobial activities, and phyco-pigment contents of three microalgae species: *S. obliquus*, *C. vulgaris*, and *S. platensis*. These species were selected based on their reported biological activities and their potential to provide valuable compounds for health and industrial applications. The primary objectives of this research were to (1) assess the antioxidant capacity of these extracts using established assays such as FRAP and DPPH, (2) analyze the phyco-pigment content, including chlorophylls, carotenoids, and phycobiliproteins, and (3) evaluate the antimicrobial efficacy of microalgae extracts against various pathogenic microorganisms. Additionally, we employed Gas Chromatography-Mass Spectrometry (GC-MS) to identify the specific bioactive compounds present in microalgae extracts, providing insights into the molecular basis of their biological activities. By identifying key bioactive compounds and evaluating their biological properties, we hope to highlight the value of microalgae in promoting health and sustainability.

MATERIALS AND METHODS

Microalgae

For this investigation, three microalgae species were chosen: *Scenedesmus obliquus, Chlorella vulgaris,* and *Spirulina platensis*. The microalgae were obtained as dry powder from Egypt's National Research Center's Algal Biotechnology.

Preparation of microalgal extracts

The algae extracts were obtained by weighing 5 grams of powdered algae into a 250ml flask with 100ml of 96% ethanol. The extraction was done using an ultrasonic water bath at 25°C for two hours. The samples were filtered after extraction, and a rotary evaporator set to 40°C was used to evaporate the solvent **(El-Chaghaby *et al.*, 2019)**.

DPPH radical scavenging assay

The antioxidant activity of the extracts was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay based on the procedure outlined by **Subri and Zin (2020)**. The reduction of the DPPH free radical was measured by reading the absorbance at 517nm. Briefly, several dilutions of microalgae extracts (50,100,150, and 200mg/ l) were prepared using methanol. A test tube containing each diluted sample was combined with 2 milliliters of 200mg/ l DPPH and left at room temperature for 20 minutes in the dark. Measurements of absorbance against methanol were made spectrophotometrically at 517nm **(Wu *et al.*, 2022)**.

Ferric reducing antioxidant power (FRAP) assay

The reducing power of the extracts was determined using the FRAP assay. One milliliter of each sample solution (50,100,150, and 200mg/ L) was combined with phosphate buffer (2.5mL, 0.2 mol/l, pH 6.6) to find the reducing power of the three microalgae. Afterward, 2.5mL of potassium ferric cyanide (10g/ l) were added. Following a 20-minute incubation period at 50°C, 2.5mL of 10% tri-chloro acetic acid was added, and the mixture was centrifuged for 10 minutes at 3000 rpm. The absorbance was measured at 700nm against a blank (methanol) after the upper layer of solution (2.5ml) was combined with distilled water (2.ml) and ferric chloride (0.5mL, 1g/ L). Growing reducing power was indicated by the reaction mixture's increasing absorbance. Every test was run three times, and the mean value was determined **(El-Chaghaby *et al.*, 2024)**.

Pigment content analysis

Chlorophyll-a and carotenoid quantification

The concentrations of chlorophyll a, chlorophyll b, and total carotenoids in the microalgal extracts were determined spectrophotometrically according to **Chikhoune *et al*. (2024)** .The absorbance of microalgal extracts was determined at 663 and 645nm using a UV-Vis spectrophotometer for two types of chlorophyll a and b. The absorbance measurement of carotenoid content was done at 470nm.

b- Phycobiliprotein quantification

The phycobiliprotein content of the microalgal extracts was determined according to the standard spectrophotometric method **(Sibaoueih *et al.*, 2021)**. Algal biomass (100mg) was suspended in 10mL of 0.1 M phosphate buffer (pH 6.8) and was subjected to repeated freeze-thaw cycles to extract the phycobiliproteins. The absorbance was measured at 620nm for C-phycocyanin, 652nm for allophycocyanin, and 562nm for phycoerythrin.

Antimicrobial activity assay

The antimicrobial properties of microalgal extracts were tested against four bacterial and two fungal strains using the agar well diffusion technique. This method was employed to evaluate the extracts' efficacy against a range of pathogenic microorganisms, including Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*), and fungi (*Candida albicans* and *Aspergillus niger*). Agar wells with a diameter of 6mm were filled with 10µL of the extract at a concentration of 50mg/ mL. The plates were incubated at 37°C for 24 hours for bacterial cultures and 48 hours for fungal cultures. The antimicrobial activity was determined by measuring the inhibition zones in millimeters **(Balouiri *et al.*, 2016; Ibrahim *et al.*, 2020)**

GC-MS analysis of bioactive compounds

The chemical composition of the extracts was determined using Gas Chromatography-Mass Spectrometry (GC-MS). The analysis utilized an Agilent 7890A gas chromatograph paired with an Agilent 7000A MS/MS system.

Separation was achieved using an HP-5MS capillary column (30m × 0.25mm, 0.25µm film thickness). The oven temperature was programmed to increase from 60 to 300°C at a rate of 10°C per minute, with a final hold of 10 minutes. The injector temperature was maintained at 250°C, and helium served as the carrier gas at a flow rate of 1mL/ min. Mass spectrometry conditions included an ion source temperature of 250°C

and an electron ionization energy of 70eV, with mass spectra collected in the 50–550 m/z range. Compound identification was conducted by comparing the obtained mass spectra to those in the NIST 17 (National Institute of Standards and Technology) and Wiley 275 databases.

Statistical analysis

All experiments were conducted in triplicate, and the results were presented as the mean ± standard deviation (SD). Statistical analysis was carried out using SPSS software.

RESULTS AND DISCUSSION

Antioxidant capacity of algal extracts

DPPH radical scavenging

The DPPH radical scavenging activity of the three microalgae species *S. obliquus, C. vulgaris*, and *S. platensis,* as measured at different extract concentrations (50, 100, 150, and 200mg/ L) is shown in Fig. (1) and the results are expressed as percentage inhibition of DPPH radicals. The DPPH assay demonstrated that the radical scavenging activity of the microalgae increased with concentration for all three species, indicating a dose-dependent antioxidant effect. *S. obliquus* demonstrated the second-highest DPPH scavenging activity, with 69.01% inhibition at 200mg/ L. Though slightly lower than *S. platensis*, *S. obliquus* showed a marked increase in activity at higher concentrations, particularly between 150 and 200mg/ l, where its activity rised from 56.42 to 69.01%. This suggests that the antioxidant compounds in *Scenedesmus* may become more effective at higher doses. *C. vulgaris* exhibited the lowest DPPH scavenging activity across all concentrations, with 64.54% inhibition at 200mg/ L. Although *Chlorella* demonstrated moderate antioxidant activity, its performance was consistently lower than both *Spirulina and Scenedesmus,* particularly at lower concentrations.

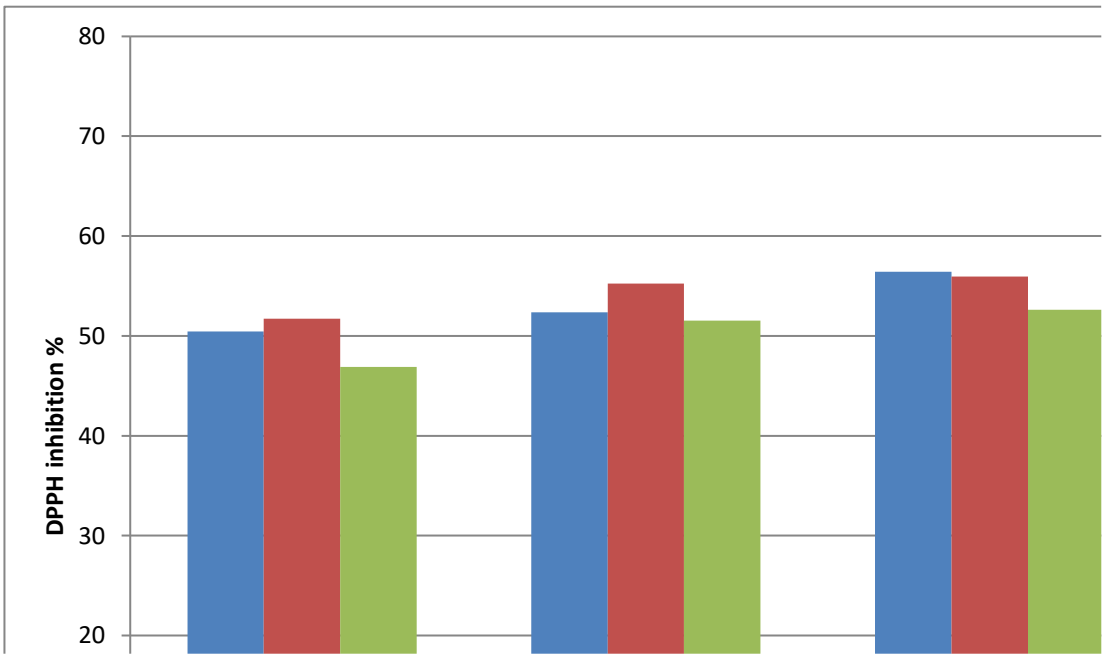

Fig. 1. DPPH scavenging activity of three microalgae extracts, *Scenedesmus obliquus* (SC), *Spirulina platensis* (Spi) and *Chlorella vulgaris* (ChI)

S. platensis consistently exhibited the highest DPPH radical scavenging activity across all concentrations, achieving 70.35% inhibition at 200mg/ L. This suggests that *Spirulina* possesses potent antioxidant compounds capable of neutralizing free radicals effectively. The activity of *Spirulina* was particularly notable at 50mg/ L, where it showed stronger inhibition (51.71%) compared to *Scenedesmus* (50.44%) and *Chlorella* (46.89%).

In summary, *Spirulina platensis* demonstrated the highest overall antioxidant capacity based on DPPH activity, making it a promising candidate for applications where strong radical scavenging is desired. *S. obliquus*, while slightly less effective, also showed significant antioxidant potential, particularly at higher concentrations. *C. vulgaris*, though moderately effective, may require optimization or higher doses to achieve similar antioxidant benefits to the other two species. In our previous work it was demonstrated that *S. platensis* was the highest one in total antioxidant activity followed by *C. vulgaris* and *S. obliquus* **(El-Chaghaby *et al.*, 2019)**.

Ferric reducing power activity

The ferric reducing antioxidant power (FRAP) assay was used to evaluate the reducing power of three microalgae species, *S. obliquus*, *S. platensis*, and *C. vulgaris* at different concentrations (50, 100, 150, and 200mg/ L). The results are presented in Fig. (2) as absorbance values, which correlate to the reducing power of the samples.

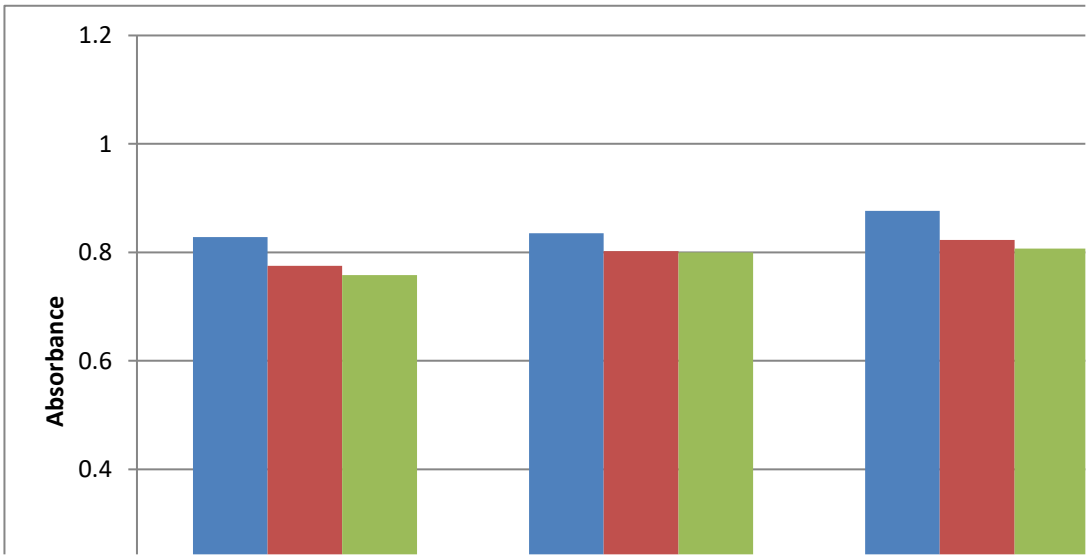

Fig. 2. FRAP assay of three microalgae extracts, *Scenedesmus obliquus* (Sc), *Spirulina platensis* (Spi) and *Chlorella vulgaris* (Chl)

The results of FRAP assay revealed that *S. obliquus* exhibited the highest ferric-reducing power across all concentrations, with absorbance values increasing steadily as the concentration increased. At 200mg/ L, *S. obliquus* showed a markedly higher absorbance (1.1239) compared to both *S. platensis* (0.829) and *C. vulgaris* (0.8774), indicating its superior antioxidant activity. This suggests that *S. obliquus* has a greater ability to donate electrons and reduce ferric ions, this could be linked to its elevated levels of bioactive compounds, including phenolics and flavonoids, which are well-known for their antioxidant properties.

C. vulgaris showed moderate ferric-reducing power, with absorbance values closely following those of *S. platensis* at lower concentrations but surpassing it slightly at the highest concentration (200mg/ L). This increase in reducing power at higher concentrations suggests that *C. vulgaris* also possesses antioxidant compounds, though at a lower potency than *S. obliquus*.

Spirulina platensis exhibited the lowest reducing power among the three algae at all concentrations. While *S. platensis* showed a slight increase in absorbance from 50 to 150mg/ L, its reducing power plateaued at higher concentrations, with a minimal difference between 150 and 200mg/ L. This lower performance compared to *S. obliquus* and *C. vulgaris* may be due to differences in the type and quantity of antioxidant compounds present in *S. platensis*, such as phycocyanin, which may not be as effective in reducing ferric ions as the compounds found in the other algae.

In summary, *S. obliquus* exhibited the highest ferric-reducing power, suggesting it could be a more potent source of natural antioxidants compared to *S. platensis* and *C. vulgaris*.

The phyco-pigment contents

Chlorophyll and carotenoids content

The chlorophyll and carotenoid content of the three selected microalgae are presented in Table (1).

Table 1. Pigments in microalgae extracts

Microalgae type	Chlorophyll (a) (mg/L)	Chlorophyll (b) (mg/L)	Carotenoids (mg/L)
Spirulina platensis	11.4584 ±0.1047	2.1752 ±0.3305	4.1336 ±0.1737
Chlorella vulgaris	5.2320 ±0.0116	2.0861 ±0.1933	1.4602 ±0.0789
Scenedesmus obliquus	7.2677 ±0.0353	5.0122 ±0.1525	1.2541 ±0.0742

Data are presented as mean values ±standard deviation

Scenedesmus obliquus showed intermediate chlorophyll content (7.27mg/ L) and the highest concentration of chlorophyll b (5.01mg/ L). The higher ratio of chlorophyll b to chlorophyll a in *Scenedesmus obliquus* indicates its adaptation to low-light environments, as chlorophyll b extends the range of light absorption for photosynthesis. The carotenoid content of *S. obliquus* (1.25mg/ L) was lower compared to *Spirulina*, suggesting a lesser role in photo-protection or anti-oxidation. As previously reported, *Scenedesmus obliquus* has chlorophylls of a and b series nearly about 8.3mg/ g **(Fernandes *et al.*, 2021)**.

Chlorella vulgaris had the lowest chlorophyll a (5.23mg/ L) and carotenoid (1.46mg /L) content among the three species, though it exhibited comparable levels of chlorophyll b (2.09mg/ L) to *Spirulina*. This suggests that *Chlorella vulgaris* is less efficient at light absorption compared to *Spirulina*, but it can still perform photosynthesis efficiently in moderate light environments due to its balanced chlorophyll content.

Spirulina platensis exhibited the highest levels of chlorophyll a (11.46mg/ l) and carotenoids (4.13mg/ l) among the three algae. This is consistent with the known pigmentation of *Spirulina*, which is rich in chlorophyll and carotenoids due to its cyanobacteria nature, enabling efficient light absorption for photosynthesis. The high carotenoid content, in particular, contributes to its antioxidant properties, as carotenoids are effective at scavenging free radicals. Conversely, it exhibits a higher carotenoid content compared to findings from earlier studies. **(Sibaoueih *et al.*, 2021; Pan-utai *et al.*, 2022)**.

Phycobiliprotein content

Phycobiliprotein content of the three selected microalgae is summarized in Table (2). *Scenedesmus obliquus* had minimal amounts of phycobiliproteins, with C-phycocyanin (0.14mg/ g), allophycocyanin (0.12mg/ g), and phycoerythrin (0.05mg/ g). These low levels suggest that *Scenedesmus obliquus* relies more on chlorophyll and carotenoids for light absorption and photo protection, as opposed to phycobiliproteins, which are more abundant in cyanobacteria like *Spirulina* **(Aizpuru & González-Sánchez 2024)**.

Table 2. Phycobiliproteins in microalgal extracts

Microalgae type	C-phycocyanin (mg/g)	allophycocyanin (mg/g)	phycoerythrin (mg/g)
Spirulina platensis	20.2997 ±0.0632	9.4623 ±0.1693	3.5004 ±0.0842
Chlorella vulgaris	1.9146 ±0.0245	0.3982 ±0.0142	0.0716 ±0.0057
Scenedesmus obliquus	0.1395 ±0.0044	0.1185 ±0.0080	0.0520 ±0.0039

Data are presented as mean values ±standard deviation

Chlorella vulgaris contained moderate levels of C-phycocyanin (1.91mg/ g), with very low concentrations of allophycocyanin (0.40mg/ g) and phycoerythrin (0.07mg/ g). Although *Chlorella vulgaris* is not a cyanobacterium, it still produces minor amounts of phycobiliproteins, which likely contribute to its antioxidant properties.

Spirulina platensis had by far the highest phycobiliprotein content, with C-phycocyanin (20.30mg/ g), allophycocyanin (9.46mg/ g), and phycoerythrin (3.50mg/ g).

Phycocyanin, the signature blue pigment in *Spirulina*, is known for its potent antioxidant, anti-inflammatory, and immune-modulating properties, which makes *Spirulina* particularly valuable for health-related applications. *Spirulina platensis* exhibits higher concentrations of phycobiliproteins compared to those reported in earlier research **(Sibaoueih *et al.*, 2021)**. The pigment levels in *Spirulina platensis* are influenced by various environmental factors, including pH, temperature, salinity, and light intensity **(Nahid *et al.*, 2023)**.

Antimicrobial activity

The antimicrobial activity of the three microalgae species *Scenedesmus obliquus* (1), *Chlorella vulgaris* (2), and *Spirulina platensis* (3) was evaluated against five different pathogenic microorganisms, including *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Candida albicans*, and *Aspergillus niger*. The antimicrobial data are presented in Table (3).

Table 3. Antimicrobial activity of microalgae extracts

Sample / Pathogenic microorganism	*Scenedesmus obliquus*	*Chlorella vulgaris*	*Spirulina platensis*	Control
Bacillus subtilis (ATCC 6633)	26±0.1	26±0.1	32±0.1	29±0.1
Staphylococcus aureus (ATCC 6538)	29±0.2	25±0.2	25±0.1	25±0.1
Escherichia coli (ATCC 8739)	24±0.2	19±0.1	16±0.2	19±0.1
(13883) *Klebsiella . pneumoniae ATCC*	18±0.1	20±0.2	22±0.2	20±0.1
Candida albicans (ATCC 10221)	27±0.1	25±0.1	28±0.2	19±0.3
Asperagillus Nigar (ATCC:16404)	NA	NA	NA	25±0.1

*NA: No activity, control for Bacteria was Gentamycin and for fungi was fluconazole.

* Zone of inhibition is expressed as mean ± SD (mm), 100µL Samples and Control were inoculated each well at 50mg/mL concentration.

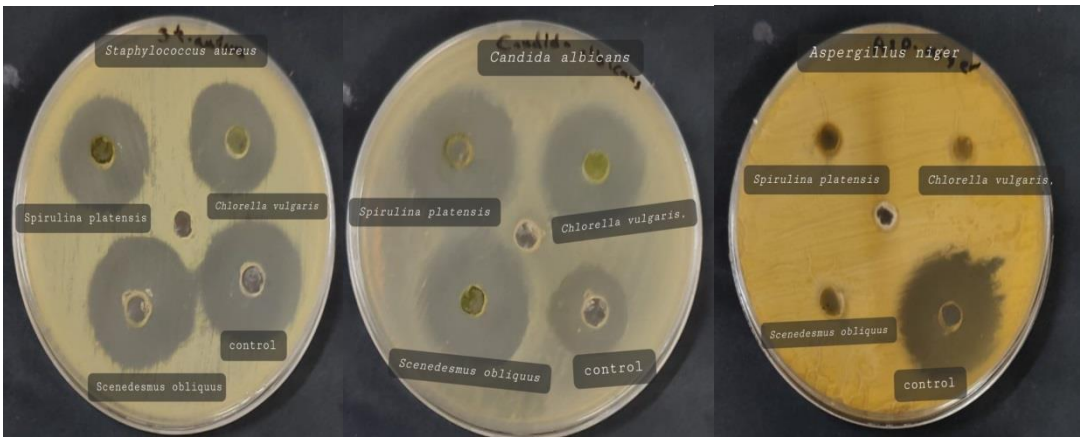

Fig. 3. Antimicrobial effects of microalgae

In the case of Gram-positive bacteria *Bacillus subtilis*, *Spirulina platensis* extract showed the highest inhibition zone (32±0.1mm), surpassing both *Chlorella vulgaris* (26±0.1mm) and *Scenedesmus obliquus* (26±0.1mm). The control (Gentamycin) exhibited an inhibition zone of 29± 0.1mm, slightly lower than that of *Spirulina platensis* but higher than the other two microalgae species.

Scenedesmus obliquus had the strongest activity against *Staphylococcus aureus* with an inhibition zone of 29± 0.2mm, compared to 25± 0.2mm for *Chlorella vulgaris* and 25± 0.1mm for *Spirulina platensis*. The control sample showed similar results to *Spirulina platensis* and *Chlorella vulgaris*, with an inhibition zone of 25± 0.1mm.

For Gram-negative bacteria, *Scenedesmus obliquus* demonstrated the highest antimicrobial activity against *Escherichia coli* with an inhibition zone of 24± 0.2mm, followed by *Chlorella vulgaris* (19± 0.1mm) and *Spirulina platensis* (16± 0.2mm).While the *Chlorella vulgaris* extract's inhibition zones were significantly similar to those of Gentamicin.

Spirulina platensis showed the strongest activity with an inhibition zone of 22± 0.2mm against *Klebsiella pneumoniae*, followed by *Chlorella vulgaris* (20± 0.2mm) and *Scenedesmus obliquus* (18± 0.1mm). Gentamicin showed an inhibition zone of 20± 0.1mm, similar to *Chlorella vulgaris*.

Scenedesmus obliquus showed the highest efficacy against *Escherichia coli* and *Staphylococcus aureus*, suggesting that its antimicrobial compounds may have specific effectiveness against Gram-negative bacteria and certain Gram-positive strains **(Shevelyuhina *et al.*, 2022)**. *Chlorella vulgaris* generally exhibited moderate antimicrobial activity, with some variation across different microorganisms. Its bioactive compounds, such as chlorophyll and carotenoids, may contribute to this effect, though at lower potency compared to *Spirulina* and *Scenedesmus*. On the other hand, previous study revealed that the effectiveness of the crude extract of *Chlorella vulgaris* against

both Gram-negative and Gram-positive bacteria may be attributed to the presence of Chlorellin, a mixture of fatty acids **(Hussein *et al.*, 2018)**.

Spirulina platensis demonstrated the most potent antimicrobial activity, particularly against *Bacillus subtilis* and *Candida albicans*. This might be attributed to its rich composition of bioactive compounds, such as phycocyanin and polysaccharides, which are known to possess antimicrobial properties **(Alshuniaber *et al.*, 2021)**.

The observed antibacterial effects of the selected microalgae suggest potential utility as a supplementary or alternative treatment, especially considering the rising concern over antibiotic resistance. This is consistent with previous studies by **Alshuniaber *et al.* (2021)** and **Little *et al.* (2021)** who assessed that green microalgae have had to adapt to conditions that exclude many other organisms including bacteria to survive.

Regarding fungi, the *Spirulina platensis* extract exhibited significant activity against *Candida albicans* (28±0.2mm), which was the highest among the tested microorganisms, while *Scenedesmus obliquus* (27±0.1mm) and *Chlorella vulgaris* (25±0.1mm) showed slightly lower activity. The control sample (fluconazole) had a significantly lower inhibition zone of 19±0.3mm.

This suggests strong antifungal properties that could be exploited in the development of natural antifungal treatments. Interestingly, none of the microalgae showed activity against *Aspergillus niger*, indicating a potential limitation in their antifungal spectrum. The control sample consistently showed antimicrobial activity across all organisms, with comparable or lower inhibition zones to the microalgae species, indicating the potential of these microalgae extracts as natural antimicrobial agents.

Overall, the antimicrobial efficacy of microalgae extracts against various microorganisms highlights its potential as a natural antimicrobial agent. The bioactive components like fatty acids, flavonoids, and phenolic acids inside microalgae likely contributes to its antimicrobial properties **(Kılıç *et al.*, 2022)**. These findings support the use of microalgae extracts in food and feed additives to enhance safety and extend shelf life by inhibiting the growth of pathogenic microorganisms. Additionally, evaluating the synergistic effects of microalgae extracts with conventional antibiotics could provide valuable insights into developing more effective antimicrobial therapies **(Sukhikh *et al.*, 2022)**. The variation in antimicrobial activity among the different algae may be attributed to differences in their biochemical composition, environmental conditions during cultivation, and extraction methods used **(Little *et al.*, 2021)**.

Microalgae bioactive components identified by GC-MS

GC-MS analysis of *Scenedesmus obliquus*

The GC-MS analysis of *Scenedesmus obliquus* provided a detailed profile of its bioactive compounds, revealing a diverse range of fatty acids, flavonoids, and other phytochemicals that are likely responsible for its observed antimicrobial and antioxidant activities. The key antioxidant and antimicrobial compounds identified in the microalgae extract are listed in Table (4) and Fig. (4).

The compounds included fatty acids such as cis-Vaccenic acid. These unsaturated fatty acids and their derivatives exhibit significant antioxidant properties by scavenging free radicals, thereby preventing oxidative damage to cells and tissues. Additionally, they possess antimicrobial effects against a wide range of bacterial and fungal species. In the present study, the total fatty acids in *Scenedesmus obliquus* extract accounted for approximately 74% of the area sum%. cis-Vaccenic acid (omega-7) at 27.02% was identified as the predominant fatty acid, comprising 36% of the area sum%, followed by Methyl γ-linolenate (omega-6) derivative at 16.8%, Arachidonic acid at 14%, 9-Octadecenoic acid (Z)-, methyl ester at 7.48%, and cis-10-Nonadecenoic acid at 3.18%. High levels of poly-unsaturated fatty acids, particularly Methyl γ-linolenate, cis-Vaccenic acid, and Arachidonic acid, correlates well with the strong antioxidant properties observed in the FRAP and DPPH assays conducted for *Scenedesmus obliquus*. These compounds are known to neutralize free radicals and to inhibit lipid peroxidation, providing protective effects against oxidative stress. The significant antioxidant activity observed in the DPPH assay is likely a result of these fatty acids working synergistically **(da Silva *et al.*, 2021)**. The fatty acid profile of *Scenedesmus obliquus* observed in this study is consistent with findings reported by other researchers **(da Silva *et al.*, 2021; Hariram *et al.*, 2022)**. The study carried out by ***da Silva et al.* (2021)** assessed that high amount of palmitic acid (> 29%) oleic (> 25%) and linolenic (> 17%). **Rocha *et al.* (2019)**, reported high levels of linolenic (> 15%), oleic (> 14%), and linoleic acids (> 10%) and showed that the cultivation method has a significant impact on the fatty acid profile of *Scenedesmus obliquus.*

The extract was also found to contain different terpenoids e.g. squalene which is considered as a natural antioxidant that prevents lipid peroxidation, supporting cellular health. It was detected in *Scenedesmus obliquus* extract (0.54%) area sum %. In addition to its antioxidant effects, squalene is also known for its ability to enhance skin health and support the immune system. In terms of antimicrobial activity, the presence of γ-Terpineol, dihydro- at 2.48% and cis-10-Nonadecenoic acid provides strong antimicrobial properties.

GC-MS analysis of *Chlorella vulgaris*

The GC-MS results of *Chlorella vulgaris* revealed a variety of bioactive compounds, predominantly fatty acids, and their derivatives, alkanes, and sterols, as presented in Fig. (5) and listed in Table (5). These compounds play critical roles in the organism's biological activity, contributing to its antimicrobial, antioxidant, and other bio-functional properties. The high levels of fatty acid methyl esters, such as pentadecanoic acid (21.75%), 14-methyl-, methyl ester, and 9-hexadecenoic acid methyl ester (14.48%), suggest that the responsibility of these components for the antimicrobial effects seen in the agar well diffusion assays **(Koley *et al.*, 2024)**.

Table 4: Active compounds in *Scenedesmus obliquus* extract detected by GC/MS

RT(min)	Compound name	Area sum%
7.58	Octadecane, 6-methyl-	0.56
8.43	4,8-Decadienal, 5,9-dimethyl-	0.91
8.72	7-Hexadecenal, (Z)-	0.5
9.31	γ-Terpineol, dihydro-	2.48
9.65	Undecanoic acid	1.42
10.58	2,6-Octadiene, 2,6-dimethyl-	1.54
11.22	Tetradecane, 2,6,10-trimethyl-	0.47
12.29	Squalene	0.54
12.92	cis-Z-α-Bisabolene epoxide	0.63
14.21	Heptadecane	1.87
15.08	Tetradecanoic acid, 12-methyl-, methyl ester	0.5
15.14	Estra-1,3,5(10)-trien-17β-ol	1.53
15.4	10-Octadecenoic acid, methyl ester	0.72
15.51	11,14-Eicosadienoic acid, methyl ester	1.06
15.6	2-Pentadecanone, 6,10,14-trimethyl-	1.24
15.9	cis-10-Nonadecenoic acid	3.18
16.07	9-Hexadecenoic acid, methyl ester, (Z)-	1.74
16.27	Pentadecanoic acid, 14-methyl-, methyl ester	1.26
16.8	6,9,12-Octadecatrienoic acid, methyl ester	3.11
16.9	9-Octadecenoic acid (Z)-, methyl ester	7.48
17.37	Linoleic acid ethyl ester	0.96
17.51	Methyl γ-linolenate	16.81
18.5	cis-Vaccenic acid	27.02
18.71	Cholic acid	5.37
19.4	Arachidonic acid	14.07
19.6	1-Heptatriacotanol	3.05

Table 5. Active compounds in *Chlorella vulgaris* extract detected by GC/MS

RT(min)	Compound Name	Area sum %
7.58	Octadecane, 6-methyl-	0.77
8.43	4,8-Decadienal, 5,9-dimethyl-	0.54
8.72	7-Hexadecenal, (Z)-	0.49
11.22	Tetradecane, 2,6,10-trimethyl-	0.5
12.29	Squalene	0.33
12.92	cis-Z-α-Bisabolene epoxide	0.3
13.29	Hexadecane	0.45
14.21	Heptadecane	13.55
14.52	Myristic acid, methyl ester	0.64
15.08	Tetradecanoic acid, 12-methyl-, methyl ester	0.29
15.14	Estra-1,3,5(10)-trien-17β-ol	0.36
15.4	10-Octadecenoic acid, methyl ester	0.24
15.51	11,14-Eicosadienoic acid, methyl ester	0.39
15.6	2-Pentadecanone, 6,10,14-trimethyl-	0.48
15.9	cis-10-Nonadecenoic acid	0.49
16.07	9-Hexadecenoic acid, methyl ester, (Z)-	14.48
16.27	Pentadecanoic acid, 14-methyl-, methyl ester	21.75
16.8	6,9,12-Octadecatrienoic acid, methyl ester	0.59
16.9	9-Octadecenoic acid (Z)-, methyl ester	0.66
17.37	Linoleic acid ethyl ester	0.27
17.51	Methyl γ-linolenate	11.19
17.64	Oleic acid	17.41
17.8	Palmitic acid	8.26
17.9	Heptadecanoic acid, 16-methyl-, methyl ester	2.82
18.5	cis-Vaccenic acid	0.36
19.4	Arachidonic acid	0.35
19.6	1-Heptatriacotanol	0.78
19.92	Stigmasterol	1.27

Table 6. Active compounds in *Spirulina platensis* extract detected by GC/MS

RT(min)	Compound name	Area sum %
6.179	Isoflavone, 6,7-dimethoxy-	0.82
7.5	Octadecane, 6-methyl-	0.83
10.03	D-Verbenone	0.72
11.22	Hexa-hydro-farnesol	0.44
12.28	4-Methyldocosane	0.7
12.9	Dihydro-3-oxo-β-ionol	1.01
13.29	Hexadecane	0.9
14.25	Heptadecane	15.8
14.5	Myristic acid, methyl ester	0.72
15.07	Pentadecanoic acid, methyl ester	0.65
15.4	16-Octadecenoic acid, methyl ester	0.23
15.5	Phytol	0.74
15.6	9E)-9-Octadecenoic acid	0.31
15.87	Undecanal	0.75
16.08	Palmitoleic acid	7.06
16.27	Stearic acid	18.89
17	Oleic Acid	1.46
17.6	Linolenic acid	10.94
17.64	epi-Cedrol	16.96
17.81	(-)-Gallocatechin	8.53
17.86	Kaempferol-7-O-neohesperidoside	3.37
18.04	Methyl arachidonate	0.94
18.21	Squalene	0.95
18.48	5,7,3'-Trimethoxyflavone	1.05
18.71	Cholic acid	0.51
19.15	Luteolin 6,8-c-diglucoside	0.79
20.2	β-Sitosterol	0.88
20.8	cis-Nerolidol	0.55
21.45	β-Ionone	2.51

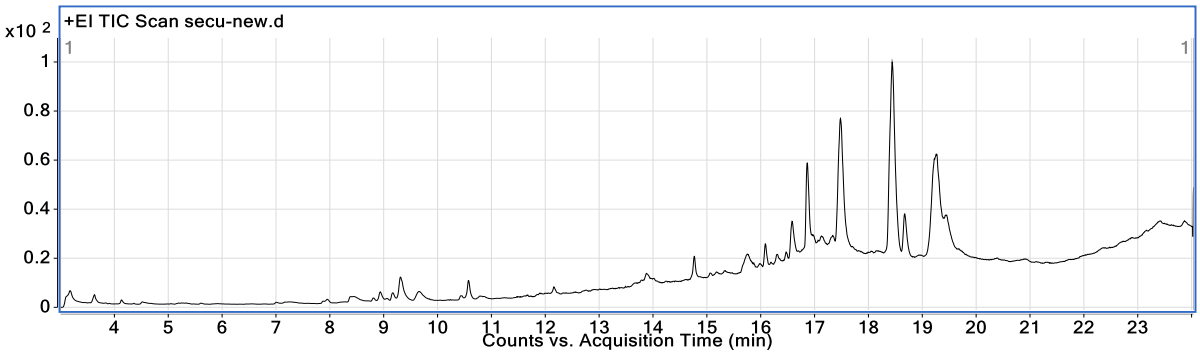

Fig. 4. GC chromatogram of *Scenedesmus obliquus* extract

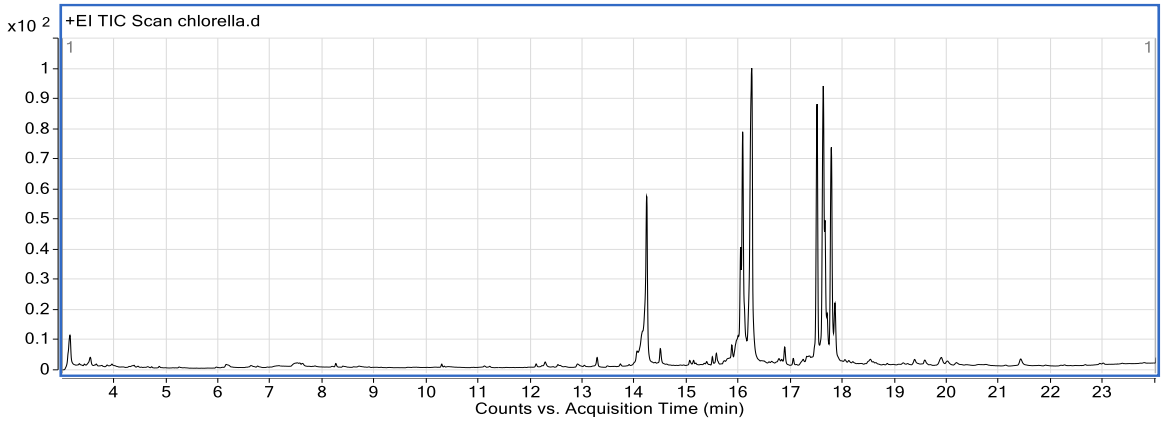

Fig. 5. GC chromatogram of *Chlorella vulgaris* extract

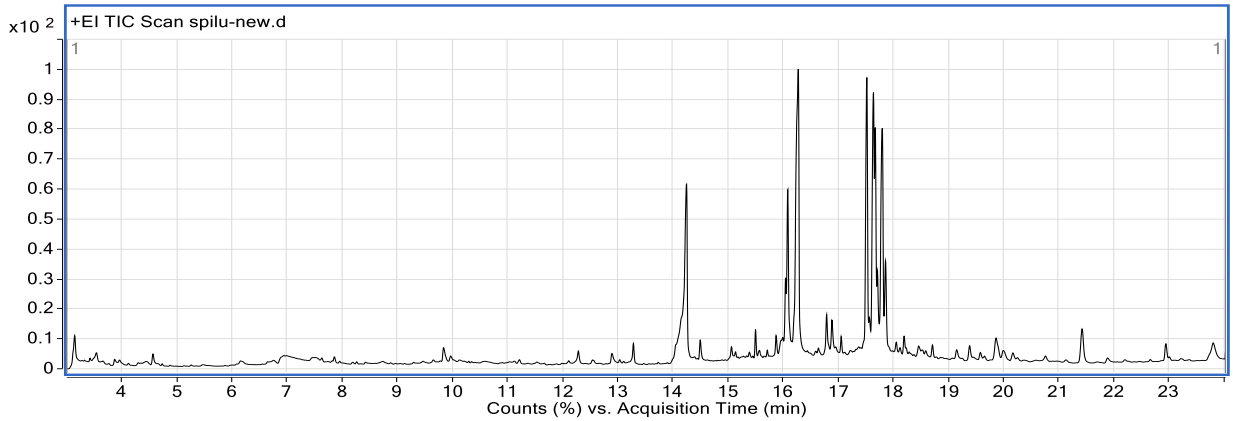

Fig. 6. GC chromatogram of *Spirulina platensis* extract

The presence of significant amounts of oleic acid (17.4%), methyl γ-linolenate (11.19%), and palmitic acid (8.26%) supports the antioxidant properties of *Chlorella vulgaris*, as these fatty acids are famous for their capability to neutralize free radicals and ban oxidative stress. Their abundance in the algal extract may explain the strong antimicrobial activity observed, particularly against Gram-positive bacteria like *Staphylococcus aureus*. A study using GC-MS analysis identified the chemical composition of the methanol extract of *Chlorella vulgaris*, revealing the presence of eicosanoic acid, oleic acid, n-hexadecanoic acid, octadecenoic acid, and pentadecanone **(Perveen *et al.*, 2022)**.

The extract was also found to contain Heptadecane (13.55%). Heptadecane is a long-chain alkane known for its antimicrobial properties. The antimicrobial ability of Heptadecane presented in this extract has previously been published **(Perveen *et al.*, 2022)**. The extract was also found to contain different terpenoids e.g. squalene (0.33%) and Stigmasterol (1.27%) which is a phytosterol known for its anti-inflammatory, cholesterol-lowering, and antioxidant effects. Phytosterols are often found in algae and are important for their potential therapeutic applications. Overall, the GC-MS analysis indicates that *Chlorella vulgaris* contains a range of bioactive compounds that could contribute to its potential therapeutic effects, particularly in antimicrobial and antioxidant applications **(Kılıç *et al.*, 2022)**.

GC-MS analysis of *Spirulina platensis*

The GC-MS analysis of *Spirulina platensis* reveals the presence of a diverse array of bioactive compounds, including fatty acids, sterols, flavonoids, and terpenes suggesting significant contributions to the antimicrobial and anti-inflammatory properties of the algae presented in Fig. (6) and listed in Table (6). In the present study, the total fatty acids in *Spirulina platensis* extract accounted for approximately 40% of the area sum. Stearic acid (18.89%) was identified as the predominant fatty acid, comprising 47% of the area sum%. Stearic acid exhibits antimicrobial properties, which could contribute to the biological activity of *Spirulina platensis* extracts. Linolenic acid was also detected at 10.94% area sum. It's an essential omega-3 fatty acid with well-known anti-inflammatory properties **(Zaki *et al.*, 2022)**. Palmitoleic acid is a monounsaturated fatty acid that has shown antimicrobial and anti-inflammatory properties was detected at 7.06% area sum, followed by oleic acid (1.46%). A previous study assessed that there were five predominant fatty acids found in *Spirulina platensis*, including hexadecanoic acid (93.92%), methane sulfonyl acetic acid (3.93%), pentadecanoic acid (0.89%), octadecadienoic acid (0.71%), and tridecanoic acid (0.52%) of the total peak area **(Zaki *et al.*, 2022)**.

The GC-MS results also indicated the presence of several terpenes, including epi-Cedrol (16.96%), β-Ionone (2.51%), Dihydro-3-oxo-β-ionol (1.01%), phytol (0.74%), and cis-Nerolidol (0.55%). These terpenes, known for their antimicrobial properties, contribute to the extract's ability to inhibit microbial growth, particularly epi-Cedrol, which is considered a sesquiterpene alcohol known for its strong anti-inflammatory and antimicrobial activities. Its significant presence in *Spirulina platensis* highlights its potential in medicinal applications, especially in managing inflammation and microbial infections.

CONCLUSION

This study shows that *Spirulina platensis*, *Scenedesmus obliquus*, and *Chlorella vulgaris* exhibit notable antioxidant and antimicrobial activities, which offers potential applications as natural additives in food and feed as health-promoting agents. *Spirulina platensis* had the strongest DPPH antioxidant activity and significant antimicrobial effects, especially against various bacteria and fungi, likely due to its high phycocyanin and chlorophyll content. *Scenedesmus obliquus* showed the highest ferric reducing power, attributed to its high levels of chlorophyll b and fatty acids, which contribute to its antioxidant and targeted antimicrobial activity. While *Chlorella vulgaris* demonstrated moderate bioactivity, its fatty acids and unique compounds make it valuable in specific applications. Overall, the GC-MS analysis confirmed that these microalgae contain bioactive compounds with potential uses in pharmaceuticals, food preservation, and antimicrobial resistance management.

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REFERENCES

Aizpuru, A. and González-Sánchez, A. (2024). Traditional and new trend strategies to enhance pigment contents in microalgae. World J. Microbiol. Biotechnol., 40(9): 1–26. https://doi.org/10.1007/s11274-024-04070-3

Alshuniaber, M. A.; Krishnamoorthy, R. and AlQhtani, W. H. (2021). Antimicrobial activity of polyphenolic compounds from *Spirulina* against food-borne bacterial pathogens. Saudi J. Biol. Sci., 28(1):459–464. https://doi.org/10.1016/j.sjbs.2020.10.029

Balouiri, M.; Sadiki, M. and Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. Journal of pharmaceutical analysis, 6(2): 71–79. https://doi.org/10.1016/j.jpha.2015.11.005.

Chikhoune, A.; Ghazi, A. and Adjadj, F. (2024). Bioactive Potential of Milk Thistle (*Sylibum marianum*) Seeds and Applicability of Its Edible Oil in Food Processing †. Biol. Life Sci. Forum., 36(2): 1–7.

da Silva, M. E. T.; Martins, M. A.; de Oliveira Leite, M.; Milião, G. L. and Coimbra, J. S. dos R. (2021). Microalga *Scenedesmus obliquus*: extraction of bioactive compounds and antioxidant activity1. Rev. Cienc. Agron., 52(2): 1–8. https://doi.org/10.5935/1806-6690.20210036

El-Chaghaby, G.A.; Rashad, S.; Abdel-Kader, S., F.A.; Rawash, E.-S. and Abdul Moneem, M. (2019). Assessment of phytochemical components, proximate composition and antioxidant properties of *Scenedesmus obliquus*, *Chlorella vulgaris and Spirulina platensis* algae extracts. Egypt. Aquat. Biol. Fish., 23(4): 521–526. https://doi.org/10.21608/ejabf.2019.57884

El-Chaghaby, G. A.; Rawash, E. S. A. and Rashad, S. (2024). Effect of Edible Coating Prepared from *Aloe Vera* Incorporated with Lemongrass Extract on the Antioxidant Properties of the Nile Tilapia Fish Fillets Under Refrigerated Storage. Egypt. Aquat. Biol. Fish., 28(1): 987–1001. https://doi.org/10.21608/ejabf.2024.339450

El-Chaghaby, G. A.; Shehta, H. A.; Rashad, S.; Rawash, E. S. A. and Eid, H. R. (2024). Evaluation of the antioxidant and antimicrobial activities of the spent coffee extracts and their applications as natural food preservatives of chicken fillets. Nov. Res. Microbiol. J., 8(1): 2303–2319. https://doi.org/10.21608/nrmj.2024.339900

Fernandes, A. S.; Nascimento, T. C.; Pinheiro, P. N.; de Rosso, V. V.; de Menezes, C. R.; Jacob-Lopes, E. and Zepka, L. Q. (2021). Insights on the intestinal absorption of chlorophyll series from microalgae Andr e. Int. Food Res.J., 140(December 2020). https://doi.org/10.1016/j.foodres.2020.110031

Hariram, V.; John, J. G.; Sangeethkumar, E.; Gajalakshmi, B.; Ramanathan, V.; Vinothkumar, M.; Selvakumar, R. and Balachandar, M. (2022). *Scenedesmus obliquus* and *Chlorella vulgaris* - A Prospective Algal Fuel Source. NEPT., 21(5): 2129–2139. https://doi.org/10.46488/NEPT.2022.v21i05.009

Hussein, H. J.; Naji, S. S. and Al-Khafaji, N. M. S. (2018). Antibacterial properties of the *Chlorella vulgaris* isolated from polluted water in Iraq. J. **Pharm.** Sci. Res., 10(10): 2457–2460.

Ibrahim, H.A.H.; Shaaban, M.T.,; Hanafi, A.A.M. and Abdelsalam, K.M. (2020). Inhibition of bacteria isolated from human specimens by selected marine-origin extracts. Egypt. J. Exp. Biol. (Bot.), 16(1): 91-103. DOI: 10.5455/egyjebb.20200509032540

Kılıç, N. K.; Safran, E. and Dönmez, G. (2022). Antimicrobial Characteristics of Bioactive Compounds By a Thermotolerant Microalga *Chlorella Vulgaris* Isolated From Thermal Springs of Turkey. J. microbiol., biotechnol. food sci., 11(6): 4–8. https://doi.org/10.55251/jmbfs.2285

Koley, R.; Mahato, B.; Hazra, K.; Khatun, A.; Ghorai, R.; Adhikari, A. Debnath, B. and Das, S. (2024). Microalgae *Chlorella Vulgaris* isolation and GCMS Analysis of Phytocomponent Methanolic Extracts. Tuijin Jishu/ J. Propuls. Technol., 45(1): 1001–4055.

Little, S. M.; Senhorinho, G. N. A.; Saleh, M.; Basiliko, N. and Scott, J. A. (2021). Antibacterial compounds in green microalgae from extreme environments: A review. Algae, 36(1):61–72. https://doi.org/10.4490/algae.2021.36.3.6

Nahid, N.; Azam, A.; Ejhieh, N. and Reza, A. (2023). Intensified Phycobiliprotein Extraction from *Spirulina Platensis* by Freezing and Ultrasound Methods. IRAN J CHEM CHEM ENG., 42(2): 601–617. https://doi.org/10.30492/IJCCE.2022.541486.4992

Naik, B.; Mishra, R.; Kumar, V.; Mishra, S.; Gupta, U.; Rustagi, S.; Gupta, A. K.; Preet, M. S.; Bhatt, S. C. and Rizwanuddin, S. (2024). Micro-algae: Revolutionizing food production for a healthy and sustainable future. J. Agric. Food Res.,15(2):100939. https://doi.org/10.1016/j.jafr.2023.100939

Pan-utai, W.; Iamtham, S.,; Boonbumrung, S. and Mookdasanit, J. (2022). Improvement in the Sequential Extraction of Phycobiliproteins from *Arthrospira platensis* Using Green Technologies. Life, 12(11): 1–19. https://doi.org/10.3390/life12111896

Perveen, K.; Bukhari, N. A.; Al Masoudi, L. M.; Alqahtani, A. N.; Alruways, M. W. and Alkhattaf, F. S. (2022). Antifungal potential, chemical composition of *Chlorella vulgaris* and SEM analysis of morphological changes in Fusarium oxysporum.Saudi J. Biol. Sci. 29(4): 2501–2505. https://doi.org/10.1016/j.sjbs.2021.12.033

Rocha, D. N.; Martins, M. A.; Soares, J.; Vaz, M. G. M. V.; de Oliveira Leite, M.; Covell, L. Mendes, L. B. B. (2019). Combination of trace elements and salt stress in different cultivation modes improves the lipid productivity of *Scenedesmus* spp. Bioresource Technol., 289(June), 121644. https://doi.org/10.1016/j.biortech.2019.121644

Shevelyuhina, A.; Babich, O.; Sukhikh, S.; Ivanova, S.; Kashirskih, E.; Smirnov, V.; Michaud, P. and Chupakhin, E. (2022). Antioxidant and Antimicrobial Activity of Microalgae of the Filinskaya Bay (Baltic Sea). Plants, 11(2264): 1–15.

Sibaoueih, M.; Nasser, B.; Eddoha, R.; Essamadi, A. and El, B. (2021). Chemical characterization and nutritional value of *Spirulina platensis* cultivated in natural conditions of Chichaoua region (Morocco) c. j.sajb, 141: 235–242. https://doi.org/10.1016/j.sajb.2021.05.006

Subri, M. and Zin, M. (2020). Antioxidative properties and proximate analysis of spent coffee ground (SCG) extracted using ultrasonic-methanol assisted technique as a potential functional food ingredient. food research, 4(June): 636–644.

Sukhikh, S.; Prosekov, A.; Ivanova, S.; Maslennikov, P.; Andreeva, A.; Budenkova, E.; Kashirskikh, E., Tcibulnikova, A., Zemliakova, E., Samusev, I. and Babich, O. (2022). Identification of Metabolites with Antibacterial Activities by Analyzing the FTIR Spectra of Microalgae. Life, 12(9):1–18. https://doi.org/10.3390/life12091395

Wu, H.; Lu, P.; Liu, Z.; Sharifi-Rad, J. and Suleria, H. A. R. (2022). Impact of roasting on the phenolic and volatile compounds in coffee beans. Food sci. nutr., 10(7): 2408–2425. https://doi.org/10.1002/fsn3.2849

Zaki, N. H.; Ali, A. M.; Al-rubaiee, G. H. and Alhammer, A. H. (2022). Anti-bacterial and Anti-tumoral Activities of Spirulina Platensis Extracellular Extract. Malays. J. Med. Res., 18(7): 11–16.