Bull. Pharm. Sci., Assiut University, Vol. 47, Issue 2, 2024, pp.899-916.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/

BULL. PHARM. SCI. Assiut Univ.

PHENOLIC CONTENT, ANTIOXIDANT, AND ANTIBACTERIAL ACTIVITIES OF HYDROMETHANOLIC EXTRACT OF *LIMNOSPIRA FUSIFORMIS* TL03 ISOLATED FROM TELAMINE LAKE, NORTHWEST ALGERIA

Belkacem.Guenachi[1,2,3*], Hakima. Mefti Korteby[1], Lynda. Lamari [3]

[1]*Blida 1 University, Laboratory of Aromatic and Medicinal Plants Research, Natural Sciences and Life Faculty, Blida, Algeria*
[2]*National Center for Research and Development of Fisheries and Aquaculture (CNRDPA), Bou Ismail, Algeria*
[3]*Biology Laboratory of Microbial Systems, Higher Normal School of Kouba, Algiers, Algeria*

This study was conducted to evaluate the total phenolic, flavonoid content, antioxidant, and antibacterial activity of hydomethanolic Limnospira fusiformis TL03 .

The total phenolics and flavonoids were determined by using the Folin-Ciocalteu and aluminum chloride methods, respectively. The YL 9100 HPLC was used to identify the phenolic compounds.

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity and beta-carotene-linoleic acid tests were used to evaluate antioxidant activity in vitro. The antimicrobial activities were tested against six bacteria using the disc diffusion method and the agar dilution method.

The total phenolic and flavonoids contained in the hydromethanolic extract were 18.41 ±0.18 mg GAE/g DW and 2.11 ±0.12 mg quercetin/g DW, respectively. The result of polyphenol analysis showed the presence of: Ascorbic acid, Gallic acid, Luteolin , Quercetine, Kaempferol.

This extract shows strong DPPH radical scavenging and β-carotene bleaching activities with a percentage of 86.19±1.61% and 85.87±1.65%, respectively, in addition to activity antibacterial, in which the zone inhibition diameters (ZID) range from 33.33±1.53 to 11±1 mm, with the higher value antibacterial activity (33.33±1.53 mm) registered against S. aureus and the lower minimum inhibitory concentrations (MIC) observed against K pneumonia (0.78 mg/ml). In conclusion, it was revealed that the hydromethanolic extract of L. fusiformis TL03. has significant antioxidant and antibacterial properties

Keyswords: *Limnospira fusiformis TL03, Total phenolic and flavonoid, HPLC, DPPH, Beta carotene, Antibacterial activity*

INTRODUCTION

There is growing interest in the global health community about oxidative stress and the spread of infectious diseases. The oxidative stress, caused by a variety of stressors and free radical inducers, may be responsible, to varying degrees, for the onset and/or development of many diseases, including cancer, diabetes, metabolic disorders, atherosclerosis, and cardiovascular disease [1]. The treatment of oxidative stress through the long-term use of synthetic antioxidants may cause some health problems, such as skin allergies, digestive problems, and, in some cases, an increased risk of cancer[2-6]. The global outbreak of infectious bacterial diseases with the widespread prevalence of antibiotic-resistant bacteria poses another threat to human and animal health.

Plants are a sustainable source of bioactive substances that have been used as

Received : 28/5/2024 & Accepted : 25/9/2024

*Corresponding author: Guenachi. Belkacem, E-mail: bguenachi@gmail.com

folk medicines by humans since ancient times [7,8]to treat various diseases, and also their diversity of chemical compounds has been used in the production of various drugs. Microalgae and Cyanobacteria are also valuable sources of bioactive compounds, with a tenfold greater diversity of bioactive substances compared to land plants[9].

In the recent years, there has been an increased interest in microalgae and cyanobacteria research due to their wide biotechnological applications including, wastewater treatment, carbon dioxide fixation, production of biodiesel and methane[10-16]. In addition in agriculture as biofertilizers[17, 18] and biostimulants[19].

Cyanobacterium (Limnospira and / or Arthrospira), commercially known as Spirulina[20-22], is one of the most produced microalgae and cyanobacteria worldwide[23;] about 30% of the world global algal biomass production comes from the genus *Arthrospira*[24]. The global production was 89,000 tons in 2016 (FAO, 2018)[25]. Spirulina has been consumed by humans since ancient civilizations such as the Aztecs, who were the first to realize the nutritional value of these organisms[26 -27] and is still traditionally consumed in Africa, near Lake Chad [28].

Spirulina have high nutritional value[29]; it contains a high amount of proteins with all essential amino acids[30-35] and essential fatty acids, minerals, pigments, vitamins, and phenolic compounds. It was proven to be used as a food supplement, animal feed, cosmetics, and natural coloring ; food colors [36-42] .Furthermore, the biological and therapeutic study of *spirulina* has also received a lot of attention due to the fact that it contains many bioactive compounds with therapeutic properties[27,41, 43, 45- 51] including weight control, intestinal flora, anticancer and immune modulating activities, Anti-inflammatory, antioxidant, antiviral, and antibacterial[41,52, 53].

In this paper, the first study on the biological activity of *Limnospira fusiformis* TL03, recently isolated from Telamine Lake in the northwest of Algeria. Therefore, the current study was carried out to assess the antioxidant activities of *Limnospira fusiformis* TL03 hydromethanolic extract and the characterization of its polyphenolic compounds in addition to evaluate its antimicrobial activity using agar well-diffusion assay and minimum inhibition concentration (MIC) against selected Gram positive and Gram negative bacteria.

MATERIAL AND METHODS

Chemicals Carrageenan, linoleic acid, ascorbic acid, butylated hydroxytoluene, Tween 40, potassium ferricyanide, DPPH, Ferric chloride, β-carotene, Methanol, Galic acid, Rutin, Vanillic acid, Flavone, Galangin, Caffeine, Stearic acid, Tannic acid, Myricetin, Alpha-tocopherol, Morin, Apigenin, Diosmin, Catechin, Tangeretin, 5-Hydroxy-flavon, Chrysin, Kaemferide, Robinin, 3-Hydroxy-flavon, Acacetin, Hesperetin, Quercetin, 6.methoxyflavone, Aspirin, Acetic acid used in the experiment were procured from Sigma (Sigma-Aldrich, Germany).

Biological material

Cyanobacterium strain: The cyanobacterium *Limnospira. fusiformis* TL03 (accession number MZ215991.1) was isolated from Telamine Lake in northwest Algeria.

L. fusiformis TL03 Culture conditions

L. fusiformis TL03 was cultured in Bleu Green medium BG11[54], where the pH was adjusted to 9.5 and the medium was autoclaved at 121 °C for 20 min.

The incubation was performed under controlled conditions: a temperature of 30 ± 2 °C and continuous light of 2.7 Klux provided by white fluorescent tubes. The mixing of the cells was carried out by aeration pump in order to keep the cells in suspension and to avoid their agglomeration. After incubating for 20 days, the cells were harvested, washed with distilled water, and dried using a lyophilizer. (ALPHA 1- 2 LD plus).

Bacterial strains

The tested bacteria were provided by the Microbial Systems Biology Laboratory, Kouba Higher Normal School of Kouba, Algeria. The antibacterial activity of hydromethanolic extracts of *L. fusiformis* TL03 was tested against six strains of pathogenic bacteria **(Table 1)**.

Table 1 : Tested bacterial strains.

Bacteria	Strains	Origin
Bacteria gram positive	*Bacillus subtilis* (*B. subtilis*)	ATCC 6633
	Staphylococcus aureus (*S. aureus*)	CIP 7625
	Listeria monocytogenes (*L. monocytogenes*)	CIP 82110
Bacteria gram negative	*Escherichia coli* (*E. coli*)	ATCC 10536
	Pseudomonas aeruginosa(*P. aeruginosa*)	CIP A22
	Klepseilla pneumonia (*K. pneumonia*)	CIP 82.91

ATCC : American Type Culture Collection, CIP : Collection de l'Institut Pasteur (Institut Pasteur Collection).

Extract preparation

A crude hydromethanolic extract of *L. fusiformis* TL03 was prepared by macerating 2 g of freeze-dried algae in 100 mL of methanol/water (7 v: 3 v). The mixture was kept at room temperature in the dark for 24 hours and then filtered through Wathman No. 1 paper. These procedures were repeated three times to extract the maximum amount of compounds. The three filtrates were collected in an opaque glass vial and then dried at 40 °C using a rotary vacuum evaporator (Buchi R-210). The dried extracts were stored at 4°C in the dark until use.

Total phenolic assay

The total phenolic content of the extract was assessed using spectrophotometric methods[55] based on the Folin-Ciocalteu reagent. 3.9 ml of distilled water was combined with 0.1 ml of diluted hydromethanolic crude extract (10 mg/mL), and the mixture was thoroughly mixed with 0.25 mL of Folin-Ciocalteu reagent for 3 min. Then, 0.75 mL of 20% (w/v) sodium carbonate (Na_2CO_3) was added to the mixture. After thorough mixing, samples were incubated in a water bath at 40 °C for 40 min. The absorbance was measured at 765 nm against a blank sample consisting of distilled water instead of the diluted sample. The total phenolic content of the extracts was determined and expressed as mg of Gallic acid equivalents (GAE) per gram of sample in dry weight (mg GEA/g) using the regression equation of the calibration curve. The analysis was conducted three times.

Equation: y = 0.0795X - 0.1033

Total flavonoid content

The total content of flavonoids in the crude extract was determined by the aluminum chloride colorimetric method [56] Briefly, 0.75 mL of hydromethanolic crude extract (10 mg/mL) was made up to 1.5 mL with 2% $AlCl_3$ solution methanol. The mixture was well mixed before incubation at room temperature in the dark for 10 minutes. The mixture absorbance was measured at 440 nm against the standard containing a diluted solution of $AlCl_3$.

A calibration curve for quercetin was used to determine the total flavonoid concentration and the result was expressed as milligram equivalents of quercetin per gram of dry microalgae (mg QE/g). Equation: Y = 0.5658X – 0.1155

Polyphenol analysis

The HPLC analysis of crude hydromethanolic extract of *L. fusiformis* TL03 was performed using a Young Lin YL 9100 HPLC chromatograph with a UV-Vis detector (YL9120). The separation was achieved by a reversed-phase Agilent XDB Eclipse C8 column (250 mm/4.6 mm/5 m). The mobile phase was bi-distilled water enriched with 0.1 % acetic acid (Eluent A) and gradient methanol (Eluent B). The flow rate was adjusted at 1 mL/min, the temperature was regulated at 30 °C, and the injection volume was 20 µL. The HPLC gradient elutiens were as follows: 0–50 min, 95% elution A and 5% elution B; 50–60 min, 5% A and 95% elution B. The chromatograms were recorded at 254 and 280 nm.

Each compound of phenolic acids and flavonoids contained in hydromethanolic extract was identified by comparing its

retention time (Rt) and the UV spectra to those of the following standards (Galic acid, Rutin, Vanillic acid, Ascorbic acid, Flavone, Galangin Caffeine, Stearic acid, Tannic acid, Myricetin, Alpha-tocopherol, Morin, Apigenin, Diosmin, Catechin, Tangeretin**,** 5-Hydroxy-flavon, Chrysin, Kaemferide, Robinin, 3-Hydro-flavon, Acacetin, Hesperetin, Quercetin, and 6.methoxy-flavon, Xanthotoxine, Luteolin, Kaempferol)

Antioxidant Activity

DPPH radical scavenging activity

The DPPH assay was performed according to Tep [57], the Crude hydromethanolic extracts (10 mg/ml) were prepared in a series of dilutions (0.125, 0.250, 0.5, 1, 2, 3, 4, and 5 mg/ml) by adding methanol.

An equal volume of DPPH solution dissolved in methanol (0.004%) was added to 1.5 ml of each diluted test extract (0.125 to 5 mg/ml). After vigorous mixing for 10 seconds, the solution was incubated at room temperature in the dark for 30 minutes. The absorbance was read at a wavelength of 517 nm. Reference standards of BHT, Ascorbic acid, Trolox, Vitamin E were used as benchmarks.

All samples were performed in triplicate.

The following formula was used to determine the percentage of free radical DPPH inhibition

$$(I\ \%) = [(Ac - As)/ (Ac)] \times 100\ [Tep]^{57.}$$

Where As is the sample absorbance and Ac represents the absorbance of the control reaction, which uses all the reagents except the test substance.

The IC50 value was used to determine the antiradical activity of the samples; this value is the concentration of the sample required for 50% inhibition of DPPH radicals

Beta-carotene determination

Two mg of beta-carotene was dissolved in 10 ml of chloroform, and 2 ml of this solution was added to a vial previously containing 40 mg of linoleic acid and 400 mg of Tween 80. The mixture was agitated, and then the chloroform was completely evaporated using a rotary vacuum evaporator. Afterward, 100 ml of distilled water was added to the residue. After thoroughly mixing, a volume of 4.8 mL of the obtained emulsion was transferred to test tubes, each one containing 0.2 mL of the extract (10 mg/mL) or the same volume of control antioxidants (BHT).

The tubes were placed in a water bath at 50 °C and the absorbance was measured at 470 nm at intervals of 30, 60, 90 and 120 minutes. Samples were performed in triplicate

The bleaching rate of beta-carotene (R) is calculated according to Al-Shaikhan[58] as follows:

$$Rt = In\ (Abst0/Abst)/t$$

Rt: the bleaching rate of beta-carotene at times (30, 60, 90, and 120 minutes); In: natural logarithm; Abst0 corresponds to the initial absorbance of the emulsion immediately after sample preparation (t = 0 minutes); Abst: the absorbance of the emulsion at times (30, 60, 90, and 120 minutes). The percentage of antioxidant activity is calculated using the following equation:

$$\text{Antioxidant activity \%} = [(\text{R control} - \text{R sample})/\text{R control}] \times 100.$$

R control and R sample are the average rates of control and sample (extract) bleaching, respectively.

Antibacterial activity test

Two methods were used to evaluate the antimicrobial activity of crude hydromethanolic extract of L. fusiformis TL03: disc diffusion and *agar dilution method*

Disc diffusion method

Disc diffusion preparation

The disc diffusion technique was used to assess the antibacterial activity of the hydromethanolic extract of *L fusiformus* TL03. Sterile Whatman No. 1 discs (5.5 mm in diameter) were impregnated with 10 µl of hydromethanolic extracts (50 mg/ml).

Ampicillin (1 mg/mL) was used as a control

The tested bacterial strains were plated individually on nutrient agar. After 18 hours of incubation at 35°C, the colonies were transferred to saline (0.9% NaCl) to obtain a suspension turbidity equivalent to 1.5 x 108 CFU/mL [Alagawany] [59]

One hundred microliters of bacterial aliquots were spread on the surface of Mueller-

Hinton agar (MHA) Petri dishes and then, three sterilized dried paper discs containing crude hydromethanolic extract or control (ampicillin) were placed equidistantly on the surface of agar Petri dishes (90 mm diameter) for each bacterial strain tested. Prior to incubation, the Petri dishes were placed in the dark at 4°C for 1 hour to allow the hydromethanolic extract and the ampicillin to spread. Then the plates were incubated at 37 °C for 24 h, and the inhibition zones diameters (mm) around the discs were measured by the transparent ruler.

***Agar dilution method:*Minimum inhibition concentration (MIC)**

From the initial concentration of crude hydromethanolic extract (50 mg/ml), a series of double dilutions (25, 12.5, 6.25, 3.125, 1.56, 0.78 mg/ml) were prepared by addition to Mueller-Hinton broth. The control was prepared using the same method as described above with double dilution of ampicillin (0.5, 0.25, 0.125, 0.0625, 0.032, and 0.016 mg/mL).

One hundred µL of each tested bacterial cell suspension (1.5 108 CFU/ml) was placed separately on the Petri agar surface. All plates were incubated at 37 °C for 24 hours.

The MIC values were defined as the lowest concentration of antibacterial agents that inhibited the growth of bacteria [Ashour] [60]

Statistical analysis

The statistical analysis was conducted using IBM SPSS 25.0 software. The data were presented as means ± standard error (S.D.). The differences between groups were analyzed using a one-way analysis of variance (ANOVA) test, followed by the Tukey test.

RESULTS AND DISCUSSION

Results

Quantification of TPC and TFC

This study showed that the hydromethanol extract of *L fusiformis* TL03 contained a high total phenolic and flavonoid. The total phenolic and flavonoid compounds were 18.41±0.18 GAE mg/g and 2.11±0.12 QE mg/g DW, respectively.

Polyphenol analysis

The phenolic compounds of the hydromethanolic extract of *L. fusiformis* TL03 were investigated by HPLC chromatography.

The phenolic compounds contained in the extract were identified by comparing the retention time and UV spectra of the peaks obtained from the extract with those of the standards.

The results of HPLC analysis of the crude extract showed the presence of seven compounds including: Ascorbic acid, Gallic acid, Luteolin, Quercetin, Kaempferol. **(Fig. 1, Table 2)**.

Antioxidants activities

In this study, two assays (DPPH and beta-carotene/linoleic acid test) were used for the evaluation of the antioxidant activity of the hydromethanolic extract of L. fusiformis TL03 and the results are shown in **Table (3)**.

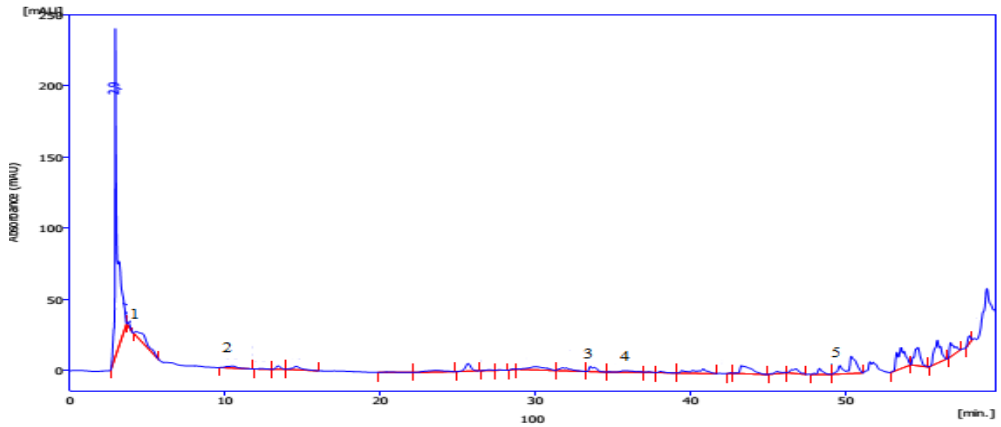

Fig.1: HPLC chromatogram of *L. fusiformis* TL03 hydromethanolic extracts. Phenolic compounds identified: (1): Ascorbic acid, (2): Gallic acid, (3): Luteolin, (4): Quercetine, (5): Kaempferol.

Table 2: The phenolic compounds identified by HPLC-DAD in hydromethanolic extract of *L. fusiformis* TL03.

N0	Compound	Area	Retention time	Nature of compound
1	Ascorbic acid	0.5	3.66	Vitamine C
2	Gallic acid	1	10.34	phenolic acid.
3	Luteolin	1.6	33.53	Flavon
4	Quercetine	1	35.703	Flavonol
5	Kaempferol	0.1	49.303	Flavonol

Table 3: Antioxidant activity (DPPH assay) and IC_{50} value in hydromethanolic extract of L.fusiformis TL03.

Extracts /standars	IC50 (µg ml $^{-1}$)DPPH	% inhibition of DPPH
Hydromethanolic extract	470	86.19 ± 1.61 (a)
BHT	72,16 ± 0,1	82,36 ± 0,94 (b)
Ascorbic acid	4 ± 0,1	98,54 ± 0,25©
Trolox	6,86 ± 0,05	98,03 ± 0,09©
vitamine E	9,55 ± 0,07	94,08 ± 0,1(d)

DPPH

The crude hydromethanolic extract of *L fusiformis* TL03 show strong antioxidant activity with percentage inhibition of DPPH radical reached to 86.19±1.61 %.

Comparing this result with the standards (antioxidant references), the percentage of free radical inhibition of the extract was higher than that of BHT (82.36 ± 0.94%), while it was lower than that of other standards such as Ascorbic acid (98.54 ± 0.25%), Trolox (98.03 ± 0.09%), and Vitamin E (94.08 ± 0.1%).

The IC50 value for TL03 hydromethanolic extracts was 470 µg/ml. While their values were 72.16 ± 0.1, 4 ± 0.1, 6.86 ± 0.05 and 9.55 ± 0.07 µg.ml-1 for -BHT, ascorbic acid, Trolox and vitamin E, respectively.

Beta carotene

The results of the percentages of antibleaching activities showed that the hydromethanolic extract of Limnospira fusiformis was significantly more effective in inhibiting the oxidation of linoleic acid (60. 35 ± 1.33 %) ($p< 0.05$) than that of BHT (52.5 ±0.82 %):

Antibacterial activities

The antibacterial activities of crude hydromethanolic extract of *L. fusiformus* TL03 were evaluated against six strains of bacteria by measuring the diameter of the inhibition zones, and the results were registered in **Table 4**.and **Fig. 2**.

The tested bacterial strains revealed different results, in which the diameters of the inhibition zone (ZID) ranged from 16.33±0.58 to 33.33±1.53 mm at a concentration of 50 mg/ml. The highest mean of zone inhibition diameter was registered against the Gram-positive bacterium *S. aureus (33.33±1.53 mm)*, followed by *B. subtilis* (16.67±0.58 mm) and *L. monocytogenes* (16.33±0.58 mm).

For Gram-negative bacteria, the highest value of zone inhibition diameter (16.33±1.15mm) against *K. pneumonia was* followed by *P. aeruginosa* (14.67±0.58 mm) and the lowest *E. coli* (11±1mm)

Table 4: Antibacterial activity of crude hydromethanolic extract of *L. fusiformus* TL03 tested by disc diffusion and Agar dilution method.

Bacterial strains		*L. fusiformis TL03* extract		Ampicilline	
		ZID(mm)	MIC(mg/ml)	ZID(mm)	MIC(mg/ml)
Bacteria gram positive	*B.* subtilis	16.67±0.58(a)	6.25	19.67± 0.58(d)	< 0.016
	S. aureus	33.33±1.53(b)	6.25	40±00(e)	0.5
	L. monocytogenes	16.33±0.58(a)	6.25	35±00(b)	0.5
Bacteria gram negative	*E. coli*	11±1(c)	12.5	24.33±1.15(f)	<0.016
	P. aeruginosa	14.67±0.58(a)	1.56	9.67±0.58(c)	< 0.016
	K. pneumonia	16.33±1.15(a)	0.78	40.33±0.58(e)	0.25

MIC: Minimum inhibitory concentration, ZID: zone inhibition diameter.
Different letters indicate significant difference (P< 0.5) . Error bar represents standard error (n = 4).

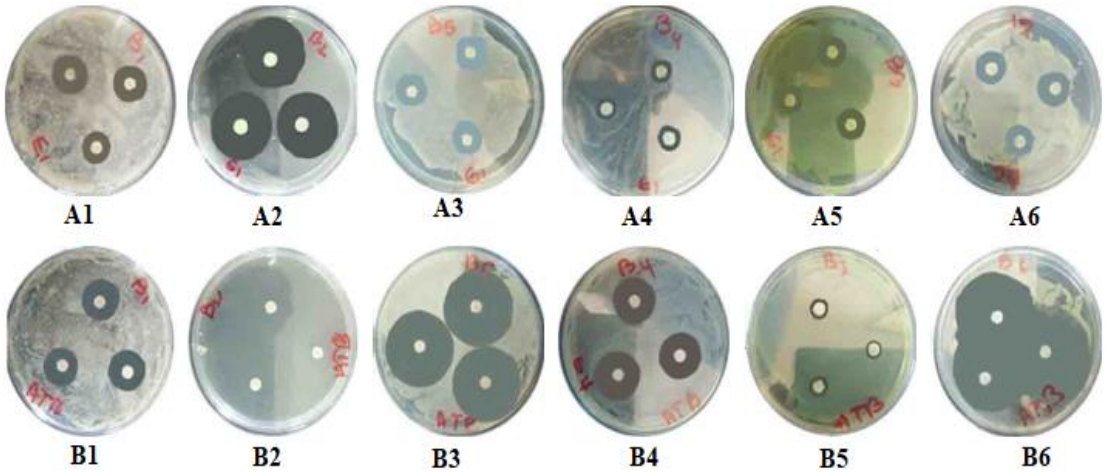

Fig. 2: Zone inhibition diameters (ZID) of the hydromethanolic extract of *L. fusiformis TL03* (A) and the antibiotic Ampicilline (B) against six bacterial strains. 1: *B. subtilis*; 2: *S. aureus*; 3: *L. monocytogenes*; 4: *E. coli*; 5: *P. aeruginosa*; 6: *K. pneumonia*.

Diffusion method

The minimum inhibitory concentrations (MIC) of L. fusiformus TL03 **extracts** are shown in **Table 4** and **Fig. 3**

The results showed that the MICs (minimum inhibitory concentrations) of the methanolic extract**(Table 5)** ranged from 0.78 mg/ml to 12.5 mg/ml. The lowest MIC value (0.78 mg/ml) was registered against *K. pneumonia,* followed by *P. aeruginosa* (1.56 mg/ml. whereas the highest value (12.5 mg/ml) was against *E. coli.* However, the MIC of this extract was 6.25 mg/ml against three bacteria gram+, *B. subtilis* , *S. aureus* and *L. monocytogenes*. The MIC of ampicillin ranged from 0.5 mg/ml to less than 0.016 mg/ml, where the highest value was registered against *S. aureus* and *L. monocytogenes,* followed by 0.25 mg/ml against *K. pneumonia,* while the lowest value (less than >0.016 mg/ml) was obtained against *B. subtilis*, *P. aeruginosa*, and *E. coli.*

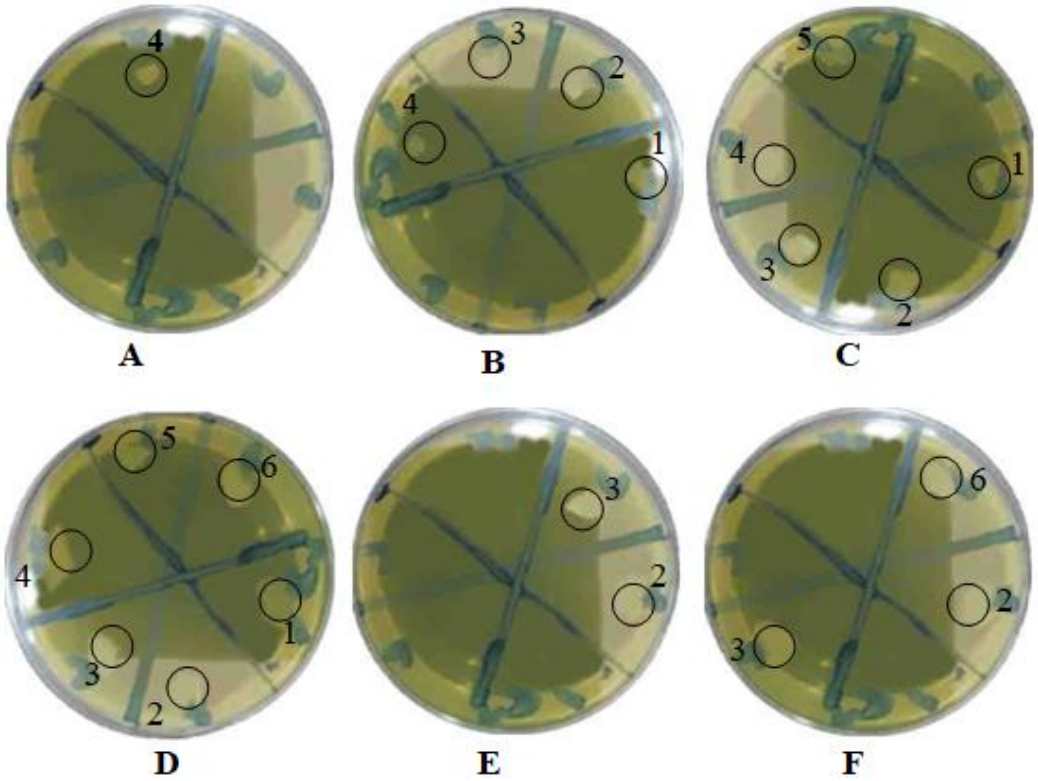

Fig. 3: Minimum inhibition concentration of the hydromethanolic extract of *L. fusiformis TL03* (A-D) and the antibiotic Ampicilline (E-F) against six bacterial strains. 1: *B. subtilis*; 2: *S. aureus*; 3: *L. monocytogenes*; 4: *E. coli*; 5: *P. aeruginosa*; 6: *K. pneumonia*.
A : 12.5 mg /ml , B : 6.25 mg /ml, C: 1.56 mg /ml, D: 12.5 mg /ml, E: 0.5 mg /ml, F: 0.016 mg /ml.

Table 5: Antibacterial activity of crude hydromethanolic extract of *L. fusiformus* TL03 **tested by** disc diffusion and Agar dilution method.

Bacterial strains		*L. fusiformis TL03* extract		Ampicilline	
		ZID(mm)	MIC(mg/ml)	ZID(mm)	MIC(mg/ml)
Bacteria gram positive	*B.* subtilis	16.67±0.58(a)	6.25	19.67± 0.58(d)	< 0.016
	S. aureus	33.33±1.53(b)	6.25	40±00(e)	0.5
	L. monocytogenes	16.33±0.58(a)	6.25	35±00(b)	0.5
Bacteria gram negative	*E. coli*	11±1(c)	12.5	24.33±1.15(f)	<0.016
	P. aeruginosa	14.67±0.58(a)	1.56	9.67±0.58(c)	< 0.016
	K. pneumonia	16.33±1.15(a)	0.78	40.33±0.58(e)	0.25

MIC: Minimum inhibitory concentration, ZID: zone inhibition diameter.
Different letters indicate significant difference (P< 0.5) .Error bar represents standard error (n = 4).

Discussion

Phenolic compounds are secondary metabolites essential for plant physiological processes, stress responses, and survival under harsh conditions [61]. The phenolic content of *S. platensis* is also influenced by a number of parameters, such as algal species, origin, culture conditions, stress factors, time of biomass collection, and age, as well as the methods of their extraction and solvents utilized[62]. They are classified as simple phenols, phenolic acids, flavonoids, xanthones, stilbenes, and lignans[63,64]. Flavonoids and phenolic acids are the main classes of phenolic compounds reported in *Spirulina*[43].

The total phenol content in the hydromethanolic extract of *L. fusiformis* TL03 agrees well with the results of other previously reported spirulina species[63, 65 ,66], while being higher than those documented in several research[67-73].

Total contents of flavonoids

By comparing our findings of total flavonoids in this study (2.11 ± 0.12 QE mg/g DW) with those reported by several authors, we noticed that the level of flavonoids in the hydromethanolic extract of the examined algae was higher than that found in other studies.

[Bellahcen][67] found that the total contents of flavonoids in ethanolic and aqueous extracts (0.21 ± 0.01 and 0.15 ± 0.01 mg quercetin/g dw, respectively) of Moroccan *Spirulina.* [Thangaraj][70] reported that the total flavonoids in the methanolic extract of *Arthospira platensis* (*spirulina*) were 1.42 ± 0.05 mg/g dry weight.

Other studies reported that the amount of flavonoids present in a *spirulina* extract was determined to be 142.23 mg quercetin/kg of extract[71] and 176.3 ± 7.65 mg QE/100 g in the aqueous extract of *spirulina* powder[65].

On the other hand, some studies indicated a high amount of total flavonoids compared to our findings. [Zainoddin][64] declared that the total flavonoid content in *spirulina* was 11.19 ± 0.07 mg QE/g DW. [Seghiri][69] found the content in the methanolic extract of Moroccan *Spirulina* to be 15.60 ± 2.74 mg RE/g dw.

Hplc Analysis

The polyphenol chromatographic analysis of the crude hydromethanolic extract of *L. fusiformis* TL03 revealed the presence of various compounds, of which five were identified **(Fig 1 and Table**

2). These results are similar to those of previous studies in which the authors mentioned the presence of ascorbic acid, gallic acid, luteotin, quercetin, kaempferol, and other phenolic compounds in different species of *spirulina*[67-69, 74-76, 78, 80].

Antioxydant Activities

DPPH

The DPPH assay results of this study **(Table 3)** showed that the hydromethanolic extracts of the TL03 strain possessed strong radical scavenging activity. The result of this study is similar in part to other study on the effective scavenging activity of DPPH radicals in *Spirulina platensis* extract[81], where the inhibition values of extracts prepared from 50%, 70%, and 96% methanol/water were 91.81%, 78.94%, and 58.61%, respectively[80]. In agreement, other study recorded the high percentage inhibition of DPPH radicals (97.37, 89.47, and 77.66%) in methanolic extract of *S. obliquus*, *B. eriensis*, followed by *P. pyrenoidosa*, respectively [74].

While the DPPH assay finding is greater than that of a methanolic extract of *Arthrospira platensis* reported by GHEDA [82] and those reported for green microalgae including *Chlorella sp.*E53 , *Chlorella sp.*ED53 and *Chlorococcum sp.*C53[83].

The potent antiradical activity may be due to the presence of phycobilin pigments (C-phycocyanin, allophycocyanin, and C-phycoerytherin) as well as phenolic and flavonoid compounds in the methanolic extract of *Arthrospira*[84].

The obtained results of IC 50 are higher compared to those mentioned by Abd El-Baky[77], who reported that the IC 50 of extracts derived from *S. maxima* cultured in Zarrouk media containing 2.5, 1.875, 1.25, 0.625, and 0 NaNO3 g/L revealed an IC50 value of 30.0, 28.0, 26.0, 23.0, and 22.0 µg /ml, respectively. Furthermore, the inhibition percentage of this study was higher than those (ranging from 60.33 ± 1.12 to 81.14 ± 1.25%) reported in different media of the same study.

Beta carotene

Carotenoids are important groups of pigments (red, orange, or yellow colors) that are present in plants, fungi, bacteria, and algae and cyanobacteria[85,86]. They are also key metabolites for human nutrition and health[87].

Natural carotenoids (lutein, β-carotene, and lycopene) have beneficial effects on both human and animal health[88,89]. B-carotene remains their essential source of vitamin A, which is necessary for the functions of the retina. In addition, it possesses anticancer, and antiviral and antibacterial properties[90].

The effectinesse of hydromethanolic extract to inhibit β-carotene bleaching was 85.87 ± 1.65% at 10mg/mL **(Table 4)**. The result of

this study of beta-carotene bleaching **(Table 4)** was consistent with that reported in *Spirulina* extract produced from various solvents (ethyl acetate, hexane, and chloroform extracts), in which *β*-carotene bleaching rates were 93.64±0.25%, 73.36±0.35%, and76.77±0.12%, respectively[91].

Antibacterial Activities

Several studies have investigated the antimicrobial activity of *Spirulina* extracts against the bacterial strains tested in this study, in addition to other pathogenic bacterial strains. *B. cereus*[68,92] and *S. aureus*[68,93-99], *L. monocytogenes*, *E. coli*[68, 93-95, 97, 99, 100], *P. aeruginosa*[93, 94, 97] and *K. pneumonia*[94, 95, 97, 99]. Those authors reported that there are different levels of effectiveness depending on the algae species, culture condition, process, and solvents used for the extraction.

Our findings **(Table 5)** showed that *L fusiformis* TL03 extracts had strong antibacterial activity against *S. aureus, B. cereus, K. pneumonia, P. aeruginosa,* and *L. monocytogenes,* whereas they had less activity against *E. coli*.

These results are also in agreement with previous studies[99,101, 102], which confirmed that the methanol extract of *Spirulina platensis* showed high activity against several positive and negative gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonaeruginosa*, *Klebsiella pneumoniae, Streptococcus pyogenes, Streptococcus epidermidis, Proteus mirabilis, Salmonella typhi,* and *Shigella flexneri*).

According to Abdel-Moneim[103], The methanolic extract of *Spirulina platensis* exhibited greater total phenolic content, antioxidant activity, and antibacterial activity in comparison to other extracts (hexane and acetone extracts). Furthermore, it has been noted that the methanolic extract of *Spirulina platensis* has potent antimicrobial and antifungal activities against some pathogenic microbial strains[104].

Martelli[68] demonstrated similar results as our findings, where the hydroethanolic extracts of *A. platensis* showed the highest antimicrobial activity (ZID = 15 mm) against gram-positive bacteria (*L. monocytogenes, S. aureus, and B. cereus*) and interesting inhibition results for gram-negative bacteria (*E. coli, Salmonella* spp.).

Kaushik[99] reported that the ZID of methanolic extract of *Spirulina platensis against* three bacteria (*S. typhi*, *P. aeruginosa*, *E. coli*, *S. aureus*) ranged from 11.52±.18 to 15.21±.1mm but no activity was observed against *K. pneumonia.*

Abdel-Moneim[103] stated Reported that the strong antimicrobial activity of the methanolic extract may be due to its high phenolic content.

According to some published papers[105,106], the antimicrobial activities of algae against a variety of bacteria and fungus are linked to polyphenols, terpenes, flavonoids, alkaloids, pigments,amino acids, polysaccharides (such as depolymerized fucoidans), lipids, and other lipid-soluble substances.

Spirulina bioactive compounds can damage bacterial cell integrity by increasing membrane permeability, which leads to loss of cytoplasmic content and then cell death[103]. On the other hand, Demule[107] reported that the antibacterial activity of the methanolic extract of *S. platensis* is caused by the presence of a significant amount of linolenic acid.

The minimum inhibitory concentration (MIC) of hydromethanolic extract of *L* fusiformis TL03 **(Table 5)** *ranging* from **0.78 to 12.56 mg/ml.** These results agree in part with previous studies by Abdel-Moneim[103], which recorded that the MIC values of *Spirulina* extracts (methanol, hexane, and acetone) against tested bacteria ranged from 1.2 to 10 mg/ml, with the lowest level MIC in methanol extracts (1.2 to 2 mg/ml).

These data are also in agreement with the previous study by Usharani[102], who reported that the MIC value of methanolic extract of *Spirulina platensis against eleven bacterial strains (Staphylococcus aureus, Streptococcus epidermidis, Streptococcus pyogenes, Bacillus cereus, Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa, Vibrio cholerae, Salmonella typhi, Klebsiella pneumoniae, and Shigella flexneri*) ranged from 1.25 mg/ml to 5 mg/ml.

Kaushik reported that .The MIC of methanol extract of *Spirulina platensis* against *S. aureus* and *E. coli* were 128 μg /ml and 256 μg /ml, respectively.

Conclusion

In conclusion, the results of this work showed that the methanolic extract of *L. fusiformis* TL03 contains a variety of bioactive compounds and has potent antioxidant and antibacterial properties against several pathogenic bacteria. Consequently, further research should be conducted to identify, isolate and characterize the molecules responsible for these activities, which may be used as alternatives to antibiotics and other conventional chemical drugs to treat various diseases. It is useful to conduct further studies to investigate its benefits in various pharmaceutical and food industry applications.

REFERENCES

1. Y. Taniyama and K. K. Griendling, "Reactive oxygen species in the vasculature:molecular and cellular mechanisms", ***Hypertension***, 42(6), 1075–1081 (2003).
2. S. H. Jeong, B. Y. Kim, H. G. Kang, H. O. Ku and J. H. Cho, "Effects of butylated hydroxyanisole on the development and functions of reproductive system in rats", ***Toxicology***, 208(1), 49–62(2005).
3. A. B. Engin, N. Bukan, O. Kurukahvecioglu, L. Memis and A. Engin, "Effect of butylated hydroxytoluene (E321) pretreatment versus l-arginine on liver injury after sub-lethal dose of endotoxin administration", ***Environ Toxicol Pharmacol***, 32, 457–464 (2011)
4. A. Botterweck, H. Verhagen, R. Goldbohm, J. Kleinjans and P. V. D. Brandt, "Intake of butylated hydroxyanisole and butylated hydroxytoluene and stomach cancer risk: results from analyses in the Netherlands Cohort Study", ***Food Chem Toxicol***, 38(7), 599–605(2000).
5. S. Randhawa and S. L. Bahna, "Hypersensitivity reactions to food additives", ***Curr Opin Allergy Clin Immunol***, 9(3), 278–283(2009).
6. M. Bleve, L. Ciurlia, E. Erroi, G. Lionetto, *et al.*, "Lionetto An innovative method for the purification of anthocyanins from grape skin extracts by using liquid and sub-critical carbon dioxide", ***Sep Purif Technol,*** 64(2), 192–197(2008).
7. L. Angiolella, G. Sacchetti and T. Efferth, "Antimicrobial and antioxidant activities of natural compounds", ***Evid Based Complement Alternat Med***, 2018(1), 1–3 (2018).
8. N. Chaachouay and L. Zidane, "Plant-Derived Natural Products: A Source for Drug Discovery and Development", ***Drugs Drug Candidates***, 3(1), 184–207 (2024).
9. W. Fu, D. R. Nelson, Z. Yi, M. Xu, B. Khraiwesh, K. Jijakli and K. Salehi-Ashtiani, "Bioactive compounds from microalgae: Current development and prospects", ***Studies In Natural Products Chemistry***, 54, 199–225 (2017).
10. M. A. Zaki, M. Ashour, A. M. Heneash, M. M. Mabrouk, A. E. Alprol, H. M. Khairy and M. E. Elshobary, "Potential Applications of native cyanobacterium isolate (Arthrospira platensis NIOF17/003) for biodiesel production and utilization of its byproduct in marine rotifer (Brachionus plicatilis) production", ***Sustainability***, 13(4), 1769(2021).
11. M. A. Rahman, M. A. Aziz, R. A. Al-Khulaidi, N. Sakib and M. Islam, "Biodiesel production from microalgae Spirulina maxima by two step process: Optimization of process variable", ***JRRAS***, 10(2), 140–147(2017).
12. S. S. Mostafa and N. S El-Gendy, "Evaluation of fuel properties for microalgae Spirulina platensis bio-diesel and its blends with Egyptian petro-diesel", ***Arab J Chem***, 10(2), 2040–2050 (2017).
13. U. Rajak, P. Nashine and T. N. Verma, "Characteristics of microalgae spirulina biodiesel with the impact of n-butanol addition on a CI engine", ***Energy***, 189, 116311(2019).
14. M. E. Murad and M. F. Al-Dawody, "Biodiesel production from spirulina microalgae and its impact on diesel engine characteristics" ***Rev Al-Qadisiyah J Eng Sci***, 13, 158–166(2020).
15. E. G. D. Morais, A. P. A Cassuriaga, N. Callejas, N. Martinez, I. Vieitez, I. Jachmanián and J. A. V. Costa, "Evaluation of CO 2 biofixation and biodiesel production by Spirulina

(Arthospira) cultivated in air-lift photobioreactor", ***Braz Arch Biol Technol***, 61, 18161339 (2018).

16. A. Rempel, F. de Souza Sossella, A. C. Margarites, A. L.Astolfi, R. L. R. Steinmetz, A. Kunz, and L. M. Colla, "Bioethanol from Spirulina platensis biomass and the use of residuals to produce biomethane: An energy efficient approach", ***Bioresour Technol***, 288, 121588(2019).
17. R. Dinesh kumar, M. Duraimurugan, N. Sharmiladevi, L. P. Lakshmi, A. A. Rasheeq, A. Arumugam and P. Sampathkumar, " Microalgal liquid biofertilizer and biostimulant effect on green gram (Vigna radiata L) an experimental cultivation", ***Biomass Conv Bioref***, 1–21, (2020).
18. F. Arahou, I. Lijassi, A. Wahby, L. Rhazi, M. Arahou and I. Wahby, "Spirulina-Based Biostimulants for Sustainable Agriculture: Yield Improvement and Market Trends", ***BioEnergy Research***, 16(3), 1401–1416 (2022).
19. C. Wuang, M. C. Khin, P. Q. D. Chua and Y. D. Luo, "Use of Spirulina biomass produced from treatment of aquaculture wastewater as agricultural fertilizers", ***Algal Research***, 15, 59–64(2016).
20. M. Castillo, G. Guevara, S. Baldanta, P. S. Rodríguez, L. Agudo, J. Nogales and J. M. N. Llorens, "Characterization of Limnospira platensis PCC 9108 RM and CRISPR-Cas systems", ***Microbiol Res***, 279, 127572 (2024).
21. M. Pelagatti, G. Mori, S. Falsini, R. Ballini, L. Lazzara and A. Papini, "Blue and Yellow Light Induce Changes in Biochemical Composition and Ultrastructure of Limnospira fusiformis (Cyanoprokaryota)", ***Microorganisms***, 11(5), 1236(2023).
22. T. Roussel, S. Halary, C. Duval, B. Piquet, J. P. Cadoret, L.Vernès and B. Marie, "Monospecific renaming within the cyanobacterial genus Limnospira (Spirulina) and consequences for food authorization", ***J Appl Microbiol***, 134(8), lxad 159(2023).
23. S. C. Silva, I. C. Ferreira, M. M. Dias and M. F. Barreiro, "Microalgae-derived pigments: A 10-year bibliometric review and industry and market trend analysis", ***Molecules***, 25(15), 3406(2020).
24. W. A. Metekia, A. G. Usman, B. H. Ulusoy, S. I. Abba and K. C. Bali, "Artificial intelligence-based approaches for modeling the effects of spirulina growth mediums on total phenolic compounds", ***Saudi J Biol Sci***, 29(2), 1111–1117(2022).
25. FAO. 2018,"The State of World Fisheries and Aquaculture: Meeting the sustainable development goals", ***Rome Food and Agriculture Organization of the United Nations,*** 2018(2018).
26. S. A. Pareek, B. R. Kasvan and N. Singh, "Effect of a novel dietary supplement Khejri, and Spirulina supplementation on lipid profile in cricket players", ***Front Sports Act Living***, 4, 1075388(2023).
27. R. K. Singh, S. P. Tiwari, A. K. Rai and T. M. "Mohapatra, Cyanobacteria: an emerging source for drug discovery", ***J Antibiot***, 64(6), 401–412(2011).
28. M. Carcea, M. Sorto, C. Batello, V. Narducci, A. Aguzzi, E. Azzini and V. Turfani, "Nutritional characterization of traditional and improved dihé, alimentary blue-green algae from the lake Chad region in Africa", ***LWT-Food Science and Technology***, 62(1), 753–763 (2015).
29. M. M. Ramírez-Rodrigues, C. Estrada-Beristain, J. Metri-Ojeda, A. Pérez-Alva, and D. K. Baigts-Allende," Spirulina platensis protein as sustainable ingredient for nutritional food products development", ***Sustainability***, 13(12), 6849(2021).
30. M. Raczyk, K. Polanowska, B. Kruszewski, A. Grygier and D. Michałowska, "Effect of Spirulina (Arthrospira platensis) Supplementation on Physical and Chemical Properties of Semolina (Triticum durum) Based Fresh Pasta", ***Molecules***, 27(2), 355(2022).
31. F. Jung, A. Krüger-Genge, P. Waldeck and J. H. Küpper, "Spirulina platensis, a super food", ***J Cell Biotechnol***, 5(1), 43–54(2019).

32. V. Saharan and S. Jood, "Nutritional composition of Spirulina platensis powder and its acceptability in food products", ***Int J Adv Res*** , 5(6), 2295–2300(2017).
33. S. Priyanka, R. Varsha, R. Verma and S. B. Ayenampudi, "Spirulina: a spotlight on its nutraceutical properties and food processing applications", ***J Microbiol Biotech Food Sci*** , 12(6), e4785 (2023).
34. A. M. Sharoba, "Nutritional value of spirulina and its use in the preparation of some complementary baby food formulas", ***J Dairy Sci Biotechnol***, 5(8), 517–538(2014).
35. N.Seyidoglu, S. Inan and C. A Aydin, "prominent superfood: Spirulina platensis. In Superfood and Functional Food the Development of Superfoods and Their Roles as Medicine", ***Intech Open London UK***, 22(1), 1–27(2017).
36. K. D. Athiyappan, W. Routray and B. Paramasivan, "Phycocyanin from Spirulina: A comprehensive review on cultivation, extraction, purification, and its application in food and allied industries", ***Food and Humanity***, 100235 (2024).
37. J. J. Oliveira and H. Ribeiro, "Food market trends: the cases of spirulina and bee pollen 56th", ***Int Sci Conf Econ Soc Dev,*** 246–258(2020).
38. F. Shahidi and C. Alasalvar, (Eds.), "Handbook of functional beverages and human healt", ***CRC Press,*** 11, (2016).
39. S. Bleakley and M. Hayes, "Algal proteins: extraction, application, and challenges concerning production", ***Foods***, 6(5), 33 (2017).
40. M. Tavakoli, M. B. H. Najafi and M. Mohebbi, " Effect of the milk fat content and starter culture selection on proteolysis and antioxidant activity of probiotic yogurt", ***Heliyon***, 5(2), e01204(2019).
41. N. K. AlFadhly, N. Alhelfi, A. B. Altemimi, D. K. Verma, F. Cacciola and A. Narayanankutty, "Trends and technological advancements in the possible food applications of Spirulina and their health benefits", ***Molecules***, 27(17), 5584(2022).
42. D. K. Koli, S. G. Rudra, A.Bhowmik and S. Pabbi,"Nutritional, functional, textural and sensory evaluation of Spirulina enriched green pasta: A potential dietary and health supplement", ***Foods***, 11(7), 979 (2022).
43. D. G. Bortolini, G. M. Maciel, I. D. A. A. Fernandes, A. C. Pedro, F. T. V. Rubio, I. G. Brancod and C. W. I. Haminiuk, "Functional properties of bioactive compounds from Spirulina spp.: Current status and future trends", ***Food Chem Mol Sci***, 100134 (2022).
44. R. Fernandes, J.Campos, M.Serra, J. Fidalgo, H. Almeida, A. Casas and A. I. Barros, Exploring the benefits of phycocyanin: From Spirulinacultivation to its widespread applications, ***Pharmaceuticals***, 16(4), 592 (2023).
45. G. Gentscheva, K. Nikolova, V. Panayotova, K Peycheva, L. Makedonski, P Slavov and I Yotkovska,"Application of Arthrospira platensis for Medicinal Purposes and the Food Industry A Review of the Literature", ***Life***, 13(3), 845 (2023).
46. S. Kumar, V. K. R.Surasani, A. Singh, S. M. Singh, Hauzoukim, L. N. Murthy and K. G. Baraiya , "Bioactive compounds from micro-algae and its application in foods : a review", ***Discov Food***, 4(1), 4-27 (2024).
47. M. H. Saad, E. M. El-Fakharany, M. S. Salem and N. M. Sidkey,"The use of cyanobacterial metabolites as natural medical and biotechnological tools", ***J Biomol Struct Dyn***, 40(6), 2828-2850(2022).
48. S. Hassan, R. Meenatchi, K. Pachillu, S. Bansal, P. Brindangnanam, J. Arockiaraj and J. Selvin, "Identification and characterization of the novel bioactive compounds from microalgae and cyanobacteria for pharmaceutical and nutraceutical applications", ***J Basic Microbiol***, 62(9), 999-1029 (2022).
49. S. A. Khalifa, E. S. Shedid, E. M. Saied, A. R. Jassbi, F. H. Jamebozorgi, M. E. Rateb and H. R. El-Seedi, "Cyanobacteria—From the oceans to the potential biotechnological and biomedical applications", ***Mar Drugs***, 19(5), 241(2021).
50. S. Kini, M. Divyashree, M. K. Mani and B. S. Mamatha, "Algae and cyanobacteria

as a source of novel bioactive compounds for biomedical applications", ***Adv cyanobact Biol***, 173-194 (2020).
51. S. Vijayakumar and M. Menakha, "Pharmaceutical applications of cyanobacteria — A review ", ***J Acute Med***, 5(1), 15-23(2015).
52. B. Thevarajah, G. K. S. H.Nishshanka, M. Premaratne, P. H. V. Nimarshana, D. Nagarajan, J. S Chang and T. U. Ariyadasa, "Large-scale production of Spirulina-based proteins and c-phycocyanin: A biorefinery approach", ***Biochem Eng J***, 185, 108541(2022).
53. G. M. Hamad, A. El-Baky, M. M. Sharaf and A. A. Amara,"Volatile compounds, fatty acids constituents, and antimicrobial activity of cultured Spirulina (Arthrospira fusiformis) isolated from Lake Mariout in Egypt", ***Sci World J vol***, 2023(2), 9919814 (2023).
54. M. M. Allen and R. Y. Stanier, "Growth and division of some unicellular blue-green algae", ***J Gen Microbiol***, 51(2), 199-202(1968).
55. S. V. L.ingleton and J. A. Rossi, "Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents", ***Am J Enol Vitic***, 16(3), 144-158 (1965).
56. J. L. C. Lamaison and A. Carnet, "Teneurs en Principaux Flavonoides des fleurs de Crataegus Monogyna Jacq et de Crataegus Laevigata (Poiret D. C) en Fonction de la Vegetation. Pharmaceutica", ***Pharm Acta Helv***, 65, 315-320 (1990).
57. B. Tepe, M. Sokmen, H. A. Akpulat and A. Sokmen, "Screening of the antioxidant potentials of six Salvia species from Turkey", ***Food Chem***, 95(2), 200-204(2006).
58. M. Al-Saikhan, L. Howard and J. C. Miller," Antioxidant activity and total phenolics in different genotypes of potato (Solanum tuberosum, L.)", ***J Food Sci***, 60(2), 341-343(1995).
59. M Alagawany, M. Madkour, E. M. T. l-Saadony,and, F. M. Reda," Paenibacillus polymyxa (LM31) as a new feed additive: Antioxidant and antimicrobial activity and its effects on growth, blood biochemistry, and intestinal bacterial populations of growing", ***Anim Feed Sci Technol***, 276(2), 114920 (2021).
60. E. A. Ashour, M. E. A. El-Hack, M. E. Shafi, W. Y. Alghamdi, A. E. Taha, A. A. Swelum and M. T. El-Saadony,"Impacts of green coffee powder supplementation on growth performance, carcass characteristics, blood indices, meat quality and gut microbial load in broilers", ***Agriculture***, 10(10), 457, (2020).
61. J. Cichoński and G. Chrzanowski, "Microalgae as a source of valuable phenolic compounds and carotenoids", ***Molecules***, 27(24), 8852(2022).
62. K. Korzeniowska, B. Łęska and P. P. Wieczorek, "Isolation and determination of phenolic compounds from freshwater Cladophora glomerata", ***Algal Res***, 48, 101912 (2020).
63. M. M. Vuolo, V. S. Lima and M. R. M. Junior, "Phenolic compounds: Structure, classification, and antioxidant power. Bioactive compounds: health benefits and potential applications, 33-50 (2019).
64. T. Papalia, R. Sidari and M. R. Panuccio, "Impact of different storage methods on bioactive compounds in Arthrospira platensis biomass", ***Molecules***, 24(15), 2810(2019).
65. A. M. Elbaz, A. M. Ahmed, A. Abdel-Maqsoud, A. M. Badran and A. M. E. Abdel-Moneim, "Potential ameliorative role of Spirulina platensis in powdered or extract forms against cyclic heat stress in broiler chickens", ***Environ Sci Pollut Res Int***, 29(30), 45578-45588 (2022).
66. H. A. H Zainoddin, A. Hamzah, Z. Jamari and W. A. W. Omar, "Chemical profiles of methanolic extracts from two species of microalgae, Nannochloropsis sp. and Spirulina sp.", ***Pertanika J Trop Agric Sci***, 41(3), 1085-1096 (2018).
67. T. O. Bellahcen, A. AAmiri, I. Touam, F. Hmimid, A. E. Amrani, A. Cherif and M. Cherki, "Evaluation of Moroccan microalgae: Spirulina platensis as a potential source of natural antioxidants", ***J Complement Integr Med***, 17(3), 20190036 (2020).

68. F. Martelli, M. Cirlini, C. Lazzi, E. Neviani and V. Bernini, "Edible seaweeds and spirulina extracts for food application: In vitro and in situ evaluation of antimicrobial activity towards foodborne pathogenic bacteria", ***Foods***, 9(10), 1442 (2020).
69. R. Seghiri, M. Kharbach and A. Essamri, "Functional composition, nutritional properties, and biological activities of Moroccan Spirulina microalga", ***J Food Qual***, 2019(3), 11 (2019).
70. M. Thangaraj, B. P. Saravana, J. Thanasekaran, S. Joen-Rong, M. Manubolu and K. Pathakoti, "Phytochemicals of algae, Arthospira platensis (spirulina) Chlorella vulgaris (chlorella) and Azolla pinnata (azolla)", ***GSC Biol Pharm Sci***, 19(2), 023-043 (2022)..
71. G. A. El-Chaghaby, S Rashad, S. F. Abdel-Kader, , E. S. A. Rawash and M. Abdul Moneem, "Assessment of phytochemical components, proximate composition and antioxidant properties of Scenedesmus obliquus, Chlorella vulgaris and Spirulina platensis algae extracts", ***Egypt J Aquat Biol Fish***, 23(4), 521-526 (2019).
72. A. Salamatullah," Characterization of extraction methods to recover phenolic-rich antioxidants from blue green algae (spirulina) using response surface approaches", (2014).
73. A. Kumar, D Ramamoorthy, D. K. Verma, A. Kumar, N. Kumar, K. R. Kanakn and K. Mohan, "Antioxidant and phytonutrient activities of Spirulina platensis", ***Energy Nexus***, 6, 100070 (2022).
74. M. Guldas, S. Ziyanok, Y. Sahan, E. Yildiz and O. Gurbuz, "Antioxidant and anti-diabetic properties of Spirulina platensis produced in Turkey", ***Food Sci Technol***, 41(3), 615-625 (2020).
75. M. Akbarizareh, H. Ofoghi, and M. Hadizadeh, "Assessment of Phenolic Components of the microalgae Spirulina platensis using two methods of Chromatography, TLC and HPLC", ***J Mar Biol***, 2019, (2014).
76. A. Pagnussatt, E. M. Del Ponte, J. Garda-Buffon and E. Badiale-Furlong, "Inhibition of Fusarium graminearum growth and mycotoxin production by phenolic extract from Spirulina sp", ***Pestic Biochem Physiol***, 108(1), 21-26 (2014).
77. H. H. Abd El-Baky, F. K. El Baz and G. S. El-Baroty, "Production of phenolic compounds from Spirulina maxima microalgae and its protective effects in vitro toward hepatotoxicity model", ***Afr J PharmacyPharmacol***, 3(4), 133-139 (2009).
78. S. C. Sahin, "The potential of Arthrospira platensis extract as a tyrosinase inhibitor for pharmaceutical or cosmetic applications", ***S Afr J Bot***, 119, 236-243 (2018).
79. H. Farg, A. El-Makhzangy and A. G. El-Shawaf," Chemical and technological studies on micro algae extracts and its utilization in some food products. 1- Chemical, biochemical characteristics and nutritional value of spirulina algae.", ***J Product Dev***, 26(1), 107-117 (2021).
80. N. H. El-Tablawy, H. A .Mansour and A. E. S. M. Shaaban, "Antioxidant activities of some edaphic algae in Egypt", ***Beni-Suef univ j basic appl Sci***, 9(1), 1-11 (2020).
81. P. H. Riyadi, E. Susanto, A. D. Anggo, M. H. Arifin and L. Rizki,"Effect of methanol solvent concentration on the extraction of bioactive compounds using ultrasonic-assisted extraction (UAE) from Spirulina platensis", ***Food Res***, 7(3), 59-66 (2023).
82. S. Gheda, E. H. Abd El-Zaher, A. M. Abou-Zeid, N. A. Bedair and L. Pereira, "Potential Activity of Arthrospira platensis as Antioxidant, Cytotoxic and Antifungal against Some Skin Diseases: Topical Cream Application", ***Mar Drugs***, 2023(21), 160 (2023).
83. W. Choochote, L. Suklampoo and D. Ochaikul, "Evaluation of antioxidant capacities of green microalgae", ***J Appl Phycol***, 26(1), 43-48 (2014).
84. E. A. Shalaby and S. M. Shanab, "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 (2013).

85. A. Hermanns, X. Zhou, Q. Xu, Y. Tadmor and L. Li, "Carotenoid pigment accumulation in horticultural plants", ***Hortic* Plant *J***, 6(6), 343-360 (2020).
86. T. Bhatt and K. Patel," Carotenoids: potent to prevent diseases review", ***Nat Prod Bioprospect***, 10(3), 109-117 (2020).
87. I. Sharma, N. Khare and A. Rai, "Carotenoids: Sources, Bioavailability and Their Role in Human Nutrition",In Dietary Carotenoids-Sources, Properties, and Role in Human Health. IntechOpen, (2024).
88. J. Amengual, E. Gouranton, Y. G J van Helden, S. Hessel, J. Ribot, E. Kramer and J. von Lintig, "Beta-carotene reduces body adiposity of mice via BCMO1", ***PloS One***, 6(6), e20644 (2011).
89. J. von Lintig, S. Hessel, A. Isken, C. Kiefer, J. M. Lampert, O. Voolstra and K. Vogt, "Towards a better understanding of carotenoid metabolism in animals", ***Biochim Biophys Acta***, 1740(2), 122-131 (2005)..
90. N. F. Gloria, N. Soares, C. Brand, F. L. Oliveira, R. Borojevic and A. J. Teodoro," Lycopene and beta-carotene induce cell-cycle arrest and apoptosis in human breast cancer cell lines", ***Anticancer Res***, 34(3), 1377-1386(2014).
91. H. E. A. Ali, S. M. M. Shanab, M. A. M. Abo-State, E. A. A. Shalaby, U. M. N. El Demerdash and M. A. Abdullah, "Evaluation of antioxidants, pigments and secondary metabolites contents in Spirulina platensis", ***Appl Mech Mater***, 625, 160-163 (2014).
92. K. Velichkova, I. Sirakov, N. Rusenova, G. Beev, S. Denev, N. Valcheva and T. Dinev, "In vitro antimicrobial activity on Lemna minuta, Chlorella vulgaris and Spirulina sp. extracts", ***Fresenius Environ Bull***, 27(8), 5736-5741(2018).
93. A. Karray, N. Krayem, H. B. Saad and A. Sayari,"Spirulina platensis, Punica granatum peel, and moringa leaves extracts in cosmetic formulations: an integrated approach of in vitro biological activities and acceptability studies", ***Environ Sci Pollut Res Int***, 28(7), 8802-8811(2021).
94. V. Sankarapandian, K. Nitharsan, K. Parangusadoss, P. Gangadaran, P. Ramani, B. A. Venmathi Maran and M. P. Jogalekar, "Prebiotic Potential and Value-Added Products Derived from Spirulina laxissima SV001—A Step towards Healthy Living", ***BioTech***, 11(2), 13 (2022).
95. M. A. Alshuniaber, R. Krishnamoorthy and W. H. Alqhtani,"Antimicrobial activity of polyphenolic compounds from Spirulina against food-borne bacterial pathogens", ***Saudi J Biol Sci***, 28(1), 459-464 (2021).
96. A. A. Al-Ghanayem,"Antimicrobial activity of Spirulina platensis extracts against certain pathogenic bacteria and fungi", ***Adv Biores***, 8(6), 96-101 (2017).
97. S. Dvl, C. Sreenath Kumar and R. Rengasamy, "Purified C-phycocyanin from Spirulina platensis (Nordstedt) Geitler: a novel and potent agent against drug resistant bacteria", ***World J Microbiol Biotechnol***, 27(4), 779-783 (2011).
98. S. Santoyo, M. Herrero, F. J. Senorans, A. Cifuentes, E. Ibáñez and L. Jaime," Functional characterization of pressurized liquid extracts of Spirulina platensis", ***Eur Food Res Technol***, 224(1), 75-81 (2006).
99. P. Kaushik and A. Chauhan, "antibacterial activity of laboratory grown culture of In vitro Spirulina platensis***", Indian J Microbiol***, 48(3), 348-352 (2008).
100. J. A. Mendiola, L. Jaime, S. Santoyo, G. Reglero, A. Cifuentes, E. Ibañez and F. J. Señoráns, "Screening of functional compounds in supercritical fluid extracts from Spirulina platensis", ***Food chem***, 102(4), 1357-1367 (2007).
101. S. F. Gheda and G. A. Ismail, "Natural products from some soil cyanobacterial extracts with potent antimicrobial, antioxidant and cytotoxic activities", ***An Acad Bras Cienc***, 92(2), e20190934 (2020).
102. G. Usharani, G. Srinivasan, S. Sivasakthi and P. Saranraj, "Antimicrobial activity of Spirulina platensis solvent extracts against pathogenic bacteria and fungi", ***Biol Res***, 9(5), 292-298(2015).
103. A. M. E. Abdel-Moneim, M. T. El-Saadony, A. M. Shehata, A. M. Saad, S. A. Aldhumri, S. M. Ouda and N. M.

Mesalam,"Antioxidant and antimicrobial activities of Spirulina platensis extracts and biogenic selenium nanoparticles against selected pathogenic bacteria and fungi", ***Saudi J Biol Sci***, 29(2), 1197-1209 (2022).

104. M. Hetta, R. Mahmoud, W. El-Senousy, M. Ibrahim, G. El-Taweel and G. Ali, "Antiviral and antimicrobial activities of Spirulina platensis", ***World J Pharm Pharm Sci***, 3(6), 31-39 (2014).
105. S. Afzal, A. K. Yadav, A. K. Poonia, K. Choure, A. N. Yadav and A. Pandey,"Antimicrobial therapeutics isolated from algal source: retrospect and prospect", ***Biologia,*** 78(2),291-305 (2023).
106. M. Bartolomeu, C. Vieira, M. Dias, T. Conde, D. Couto, D. Lopes and M. R. Domingues, "Bioprospecting antibiotic properties in photodynamic therapy of lipids from Codium tomemtosum and Chlorella vulgaris", ***Biochimie***, 203, 32-39 (2022).
107. M. C. Z. Demule, G. Z. Decaire and M. S. Decano, "Bioactive substances from Spirulina platensis (Cyanobacteria)", ***Phyton-Int J Exp Bot***, 58, 93-96(1996).

Bull. Pharm. Sci., Assiut University, Vol. 47, Issue 2, 2024, pp.899-916.

نشرة العلوم الصيدلية
جامعة أسيوط

BULL. PHARM. SCI. Assiut Univ.

المحتوى الفينولي والأنشطة المضادة للأكسدة والمضادة للبكتيريا للمستخلص الهيدروميثانولي لنبات ليمنوسبيرا فيوزيفورميس لت ٠٣ المعزول من بحيرة التلامين، شمال غرب الجزائر

جيناتشي. بلقاسم[١،٢،٣*] – قرطبي مفتي. حكيمة[١] – العماري ليندة لعماري[٣]

[١]جامعة البليدة ١ ، مخبر أبحاث النباتات العطرية والطبية، كلية العلوم الطبيعية والحياة، البليدة، الجزائر

[٢] المركز الوطني للبحث والتنمية فى الصيد البحري وتربية المائيات(CNRDPA) ، بو إسماعيل، الجزائر

[٣]المختبر البيولوجي للأنظمة الميكروبية، المدرسة العليا العادية بالقبة، الجزائر العاصمة، الجزائر

أجريت هذه الدراسة لتقييم المحتوى الفينولي الكلى والفلافونويد والنشاط المضاد للأكسدة والمضاد للبكتيريا للمستخلص الهيدروإيثانولي للطحلب الأخضر المزرق ليمنوسبيرا فيوزيفوريمس لت ٠٣ . وقد تم تحديد إجمالي الفينولات والفلافونويدات فى المستخلص وذلك باستخدام طريقتي فولين–سيكالتو وكلوريد الألومنيوم، على التوالي. و تم استخدام تقنية YL 9100 HPLC للتعرف على المركبات الفينولية. وتم استخدام ٢، ٢–ثنائي فينيل –١ بيكريل هيدرازيل (DPPH) لتحديد نشاط الشوارد الجذرية واختبارات حمض اللينوليك بيتا كاروتين لتقييم نشاط مضادات الأكسدة في المختبر. وتم اختبار النشاط المضاد للميكروبات ضد ستة أنواع من البكتيريا باستخدام طريقة الانتشار القرصي وطريقة تخفيف الأجار. و كان إجمالي الفينول والفلافونويدات الموجودة في المستخلص الهيدروميثانولي ١٨,٤١ ± ٠,١٨ مجم حمض الجاليك / جم DW و٢,١١ ± ٠,١٢ مجم كيرسيتين / جمDW ، على التوالي. و أظهرت نتيجة تحليل البوليفينول وجود: حمض الأسكوربيك، حمض الجاليك، للوتيولين، كيرسيتين، كيمبفيرول .

وأظهر هذا المستخلص نشاطًا قويًا لتطهير جذري DPPH وتبييض البيتا كاروتين بنسبة ٨٦,١٩ ± ١,٦١٪ و ٨٥,٨٧ ± ١,٦٥٪ على التوالي، بالإضافة إلى النشاط المضاد للبكتيريا، حيث تتراوح أقطار تثبيط المنطقة (ZID) من ٣٣,٣٣ ± ١,٥٣ إلى ١١±١ ملم، وك١لك أظهر نشاط مضاد للجراثيم ذو قيمة أعلى (٣٣,٣٣±١,٥٣ ملم) ضد المكورات العنقودية الذهبية والحد الأدنى من التركيزات المثبطة (MIC) التي لوحظت ضد بكتريا الالتهاب الرئوي ٠,٧٨) ملجم / مل). وأخيرا تم التوصل إلى أن المستخلص الهيدروميثانولي لنبات ليمنوسبيرا فيوزيفورميس لت ٠٣ له نشاط مضاد للأكسدة ومضاد للبكتيريا.