DJS Vol. 50(1)(2025) –pp. 129-146 ISSN: 1012-5965

Delta Journal of Science

Available online at
https://djs.journals.ekb.eg/

Research Article

BOTANY

In Vitro Assessment of the Antimicrobial and Antioxidant Properties of *Spirulina platensis* Extracts Against Diverse Bacterial Isolates

Nada Rageh*; Enas M. El-Ballat; Sobhy E. Elsilk and Mostafa M. El-Sheekh

Botany and Microbiology Department, Faculty of Science, Tanta University, Egypt.

***Corresponding author:** Nada Rageh **E-mail:** nada.rageh2024@gmail.com

Received: 6/ 6/ 2025 **Accepted:** 23/ 6/ 2025

KEY WORDS

Spirulina platensis, Antimicrobial, Antioxidant , in vitro, bacteria isolates

ABSTRACT

Arthrospira platensis (synonym: Spirulina platensis) has a wide range of activities, notably antibacterial property against bacterial pathogens. Therefore, they can be considered as functional components in natural alternatives to antibiotics. The current study aims to evaluate the antibacterial and antioxidant potentials of *S. platensis* extract against different bacteria isolates isolated from wound infections, and food sources. The extraction of *spirulina* was performed using a variety of solvents including 70% ethanol, 70% methanol, and cold and hot distillation water. Ethanolic extracts of *Spirulina* showed higher antimicrobial activity than methanolic extracts using agar well diffusion method, with inhibition zones ranging from 22.67 to 38.67 mm. The most resistant bacterial isolate was identified using 16s rRNA gene sequencing as *P. aeruginosa* with GenBank accession no. NR_114471. Moreover, the antioxidant activity of *Spirulina* extracts was performed using DPPH radical scavenging activity. The results showed that aqueous hot extract of *Spirulina* recorded high levels of antioxidant activity after 72 hr (81.78±2.19). As a conclusion, *in vitro* results of antibacterial activity of *spirulina* extracts proved that *S. platensis* could be of potential value in production of antimicrobial agents which could be effective when compared with contemporary antimicrobial compounds. Hot water extracts from *S. platensis* had high potential of antioxidant activity *in vitro* and it can be used as natural antioxidants.

Introduction

Bacterial infection is one of the most common clinical diseases that can affect many tissues and organs of the human body **(Wheatley e*t al.,* 2022).** Clinically, antibiotics are used to treat pathogenic bacteria that have progressively become resistant to more and more antibiotics. Concurrently, the "last line of defense" medicines polymyxin, vancomycin, and others have also produced multi-drug-resistant (MDR) bacteria **(Wang *et al.,* 2022).** Medical researchers have pointed out that about 50% of the world's antibiotics are misused each year, and over 80,000 people in China currently die indirectly or directly from antibiotic misuse in China each year.

The Global Antimicrobial Resistance and Use Surveillance System (GLASS) of the World Health Organization (WHO) reported significant antibiotic resistance in 500,000 probable bacterial infections across 22 nations **(Li *et al.,* 2019; Antimicrobial Resistance Collaborators (ARC) *et al.,* 2022).** In 2017, the WHO identified twelve of the most antibiotic-resistant "superbugs" posing the greatest threat to human health, involving carbapenem-resistant *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Escherichia coli*. These were categorized as "urgent" level pathogens, highlighting the urgent need for the development of new antibiotics **(Tacconelli e*t al.,* 2018; Mancuso *et al.,* 2021).**

P. aeruginosa is frequently found in chronic wound infections. It is a Gram-negative bacterium known for its opportunistic pathogenicity **(Kirketerp-Møller *et al.,* 2008; Rahim *et al.,* 2017).** Of its natural resistance to numerous antibiotics and capacity to produce biofilm matrices that escape traditional medicines, *P. aeruginosa* is well-known **(Drenkard and Ausubel, 2002; Aloush *et al.,* 2006; Schaber *et al.,* 2007**; **Kamali *et al.,* 2020).** The capacity of *P. aeruginosa* to produce biofilm matrices that elude traditional antibiotics and its inherent resistance to several drugs are well-known **(WHO, 2017).**

Photosynthetic microorganisms known as microalgae use light energy to convert CO_2 into a range of proteins, lipids , and carbohydrates **(Barkia *et al.,* 2019; Saadaoui *et al.,* 2021).** Biodiversity is abundant in the marine environment **(Maharana *et al.,* 2019)** and is a potential natural source of antibacterial, anti-inflammatory, antioxidant, and anticancer products **(Malve, 2016; Patra *et al.,* 2020).** The cyanobacterium *Spirulina* spp. contains a variety of substances, including pigments, polysaccharide, lipid, and protein. Chromophores, or pigments, are incorporated into complex proteins and non-proteins such as chlorophyll, phycocyanin, allophycocyanin, and phycoerythrin in *Spirulina*. The primary pigment that gives *Spirulina* its green-blue color is called phycocyanin (PC).

The subunits α and β proteins combine to produce phycocyanin, which has various isomeric linear tetrapyrroles with double bonds (bilin chromophore). A thioether bond to a cysteine residue binds the bilin chromophore to the polypeptide. The apoprotein has twenty amino acids and can chelate Hg^{+2} and Fe^{+2} **(Borowitzka, 2009; Fernández-Rojas *et al.,* 2014).**

The purpose of this study was to investigate the *in vitro* antimicrobial and

antioxidant potential of *Spirulina platensis* extracts against diverse bacterial isolates. The pharmaceutical, medicinal and nutritional applications of the tested seaweeds were documented by assessing the antioxidant, antimicrobial effects of these seaweed extracts.

Materials and Methods

Collection of samples

This study involved ten bacterial isolates from wound infections and one from food sources. The isolates were obtained from the Tanta University of Egypt's Faculty of Science's culture collection.

Identification of bacterial isolates

Both pathogenic and environmental isolates were cultivated for 24 hours on nutrient agar in sterile conditions. Following subculturing on selective media, the isolates were incubated for 24 hours at 37°C. Following the manufacturer's recommendations, a single colony was subcultured to produce a pure culture of each isolate, and the VITEK®2 Compact System was used to identify each isolate.

Antibiotic sensitivity test

Mueller-Hinton agar was used to examine the antibiotic susceptibility of bacterial isolates using the Kirby-Bauer disc diffusion method, according to the Clinical Laboratory Standards Institute (CLSI, 2022). AMC (30 µg), CIP (5 µg), SAM (25 µg), and IPM (10 µg) were the four distinct antibiotics from the four families that were evaluated. The classic Kirby-Bauer disc diffusion method selected a subset of antibiotic discs to investigate their efficacy against the isolated bacteria **(Bauer *et al.*, 1966).** Four to five identical colonies from each bacterial isolate (overnight growth) were separately placedinto sterile distilled water and vigorously shaken to produce a 10^6 CFU/ml turbidity under sterilized conditions. The Mueller-Hinton agar plates were inoculated within fifteen minutes using a sterile cotton swab submerged in the culture suspension. To ensure full contact with the agar, antibiotic discs were distributed over the surface of the inoculation plate and carefully pressed down with sterile forceps. The plates were inverted and incubated aerobically at 37°C for 24 hours, followed by the application of the discs for 15 minutes. The diameter of each inhibitory zone that developed around the antibiotic discs was measured in millimeters. The Clinical Laboratory Standards Institute (CLSI) guidelines, which provide protocols for antibiotic testing, were followed in interpreting the results. Three categories were applied to the data: I (intermediate), S (sensitive), and R (resistant) **(CLSI, 2022).**

Preparation of *S. platensis* Extract

The algal cells of *Spirulina platensis* were obtained from the National Institute of Oceanography and Fisheries, Hydrobiology Laboratory, Al-Qanater Al-Khayriya, Egypt. The algal biomass was first dried and then ground into a fine powder. For extraction, 10 grams of dried *S. platensis* powder (representing the initial dry weight) were dissolved in 150 mL of different solvents including 70% ethanol, 70% methanol, cold distilled water, and hot distilled water. To prepare the hot water extract, 1 gram of *Spirulina platensis* powder was soaked in 30 mL of distilled water and incubated at 60 °C for 2 hours. The material was steeped in the appropriate solvents (1:15 w/v) inside a conical flask, which was then sealed with cotton wool. Afterwards, kept for two days at 20–30°C on a rotatory shaker operating at 120 rpm. After the extracts were filtered, the filtrate was heated to 45°C

in an oven to remove the solvent. To obtain a 5 mg/mL final concentration, the crude extracts were suspended in the appropriate solvents and stored at -20°C in an airtight container to prevent microbial contamination. The extract preparation stages were then repeated as previously described. Following the formula extraction yield% (W1/W2)*100, each extract's extraction yield % was calculated. W1 stands for the weight of the dried crude extract, while W2 refers to the sample weight (1 g) prior to extraction, according to **Maisuthisakul and Pongsawatmanit (2004).**

Antimicrobial activity of *S. platensis* extract

Bauer *et al.*'s well diffusion method (1966) was used to evaluate the antibacterial properties of *S. platensis* preparations. Mueller-Hinton agar (20 ml) was added to Petri plates and left to solidify. Single pure colonies of each strain with similar morphology were taken using a sterile loop, inoculated into 5 ml of sterile nutrient broth, and then cultured at 37°C for 10 to 18 hours until apparent turbidity was observed. Saline was added to the usual turbidity to achieve a cell count of 10^6 CFU/ml. In the center of dried Mueller Hinton Agar plates (an agar layer with a thickness of approximately 4 mm), 0.1 ml of each of the prior suspensions was deposited. After that, it was evenly distributed with a sterile swab and left to dry for 15 minutes at 37°C. A 6 mm diameter well was created using sterile tips with a 1-2 cm portion. 100 µl of (0.1) g/ml of various algal extracts suspended in 10% DMSO (dimethyl sulfoxide) was added to each well, and the wells were tested against various isolates of bacteria, with 100 µl of 10% DMSO serving as the control. Incubation was carried out at 37°C for 24 to 48 hours. The susceptibility of the bacteria to the tested extracts was evaluated by measuring the inhibitory zone diameter in millimeters, with values recorded as the average of three replicates.

Molecular identification of the selected bacterial isolate by 16S rRNA

Ten milliliters of nutrient broth medium were used to culture the selected bacterial isolates in sterile test tubes **(Zimbro *et al.*, 2015).** Incubation of the cultures was performed at 37°C for 48 hours. DNA was extracted from the culture utilizing the Patho Gene-Spin DNA/RNA extraction kit, which was supplied by Intron Biotechnology Company, Korea, and delivered to the Molecular Biology Research Unit at Assiut University. For polymerase chain reaction (PCR) and gene sequencing, DNA samples were sent to SolGent Company in Daejeon, South Korea. Two universal primers were used for the PCR: 1492R (5'-GGTTACCTTGTTACGACTT-3') and 27F (5'-AGAGTTTGATCCTGGCTCAG-3'). A size nucleotide marker (100 base pairs) was used for electrophoresis on a 1% agarose gel to confirm the purified PCR products (amplicons). The amplicons were sequenced after adding dideoxynucleotides (dd NTPs) to the reaction mixture. Using 27F and 1492R primers, bacterial amplicons' sense and antisense positions were sequenced **(White *et al.*, 1990).** The National Centre of Biotechnology Information (NCBI) website's Basic Local Alignment Search Tool (BLAST) was used to examine the sequences. MegAlign (DNA Star) software version 5.05 was used to

perform phylogenetic analysis of the sequences.

Antioxidant activity of *S. platensis* extract

DPPH radical scavenging activity

Using a modified version of **Yen and Chen's (1995)** approach, the purple-coloured solution of 1, 1-diphenyl 2-picrylhydrazyl radical (DPPH) was bleached to assess the *S. platensis* extracts' electron donation potential and scavenging capabilities.

Statistical analysis

Descriptive statistical analyses, such as means ± SD (standard deviation) and percentages, were performed using Microsoft Excel 2023 (Microsoft Corp., USA). The Statistical Package for the Social Sciences (SPSS v. 26, USA) was used to perform additional statistical analyses. A one-way ANOVA test was conducted to calculate the statistical significance of the results between the tested bacterial isolates and different extracts. P values below 0.05 were considered statistically significant. Specific difference between groups were analysis using Tukey's multiple comparisons test**.**

Results

Identification of bacterial isolates

According to biochemical test results, isolates 1, 2, 3, 4, and 12 were identified as *Pseudomonas aeruginosa*. In contrast, isolates 5, 6, and 7 were identified as *Staphylococcus haemolyticus,* isolates 8 and 10 were identified as *Klebsiella pneumoniae*, and isolates 9 and 11 belonged to the *Salmonella* group. **Table (1)** represents the identification of twelve isolates. Biochemical tests of the Gram-negative and Gram-positive bacteria were described in **Table (S1, S2)**.

Antibiotic sensitivity of bacterial isolates

The disc diffusion method was performed to assess the resistance of bacterial isolates to various specific antibiotics. The results were recorded as being susceptible (S), resistant (R), or intermediate (I) to the tested antibiotics based on the diameter of clear zone (mm) values, which were interpreted about **CLSI (2021)** as illustrated in **Table (2).** Most of the isolates (83%, n=10) were found to be resistant to Amoxicillin-clavulanic acid (AMC), while 58% (n=7) showed resistance to Ampicillin/sulbactam (SAM). Imipenem (IPM) was the most susceptible antibiotic, as 83% (n=10) of isolates were sensitive to IPM. Most of the isolates (83%, n=10) were intermediate to Ciprofloxacin (CIP).

Antimicrobial activity of *S. platensis* extracts

Well diffusion results, represented in **Fig. (1, 2)**, showed that Gram-negative bacteria (*K. pneumoniae)* with inhibition zones (35.67±1.25) proved to be more susceptible to *Spirulina* methanolic extract than Gram-positive bacteria (*S. haemolyticus*) with inhibition zones (30±1.63). Our results demonstrated that each extract had a different antibacterial activity against each tested bacteria. With inhibition zones that extend from 22.67 to 38.67 mm, the ethanolic extract of *Spirulina* exhibited greater antibacterial activity than the methanolic extract. Hot and cold water extract didn’t show any antimicrobial effect against all tested bacteria. Methanolic and ethanolic extracts were performed after 48, 72, and 96 hr. Extraction after 96 hr recorded a better effect on the tested bacteria; with ethanolic extract, four isolates (3, 8, 10, and 12) showed a significant difference

between the 3 extraction periods. Meanwhile, methanolic extract recorded a considerable difference between different extraction periods, with most isolates, as shown in **Fig. 3 (a, b).**

Table (1): Identification of the bacterial isolates by the automated VITEK2 compact system

Isolate number	Identification
1	*Pseudomonas aerginosa*
2	*Pseudomonas aerginosa*
3	*Pseudomonas aerginosa*
4	*Pseudomonas aerginosa*
5	*Staphylococcus haemolyticus*
6	*Staphylococcus haemolyticus*
7	*Staphylococcus haemolyticus*
8	*Klebsiella pneumoniae*
9	*Salmonella* group
10	*Klebsiella pneumoniae*
11	*Salmonella* group
12	*Pseudomonas aerginosa*

Table (2): Antimicrobial susceptibility of bacterial isolates (inhibition zone (mm) ± S.D

Tested bacteria	Antibiotics			
	AMC (13-18)	SAM (11-15)	IPM (15-19)	CIP (18-25)
	Mean of inhibition Zone (mm ± S.D)			
***P. aeruginosa*(1)**	(R)	(R)	(S)	(I)
***P. aeruginosa*(2)**	(R)	(R)	(S)	(I)
***P. aeruginosa*(3)**	(R)	(R)	(S)	(I)
***P. aeruginosa*(4)**	(I)	(S)	(R)	(I)
***S. haemolyticus* (1)**	(R)	(R)	(S)	(I)
***S. haemolyticus* (2)**	(R)	(R)	(S)	(I)
***S. haemolyticus* (3)**	(R)	(R)	(S)	(S)
***K. pneumoniae* (1)**	(R)	(I)	(I)	(I)
***Salmonella* group(1)**	(R)	(I)	(S)	(I)
***K. pneumoniae* (2)**	(R)	(R)	(S)	(I)
***Salmonella* group(2)**	(R)	(S)	(S)	(S)
***P. aeruginosa* (5)**	(S)	(S)	(S)	(I)

For AMC; R≤13, S ≥18; **For SAM;** R≤ 11, S ≥ 15
For IPM; R≤ 15, S ≥ 19**; For CIP;** R≤ 18, S ≥ 2

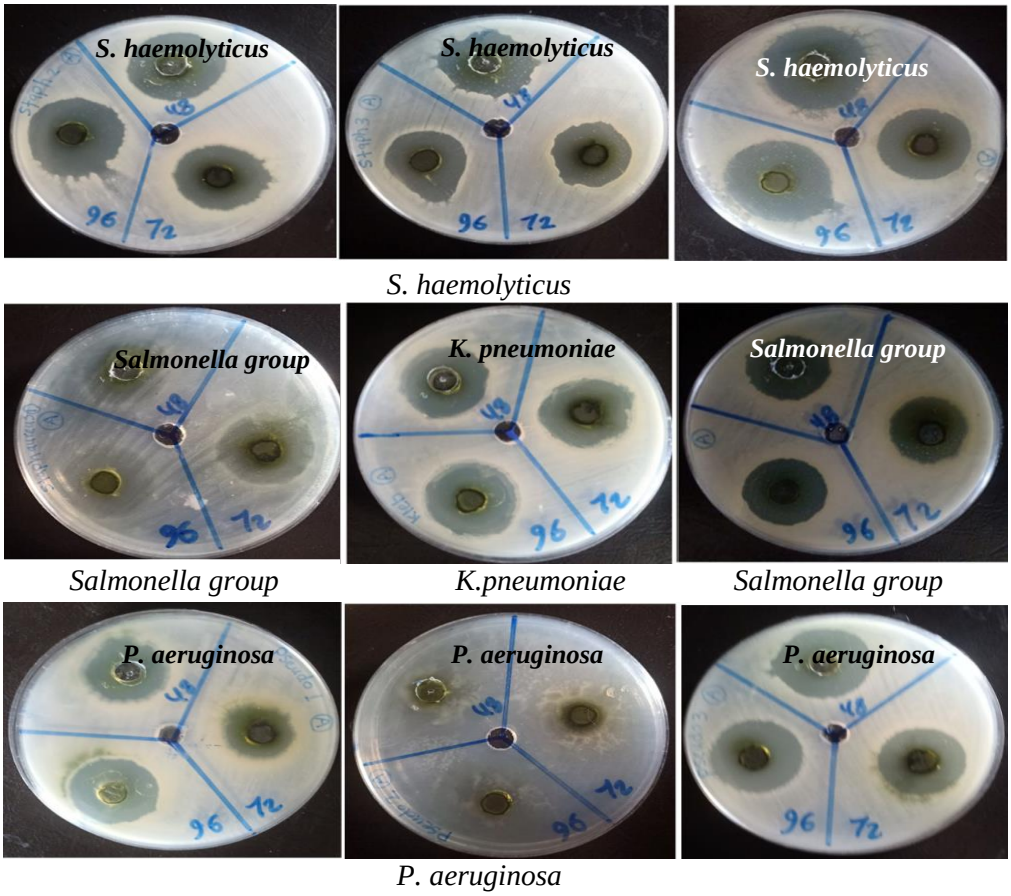

Fig.(1): Effect of methanolic extracts of *Spirulina* on the tested bacteria

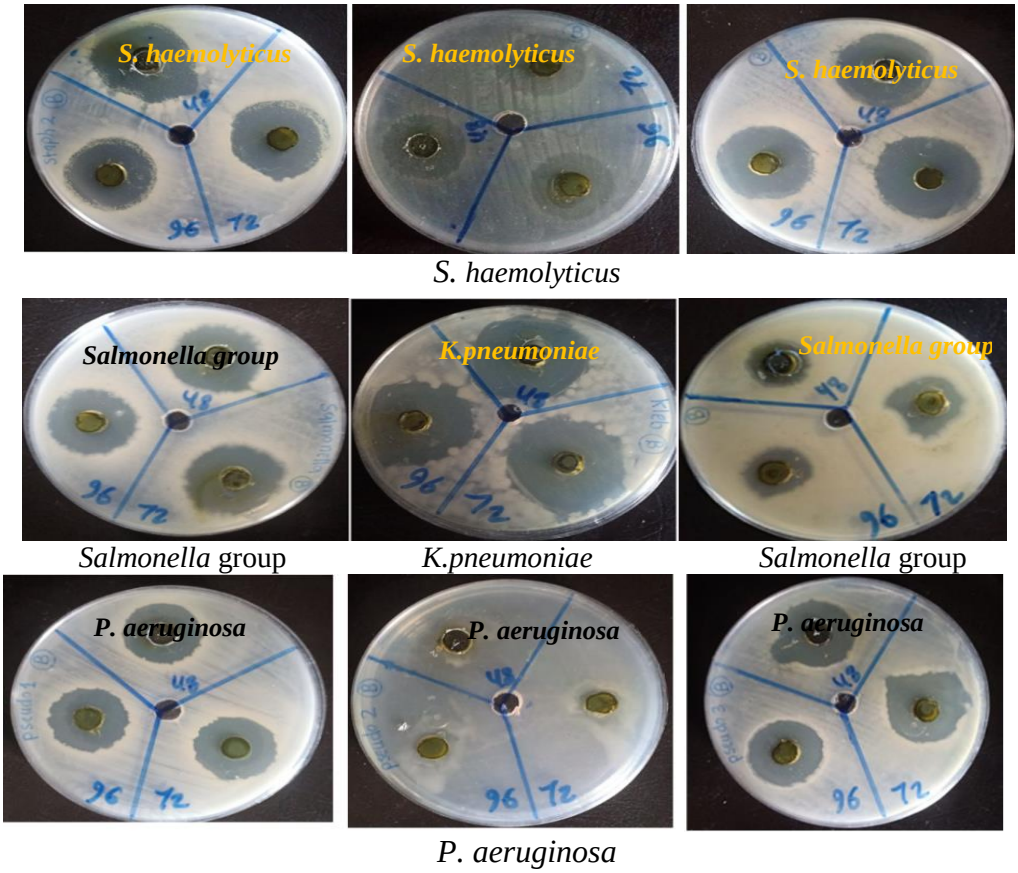

Fig. (2): Effect of ethanolic extracts of *Spirulina* on the tested bacteria

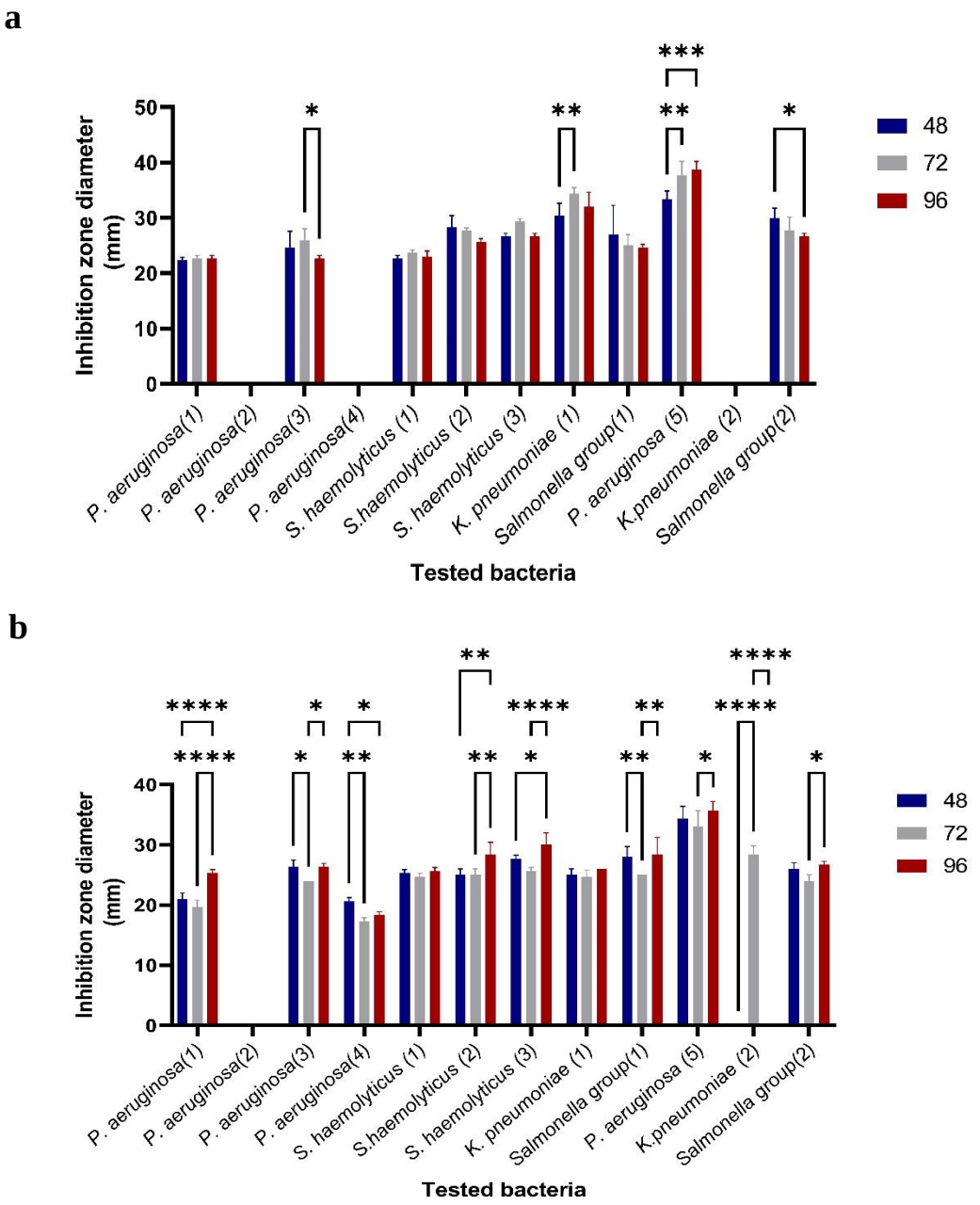

Fig. (3): Inhibition zones of Spirulina extracts against tested bacteria (a) Spirulina methanolic extracts, and (b) Spirulina ethanolic extracts. Data are presented as mean ± SD. Asterisks represent the significant difference between the three extraction periods among different isolates using two-way ANOVA, P value < 0.05

ANOVA table For *Spirulina* ethanolic extracts	**SS**	**df**	**MS**	**F (DFn, DFd)**	**P value**
Interaction	126.4	22	0.05433	F (22, 72) = 2.351	P=0.0035
Time	16748	11	0.1424	F (11, 72) = 622.8	P<0.0001
Bacterial isolates	17.56	2	0.8341	F (2, 72) = 3.591	P=0.0326
Residual	176.0	72	0.001229		
ANOVA table For *Spirulina* methanolic extracts	**SS**	**df**	**MS**	**F (DFn, DFd)**	**P value**
Interaction	1761	22	80.06	F (22, 72) = 58.42	P<0.0001
Time	8332	11	757.5	F (11, 72) = 552.7	P<0.0001
Bacterial isolates	22.74	2	11.37	F (2, 72) = 8.297	P=0.0006
Residual	98.67	72	1.370		

**SS (sum of squares), df (degrees of freedom), MS (mean square), and P values

Molecular identification of the selected bacterial isolates

Molecular identification of the selected bacterial isolate (no. **2**) through 16S rRNA. *P. aeruginosa* was the most resistant bacterium to the tested extracts. The identification of *P. aeruginosa* by the VITEK2 compact system was verified by using PCR to molecularly identify the 16s rRNA gene sequence. The results of identification and phylogenetic trees were as shown in **Fig. (4).** This strain showed 98.29% - 100% identity and 99% - 100 % coverage with many strains of the same species, such as the type of material *P. aeruginosa* ATCC 10145 with GenBank accession no. NR_114471. The tree contains *Staphylococcus aureus* as an outgroup strain, where S stands for *Staphylococcus* and P for *Pseudomonas*.

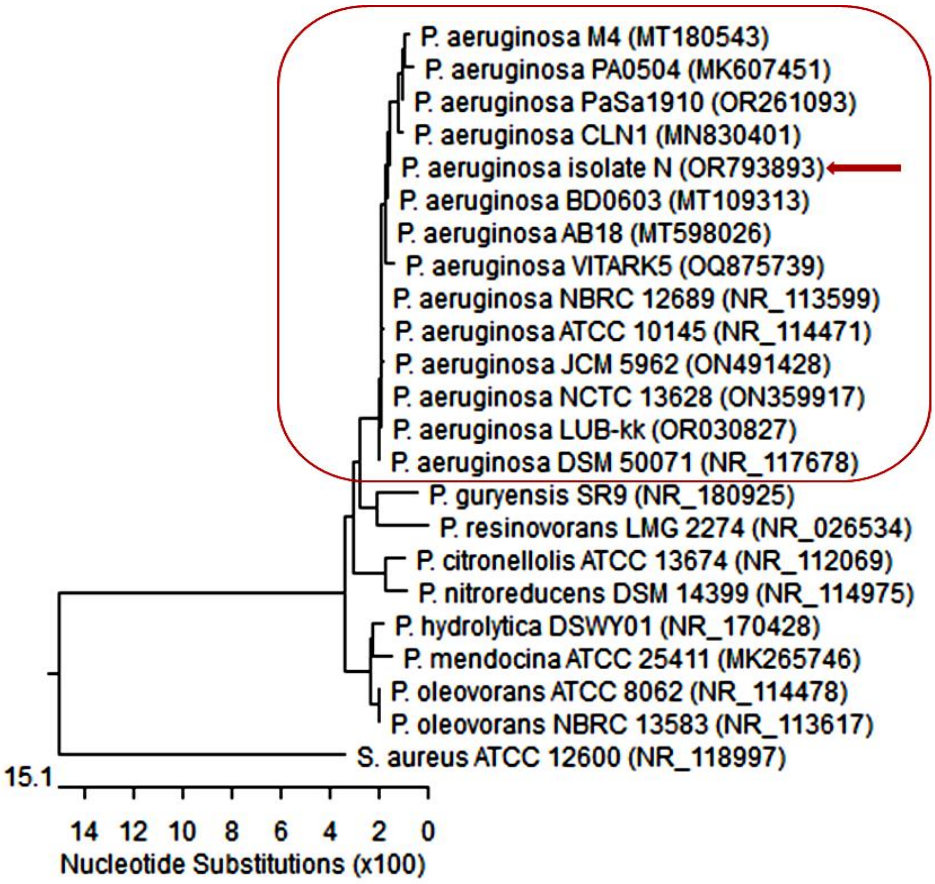

Fig. (4): Phylogenetic tree based on 16S rDNA sequences of the bacterial strain isolated in the present study (*Pseudomonas aeruginosa* isolate N, arrowed) aligned with closely related strains accessed from the GenBank

Antioxidant activity

DPPH antioxidant assay

The antioxidant activity of the *Spirulina* extract was demonstrated using the DPPH assay. Results in **Table (3)** showed the most vigorous DPPH radical scavenging activity of *Spirulina* aqueous hot extract (81.78±2.19) after 72 hr.

Among *Spirulina* extracts, it could be observed that aqueous extract (hot extract after 72 hr followed by cold extract after 96 hr) exhibited higher DPPH radical inhibition. Asterisks represent the significant difference between the three extraction periods among different solvents using two-way ANOVA was shown in **Fig. (5).**

Table (3): The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) antioxidant assay of *Spirulina* algal extracts

Solvent	Time	*Spirulina*
Methanol	**48**	16.49 ±3.55
	72	11.62±8.01
	96	24.76±12.04
Ethanol	**48**	12.17±8.77
	72	23.65±9.46
	96	43.4±9.19
Hot H_2O	**48**	21.61±12.2
	72	**81.78±2.19**
	96	21.06±9.03
Cold H_2O	**48**	45.16±27.29
	72	72.27±2.71
	96	**73.96±0.1**

ANOVA table For *Spirulina* ethanolic extracts	SS	df	MS	F (DFn, DFd)	P value
Interaction	126.4	22	0.05433	F (22, 72) = 2.351	P=0.0035
Time	16748	11	0.1424	F (11, 72) = 622.8	P<0.0001
Bacterial isolates	17.56	2	0.8341	F (2, 72) = 3.591	P=0.0326
Residual	176.0	72	0.001229		

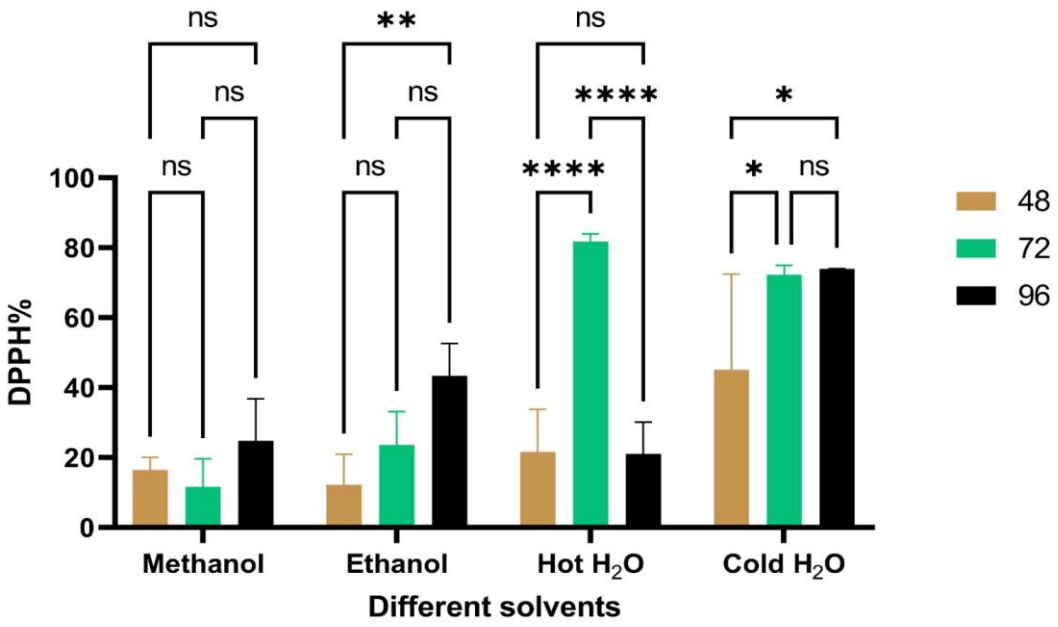

Fig. (5): DPPH-scavenging activity of *Spirulina* extract. Data presented as mean ± standard deviation (n = 3). Asterisks represent the significant difference between the three extraction periods among different solvents using two-way ANOVA, P value < 0. 05.

Discussion

There is worldwide concern over the rising number of reported cases of antibiotic resistance **(Prestinaci *et al.*, 2015; Yap *et al.*, 2019).** Developing novel medicines or substances with antibacterial activity is vital and critical for addressing this problem **(Chanda and Rakholiya, 2011).** The most common Gram-negative bacterium belonging to the Pseudomonadaceae family is *P. aeruginosa.* The number of multidrug-resistant (MDR) infections has increased globally. An extraordinary rise in *P. aeruginosa* has presented significant challenges to clinical treatment and public health **(Peng *et al.*, 2015; Yang *et al.*, 2023).** According to the European Centre for Disease Prevention and Control (ECDC), the prevalence of MDR *P. aeruginosa* varies by location, with someareas experiencing a higher incidence than others. This illustrates the diversity of regions and the importance of customizing solutions for each **(WHO, 2022).**

The bacterial isolates used in this study include *Pseudomonas aeruginosa*, *Staphylococcus haemolyticus, Klebsiella pneumoniae*, and *Salmonella* spp. They are all clinically significant pathogens known for their increasing resistance to antimicrobial agents. *P. aeruginosa* is a major opportunistic pathogen associated with wound and hospital-acquired infections and has exhibited a worrying trend of antimicrobial resistance in recent years **(Alatoom *et al.*, 2024).** *S. haemolyticus*, though often overlooked, has emerged as a multidrug-resistant organism, particularly in nosocomial environments, and shows a strong ability to form biofilms and persist under selective pressure **(Shoaib *et al.*, 2023).** *K. pneumoniae* is a key contributor to multidrug-resistant infections, especially those related to carbapenemase production, which poses a critical threat to global health systems **(Ding *et al.*, 2023).** *Salmonella* spp. remains one of the most important foodborne pathogens worldwide, with environmental reservoirs and antimicrobial resistance playing a crucial role in their persistence and transmission **(Billah and Rahman, 2024).** The presence of these isolates in the current study provides a relevant and challenging spectrum of test organisms to evaluate the antimicrobial potential of natural agents such as *Spirulina platensis*.

This work sought to assess the bioactive properties of algae and their antioxidant activity for potential applications in the clinical field. Numerous studies have examined the antimicrobial properties of microalgae extracts **(Chanda and Rakholiya, 2011; de Morais *et al.*, 2015).** Compared to water-based techniques, organic solvents consistently provide superior extraction efficiency for compounds with antibacterial properties **(Lima-Filho *et al.*, 2002).** According to the results, methanol and ethanol were the best solvents for extracting active components. Although theethanol extract showed antibacterial activity against several pathogens, many of the studied solvents were effective against all species. The presence of bioactive metabolites that dissolve in ethanol but not in diethyl ether may explain this **(Tüney *et al.*, 2006).** As stated by **Abedin and Taha (2008)**, *Spirulina platensis* extracts in acetone and diethyl ether exhibited the most potent antibacterial efficacy against *P.*

aeruginosa and *Bacillus subtilis*. Additionally, in the study by **Santoyo et al. (2006)**, petroleum ether and hexane extracts were significantly more active than ethanolic extracts. One well-documented technique for isolating the active antibacterial components from microalgae is methanol extraction **(Zea-Obando *et al.*, 2018; Patil and Kaliwal, 2019).**

Based on the current study's outcomes, *Spirulina*'s ethanolic extract demonstrated greater antibacterial activity than methanolic extracts, with inhibition zones ranging between 22.67 and 38.67 mm. Methanolic and ethanolic extractions were performed after 48, 72, and 96 hours; extraction after 96 hours showed improved effects on the tested bacteria. The methanolic extract showed the highest efficacy against the studied bacteria **(Gheda and Ismail, 2020).** The high total phenolic content of the methanolic extract may contribute to its potent antibacterial activity. Previous reports indicate that infections utilize similar invasion and adhesion mechanisms to invade the intestines of animals and humans. *Spirulina*'s antimicrobial action may stem from its ability to interfere with pathogen attachment and invasion, quorum sensing, biofilm formation, and motility **(Abd El-Hack e*t al.*, 2019; Abd El-Hack *et al.*, 2020; Abd El-Hack *et al.*, 2021; Abdel-Moneim *et al.*, 2020; Abou-Kassem *et al.*, 2021; Saleh *et al.*, 2021).** The bioactive constituents in *Spirulina* can disrupt bacterial cell integrity and increase membrane permeability, resulting in the loss of cytoplasmic material. Swarm motility, autoinducer AI-2 activity, and biofilm formation were all reduced in *Campylobacter jejuni* cultures exposed to specific plant-derived compounds (by 90% and 35–75%, respectively) **(Castillo *et al.*, 2014).**

In the study by **Selim et al., (2025)**, *in vivo* and *in vitro* results revealed potential antibacterial and antibiofilm activities of *A. maxima* (*spirulina*) against the tested carbapenem-resistant *K. pneumoniae* isolates. Moreover, *A. maxima* markedly decreased the inflammation that was triggered by the induced infection. Also Also**, Pianta et al. (2025**) showed that, the *Spirulina* extract could be used as an effective natural, broad-spectrum antimicrobial agent, with potential applications in the therapy of bacterial and fungal infectious diseases as a concentration of 4 g of *Spirulina* extract per 100 mL of Mueller Hinton agar completely inhibited the growth of all tested bacteria and yeasts, and suppressed dermatophytes growth by 5 $\log_{10}$ units.

Antioxidants are essential for protecting living things against persistent diseases and infections like cancer by scavenging free radicals from cell tissues. The antioxidant properties of the extracts reflect the existence of compounds that are able to engage with free radicals and donate electrons **(Tirado *et al.*, 2017).** A well-known substance that has been widely utilized as a free radical to assess the capacity of natural compounds to scavenge radicals is DPPH **(Zhong and Wang, 2010).** When antioxidants are present, the strong absorption band of DPPH, a stable free radical with a wavelength of approximately 517 nm, is reduced to its hydrazine form through hydrogen/electron donation **(Mohanasundari and Suja, 2016).**

Additionally, natural sources of antioxidants can extend the shelf life of

foods. Consequently, consuming antioxidants and adding them to food ingredients may protect both the body and the food **(Kumar and Pandey, 2013).**

Due to their large-scale cultivation in bioreactors, microalgae would provide a consistent and dependable source of natural compounds, including antioxidants. Additionally, the characteristics of microalgal cells can be modified by growing microalgae in clean nutrient media and avoiding the use of pesticides, herbicides, and other hazardous agents **(Amarowicz *et al.*, 2004).**

The present study evaluated the antioxidant activity of *Spirulina* extracts using different solvents (methanol, ethanol, and aqueous extract) through TAC and DPPH assays. The aqueous hot extract of *Spirulina demonstrat*ed a strong antioxidant effect against DPPH (81.78±2.19) after 72 hours. Consistent with our findings, with an IC50 of 45.21 mg/mL, **Agustina et al. (2021)** reported that the maximum antioxidant capacity was found in *Spirulina* spp. water extracted after being soaked for an hour. The antioxidant activity of *Spirulina* is also enhanced by chlorophyll, phenolic compounds, carotenoids, fatty acids, polysaccharides, and vitamins **(Gershwin and Belay, 2008; Asghari *et al.*, 2016).** In addition to *Spirulina's* strong nutritional content, several studies provide strong evidence of its potential medical uses. Unique natural antioxidants in *Spirulina* include phycocyanin, polyphenols, and carotenoids **(Estrada *et al.*, 2001; Park *et al.*, 2018; Abdel-Moneim *et al.*, 2022).**

Shalaby and Shanab (2013) used DPPH and ABTS radical scavenging methods to determine the antioxidant activities of three *Spirulina platensis* extracts: water, 50% aqueous methanol, and absolute methanol. At 200 ug/mL, the water extract exhibited the maximum antioxidant activity (95.3%), followed by aqueous methanol (68.41%) and absolute methanol (89.61%) after 30 minutes of incubation.

The aqueous extract of *S. platensis* showed increased antioxidant and antiradical activity using the DPPH method. This could be primarily because of the high concentration of phycobiliprotein pigments (8.23 mg/g), which are well-known for their strong antiradical properties **(Bougatef et al., 2024).** Additionally, water extract contains fewer phenolic components (about half as much as methanol) and phytochemicals (saponin and anthraquinone), which work in combination with phycobilin to provide water extract's significantly greater antioxidant and antiradical properties **(Shalaby and Shanab, 2013)**.

Conclusion

In vitro results of the antibacterial activity of *Spirulina* extracts proved that *S. platensis* may be useful in the production of antimicrobial agents that, when compared to modern antimicrobial compounds, may be effective. Hot water extracts from *S. platensis* demonstrated high potential of antioxidant activity *in vitro*, and they could be used as natural antioxidants. Therefore, further *in vivo* studies can be performed to evaluate the in *vivo* effects of these extracts and assess their potential for pharmaceutical application.

References

Abd El-Hack, M. E., Abdelnour, S. A., Abd El-Moneim, A. E. E., Arif, M., Khafaga, A., Shaheen, H., Samak, D.,

and Swelum, A. A. (2019). Putative impacts of phytogenic additives to ameliorate lead toxicity in animal feed. *Enviro. Sci. Pollut. Res. Int*., 26(23): 23209‑23218.

Abd El-Hack, M. E., El-Saadony, M. T., Shafi, M. E., Qattan, S. Y. A., Batiha, G. E., Khafaga, A. F., Abdel-Moneim, A. E., and Alagawany, M. (2020). Probiotics in poultry feed: A comprehensive review. *J. Anim. Physiol. Anim. Natr*., *104*(6): 1835‑1850.

Abd El-Hack, M. E., El-Saadony, M. T., Shehata, A. M., Arif, M., Paswan, V. K., Batiha, G. E., Khafaga, A. F., and Elbestawy,A. R. (2021). Approaches to prevent and control Campylobacter spp. colonization in broiler chickens: a review. *Environ. Sci. Pollut. Res. Int*., *28*(5): 4989‑5004.

Abdel-Moneim, A. E., Elbaz, A. M., Khidr, R. E., and Badri, F. B. (2020). Effect of in Ovo Inoculation of Bifidobacterium spp. on Growth Performance, Thyroid Activity, Ileum Histomorphometry, and Microbial Enumeration of Broilers. *Probiotics Antimicrob. Proteins*, *12*(3): 873‑882.

Abdel-Moneim, A. E., Shehata, A. M., Mohamed, N. G., Elbaz, A. M., and Ibrahim, N. S. (2022). Synergistic effect of Spirulina platensis and selenium nanoparticles on growth performance, serum metabolites, immune responses, and antioxidant capacity of heat-stressed broiler chickens. *Biol. Trace Elem. Res*., *200*(2): 768‑779.

Abedin, R. M., and Taha, H. M. (2008). Antibacterial and antifungal activity of cyanobacteria and green microalgae. Evaluation of medium components by Plackett-Burman design for antimicrobial activity of Spirulina platensis. *Glob. J. Biotechnol. Biochem.*, *3*(1): 22-31.

Abou-Kassem, D. E., Elsadek, M. F., Abdel-Moneim, A. E., Mahgoub, S. A., Elaraby, G. M., Taha, A. E., Elshafie, M. M., Alkhawtani, D. M., Abd El-Hack, M. E., and Ashour, E. A. (2021). Growth, carcass characteristics, meat quality, and microbial aspects of growing quail fed diets enriched with two different types of probiotics (Bacillus toyonensis and Bifidobacterium bifidum). *Poult. Sci*, *100*(1): 84‑93.

Agustina, S., Aidha, N. N., Oktarina, E., and Kurniati, N. F. (2021). Evaluation of antioxidant and wound healing activities of Spirulina sp. extract. *Egypt. J. Chem*., *64*(8): 4601-4610.

Aloush, V., Navon-Venezia, S., Seigman-Igra, Y., Cabili, S., and Carmeli, Y. (2006). Multidrug-resistant *Pseudomonas aeruginosa*: risk factors and clinical impact. *Antimicrob. Agents Chemother*, *50*(1): 43‑48.

Amarowicz, R., Pegg, R. B., Rahimi-Moghaddam, P., Barl, B., and Weil, J. A. (2004). Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food chem*,, *84*(4): 551-562.

Antimicrobial Resistance Collaborators (ARC) (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet (London, England)*, *399*(10325): 629‑655.

Arthrospira platensis (Syn.: Spirulina platensis) Extract. *Nat. Prod. Commun*, *20*(1): 1934578X251314702.

Barkia, I., Saari, N., and Manning, S. R. (2019). Microalgae for High-Value Products Towards Human Health and Nutrition. *Mar. Drugs*, *17*(5): 304.

Bauer, A. W., Kirby, W. M., Sherris, J. C., and Turck, M. (1966). Antibiotic susceptibility testing by a standardized

single disk method. *Am. J. Clin. Pathol*., *45*(4): 493‑496.

Borowitzka, M. A. (2009). ME Gershwin, A. Belay (eds). Spirulina in human nutrition and health: CRC Press, Boca Raton, 2008, 312 pp.

Bougatef, H., Hadrich, F., Gazbar, M., Sila, A., Chamkha, M., and Bougatef, A. (2024). Development of a novel method for the extraction of phycocyanin pigment from Spirulina platensis and assessment of its antioxidant, antimicrobial, and anticancer activities. *Biomass Convers. Biorefin*, *15*(5):8001-8013.

Castillo, S., Heredia, N., Arechiga-Carvajal, E., and García, S. (2014). Citrus extracts as inhibitors of quorum sensing, biofilm formation and motility of Campylobacter jejuni. *Food Biotechnol*, *28*(2): 106-122.

Chanda, S., and Rakholiya, K. (2011). Combination therapy: synergism between natural plant extracts and antibiotics against infectious diseases. Microbiol. book ser.

Clinical and Laboratory Standards Institute. (2022). Performance standards for antimicrobial susceptibility testing. *31st Ed. Wayne (PA): CLSI:* 2022.

De Morais, M. G., Vaz, B.daS., de Morais, E. G., and Costa, J. A. (2015). Biologically Active Metabolites Synthesized by Microalgae. *Biomed. Res. In.t, 2015*: 835761.

Drenkard, E., & Ausubel, F. M. (2002). *Pseudomona*s biofilm formation and antibiotic resistance are linked to phenotypic variation. *Nature*, *416*(6882): 740‑743.

Estrada, J. P., Bescós, P. B., and Del Fresno, A. V. (2001). Antioxidant activity of different fractions of *Spirulina platensis* protean extract. *II farmaco*, *56*(5-7): 497-500.

Fernández-Rojas, B., Hernández-Juárez, J., and Pedraza-Chaverri, J. (2014). Nutraceutical properties of phycocyanin. *J. Funct. Foods*, *11*: 375-392.

Gershwin, M. E., and Belay, A. (Eds.). (2007). *Hum. Nutr. Health*. CRC press.

Gheda, S. F., and Ismail, G. A. (2020). Natural products from some soil cyanobacterial extracts with potent antimicrobial, antioxidant and cytotoxic activities. *An. Acad. Bras. Cienc*, *92*(2): e20190934.

Kamali, E., Jamali, A., Ardebili, A., Ezadi, F., and Mohebbi, A. (2020). Evaluation of antimicrobial resistance, biofilm-forming potential, and the presence of biofilm-related genes among clinical isolates of *Pseudomonas aeruginosa. BMC Res. notes*, *13*(1): 27.

Kirketerp-Møller, K., Jensen, P. Ø., Fazli, M., Madsen, K. G., Pedersen, J., Moser, C., Tolker-Nielsen, T., Høiby, N., Givskov, M., and Bjarnsholt, T. (2008). Distribution, organization, and ecology of bacteria in chronic wounds. *J. Clin. Microbiol*, *46*(8): 2717‑2722.

Kumar, S., and Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: an overview. *Sci. World J*., *2013*(1): 162750.

Li, X., Bai, H., Yang, Y., Yoon, J., Wang, S., and Zhang, X. (2019). Supramolecular Antibacterial Materials for Combatting Antibiotic Resistance. *Adv. Mater (Deerfield Beach, Fla.)*, *31*(5): e1805092.

Lima-Filho, J. V. M., Carvalho, A. F., Freitas, S. M., and Melo, V. M. (2002). Antibacterial activity of extracts of six macroalgae from the northeastern Brazilian coast. *Braz. J. Microbiol*, *33*, 311-314.

Maharana, D., Saha, M., Dar, J. Y., Rathore, C., Sreepada, R. A., Xu, X. R., ... and Li, H. X. (2020). Assessment of micro and macroplastics

along the west coast of India: Abundance, distribution, polymer type and toxicity. *Chemosphere, 246*:125708.

Maisuthisakul, P. O., and Pongsawatmanit, R. (2004). Effect of sample preparation methods and extraction time on yield and antioxidant activity from kradonbok (Careya sphaerica Roxb.) leaves. *Agri. Nat. Resour, 38*(5): 8-14.

Malve H. (2016). Exploring the ocean for new drug developments: Marine pharmacology. *J. Pharm. Bioallied Sci*., *8*(2): 83‑91.

Mancuso, G., Midiri, A., Gerace, E., and Biondo, C. (2021). Bacterial Antibiotic Resistance: The Most Critical Pathogens. *Pathogens (Basel, Switzerland), 10*(10): 1310.

Mohanasundari, L., and Suja, S. (2016). Antioxidant and free radical scavenging activity of the mixture of ethanolic extracts of Alpinia speciosa and Alpinia calcarata rhizome. *Int. J. Pharm. Pharm. Sci, 8*(8):164-70.

Patil, L., and Kaliwal, B. B. (2019). Microalga Scenedesmus bajacalifornicus BBKLP-07, a new source of bioactive compounds with in vitro pharmacological applications. *Bioprocess Biosyst. Eng., 42*(6): 979‑994.

Park, J. H., Lee, S. I., and Kim, I. H. (2018). Effect of dietary Spirulina (Arthrospira) platensis on the growth performance, antioxidant enzyme activity, nutrient digestibility, cecal microflora, excreta noxious gas emission, and breast meat quality of broiler chickens. *Poult. Sci*., *97*(7): 2451-2459.

Patra, S., Praharaj, P. P., Panigrahi, D. P., Panda, B., Bhol, C. S., Mahapatra, K. K., Mishra, S. R., Behera, B. P., Jena, M., Sethi, G., Patil, S., Patra, S. K., and Bhutia, S. K. (2020). Bioactive compounds from marine invertebrates as potent anticancer drugs: the possible pharmacophores modulating cell death pathways. *Mol. Biol. Rep., 47*(9): 7209‑7228.

Peng, Y., Shi, J., Bu, T., Li, Y., Ye, X., Chen, X., and Yao, Z. (2015). Alarming and increasing prevalence of multidrug-resistant Pseudomonas aeruginosa among healthcare-associated infections in China: A meta-analysis of cross-sectional studies. *J. Glob. Antimicrob. Resist, 3*(3): 155‑160.

Pianta, E., Günnewich, N., Zimmermann, C., Günther, P., Petrini, O., Diaz-Miyar, J., and Fragoso-Corti, C. (2025). In Vitro Antibacterial and Antifungal Activity of an Arthrospira platensis (syn.: spirulina platensis) *Extract.Nat.prod.commun*,20(1)193457 8x251314702.

Prestinaci, F., Pezzotti, P., and Pantosti, A. (2015). Antimicrobial resistance: a global multifaceted phenomenon. *Pathog. Glob. Health, 109*(7): 309-318.

Rahim, K., Saleha, S., Zhu, X., Huo, L., Basit, A., and Franco, O. L. (2017). Bacterial Contribution in Chronicity of Wounds. *Microb. Ecol., 73*(3):710‑721.

Saadaoui, I., Rasheed, R., Aguilar, A., Cherif, M., Al Jabri, H., Sayadi, S., and Manning, S. R. (2021). Microalgal-based feed: promising alternative feedstocks for livestock and poultry production. *J. Anim. Sci. Biotechnol., 12*(1):76.

Saleh, A. A., Shukry, M., Farrag, F., Soliman, M. M., and Abdel-Moneim, A. E. (2021). Effect of Feeding Wet Feed or Wet Feed Fermented by *Bacillus licheniformis* on Growth Performance, Histopathology and Growth and Lipid Metabolism Marker Genes in Broiler Chickens. *Animals: OAJ from MDPI, 11*(1): 83.

Santoyo, S., Herrero, M., Senorans, F. J., Cifuentes, A., Ibáñez, E., and Jaime, L. (2006). Functional characterization of pressurized liquid extracts of Spirulina platensis. *Eur. Food Res. Technol*, *224*, 75-81.

Schaber, J. A., Triffo, W. J., Suh, S. J., Oliver, J. W., Hastert, M. C., Griswold, J. A., Auer, M., Hamood, A. N., and Rumbaugh, K. P. (2007). *Pseudomonas aeruginosa* forms biofilms in acute infection independent of cell-to-cell signaling. *Infect. immun*, *75*(8), 3715‑3721.

Selim, M. I., El-Banna, T., Sonbol, F., Negm, W. A., and Elekhnawy, E. (2025). Unveiling the potential of spirulina algal extract as promising antibacterial and antibiofilm agent against carbapenem-resistant Klebsiella pneumoniae: in vitro and in vivo study. *Microb. Cell Fact*, *24*(1), 7.

Shalaby, E. A., and Shanab, S. M. (2013). Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extracts of *Spirulina platensis*. *Indian J. Geo-Mar Sci.*, *42*(5):556-564.

Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D. L., Pulcini, C., Kahlmeter, G., Kluytmans, J., Carmeli, Y., Ouellette, M., Outterson, K., Patel, J., Cavaleri, M., Cox, E. M., Houchens, C. R., Grayson, M. L., Hansen, P., Singh, N., Theuretzbacher, U., … WHO Pathogens Priority List Working Group (2018). Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet. Infect. Dis.*, *18*(3): 318‑327.

Tirado, J. O., Flores, F., Maldonado, S., Mihae, R., Naranjo, B., Muñoz, D., ... and Rajeswari, B. (2017). Molecular characterization and antioxidant potential of Andean chlorophytes from Ecuador. *J. Appl. Pharm. Sci*., *7*(5): 056-060.

TÜney, İ., Cadirci, B. H., Ünal, D., and Sukatar, A. (2006). Antimicrobial activities of the extracts of marine algae from the coast of Urla (Izmir, Turkey). *Turk. J. Biotechnol*, *30*(3):171-175.

Wang, Z., Koirala, B., Hernandez, Y., Zimmerman, M., Park, S., Perlin, D. S., and Brady, S. F. (2022). A naturally inspired antibiotic to target multidrug-resistant pathogens. *Nature*, *601*(7894): 606‑611.

Wheatley, R. M., Caballero, J. D., van der Schalk, T. E., De Winter, F. H. R., Shaw, L. P., Kapel, N., Recanatini, C., Timbermont, L., Kluytmans, J., Esser, M., Lacoma, A., Prat-Aymerich, C., Oliver, A., Kumar-Singh, S., Malhotra-Kumar, S., and Craig MacLean, R. (2022). Gut to lung translocation and antibiotic-mediated selection shape the dynamics of Pseudomonas aeruginosa in an ICU patient. *Nat. Commun*, *13*(1): 6523.

White, T. J., Bruns, T., Lee, S. J. W. T., and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protoc: a guide to methods and applications*, *18*(1): 315-322.

WHO Regional Office for Europe/European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2022 ‑ 2020 data. Copenhagen: WHO Regional Office for Europe; 2022.

World Health Organization. (2017). Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery and Development of New Antibiotics; World Health Organization: Geneva, Switzerland.

Yang, A. F., Huang, V., Samaroo-Campbell, J., and Augenbraun, M. (2023). Multi-drug resistant *Pseudomonas aeruginosa*: a 2019-2020 single center retrospective case control study. *Infect. Prev. pract*, *5*(3):100296.

Yap, W. F., Tay, V., Tan, S. H., Yow, Y. Y., and Chew, J. (2019). Decoding Antioxidant and Antibacterial Potentials of Malaysian Green Seaweeds: *Caulerpa racemosa* and *Caulerpa lentillifera*. *Antibiotics (Basel, Switzerland)*, *8*(3): 152.

Yen, G. C., and Chen, H. Y. (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agri. Food Chem*, *43*(1): 27-32.

Zea-Obando, C., Tunin-Ley, A., Turquet, J., Culioli, G., Briand, J. F., Bazire, A., Réhel, K., Faÿ, F., and Linossier, I. (2018). Antibacterial Adhesion Activity of Tropical Microalgae Extracts. *Molecules (Basel, Switzerland)*, *23*(9): 2180.

Zhong, K., and Wang, Q. (2010). Optimization of ultrasonic extraction of polysaccharides from dried longan pulp using response surface methodology. *Carbohydr Polym*, *80*(1): 19-25.

Zimbro, M. J., Power, D. A., Miller, S. M., Wilson, G. E., and Johnson, J. A. (2009). Difco & BBL Manual: MMCM (Becton, Dickinson and Company, Sparks, MD).

التقييم المختبري للخصائص المضادة للميكروبات ومضادات الأكسدة لمستخلصات سبيرولينا بلاتينسيس ضد عزلات بكتيرية متنوعة

ندى راجح، د/ ايناس البلاط، ا.د/ صبحى السلك، ا.د/ مصطفى الشيخ

قسم النبات والميكروبيولوجى- كلية العلوم- جامعة طنطا

تتميز سبيرولينا بلاتنسيس بمجموعة واسعة من الأنشطة، أبرزها خاصية مضادة للبكتيريا ضد مسببات الأمراض البكتيرية. لذلك، يمكن اعتبارها مكونات وظيفية في البدائل الطبيعية للمضادات الحيوية. تهدف الدراسة الحالية إلى تقييم الإمكانات المضادة للبكتيريا ومضادات الأكسدة لمستخلص سبيرولينا بلاتنسيس ضد عزلات بكتيرية مختلفة معزولة من التهابات الجروح ومصادر الغذاء. تم استخلاص سبيرولينا باستخدام مجموعة متنوعة من المذيبات بما في ذلك ٧٠٪ إيثانول و ٧٠٪ ميثانول والماء المقطر البارد والساخن. أظهرت المستخلصات الإيثانولية لسبيرولينا نشاطًا مضادًا للميكروبات أعلى من المستخلصات الميثانولية باستخدام طرق انتشار بئر الآجار، مع مناطق تثبيط تتراوح من ٢٢.٦٧ إلى ٣٨.٦٧ مم. تم تحديد أكثر عزلة بكتيرية مقاومة باستخدام تسلسل جين ١٦ rRNA على أنها *P. aeruginosa*. علاوة على ذلك، تم إجراء النشاط المضاد للأكسدة لمستخلصات سبيرولينا باستخدام نشاط إزالة الجذور الحرة DPPH. أظهرت النتائج أن المستخلص المائي الساخن من سبيرولينا أظهر مستويات عالية من النشاط المضاد للأكسدة بعد ٧٢ ساعة (٨١.٧٨ ± ٢.١٩). وخلاصة القول، أثبتت نتائج النشاط المضاد للبكتيريا لمستخلصات سبيرولينا في المختبر أن سبيرولينا بلاتينسيس قد تكون ذات قيمة محتملة في إنتاج عوامل مضادة للميكروبات، والتي قد تكون فعالة عند مقارنتها بالمركبات المضادة للميكروبات المعاصرة. أظهرت مستخلصات الماء الساخن من سبيرولينا بلاتينسيس نشاطًا مضادًا للأكسدة عاليًا في المختبر، ويمكن استخدامها كمضادات أكسدة طبيعية.