

## Evaluation of *Spirulina* spp. Crude Extract Revealed Antimicrobial, Antioxidant and UV Photoprotective Properties

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### Abstract

The antimicrobial, UV photoprotective and antioxidant capabilities of *Spirulina* spp. methanolic extracts were determined using Fourier transform infrared spectroscopy (FTIR) and gas chromatography-mass spectrometry (GC-MS). Notable compounds were reported to be 5 fatty acid methyl esters, 4-methoxyallocinnamic acid, 2-ethylhexyl esters and bumetrizole. Moreover, these metabolites contained functionalities including hydroxyl, alkyl and carbonyl, as supported by the FTIR result. After 24 h, there was no zone of inhibition in the antibacterial activity as measured by the agar well diffusion method at doses of 30, 50 and 70 g/mL. The ABTS radical scavenging assay revealed that the methanolic *Spirulina* spp. exhibited an inhibitory concentration of 1,047.91 g/mL for antioxidant activity and SPF values of 0.40, 0.23 and 0.93 were calculated using Mansur's equation for concentrations of 30, 50 and 70 g/mL, respectively. The results of the study showed the potential of cyanobacterial extracts as a bioactive ingredient in cosmetic product development.

**Keywords:** *Spirulina* spp., Antimicrobial, UV Photoprotective, Antioxidant, Gas chromatography-mass spectrometry

### Introduction

Excessive exposure to sunlight can harm the skin and weaken the immune system due to the penetrating UV radiation. Using cosmetics that offer UV protection, like sunscreen or makeup with a sun protection factor (SPF), can reduce the detrimental effects of UV radiation on the skin. However, synthetic UV protecting ingredients in cosmetics may have adverse effects such as allergies, photo-toxicity and disruption of the endocrine system. For example, parabens found in makeup, moisturizers and creams can enter the skin and have been linked to the development of breast cancer [1]. Similarly, phthalates in skin cleansers and other personal care products are endocrine-disrupting chemicals that can negatively impact fertility [2]. Considering these drawbacks, there is a growing preference for naturally occurring bioactive compounds from biological sources.

Extracts obtained from various biological sources have demonstrated ultraviolet (UV) protection capabilities, such as plants [3,4], seaweeds [5,6] and fungi [7]. Among these sources, there is a growing interest in utilizing cyanobacteria due to their production of mycosporine-like amino acids (MAAs), which exhibit anti-UV [8] and antioxidant characteristics [9]. Cyanobacteria employ a combination of MAAs, polysaccharides, carotenoids and scytonemin as defense and tolerance mechanisms to counter the detrimental effects of solar UV radiation [10]. These mechanisms encompass antioxidant production, DNA repair, protein resynthesis and programmed cell death. Moreover, cyanobacteria produce MAAs as an adaptive response to extreme environments [11]. Among various cyanobacteria, *Spirulina* spp, a type of filamentous cyanobacterium, is recognized as a sustainable bioactive microalga. Its high concentration of phytochemicals and other bioactive components contribute to its numerous health-promoting properties [12]. Thus, this microbe is a desirable contender for developing non-hazardous and environmentally

sustainable cosmetic products.

Hence, this study aims to highlight the readily available yet highly underutilized biodiversity that represents potential opportunity for cosmetic applications. This will be done by investigating the cosmeceutical potential of *Spirulina* spp - methanolic extract through the evaluation of its antimicrobial, antioxidant and UV photoprotective activity. Moreover, identification of components presents in the extract indicate its contribution to the aforementioned properties.

## Materials and methods

### Sampling and profiling

A culture of *Spirulina* spp. was purchased from the Institute of Biological Sciences of the University of the Philippines Los Baños. A 100 µl sample was inoculated to another bottle containing 100 mL of Zarrouk's media containing NaCl, NaNO<sub>3</sub>, K<sub>2</sub>HPO<sub>4</sub> and K<sub>2</sub>SO<sub>4</sub> and was exposed to sunlight for optimum growth. Zarrouk's media was continually added to the culture bottle over time to level with the nutrients required by the growing samples. Similarly, this process was repeated to more bottles of Zarrouk's media to possess several subcultures of the *Spirulina* spp sample. Direct microscopy was performed to examine and confirm the identity of the cyanobacterium. *Spirulina* spp. can be described as a multicellular, filamentous blue-green microalgae, as shown in **Figure 1**.



**Figure 1** *Spirulina* spp. under 1,000× magnification.

### Extraction of cyanobacterial metabolites

The extraction method was adapted from a study by Silva-Stenico *et al.* [13]. Ten milliliters of the cyanobacteria sample and media mixture were pipetted into 32 centrifuge tubes for extraction. These were centrifuged at 6,000 rpm for 7 min to separate the media and sample layers. The supernatant was removed, leaving the cyanobacteria sample. Ten milliliters of methanol were added into each tube, then agitated for 30 min using the shaker to mix the contents. Depending on the sample size, this mixture would result in a light to dark green color solution. This process was repeated until all the solutions exhibited a transparent color. The solution underwent rotary evaporation to remove the methanol solvent, specifically with the rotary evaporator (IKA RV 10). The extract was then scraped from the round bottom flask using a laboratory scraper and transferred to a centrifuge tube. Both nitrogen gas and freeze-drying techniques were applied afterward to ensure the removal of any remaining methanol and completely dry the solution.

### Extract yield

To calculate the extract yield, the weight of the empty centrifuge tube and the sample were recorded and the difference was calculated to determine the extract yield. Distilled water was added to the tube and vortexed to collect the extract. The solution was then poured into an Erlenmeyer flask and more distilled water was added until the volume reached 1,000 mL. This solution served as the master culture of extracted cyanobacterial metabolites.

### Initial screening for the presence of UV photoprotective compounds using UV-Vis spectrophotometry

Before conducting the assays to examine the cosmeceutical properties of the extracted compounds, the *Spirulina* spp. extracts were subjected to UV - Vis spectroscopy to test the quantitative determination of compounds that absorb UV radiation. This was done using the Hitachi U-2000 Spectrophotometer. The extract was prepared with methanol and pipetted into a quartz cuvette and methanol was placed in another cuvette to serve as a blank reference. Absorbance was observed to determine whether the sample fell under the wavelength range of 200 - 400 nm, which is the range possessed by ultraviolet rays.

### Preparation of different extract concentrations

After the initial screening and extraction of the cyanobacterial metabolites, the gross weight of the centrifuge tube containing the extract was recorded using an analytical balance. The extract yield was obtained by subtracting the weight of the empty tube from the centrifuge tube with the extract. The test samples were then prepared by diluting the extracted sample with varying measurements of sterile distilled water to obtain 30, 50 and 70 µg/mL concentrations.

### Fourier transform infrared spectroscopy (FTIR)

After extraction, the dry cyanobacterial sample was placed and sandwiched in KBr discs to process the FTIR analysis. The spectroscopic measurement was performed using the Thermo Scientific Class 1 Nicolet 6700 FTIR in the 4,000 - 500 cm<sup>-1</sup> at 16 scans.

### Gas chromatography-mass spectrometry (GC-MS)

Dry cyanobacterial extracts (24.2 mg) were incubated with dichloromethane for 3 days. After this period, the soaked mixture was filtered and the residue was discarded. The supernatant was concentrated over nitrogen gas, which equated to 8.4 mg. The dried extract was reconstituted with 1 mL of dichloromethane, which was then filtered using a 13 mm polytetrafluoroethylene (PTFE) 0.45 µm Whatman syringe filter. Afterward, a GC-EI-MS experiment was performed using a previously published method [14]. The substantiated volatile constituents of the cyanobacterial extracts were established through retention index (RI), whenever possible and structural category employing the NIST Archive. The experiment was performed in triplicate.

### Agar well diffusion

Three skin microorganisms, namely *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*, were used to determine the antimicrobial properties of the sample solutions. The 3 microbial strains were inoculated onto Mueller Hinton broth to preculture the cells and were adjusted to 0.5 McFarland standard. The liquid culture was uniformly suspended on the surface of all Mueller Hinton Agar plates using a sterile swab to create lawn cultures. Afterward, 9 mm diameter wells were aseptically punched using sterile micropipette tips. 30 µL of the test solutions of different concentrations were pipetted into the wells in triplicates. After 24 h of incubation at 37 °C, each well's zone of inhibition formed was measured using a caliper. The positive control for the bacterial strains was gentamicin, whereas nystatin was used for the fungal strain. Sterile distilled water was used as the negative control.

### ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) Assay

The ABTS assay was conducted to evaluate the antioxidant properties of the test samples in their different concentrations. The ABTS radical cation (ABTS\*+) is formed by adding a potent oxidizing agent to the ABTS reagent, resulting in a dark, green-colored solution. A sample's capability in stabilizing radical cations is determined through its mixture with the ABTS\*+ solution, which will decolorize the potent color [15]. A concentration of 7 and 2.45 mM for ABTS and potassium persulfate was obtained. Five milliliters of both solutions were mixed to form the ABTS radical cation solution. This was placed in a dark environment for 16 h before the assay proper. The answer was diluted with ethanol until it absorbed 0.7 at 734 nm using the PerkinElmer Lambda 25 UV-Vis spectrophotometer. The extract samples (0.9 mL) were added to 2.9 mL of the ABTS\*+ solution for each concentration in triplicates. This was left to decolorize for 15 min and then recorded for absorbance. Trolox at pure concentration was used as the positive control, while ethanol was used as the negative. The absorbance values of the samples were used to compute the percent inhibition using the following equation by Sukweenadhi *et al.* [16]:

$$\text{Inhibition \%} = \frac{AB - AT}{AB} \times 100$$

AB is the absorbance of the ABTS reagent and AT is the absorbance of the test samples. The computed values were then credited as the Y values, while the sample concentration measurements were used as the X values to generate the linear regression formula. This was done to obtain the half-maximal inhibitory concentration IC<sub>(50)</sub>, which shows the concentration needed from the test sample to scavenge 50 % of the radical cations.

### Sun protection factor (SPF) assay

The *in vitro* sun protection factor (SPF) test, which is based on UV spectral transmission measurements, was used to assess the efficiency of sun protection in the extracted metabolites. A single UV beam was used to measure the spectral transmittance of the sample. Hitachi U-2900 Spectrophotometer was used. Absorption values were obtained from 290 to 320 nm (every 5 nm). Positive and negative controls were liquid sunscreen and methanol, respectively. Afterward, the Mansur equation was utilized to determine the SPF values. Mansur *et al.* [17] developed a mathematical equation to estimate the sun protection factor by an *in vitro* method using UV spectrophotometry, which in conjunction substitutes the *in vitro* way proposed by Sayre *et al.* [18].

The equation is as follows:

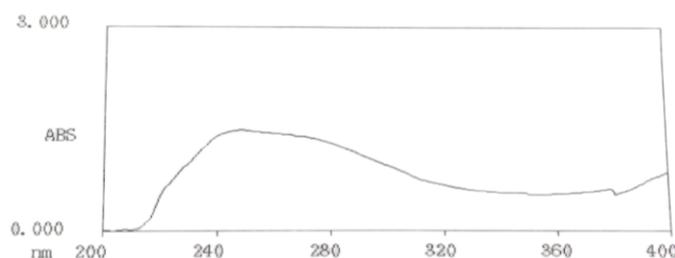
$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times abs(\lambda)$$

Wherein CF = 10 (Correction Factor), EE ( $\lambda$ ) = Erythemogenic Effect of radiation at wavelength  $\lambda$ , I ( $\lambda$ ) = Intensity of solar light at wavelength  $\lambda$  and abs ( $\lambda$ ) = Absorbance of wavelength  $\lambda$  by a solution of the preparation.

## Results and discussion

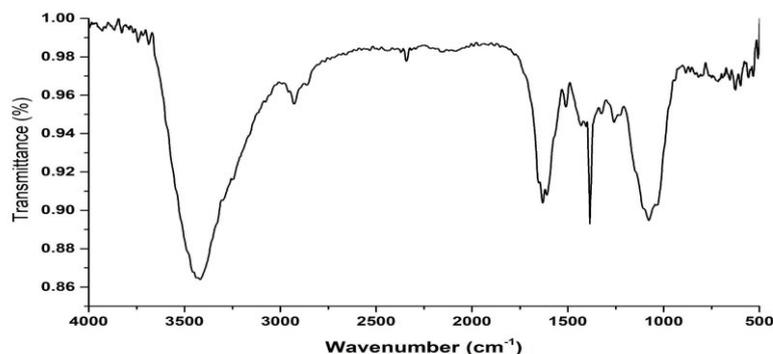
### Extract yield

The weight of the empty centrifuge tube and the sample were recorded to be 6.9234 and 7.0020 g, respectively. The difference calculated was 0.0786 g of extract yield. This was collected by adding distilled water and vortexing the sample tube. The solution was then poured into an Erlenmeyer flask and more distilled water was added until the volume reached 1,000 mL. This garnered 76.8  $\mu\text{g/mL}$  initial concentration for the extract solution. Distilled water in varying amounts was added to the solution to obtain 30, 50 and 70  $\mu\text{g/mL}$  concentrations.



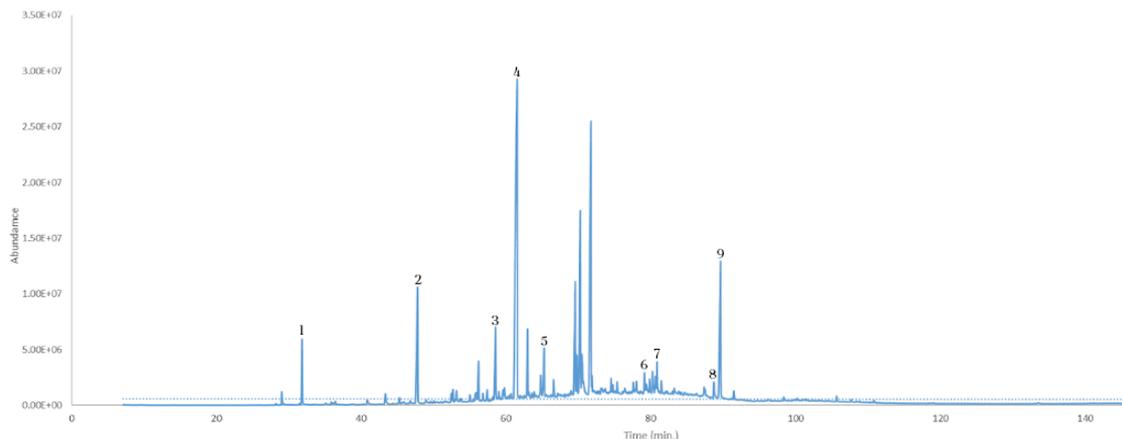
**Figure 2** Initial screening of crude cyanobacterial extract with UV-spectrophotometer.

Before commencing the assays, an initial screening was undertaken to identify compounds with UV photoprotective attributes. The cyanobacterial metabolites extracted were analyzed via UV-Vis spectroscopy, revealing an absorbance peak at 248 nm (as illustrated in **Figure 2**), categorized explicitly as UV-C.



**Figure 3** FTIR spectrum crude methanolic extract of *Spirulina* spp.

**Figure 3** FTIR spectrum of the cyanobacterial extract reveals distinctive functional groups at specific peaks. At approximately  $3,410\text{ cm}^{-1}$ , a broad peak is evident, indicating the presence of a hydroxyl group ( $-\text{OH}$ ). Furthermore, a faint  $\text{sp}^3$  alkane stretching ( $\text{C}-\text{H}$ ) is discernible at around  $2,940\text{ cm}^{-1}$ . The peak observed around  $1,600\text{ cm}^{-1}$  signifies a carbonyl stretching ( $\text{C}=\text{O}$ ), suggesting the potential existence of a carboxylic acid ( $-\text{COOH}$ ) derivative. The sharp peak around  $1,400\text{ cm}^{-1}$  observes the existence of an hydroxyl ( $-\text{OH}$ ) bending vibration. Around  $1,100\text{ cm}^{-1}$ , a strong peak is indicative of the  $\text{C}-\text{O}$  stretching vibration due to the fatty acids in the compound. Additionally, this further strengthens the likelihood of a particular  $\text{COO}^-$  carboxylate derivative from the carboxylic acid group ( $-\text{COOH}$ ).



**Figure 4** Total ion chromatogram of the volatile constituents of dried cyanobacteria extract by GC-MS Analysis.

**Table 1** Volatile constituents of the crude extract of dried cyanobacteria.

| No. | Compound   | RT (min) <sup>(a)</sup> | RI <sup>(b)</sup> | % Peak Area | Functionality               |
|-----|--|-------------------------|-------------------|-------------|-----------------------------|
| 1   | methyl dodecanoate   | 31.71 ± 0.09            | 1422 ± 0.10       | 6.9 ± 0.01  | fatty acid methyl ester     |
| 2   | methyl tetradecanoate  | 47.61 ± 0.01            | 1744 ± 0.15       | 5.0 ± 0.00  | fatty acid methyl ester     |
| 3   | 2,6-di- <i>t</i> -butyl-4-methoxyphenyl ester<br>4-azido-2-nitrobutyric acid | 56.07 ± 0.12            | 1781 ± 0.10       | 11.6 ± 0.01 | diverse functional groups   |
| 4   | methyl hexadecanoate   | 61.41 ± 0.12            | 1858 ± 0.11       | 8.6 ± 0.01  | fatty acid methyl ester     |
| 5   | methyl 14-methylhexadecanoate  | 66.43 ± 0.12            | 1985 ± 0.13       | 19.2 ± 0.01 | fatty acid methyl ester     |
| 6   | 4-methoxyallocinnamic acid 2-ethylhexyl ester                                | 79.23 ± 0.53            | 2214 ± 14.21      | 2.30 ± 0.00 | diverse functional groups   |
| 7   | benzyl butyl phthalate   | 80.71 ± 0.13            | 2241 ± 3.22       | 3.9 ± 0.01  | phthalate ester             |
| 8   | bumetrizole  | 88.52 ± 0.18            | 2426 ± 3.51       | 3.4 ± 0.00  | hydroxyphenyl benzotriazole |
| 9   | bis(2-ethylhexyl) phthalate  | 89.47 ± 0.15            | 2445 ± 2.90       | 33.9 ± 0.03 | phthalate ester             |

<sup>(a)</sup>Retention Index (HP 5ms column)

The data generated by GC-EI-MS analyses can be observed in **Figure 4** (TIC, total ion chromatogram) and **Table 1**. Five fatty acid methyl esters (FAME) were detected in the *Spirulina* spp. crude extract, specifically methyl dodecanoate, methyl tetradecanoate, methyl hexadecanoate, methyl stearate and methyl 14-methylhexadecanoate. The mass spectra of all of the FAMEs exhibited a base peak at  $74\text{ m/z}$  and a homologous series of ions at  $14\text{ m/z}$  intervals such as  $\text{m/z} = 87, 101, 115, 129, 143, 157$  and  $199$ . 2,6-di-*t*-butyl-4-methoxyphenyl ester 4-azido-2-nitrobutyric acid, which is a compound with diverse functionalities within its structure, showed a base peak at  $178\text{ m/z}$  ( $[\text{M}-\text{C}_4\text{H}_{10}]^+$ ) and a product peak at  $161\text{ m/z}$  ( $[\text{M}-\text{OH}]^+$ ). Two phthalate esters were also found: benzyl butyl phthalate and bis(2-ethylhexyl) phthalate. The base peak of benzyl butyl phthalate was  $[\text{M}-\text{C}_8\text{H}_6\text{O}_3]^+$  with an  $\text{m/z} = 149$ . The molecule bis(2-ethylhexyl) phthalate gave characteristic product peaks at  $\text{m/z} = 167$  ( $[\text{M}-2(\text{R}-\text{H})]^+$ ) and the dehydration of one water molecule from  $167\text{ m/z}$  ion to produce base peak  $\text{m/z} = 149$ . The mass spectrum of bumetrizole displayed

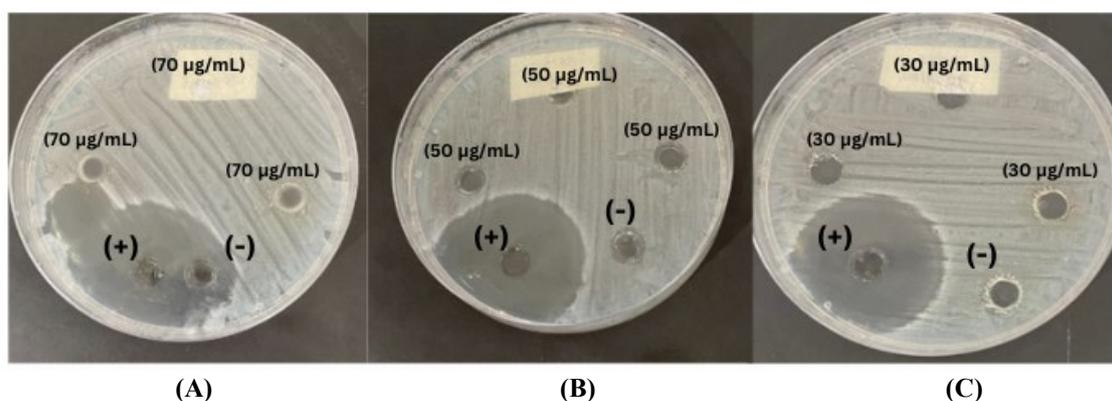
a precursor ion  $[M]^+$  + peak of  $m/z = 315$  and a base peak of  $m/z = 300$ , which signified the loss of a methyl group forming an  $[M-CH_3]^+$  ion. The retention indices were compared to the database repository ChemSpider (<http://www.chemspider.com/Default.aspx>). All the molecules gave similar retention indices except for 2,6-di-*t*-butyl-4-methoxyphenyl ester 4-azido-2-nitrobutyric acid, which could not be located in the aforementioned database or any similar sites.

Notable compounds detected by GC-MS analysis include the following: 2,6-di-*t*-butyl-4-methoxyphenyl ester 4-azido-2-nitrobutyric acid, which, according to Fisher Scientific, is known to be a free radical inhibitor, used as an antioxidant for cosmetics, vitamins and pharmaceuticals. Bis-(2-ethylhexyl) phthalate (BEHP) is a bioactive compound that can be isolated from bacteria, fungi and algae that has been reported to have potential antimicrobial properties. 4-methoxyallocinnamic acid 2-ethylhexyl esters, also referred to as octinoxates, are known for absorbing UV-B rays and, thus, often utilized as a UV filter in several cosmeceutical products (PubChem Compound Database). Lastly, according to the National Center for Advancing Translational Sciences, bumetrizole is an ultraviolet light absorber (UVA) of the hydroxyphenyl benzotriazole class, bumetrizole, which could impart light stability to plastics and other organic substrates, according to the National Center for Advancing Translational Sciences. The presence of these compounds indicates that the extract has potential, however further testing for the extract's application must be carried out to determine whether or not the concentration is sufficient to be significant.

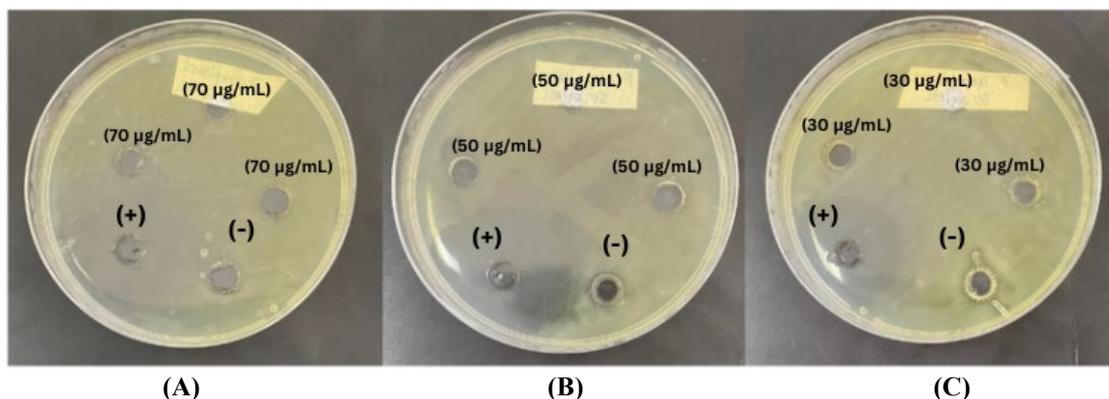
**Figure 3** shows 3 peaks at approximately 3,410, 2,940 and 1,600  $cm^{-1}$ , indicating a hydroxyl, alkyl and carbonyl group. These functional groups support the identity and properties of the compounds in the cyanobacterial extract solution by the GC-MS analysis. According to **Table 1**, 4 identified fatty acids possess a carboxylic acid in their structure. This explains the carbonyl group peak formed at 1,600  $cm^{-1}$ . Among the notable compounds discussed, the structure of 2,6-di-*t*-butyl-4-methoxyphenyl ester 4-azido-2-nitrobutyric acid and bumetrizole possess a hydroxyl and alkyl groups, while octinoxate present all of these functional groups including the carbonyl group. The acquired data from the GC-MS analysis supports the ABTS and SPF assay results, confirming the presence of metabolites with antioxidant and UV photoprotective properties.

#### Testing for antimicrobial properties by agar well diffusion

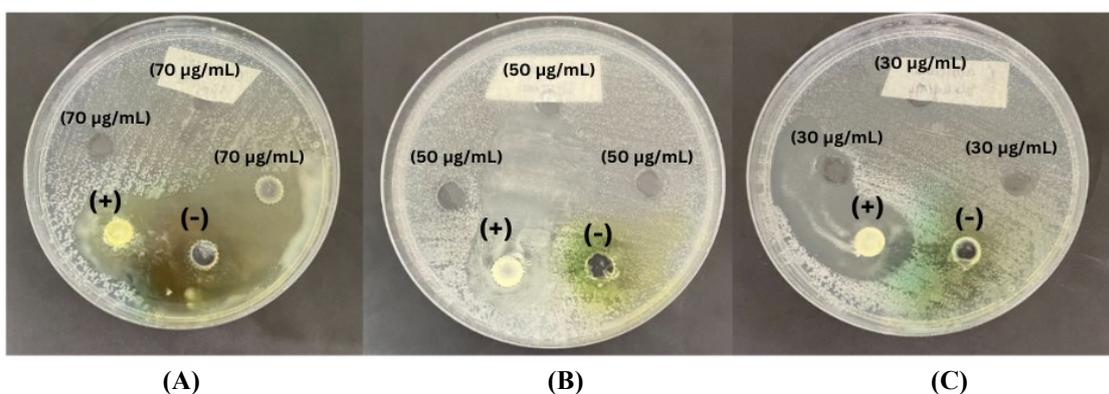
After 24 h of incubation, no inhibition zones were observed around the wells. This indicated that the *Spirulina* spp. extracts did not possess antimicrobial activity against the tested bacterial and fungal strains.



**Figure 5** Agar well diffusion method of *Spirulina* spp. crude extracts against test microorganism *Staphylococcus aureus* at varying concentrations (A) 70  $\mu g/mL$ , (B) 50  $\mu g/mL$  and (C) 30  $\mu g/mL$ .



**Figure 6** Agar well diffusion method of *Spirulina* spp. crude extracts against test microorganism *Pseudomonas aeruginosa* at varying concentrations (A) 70 µg/mL, (B) 50 µg/mL and (C) 30 µg/mL.



**Figure 7** Agar well diffusion method of *Spirulina* spp. crude extracts against test microorganism *Candida albicans* at varying concentrations (A) 70 µg/mL, (B) 50 µg/mL and (C) 30 µg/mL.

Antibiotic resistance is a serious public health issue that has risen at an alarming rate in the past few decades as bacterial pathogens tend to develop antibiotic resistance over time. Several studies regarding the antimicrobial activity of microalgae and cyanobacteria have shown promising results. The limited investigations conducted to evaluate antimicrobial levels of *Spirulina* have demonstrated that this microorganism presents novel antibiotic alternatives with encouraging potential for preventing infection [19]. To determine the levels of microbial resistance of the *Spirulina* extracts, the zone of inhibition surrounding the wells was observed and measured. Visual assessment of the plates showed the absence of zones of inhibition.

Secondary metabolites found in cyanobacterial extracts have been known to inhibit the growth of microorganisms by the following modes of activity: (1) disruption of cell membranes, (2) interference of DNA replication, (3) inhibition of enzyme activity, protein synthesis and metabolic pathways and (4) inhibition of cell wall synthesis. Antimicrobial activity depends on both cyanobacterial species as well as the solvents used for their extraction process. However, studies such as the one by Frazzini *et al.* [20] have supported methanolic extraction for cyanobacteria to have significant antimicrobial activity.

Despite previous research supporting the antimicrobial properties of *Spirulina* spp., several factors may have contributed to the absence of visible antimicrobial activity, such as the extracts' concentrations and drying up of agar wells, as shown in **Figures 5 to 7**. Although GC-MS results showed the presence of bis (2-ethyl hexyl) phthalate, a compound that has been noted for its antimicrobial activity [21], the low concentrations of the extract likely prevented it from creating a zone of inhibition. Additionally, previous research has stated that polyphenols, a large and diverse group of phytochemicals found in plant and cyanobacterial extracts, are another example of compounds responsible for strong antibacterial activity [22]. GC-MS results showed the absence of this compound. After examining the plates, it is evident that the plates were unexpectedly tilted or moved inside the incubator as the antibiotic from the positive controls spilled over.

### Testing for antioxidant properties by ABTS assay

The obtained percent inhibition values were used as the Y values, while the concentrations were X values in the acquired linear regression equation ( $y = -0.0258x + 27.536$ ). The half maximal inhibitory concentration  $IC_{(50)}$  was revealed to be 1,047.907  $\mu\text{g/mL}$  using the formula. According to **Table 2** by Jadid *et al.* [23], the test samples are considered weak antioxidants as the value is above 100. The Trolox solution attained an absorbance of 0.007328 and a percent inhibition of 98.95 %, which denotes its high antioxidant capacity.

**Table 2** Volatile constituents of the crude extract of dried cyanobacteria.

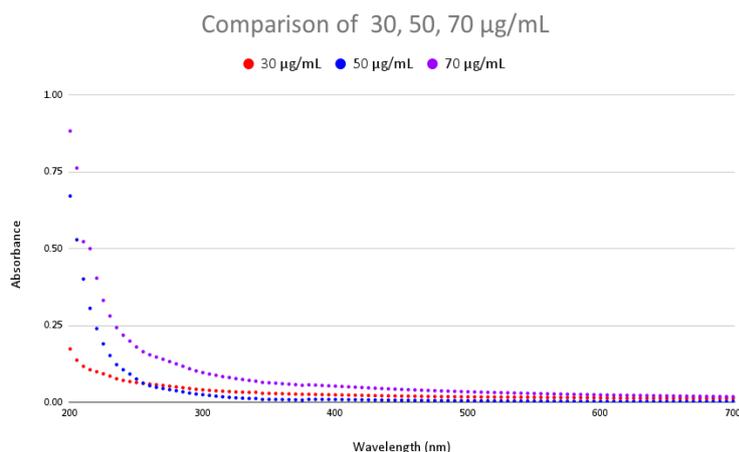
| Extract concentration | Absorbance at 734 nm | Percent inhibition (%) |
|-----------------------|----------------------|------------------------|
| 30 $\mu\text{g/mL}$   | 0.519 $\pm$ 0.036    | 25.803                 |
| 50 $\mu\text{g/mL}$   | 0.503 $\pm$ 0.034    | 28.167                 |
| 70 $\mu\text{g/mL}$   | 0.527 $\pm$ 0.015    | 4.772                  |

The garnered results of the sample solutions indicate their weak potential as a radical scavenger. This was the likely outcome as the mixture solution was minimally decolorized after 15 min while they were left to stabilize. This contrasts the result of the Trolox mixture, the positive control, that quickly lightened in color when it was combined with the ABTS radical cation. The Trolox solution was nearly transparent as its absorbance measured 0.007328, indicating a high amount of absorbed light by the instrument. This is proportional to its obtained inhibitory percentage, which was computed to be 98.95 % and thus, verifies its strong antioxidant effectivity.

The assay was conducted in triplicates per concentration solution to verify its results. When the trials were averaged, the 50  $\mu\text{g/mL}$  concentration obtained the highest percent inhibition value, followed by 30  $\mu\text{g/mL}$ , then 70  $\mu\text{g/mL}$ . Despite the support of previous studies indicating that *Spirulina* spp. possesses antioxidant properties, low concentrations of *Spirulina* extract may have minimal antioxidant activity as the concentration of bioactive compounds may be too low to scavenge free radicals effectively. The variation in data suggests that other factors may contribute to the scavenging ability of the sample. The concentration and reaction time are functions of the decolorization of the radical ABTS [24]. As the test samples were individually loaded and analyzed in the spectrophotometer, those that were tested later retained more reaction time to scavenge the radicals. Additionally, the study states the need for higher concentrations for a longer time allotment for their reaction to reach the stationary state. This explains the resulting data of the 70  $\mu\text{g/mL}$  samples having the lowest average value as they were the first to be analyzed by the UV-vis spectrophotometer. The study also proved that bioactive compounds have different rates at which they quench the ABTS\*+. Polyphenols possess strong and rapid antioxidant activity, while other bioactive compounds, such as glutathione, are considered weak within a short reaction time but are still able to quench the radical cation because of their hydrogen atom transfer mechanism. In relation to this, the extracted metabolites of the experiment may also not possess antioxidant properties or exhibit a mechanism that may take longer to implement its effect. The presence of 2,6-di-*t*-butyl-4-methoxyphenyl ester 4-azido-2-nitrobutyric acid is the only known extracted metabolite that has the ability to quench radicals. Further study on the compound must be done to determine its capabilities and properties as a radical scavenger.

### Testing for UV photoprotective properties of *Spirulina* spp. extract by SPF assay

In this research, all the 30, 50 and 70  $\mu\text{g/mL}$  extract concentrations were evaluated by UV spectrophotometry using Mansur's mathematical equation. Absorbance was measured from the range of 200 to 700 nm, but as per Mansur's equation, only the data from 290 to 230 nm was analyzed. The assay was performed in triplicate and each concentration's average absorbance was obtained and used in the equation. The SPF values were calculated as 0.395, 0.231 and 0.931, respectively. The figures below show a visual representation of the SPF values at the varying concentrations.



**Figure 8** Scatterplot of *Spirulina* spp. crude extract at 30, 50 and 70 µg/mL from 200 to 700 nm.

**Table 3** Volatile constituents of the crude extract of dried cyanobacteria.

| Extract concentration | Absorbance at 734 nm |
|-----------------------|----------------------|
| 30 µg/mL              | 0.395 ± 0.028        |
| 50 µg/mL              | 0.231 ± 0.013        |
| 70 µg/mL              | 0.931 ± 0.026        |

Whether natural or artificial, UV radiation is a form of non-ionizing radiation; prolonged exposure can result in several health risks, including cancer. Results from the initial screening of the extracted cyanobacteria metabolites allowed the researchers to confirm the presence of compounds with UV photoprotective properties. This indicated that it is possible that these compounds could exhibit antimicrobial and antioxidant as well, which provided enough evidence to allow the researchers to continue with the analysis and evaluation of the extracted metabolites. These properties were analyzed more closely later in the research. The sample peaked at 248 nm, falling under the category of UV-C (200 - 280 nm). Categories of UV radiation are classified according to their wavelength, with the short-wavelength UV-C being the most damaging type of radiation. Although natural UV-C rays are known to be absorbed by the Earth's ozone layer, humans can still be exposed to artificial UV-C radiation. Artificial sources include tanning booths, mercury vapor lighting, lasers and fluorescent lights.

The absorbance of different concentrations of the crude extract was observed from 200 to 700 nm to allow the comparison of the absorbance values and determine if any significant peaks could be detected from 200 to 400 nm. Visual assessment of the data showed activity within the 290 to 320 range and the data was analyzed to determine the SPF value. The SPF value is a quantitative measurement of the effectiveness of UV photo protectivity. The higher the SPF, the greater the effectiveness of the product in preventing sunburn. Among the samples tested, 70 µg/mL exhibits maximal absorbance higher than the other samples, which can be observed in **Table 3**. This is most likely due to the higher concentration of *Spirulina* spp. extracts; thus, a higher SPF value was calculated. It is evident that the low concentrations of the *Spirulina* extracts, which were measured in µL, contributed to the minimal SPF values.

The UV photoprotective properties of cyanobacterial extracts are generally concentration-dependent; this means that the concentration of MAAs or other secondary metabolites is directly proportional to the UV absorption capacity of the extract. Considering that relatively low concentrations were used in this research, it is highly likely that the amount of metabolites present was insufficient to effectively absorb and dissipate the UV radiation, resulting in a low SPF value. Furthermore, other compounds present in the extract, such as pigments or proteins, may affect the UV photoprotective properties of the extracts. Data variation can result from several factors, including the use of the solvent, the combination and concentration of the samples, the pH system and the emulsion's rheological properties, which can affect the UV absorption of each sample. One possible explanation for the lower SPF value at 50 µg/mL is that at higher concentrations, the extract may form aggregates, resulting in unequal distribution of the extract and

reducing absorption of UV radiation. Cyanobacteria are rich in compounds that can be used to create new products with potential applications in the cosmetic industry. Despite several studies evaluating the bioactivities of microalgae and cyanobacteria, perception of their potential use is severely limited. A systematic review paper published in 2023 stated that the majority of the articles published only mention antioxidant or anti-inflammatory properties [25].

## Conclusions

Data gathered from this research showed that the *Spirulina spp.* extracts yielded from the stated procedures significantly influenced the study. As the concentration measurements ranged in  $\mu\text{g/mL}$ , the results presented minimal affectivity that is proportional to its value. The extracts tested negative for antimicrobial properties as no zone of inhibition was formed by any of the concentrations. This could be due to several reasons, such as the low concentrations used in the experiment or the drying up of the extract solution in the agar wells. The SPF values calculated from the *in vitro* assay verify the greater potential of higher concentrations for UV photoprotective abilities, as the 70  $\mu\text{g/mL}$  solutions displayed the highest values. The 30  $\mu\text{g/mL}$  test solution achieved a higher value than the 50  $\mu\text{g/mL}$ , suggesting that other factors contribute to its potential. Finally, in this study, the *Spirulina spp.* crude extracts are classified as weak radical stabilizers because of the calculated  $\text{IC}_{(50)}$  value garnered by all concentrations. This is confirmed by the minimal decolorization observed; thus, the radical cations were slightly quenched. The cyanobacterial metabolites will exhibit higher efficacy for these properties at higher concentrations. Other factors may influence the efficacy such as the absence of certain metabolites in the sample. GCMS results revealed the compounds present from the extract solutions, confirming the presence of UV photoprotective and antioxidant compounds. In conclusion, the concentrations and identity of the metabolites are the main factors to their effectivity on these properties. The investigation on cyanobacterial metabolites for pharmaceutical and cosmeceutical purposes has been acknowledged over the years however there is still much to review regarding the factors that contribute to its efficacy. As previously mentioned, *Spirulina spp.* is a desirable contender for these purposes as it is a sustainable and rich source of bioactive ingredients. The study supports the existing knowledge of the benefits of utilizing the microalga as well as supplementing information regarding the presence of the indicated metabolites and their effectivity.

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