Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 27(6): 187 – 201 (2023)
www.ejabf.journals.ekb.eg

Fatty Acids, Minerals Composition, and Antimicrobial Activity of the Blue-Green Microalga (*Spirulina platensis*) Isolated from Qilyasan Stream, Sulaimani, Kurdistan, Iraq

Trifa K. J. Farkha
Department of Biology, College of Science, University of Sulaimani, Iraq

*Corresponding Author: trifa.jalal@univsul.edu.iq

ARTICLE INFO
Article History:
Received: Oct. 3, 2023
Accepted: Nov. 14, 2023
Online: Nov. 30, 2023

Keywords:
Fatty acid,
Minerals,
Antimicrobial activities,
Spirulina platensis,
Ethanol,
Ethyl acetate,
Microscopic

ABSTRACT

Arthrospira platensis (homotypic synonym *Spirulina platensis*) is a member of the family Microcoleaceae, which was referred to as *Spirulina platensis* throughout this paper. *S. platensis* holds a significant nutritional value due to the presence of important secondary metabolites. The current study aimed to analyze the fatty acids, minerals composition, and antimicrobial activities of *Spirulina platensis* isolated from Qliyasan stream water. For the extracts of *S. platensis*, two distinct organic solvents, ethanol, and ethyl-acetate, were utilized to achieve this purpose. Furthermore, crude extracts of *S. platensis* were evaluated *in vitro* against *Escherichia coli*, *Staphylococcus aureus*, and *Aspergillus niger* using a well diffusion assay method. According to the findings of this study, nine fatty acids were investigated using the high-performance liquid chromatography (HPLC) technique. Results revealed that $^{\alpha}$- linoleic acid C18:3 (omega 3) constituted the highest percentage (23.734%), followed by oleic acid C18:1 (17.159%). Additionally, the concentrations of 14 types of minerals were determined using an atomic absorption spectrophotometer (AAS). The highest concentration value of magnesium (Mg) was 6548µg/ gm, followed by iron (Fe) at 1250µg/ gm. The antimicrobial activity of crude extract of *S. platensis* by ethanol proved to be more efficient than ethyl-acetate against bacterial models. The highest inhibition zone diameter was recorded against *E. coli* at 16.3mm, while ethyl-acetate extract was more effective against *Aspergillus niger* with an inhibition zone diameter of 12.3mm, compared to the bacterial pathogens.

INTRODUCTION

Arthrospira platensis, a microscopic multicellular filamentous organisms, is classified within the order Ocillatoriales, the family Microcoleaceae, the class Cyanophycean, and the genus Arthrospira. Moreover, it is known by the homotypic synonym *Spirulina platensis* **(Geitler, 1925)**. *Spirulina platensis* is a type of blue-green algae that is gaining popularity around the world due to its easy cultivation, quick development, and high nutritional value as a food source for humans **..** Despite its rich

concentration of nutrients, such as proteins, vitamins, minerals, essential omega-3 and omega-6 fatty acids, *Arthrospira platensis* (*Spirulina platensis*) exhibits significantly lower percentages of total fat and cholesterol when compared to other food sources **(ALfadhly *et al*., 2022)**. Microalgae create a vast range of compounds from many metabolic pathways, such as polysaccharides, carotenoids, fatty acids, and amino acids **(Martínez-Ruizet *et al*., 2022)**, owing to the inclusion of crucial components including vital amino acids, carbohydrates, fatty acids, and minerals. *Spirulina platensis* has a high nutritional value since 50 to 70% of its dry weight is made up of protein **(Kapoor Mehta, 1993; Mazo *et al*., 2004; Soni *et al*., 2017)**. Several pharmacological actions of bioactive compounds have been documented, viz. anthelmintic, antiviral, antibacterial, and anti-inflammatory activities **(El-Sheekh *et al*., 2021)**. Due to their lack of a hard outer layer, *Spirulina* sp. in dietary supplements has a more advantageous nutritional uptake than lactobacilli and yeasts, making substances like iron, carotenoids, vitamins B1 and B2 in addition to γ-linolenic acid easier to be absorbed by the digestion system **(Martínez-Ruizet *et al.*, 2022; Taher and Saeed,2022)**. Microalgal-derived compounds have the benefit of decreasing the adverse effects of synthetic antibiotics and are thought to be inexpensive (**Falaise *et al*., 2016**). Several ecologists and scientists have studied the importance of naturally occurring algae in various ecosystems including freshwater since they may have biological effects on microorganisms, such as bacteria and fungi **(Thomas *et al*., 2021)**. Hot water extract of *S. platensis* has anticancer and antiviral impacts, as well as enhancing the innate immune system by changing the signal pathway of toll-like receptors (TLR) 2 and 4, which results in prominent maturation of macrophages **(Hirahashi *et al*., 2002)**. *Spirulina platensis* powder has a rich mineral composition, with the highest total content being potassium (1400mg.100 g−1), followed by sodium (900mg.100 g−1), phosphorus (800mg.100 g−1), calcium (700mg.100 g−1), magnesium (400mg.100 g−1), and iron (100mg.100 g−1). Furthermore, the algae are rich in selenium and cyanobacterial selenium, which exist as selenite (2%) and selenomethionine (18%). It was reported that, the high levels of calcium and magnesium were observed in *Arthrospira platensis* cultured in salt water compared to freshwater **(Sukumaran *et al*., 2014)**. The ability of *S. platensis* to reduce lipid levels and exhibit an antioxidant effects in humans with chronic obstructive pulmonary disease was addressed in the studies of **Ismail *et al*. (2015)** and **Charoenying *et al.* (2022)**. The global overuse of antibiotics has increased antibiotic-resistant bacteria, such as *Staphylococcus aureus*, *Escherichia coli*, and the resistant fungus *Aspergillus niger*. Hence, scientists are still searching for new approaches to combat these infections. An alternate method to assess antibacterial and antifungal capabilities is to use different quantities of aqueous extracts of microalga, such as Gloeocapsa species against pathogens, as indicated by **Najdenski *et al.* (2013)**. According to most studies, methanol and acetate were the best extractable organic solvents **(AL-Naqishbandy, 2020; Toma and Aziz, 2023)**. Crude extracts of *S. platensis* in methanol, petroleum ether, and acetone exhibit inhibitory zones against pathogenic

bacteria, such as *E. coli*, *S. aureus*, *Bacillus cereus*, and *Proteus mirabilis*, as well as pathogenic fungi, including *A. niger*, *A. flavus*, *A. fumigatus*, and *Candida albicans* **(Usharani *et al.*, 2015)**. Some researchers monitored the antibacterial properties of ethanol crude extract of *S. platensis* and *C. albicans* pathogenic fungi using models of multidrug-resistant *Streptococcus pyogenes* and *Salmonella typhi* pathogens **(Sdiq *et al.*, 2020)**. Antimicrobial resistance is a major public health concern of the twenty-first century since it impedes the effective prevention and treatment of an increasing number of diseases caused by pathogenic bacteria **(Potocki *et al.*, 2021)**. Several Chlorophyta species' microalgae have gained attention for being an economically significant sources of several bioactive compounds **(Kiran and Venkata, 2021)**. The current work aimed to determine the ability of the organic solvent to isolate fatty acids which have the potential as antibacterial agents against two bacteria, namely *Staphylococcus aureus* and *E. coli*, as well as a pathogenic fungus, *Aspergillus niger*.

MATERIALS AND METHODS

Description of study area

Samples were collected from four different sites on the Qilyasan stream which is in the West of Sulaymaniyah City, with an altitude of 761m above sea level, at a latitude of 35° 34′ 44.8″ North and a longitude of 45° 22′ 20.3″ East. (Fig. 1).

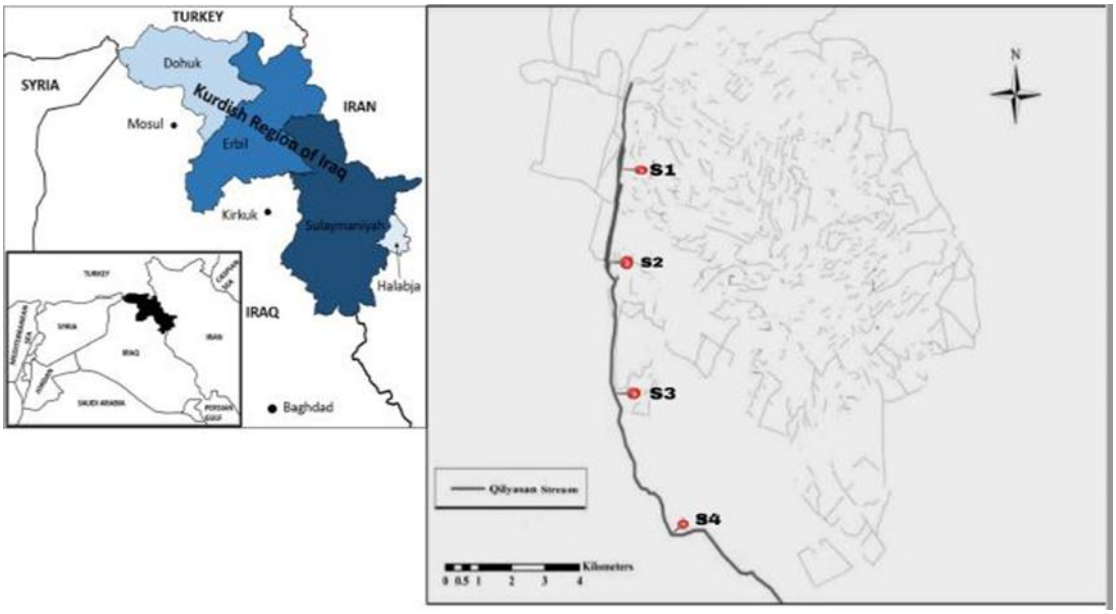

Fig. 1. Map of the Kurdistan region of Iraq on the left, and Qilyasan stream studied sites on the right side

Samples collection

Samples were monthly collected from April till the end of November 2021. Four sites were selected from the Qilyasan stream. The first station (Site 1) is near Sarchinar Spring, followed by the second (Site 2) under Qilyasan bridge; the third (Site 3) is close to Awal Village, and the fourth (Site. 4) is below the Tanjaro bridge (Table 1).

Table 1. Global position system used for reading the studied area

Station	Location	Latitude	Longitude
Site 1	Near Sarchinar Spring	35°35'18.31" N	45°22'34.11"E
Site 2	Under Qilyasan Bridge	35°34'44.31" N	45°22'35.01"E
Site 3	Close to Awal Village	35°32'8.71" N	45°22'26.75"E
Site 4	Below the Tanjaro Bridge	35°28'43.26" N	45°25'46.45"E

Cultivation and harvesting of *Spirulina plantensis*

Water samples were taken from the Qilyasan stream at four separate locations, and after being identified using a standard key, as indicated by **Wang and Zhao (2005)**, they were cultured, purified, and harvested following the method of **Sena *et al*. (2011)**. To grow the algae in a 250mL conical flask, 100mL of blue green 11(BG11) medium was added. The algal culture was grown for 16 days in a (1000 lux lighted) growth environment at 25+ 2°C, with 12/ 12-hour light-dark cycles, as described by **Stanier *et al*. (1971)**. The algal mass was centrifuged for approximately 15 minutes at 10000rpm, after which the pellet was gathered and dried in the oven at a temperature around 40°C, following the method of **Abdo *et al*. (2012)**.

Extract of *Spirulina platensis* preparation

According to the method of **Al-Qahtani and Bionobead (2019)**, 10gm of *Spirulina platensis* powder was extracted with 100ml of 70% ethanol and ethyl-acetate. Moreover, the solvents were extracted by Soxhlet at a tempreture exceeding 78°C for 3- 4 hours, following the soxhletation method as described by **Redfern *et al*. (2014)**. It is considered a more efficient process since it increases the surface contact between the solvent and the material, resulting in higher yields and more number of compounds extracted. Algal extracts were dried at 40°C using a rotary evaporator. The extracts were used for the analysis of fatty acids by HPLC technique and minerals composition by atomic absorption spectrophotometer method. These experiments were conducted in the

laboratories of the Biology Department for Advanced Research, College of Science, University of Sulaimani.

Analysis of fatty acid by high-performance liquid chromatography (HPLC)

In the analysis, the mixture of fatty acid was separated using the FLC column with a particle size of 3μm, and dimensions of (50x4.6mm I.D) C-18DB. The mobile phase consisted of acetonitrile: tetrahydrofuran (THF): 0.1% phosphoric acid in THF in a ratio of 50.4:21.6:28 (V/ V). Detection was performed using UV set at 215nm flow rate of 1.5ml/ min. The temperature was set at 40°C, and each standard used had a concentration of 25μg/ ml. The separation was carried out using a liquid chromatography on Shimazu10AV-LC system equipped with a binary delivery pump model LC-10A Shimadzu. The eluted peaks were monitored by Shimadzu SPD 10A vp detector. Specifically, an area baseline correction of 5000UV was applied, indicating that the mean area of 5000UV will give retention time on the chromatogram without clear peaks, while the area below 5000UV was considered as undetected. this baseline correction in both standard and sample was made to get a clear baseline resolution, as described by **El-Sheekh and Fathy (2009)** and **Cavonius *et al*. (2014)**.

Calculation

$$\text{Concentration of sample } \mu g/ml = \frac{\text{Area of sample}}{\text{Area of standard}} * \text{conc. of standard} * \text{dilution factor}$$

Mineral analysis

The present study aimed to screen the important minerals, such as sodium, potassium, calcium, cobalt, chromium, copper, iron, magnesium, zinc, manganese, cadmium, aluminum, nickel, and lead, using an atomic absorption spectrophotometer (AAS). The processes for analyzing minerals composition included the digestion of 1 gram of algae sample with 5ml of 10M HNO3 for dryness. Five milliliters of 10M HCl were also added to complete the digestion. After cooling at room temperature, the digested solution was diluted to 100ml with deionized water, following the method of **Paul and Muthu Sheeba (2014)** and **Pradhan *et al*. (2014)**.

Antimicrobial activity

All microbiological models utilized in this investigation were provided by Media Diagnostic Center in Erbil, Iraq-Kurdistan Region. *S. platensis* crude extract was used to treat drug-resistant strains of the pathogenic bacteria *Staphylococcus aureus* (ATCC: 25923), *Escherichia coli* (ATCC: 25922), and the pathogenic fungus *Aspergillus niger* (ATCC: 16404). The bacterial culture was inoculated on Mueller Hinton Agar, comprsing

beef infusion powder (300g /l), acid digest of casein (17.5g/ l), agar (17.0g/ l), and starch (1.5g/ l). Incubation extended for 24hrs. at 37°C, while fungal culture was inoculated on potato dextrose agar comprising of glucose (20.0g/ l), agar (15g/ l), potato infusion (4.0g/ l), and tartaric acid solution (14ml/ l), followed by incubation at 37°C. Antibiotics such as Erythromycin and Itraconazole were added to the culture medium to prevent the proliferation of pathogens that would stunt the development of the microalgae. Additionally, zones of inhibition were measured with a ruler and compared to the negative control and positive control in an agar well diffusion assay for bacteria and fungi, respectively. For the bacterial assay, erythromycin 15g was employed as a positive control, as indicated by **Abdo *et al*. (2012)** and itraconazole 15µg was used as a positive control for the fungus, following the method of **Mullis *et al*. (2019)**.

Statistical analysis

For the analysis of the data, a GraphPad Prism version 9 (GraphPad, California, USA) was used. The experimental results were expressed as mean ± standard deviation (SD). An independent t-test was used to compare between the two groups. The *P*-value was regarded as statistically significant. Significance was defined as * $P< 0.05$; ** $P< 0.01$; *** $P< 0.001$, and **** $P< 0.0001$.

RESULTS AND DISCUSSION

Determination of fatty acid

In the analysis of fatty acids in *S. plantesis*, where stearic acid was not detected, the predominant compound was ᵅ- linoleic acid C18:3(omega 3) 23,734%, followed by oleic acid at 17.159%, palmitic acid at 15.891%, and a minor presence of eicosatetraenoic acid at 6.010% (Table 2). The variation of fatty acid content in algae may be related to the environmental factors (location and habitat) and algal species, according to the extraction and transesterification methods described by **El-Sheekh and Fathy (2009)** and **Cavonius *et al*. (2014)**.

Table 2. Fatty acid (%) analysis in *Spirulina plantesis* algae

Fatty acid	Amount of fatty acid by µg/ ml	Percentage %
Capric C14:0	47,579	10.737%
Palmetic C16:0	70,415	15.891%
Oleic acid C18:1	76,036	17.159%
Stearic acid C18:1	0.000	0.000%
Linoleic acid C18:2(omega 6)	22,348	5.043%
ᵅ- liolenic acid C18:3(omega 3)	105,172	23.734%
Arachidic acid C20:3	54,104	12.210%
Decosohexaneoic acid	40,832	9.215%
Eicosopentaneoic acid	26,636	6.011%
Total	443,122	100.00%

Mineral composition

Results of the mineral composition of *S. platensis* showed that it contains sodium, potassium, calcium, cobalt, chromium, copper, iron, mMagnesium, zinc, manganese, cadmium, aluminum, nickel, and lead. The highest amount of Mg was 6548µg/ gm, followed by Fe at 1250µg/ gm, while the minimum amount was recorded for Cd at 0.92µg/ gm (Table 3). The variation of mineral concentration may be ascribed to the geochemical composition of the rock, and parent material of the studied sites. Additionally, the availability of these metals in the water is likely a contributing factor. However, it is important to consider the influence of climate conditions and the sources of pollution along the study streams. Concentration and extraction time are key factors, with intraspecific variability in the production of secondary metabolites occasionally related to seasonal variations. Discrepancies in the extraction protocols used to recover the active metabolites and differences in the assay methods may stand for the variation between our results and those reported in other studies **(Lechuga Morente, 2021)**.

Table 3. Minerals analysis of *Spirulia plantesis* algae

No.	Metals in *Spirulina* sp.	µg/ gm
1	Sodium (Na)	452
2	Potassium (K)	620
3	Calcium (Ca)	800
4	Cobalt (Co)	0.92
5	Chromium (Cr)	11.2
6	Copper (Cu)	3.21
7	Iron(Fe)	1250
8	Magnesium (Mg)	6548
9	Zinc (Zn)	82.6
10	Manganese (Mn)	52.3
11	Cadmium (Cd)	1.27
12	Aluminum (Al)	154.5
13	Nickel (Ni)	2.31
14	Lead (Pb)	3.25

Antimicrobial activity

An opportunistic pathogen, *Staphylococcus aureus*, and a coliform bacterium, *Escherichia coli*, have been carefully chosen for a clinically qualitative antibacterial assay using the Kirby-Bauer test, as cited by **Hudzicki (2009)**. The assay was conducted against both ethyl acetate and ethanol 70% crude extracts of *Spirulina platensis*. Furthermore, the bacterium *Aspergillus niger* was selected among pathogenic fungi for antifungal activity detection. The two crude extracts mentioned above (ethyl-acetate & ethanol) showed antibacterial action in varied degrees against the tested pathogenic microorganisms, as seen in Table (4). Regarding the ethyl-acetate crude extract of *S. platensis*, the inhibition zone diameters were measured at 8.5, 7.0, and 12.1mm for *E.*

coli, *Staphylococcus aureus*, and *Aspergillus niger*, respectively. Pathogenic microorganisms showed a range of reactions and sensitivities, with Gram-positive (G+ve) germs having more responses than Gram-negative (G– ve) germs. These differences occur due to the genetic or chemical structure of microbes, as indicated by **Caccamese *et al.* (1981)**, or may be traced back to cell membrane nature of the microorganisms consisting of a high level (90- 95%) of peptidoglycan, lipopolysaccharides and phospholipids (5- 10%). This composition influences the selection of a suitable medium for reactions and facilitates the entry of antibacterial factors (both bactericidal and bacteriostatic agents) into Gram-positive bacteria, leading to the disruption of the cell membrane or interference with protein biosynthesis units, such as DNA and RNA. This is in contrast to Gram-negative bacteria, where the cell membrance consists of double layers separated by a periplasmic space. The inner membrane contains of Gram-negative bacteria contains peptidoglycan (5- 10%), while the outer membrane contains phospholipids, lipoproteins, and mucopolysaccharides. This composition results in a high lipid content (90- 95%) in the Gram-negative cell membrane, making it less conductive to reactions and limiting the entry of antimicrobial agents. This, in turn, reduces the effectiveness of these agents on pathogenic microorganisimis, as noted by **AL-Samary (1999)**. Moreover, the inhibition zone diameters of ethanol extract were measured at 16.3, 7.3, and non-detected 0mm for *E. coli*, *Staphylococcus aureus*, and *Aspergillus niger*, respectively.

Table 4. Inhibition zone of antibacterial assay kirby-bauer method of both ethyl-acetate and ethanol crude extracts of (*S. platensis*) against 3 pathogenic microorganisms

	Inhibition zone (mm) of tested pathogens (mm)		
Crude extract	*E. coli*	*S. aureus*	*Aspergillus niger*
Ethyl acetate of *S.platensis*	8.5mm	7.0mm	12.1mm
70% ethanol of *S.platensis*	16.3mm	7.3mm	0mm
Erythromycin (15µg)	9.5mm	8.9mm	0mm
Itraconazole (15µg)	-	-	19.4mm

For positive control, antibacterial erythromycin (15µg) was used in the agar-diffusion assay experiment. The inhibition zone of erythromycin against *E. coli* was measured at 9.5± 0.08mm, while the ethyl-acetate crude extract for *E.coli* exhibited an inhibition zone of 8.5± 0.2mm. On the other hand, the ethanol extract demonstrated an inhibition zone of 16.3± 1.7mm, which is higher than the antibacterial activity of erythromycin. This difference is statistically significant, according to the statistical analysis conducted using GraphPad Prism, as shown in Fig (2).

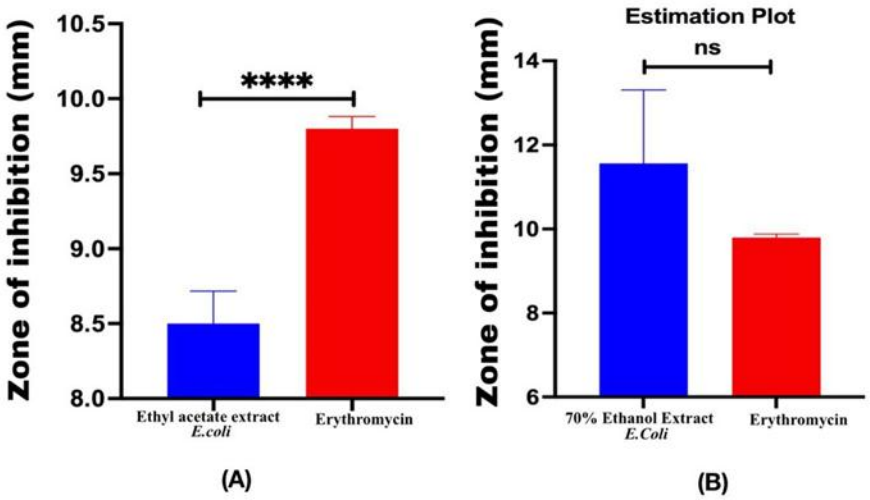

Fig. 2. Bar graph showing statistical analysis of (A) Ethyl-acetate crude extract (blue bar) on *E. coli* compared to erythromycin (Positive control, red bar) and (B) Ethanol crude extract (blue bar) on *E.coli* compared to erythromycin (Positive control, red bar)

For *Staphylococcus aureus*, the average diameters of inhibition zones created by each crude extract differed only in points (7.0± 0.08mm for ethyl acetate, and 7.3± 0.3mm for ethanol). Statistically, erythromycin showed highly significant results, compared to each crude extract that have been used (Fig. 3).

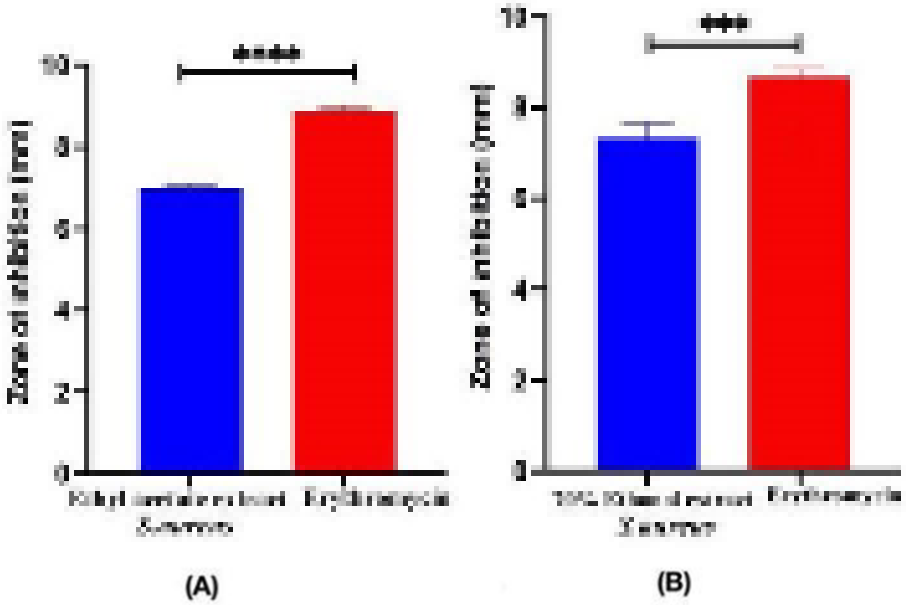

Fig.3. Bar graph showing statistical analysis of (A) Ethyl-acetate crude extract (blue bar) on *S. aureus* compared to erythromycin (Positive control, red bar) and (B) Ethanol crude extract (blue bar) on *S. aureus* compared to erythromycin (Positive control, red bar)

The ethyl-acetate extract for fungal specimen *Aspergillus niger* showed an average inhibition zone diameter of 12.1± 0.17mm; however, the ethanol extract did not show any result. In comparison, itraconazole (15µg) was used as a positive control and had an inhibitin zone diameter of 19.4± 0.1mm, which is highly significant.

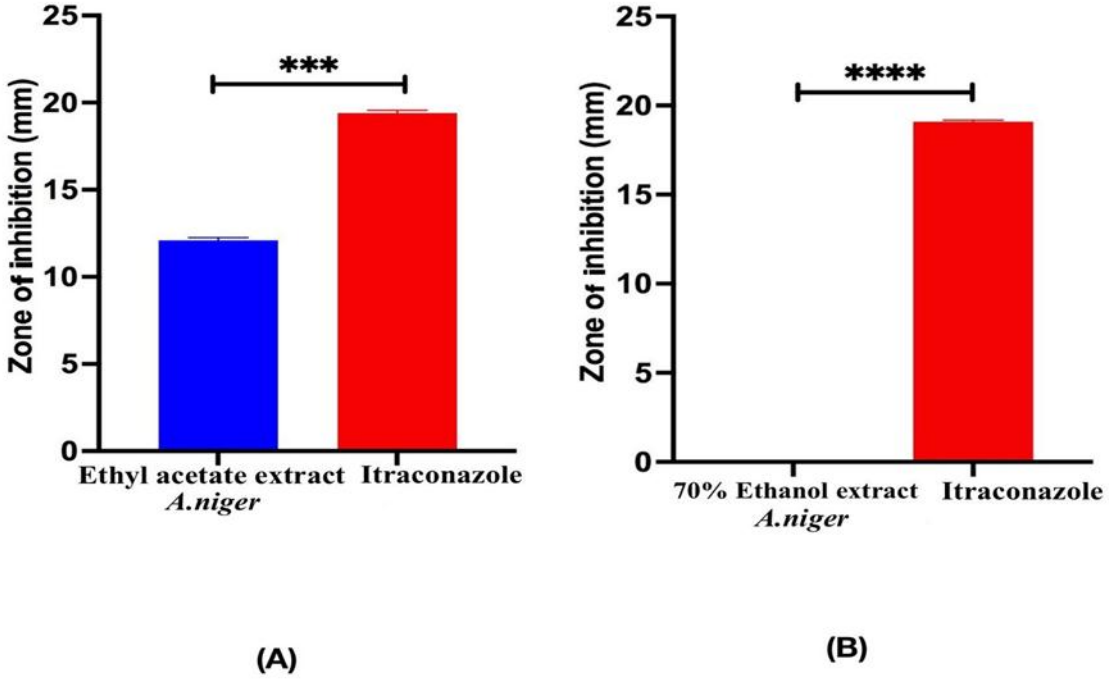

Fig. 4. Bar graph showing statistical analysis of (A) Ethyl-acetate crude extract (blue bar) on *A.niger* compared to itraconazole (Positive control, red bar) and (B) Ethanol crude extract (no effect) on *A.niger* compared to itraconazole (Positive control, red bar)

Ethanol extract was most effective on *E. coli* rather than on *Staphylococcus aureus.* Nevertheless, the ethanol extract had no effect on pathogenic *A. niger*. Alternatively, the ethyl-acetate crude extract showed both antifungal and antibacterial properties on all the pathogenic microorganisms used, thus it is not as effective as other antimicrobial agents, such as erythromycin and itraconazole. The study conducted by **Kaushik and Chauhan (2008)** which also used ethyl-acetate and 70% ethanol solvent extracts of *Spirulina platensis* against *Streptococcus pyogenes, Salmonella Typhi*, and *Candida albicans* showed that the 70% ethanol crude extract was very effective against all microbial agents. The inhibition zones were 8.7, 19.3, and 19.5mm, respectively, for each microbe, and erythromycin was used as a positive control against *S. pyogenes* (5.3mm), *Salmonella Typhi* (8.0mm) and itraconazole (12.7mm). Moreover, it was a positive control for the *C. albicans*. This means that ethanol solvent proved to be more effective on these microbes than

on *E.coli, S.aureus,* and *A.niger* used in the current study. In the study at hand, 70% ethanol showed the highest effect on the Gram–ve *E. coli*. According to a previous study by **Usharani *et al.* (2015)**, *Spirulina platensis* extracts, administered at a dosage of 5.0mg/ ml, led to a significant reduction in bacterial growth, resulting in 10- to 20mm inhibition zones. Gram-positive cocci *Streptococcus pyogenes* showed the greatest mean zone of inhibition (20± 0.4mm) when exposed to methanol crude extract of *Spirulina platensis* (5.0mg/ l), followed by *Staphylococcus aureus* (190.3mm), and *Streptococcus epidermidis* (18± 0.6mm), while Gram-negative mean inhibition zones are (19± 0.8mm), (19± 0.5mm), and (18± 0.3mm) for *Proteus mirabilis, Klebsiella pneumoniae,* and *E. coli*, respectively. In accordance with the results of the study, the methanol crude extract of *Spirulina platensis* proves more effective compared to ethyl-acetate and ethanol crude extracts used in our study since methanol is a good solvent for extraction, and it is frequently used in biology due to its polarity. Furthermore, it is capable of extracting both lipophilic and hydrophilic molecules or substances. The other advantage is that it can easily be removed at room temperature since it is highly volatile. This suggests that other organic solvents extracted from *S. platensis* have the potential to be studied and further used as antimicrobial agents. The results of a study concluded that the ethyl-acetate crude extract of *S.platensis* is effective against *S.aureus* but has no effect on *E.coli*, this disagrees with our study due to the fact that ethyl-acetate extract had no significant effect on either *S.aureus* or *E.coli* **(Fayyad *et al.*, 2022)**. Moreover, the inhibitory activity is influenced by the type of algae the effectiveness of the extraction technique, and the active intergradient **(Lechuga Morente, 2021)**.

CONCLUSION

The purpose of this study was to investigate the antibacterial properties of ethanol and ethyl-acetate crude extracts of *S. platensis* on two drug-resistant pathogenic bacteria, *Staphylococcus aureus* and *Escherichia coli*, as well as a pathogenic fungus, *Aspergillus niger*. Samples for this study were collected in the Qilyasan stream of Sulaimani City. The present study concluded that crude extracts of *Spirulina platensis* have antimicrobial activity against *E. coli, S. aureus*, and *A. niger*. Moreover, this study determined that the antimicrobial activity of crude extract of *S. platensis* by ethanol is more effective on the pathogenic bacteria used in our study than the fungal model. However, ethyl-acetate crude extract was more effective on *A.niger* than *S.aureus* and *E.coli*. Furthermore, the highest inhibition zone, measuring 16.33mm, was recorded against *Escherichia coli*, and this was achieved using a 79% ethanol solvent. This study could facilitate further exploration into the utilization of algal secondary metabolites as antibiotics and potentially developing the world of drug design by designing antibiotics made from algae.

REFERENCES

Abdo, S. M.; Hetta, M. H.; Samhan, F. A.; El-Din, R. A. S. and Ali, G. H. (2012). Phytochemical and antibacterial study of five freshwater algal species. Asian J p Sci, 11(3):109-116.

AlFadhly, N.K.Z.; Alhelfi,N.; Altemimi, A.B.; Verma, D.K.;Cacciola,F. and Narayanan kutty,A.(2022).Trends and TechnologicalAdvancements in the Possible Food Applications of Spirulina and Their Health Benefits: A Review. Molecules,27, 5584. **https://doi.org/10.3390/molecules27175584.**

Al-Naqishbandy, L. M. (2020). phycolimnological and physiological study in spring and streams within Akri District Duhok in the Kurdistan Region of Iraq. Ph.D. dissertation, Salahaddin Univer.- Coll. Sci. –Biol. Dep. Erbil/Iraq.

Al-Qahtani, W. H. ; Binobead, M. A. (2019). Anti-inflammatory, antioxidant and antihepatotoxic effects of *Spirulina platensis* against D-galactosamine induced hepatotoxicity in rats. Saudi J Biol Sci, 26(4): 647-652. https://doi.org/10.1016/j.sjbs.2018.01.003.

Al-Samary.(1999). A study of antibacterial activity of aqueous extract of *Allium sativum* L. (Liliaceae)". Eastern M H J. Vol.5, N(4):803-810

Caccamese,S.;Azzolina,R.;Furnari,G.;Cormaci,M.and Grasso,S.(1981).Antimicrobial and antiviral activities of some marine algae from eastern Sicily" Botanica Mar . Vol. (24): 365-368.

Cavonius, L. R.; Carlsson, N. G. and Undeland, I. (2014). Quantification of total fatty acids in microalgae: comparison of extraction and transesterification methods. Anal. Bioanalytic. Chem., 406: 7313-7322. https://doi.org/10.1007/s00216-014-8155-3 .

Charoenying, P.; Laosinwattana, C. and Chotsaeng, N. (2022). Allelopathic potential of subfractions from aqueous extract of *Spirulina platensis* and denatured C-phycocyanin. Curr. Appl. Sci. Technol: 10-55003. https://doi.org/10.55003/cast.2022.02.22.007.

El-Sheekh, M. M. and Fathy, A. A. (2009). Variation of some nutritional constituents and fatty acid profiles of *Chlorella vulgaris* Beijerinck grown under auto and heterotrophic conditions. Int J Bot, 5(2):153-159. https://doi.org/ 10.3923/ijb.2009.153.159.

El-Sheekh, M. M.; Ahmed, A. Y.; Soliman, A. S.; Abdel-Ghafour, S. E. and Sobhy, H. M. (2021). Biological control of soil borne cucumber diseases using green marine

macroalgae. Egyptian J of Biol P Con, 31(1): 1-7. https://doi.org/10.1186/s41938-021-00421-6.

Falaise, C.; François, C.; Travers, M. A.; Morga, B.; Haure, J.; Tremblay, R., ; Mouget, J. L. (2016). Antimicrobial compounds from eukaryotic microalgae against human pathogens and diseases in aquaculture. Marine drugs, 14(9):159. https://doi.org/10.3390/md14090159.

Fayyad, R. J.; Dwaish, A. S.; Sulman, I. M. A. and Lefta, S. N. (2022). Phytochemical profiling of hot and cold alcoholic extract from *Spirulina platensis* alga and Comparison between two extracts against multidrug-resistant bacteria. Res J Pha and Tech, 15(1): 399-404. https://doi.org/10.52711/0974-360X.2022.00066.

Geitler, L. (1925). Cyanophyceae. In: Die Süsswasser-Flora Deutschlands, Österreichs und der Schweiz. (Pascher, A. Eds) Vol.12: 1-450. Jena: Gustav Fischer.

Hirahashi, T.; Matsumoto, M.; Hazeki, K.; Saeki, Y.; Ui, M. and Seya, T. (2002). Activation of the human innate immune system by Spirulina: augmentation of interferon production and NK cytotoxicity by oral administration of hot water extract of *Spirulina platensis*. Int Im pha, 2(4):423-434. https://doi.org/10.1016/S1567-5769(01)00166-7.

Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. American Soc M, 15: 55-63.

Ismail, M.; Hossain, M. F.; Tanu, A. R. and Shekhar, H. U. (2015). Effect of spirulina intervention on oxidative stress, antioxidant status, and lipid profile in chronic obstructive pulmonary disease patients. BioMed Res Int, 486120 https://doi.org/10.1155/2015/486120.**Kapoor, R. and Mehta, U.** (1993). Effect of supplementation of blue-green alga (Spirulina) on outcome of pregnancy in rats. Plant foods for human nutrition, 43:29-35. https://doi.org/10.1007/BF01088093.

Kaushik, P. and Chauhan, A. (2008). In vitro antibacterial activity of laboratory-grown culture of Spirulina platensis. Ind J of Micr, 48:348-352. https://doi.org/10.1007/s12088-008-0043-0.

Kiran, B. R .and Venkata Mohan, S. (2021). Microalgal cell biofactory—therapeutic, nutraceutical and functional food applications. Plants, 10(5):836. https://doi.org/10.3390/plants10050836.

Lechuga Morente, D. (2021). A first study for the establishment of a source of vitamin B12 as of the genetic modification of the cyanobacteria *Arthrospira platensis*. Biomedical En:1-31 http://hdl.handle.net/10230/48221.

Martínez-Ruiz, M.; Martínez-González, C. A.; Kim, D. H.; Santiesteban-Romero, B.; Reyes-Pardo, H.; Villaseñor-Zepeda, K. R. and Parra-Saldivar, R. (2022). Microalgae bioactive compounds to topical applications products—a review. Molecules, 27(11):3512. https://doi.org/10.3390/molecules27113512.

Mazo, V. K.; Gmoshinskiĭ, I. V. and Zilova, I. S. (2004). Microalgae Spirulina in human nutrition. Voprosy pitaniia, 73(1): 45-53.

Mullis, M. M.; Rambo, I. M.; Baker, B. J. and Reese, B. K. (2019). Diversity, ecology, and prevalence of antimicrobials in nature. Front. Microbiol., 2518. https://doi.org/10.3389/fmicb.2019.02518.

Najdenski, H. M.; Gigova, L. G.; Iliev, I. I., Pilarski, P. S.; Lukavský, J. ; Okalebo, J.R.; Catha, K.W. and Woomer, P.L.(2002). Laboratory methods of soil and plant analysis: working manual, TSBF-CIAT and SACRED Africa, Nairobi, Kenya, 200.

Potocki, L.; Oklejewicz, B.; Kuna, E. ; Szpyrka, E.; Duda, M. and Zuczek, J. (2021). Application of green algal plankto *Chlorella nurekis* biomasses to modulate growth of selected microbial species. Molecules, 26(13),4038. https://doi.org/10.3390/molecules26134038 .

Pradhan, A. K.; Pradhan, N.; Sukla, L. B.; Panda, P. K. and Mishra, B. K. (2014). Inhibition of pathogenic bacterial biofilm by biosurfactant produced by *Lysinibacillus fusiformis* S9. Biop and Bios Eng, 37 (2):139-149. https://doi.org/10.1007/s00449-013-0976-5 .

Sdiq, K. H.; Salih, S. I. and Kakayi, S. T. (2020). Evaluation of *Spirulina platensis* crude extract against some pathogenic microorganisms and determination of amino acid profile by HPLC, Erbil city. (JUBPAS), 28(1): 25-34.

Sena, L.; Rojas, D.; Montiel, E.; González, H.; Moret, J. and Naranjo, L. (2011). A strategy to obtain axenic cultures of *Arthrospira spp*. cyanobacteria. World J Microbiol Biotechnol. , 27: 1045-1053. https://doi.org/10.1007/s11274-010-0549-6.

Soni, R. A.; Sudhakar, K. ;Rana, R. S. (2017). Spirulina–From growth to nutritional product: A review. Trends in Food Science and Technology, 69:157-171. https://doi.org/10.1016/j.tifs.2017.09.010.

Sukumaran, P.; Nulit, R.; Zulkifly, S.; Halimoon, N.; Omar, H.; Ismail, A.(2014). Potential of fresh POME as a growth medium in mass production of *Arthrospira platensis*. Int. J. Curr. Microbiol. Appl. Sci. 3:235–250.

Stanier, R. Y.; Kunisawa, R.; Mandel, M. C. B. G. and Cohen-Bazire, G. (1971). Purification and properties of unicellular blue-green algae (order Chroococcales). Bacteriol Rev , 35(2):171-205.

Taher,A.M. and Saeed, I. O. (2022). Bioremediation of Contaminated Soil with Crude Oil Using Two Different Bacteria. AIP Conference Proceedings, https://doi.org/10.1063/5.0094117

Thomas, N. V.; Ghafour, D. D.; Diyya, A. S. M., Ismail, R. R. and Jalal, L. K. (2021). Antibacterial effects of the organic crude extracts of freshwater algae of Sulaymaniyah, Kurdistan Region, Iraq. J. Med. Plants Res , 15(4): 178-187. https://doi.org/10.5897/JMPR2021.7117.

Toma, J. J. and Aziz, F. H. (2023). Antibacterial activity of three algal genera against some pathogenic bacteria. Baghdad Sci.J, 20(1): 0032-0032. https://orcid.org/0000-0003-0370-9421.

Usharani, G.; Srinivasan, G.; Sivasakthi, S. and Saranraj, P. (2015). Antimicrobial activity of *Spirulina platensis* solvent extracts against pathogenic bacteria and fungi. Advan. Biol. Res , 9(5):292-298. https://orcid.org/10.5829/idosi.abr.2015.9.5.9610.

Wang, Z. P. and Zhao, Y. (2005). Morphological reversion of Spirulina (Arthrospira) platensis (Cyanophyta): from linear to helical 1. Journal of Phycology, 41(3): 622-628. https://doi.org/10.1111/j.1529-8817.2005.00087.x.