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Original article

Antioxidant and antimicrobial activities of *Spirulina platensis* extracts and biogenic selenium nanoparticles against selected pathogenic bacteria and fungi

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ABSTRACT

This study investigated the antimicrobial and antioxidant activity of three *Spirulina* extracts (methanol, acetone, and hexane) and the biological selenium nanoparticles (SeNPs) fabricated by *Bacillus subtilis* AL43. The results showed that *Spirulina* extracts exhibited antimicrobial activity against tested pathogens. Besides, *Spirulina* extracts significantly scavenged ABTS and DPPH radicals in a dose-dependent manner. The methanolic extract had higher total phenolic content, antimicrobial activity, and antioxidant activity than other extracts. The selenium nanoparticles were synthesized by *Bacillus subtilis* AL43 under aerobic conditions and were characterized as spherical, crystalline with a size of 65.23 nm and a net negative charge of -22.7 . We evidenced that SeNPs possess considerable antimicrobial activity against three gram-positive, three gram-negative bacteria, and three strains from both *Candida* sp. and *Aspergillus* sp. Moreover, SeNPs were able to scavenge ABTS and DPPH radicals in a dose-dependent manner. An association was found between the total phenolic content of *Spirulina* and SeNPs and their biological activities. Our results indicate that *Spirulina* and SeNPs with significant antimicrobial and antioxidant activities seem to be successful candidates for safe and reliable medical applications.

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1. Introduction

Foodborne diseases have increased worldwide, with a noticeable public health concern (Abd El-Hack et al., 2021; Abd El-Hack et al., 2020c). Besides, the development and outbreaks of antibiotic-resistance microbes threaten human and animal health and cause a global health crisis (Abdelhady et al., 2021; Nour et al., 2021). Additionally, the antioxidant defenses in biological systems are not fully able to counter oxidative stress due to the

wide variety of stressors and free radical inducers (El-Tarabily et al., 2021). Among the novel approaches for tackling this problem are natural products, as antibiotic alternatives, that have antioxidant and antimicrobial activities (Abd El-Hack et al., 2020b; Abd El-Hack et al., 2020d; Abou-Kassem et al., 2021b; Alagawany et al., 2021a; Reda et al., 2021a). These natural products may contribute to mitigating oxidative stress via enhancing enzymic and non-enzymic antioxidants (Abd El-Hack et al., 2020c; Abdel-Moneim et al., 2021a; Abdel-Moneim et al., 2020c; Abdelnour et al., 2020a; Abdelnour et al., 2020b; Saad et al., 2021c; Saad et al., 2020b).

Spirulina platensis is an edible blue-green alga. The beneficial role of *Spirulina* in human food and domestic animal feed has received increased attention across several disciplines in recent years (Abdel-Moneim et al., 2021b; De La Jara et al., 2018; EL-Sabagh et al., 2014; Holman and Malau-Aduli, 2013). It contains high protein levels with all essential amino acids, essential fatty

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acids, minerals, pigments, carotenoids, and vitamins (Abdel-Moneim et al., 2021b; Mendiola et al., 2007). *Spirulina* was found to act as a probiotic and antioxidant agent (Abdel-Moneim et al., 2021b; Abdelkhalek et al., 2015; Bhowmik et al., 2009). Therefore, *Spirulina* is supplemented to human food and animal diets to prevent gut dysbiosis and pathogens colonization and improve antioxidant status.

Selenium is an essential trace element and has received considerable attention due to its essential functions in the biological system. Selenium is the key component of selenoproteins that is well known to be involved in animal cells' antioxidant defense system. Moreover, the antimicrobial activity of this micronutrient metalloid has been demonstrated (Cremonini et al., 2016). Nanotechnology is a burgeoning interdisciplinary approach in multiple fields of academic research. It has the ability to facilitate ground-breaking applications in human and animal health, involving pathogens resistance, antioxidant, toxin degradation, nutrient efficiency, etc. (Abd El-Hack et al., 2020b; El-Saadony et al., 2020a; Reda et al., 2021b; Reda et al., 2020). Because it is widely thought that selenium in nano form has more effective and affordable antimicrobial and antioxidant activities and safer than other forms (Abbas et al., 2021; Forootanfar et al., 2014; Ibrahim et al., 2020), it has gained much attention and wide applications in recent years. Biogenic selenium nanoparticles (SeNPs) can be synthesized using bacteria as biological catalysts, giving a safe and environmental innovation strategy for producing metal/metalloid nanoparticles with high bioactivity and low cytotoxicity and without the need to reducing and stabilizing agents (Abbas et al., 2021; Sheiha et al., 2020; Xu et al., 2018). To the best of our knowledge, limited investigations have been done to evaluate both antioxidant and antimicrobial activities of *Spirulina* and the biogenic SeNPs. Indeed, antibiotics and other chemical antimicrobials can inhibit the growth of the pathogens; however, with concerning the advantages of the high bioavailability and lower cytotoxicity to humans and animals (Cremonini et al., 2016; Kata et al., 2018), *Spirulina* and SeNPs present novel antibiotic alternatives with high potential for preventing infection in the future. The current study was undertaken to assess the antioxidant and antimicrobial activities of *Spirulina* and biogenic SeNPs in order to evaluate the potential of using them as therapeutic candidates.

2. Materials and methods

2.1. Isolation of *Spirulina*

Zarrouks medium was used to isolate and cultivate the pure culture of *Spirulina* as follow; 10 mL of 5 d old *Spirulina* was mixed with 250 mL of Zarrouks medium pH 9.5 in screw bottles then incubated at 25 °C for 10 d under continuous illumination (600–800 lx) (Zarrouk, 1966). The pure culture of *Spirulina* was obtained by streaking method on Zarrouks medium to get a single culture from this isolate. The plates were incubated at 25°C under continuous illumination (600 lx). Developed colonies were picked up and microscopically examined, and those composed of *Spirulina* cells were preserved on slants containing Zarrouks medium.

2.2. Preparation of *Spirulina platensis* extracts

The *Spirulina platensis* was obtained from Soda lake in Wadi El-Natrun, Monufia Governorate, Egypt, then was dried and powdered. Forty grams of *Spirulina* powder were homogenized in 200 mL of solvents (methanol, acetone, and hexane) and were stirred for 2 h, and the supernatants were obtained (Hassanin et al., 2020; Saad et al., 2020a). The rotary evaporator retained the

solvents, and the residues were stored at 4 °C for further analysis. All chemicals used in this work were of analytical grade.

2.3. Preparation of SeNPs

2.3.1. Isolation, screening, and identification of Se-tolerant bacterium

0.85 g of sodium selenite was dissolved in one liter of sterilized water to prepare a stock solution of sodium selenite 5 mM concentration. 1 mM and 2 mM concentrations were prepared by taking 200 and 400 mL of stock solution and diluted to a liter with sterilized water. All solutions were stored to use in further experiments. The heavy metal contaminated soil was collected from Abu-Hammad City, Wady El-Moulak village, Sharkia governorate, Egypt. 10 gm of soil were homogenized in 90 mL peptone buffer and stirred for 15 min to obtain 10⁻¹ dilution. Serial dilutions were prepared to 10⁻⁷ (Desoky et al., 2020a; Desoky et al., 2020b; Hassan et al., 2021). 100 µL of each dilution was spread over Mueller Hinton agar (MHA) plates supplemented with different sodium selenite concentrations (1, 2, and 5 mM), then incubated at 30 °C for 24 h and observed the colonies in each plate (El-Saadony et al., 2020b).

The Se-tolerant bacterium was identified based on the morphological, biochemical, and physiological tests in Bergey's Manual (DeVos et al., 2011). The identification was confirmed by and MALDI-TOF mass spectrometry (bioMérieux, Marcy l'Etoile, France) (Singhal et al., 2015).

2.3.2. Biosynthesis and characterization of biogenic SeNPs

Sodium selenite (0.17 g) was homogenized in Luria-Bertani Broth (100 mL) containing 100 µL of bacterial isolate inoculum. The conditions were adjusted to obtain the best yield of SeNPs: pH 7.2, incubation temperature 30 °C, reaction time 24 h under agitation at 150 rpm in shaking incubator. Luria-Bertani Broth (LBB) without sodium selenite was considered a control (El-Saadony et al., 2021e; Fesharaki et al., 2010; Yadav et al., 2008). The produced SeNPs were characterized using six advanced instruments. UV–Vis spectroscopy was used to estimate the optical property of the SeNPs mixture (El-Saadony et al., 2020a; Saad et al., 2021a). Fourier Transform-Infrared (FT-IR) spectroscopy ("Bruker Tensor 37, Kaller", Germany) was used to identify the potential active compounds in the SeNPs mixture (Beeke et al., 2007; El-Saadony et al., 2021b). Powder X-ray diffraction (XRD) was used to identify the crystalline nature of SeNPs (El-Saadony et al., 2021g). The shape and size of SeNPs were measured by Transmission Electron Microscopy (TEM) (JEOL 1010, Japan) (Akl et al., 2020). Size distribution and Zeta potential were estimated by Zeta sizer analysis (Nano "Z2 Malvern, Malvern Hills, UK") (El-Saadony et al., 2021c; El-Saadony et al., 2021f; Saad et al., 2021b).

2.4. Chemical studies

2.4.1. Total phenolic content

The total phenolic contents of *S. platensis* (Table 1) and biogenic SeNPs suspension (Table 2) were estimated using the Folin-Ciocalteu method (Kalagatur et al., 2018). 50 µL of each evaluated *Spirulina* extract or SeNPs suspension was mixed with 50 µL of sodium carbonate (Na₂CO₃ 7.5%, w/v) and 25 µL of diluted Folin-Ciocalteu reagent with water (1:10, v/v). The microtiter plate was placed in a microtiter plate reader (BioTek Elx808, USA) and the absorbance was read at 750 nm after 30 min. The total phenolic content was expressed as Gallic acid equivalent (µg GAE/mL).

Table 1
Total phenolic component in *Spirulina* extracts.

Solvents	Concentration (mg/mL)	Total polyphenols ($\mu\text{g GAE/mL}$)
Methanol	2.5	1120 ^{c-A,*}
	5	1403 ^{b,A,**}
	10	1592 ^{a,A,***}
Acetone	2.5	645 ^{f,B,*}
	5	747 ^{e,B,**}
	10	928 ^{d,B,***}
Hexane	2.5	337 ^{h,C,*}
	5	463 ^{g,C,**}
	10	519 ^{g,C,***}
SEM		80.53
P-values		
Solvent		<0.001
Concentration		<0.001
Solvent \times Concentration		<0.001

GAE, gallic acid equivalent, SEM, standard error of means, means in the same column with different lowercase letters indicating significant differences of the interaction, different uppercase letters indicating significant differences between *Spirulina* extracts, but different *, ** and *** indicating significant differences between concentrations of the same extract.

Table 2
Total phenolic component in biogenic selenium nanoparticles (SeNPs).

SeNPs Conc. ($\mu\text{g/mL}$)	Total polyphenols ($\mu\text{g GAE/mL}$)
100	569.0 ^e
200	852.3 ^d
300	994.0 ^c
400	1140.7 ^b
500	1381.3 ^a
SEM	72.97
P-value	<0.001

GAE, gallic acid equivalent, SEM, standard error of means, means in the same column with different letters are significantly different.

2.5. Microbial studies

2.5.1. Bacterial and fungal strains

Bacterial isolates (*Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhi*, *Escherichia coli*, and *Klebsiella pneumonia*) and fungal isolates (*Candida tropicalis*, *Candida albicans*, *Candida glabrata*, *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus fumigatus*) were obtained from Microbiology department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. Both bacterial and fungal isolates were stored at 4 °C on nutrient agar (NA) slants and Sabouraud's dextrose agar (SDA) slants, respectively.

2.5.2. Preparation of bacterial and fungal inoculum

A streak of tested bacteria slant was mixed in 5 mL of nutrient broth and then incubated at 30 °C until 1.5×10^8 CFU/mL (Alagawany et al., 2021b). A loop of tested fungi slant was mixed in 5 mL of SDA and incubated at 28 °C until 0.5 McFarland standards 2.3×10^3 (El-Saadony et al., 2019).

2.6. Antimicrobial activity

2.6.1. Disc diffusion method

The antibacterial and antifungal activities of *Spirulina* extracts and SeNPs were estimated by the disc diffusion method (El-Saadony et al., 2021d). *Spirulina* extracts were prepared at three concentrations (2.5, 5, and 10 mg/mL). 100 μL of each concentration was dissolved in 1 mL of DMSO 5%. Sterilized paper discs (6 mm) were prepared and saturated with *Spirulina* extracts.

100 μL of bacterial inoculum (1×10^8 CFU/mL) and fungal spore suspension (2.3×10^3 CFU/mL) were spread on MHA and SDA plates surface, respectively. The previously saturated discs (6 mm) were placed on both sides of MHA and SDA plates. The discs saturated with ciprofloxacin or diniconazole were positive controls. The MHA plates were incubated at 37 °C for a day, and SDA plates were incubated at 28 °C for five days (fungi). The same procedure was repeated with SeNPs concentrations (100, 200, 300, 400, and 500 $\mu\text{g/mL}$). The inhibition zones (mm) around discs were measured by the transparent ruler.

2.6.2. Minimum inhibitory concentration (MIC)

The MIC of *Spirulina* extracts and SeNPs were estimated by the microdilution method according to Ericsson and Sherris (1971). *Spirulina* extracts and SeNPs concentrations were dissolved in 5% DMSO. 500 μL of *spirulina* extracts and SeNPs concentrations were homogenized in Mueller Hinton broth (MHB) and Sabouraud dextrose broth (SDB) tubes that inoculated with 100 μL of bacterial (1.5×10^8 CFU/mL) and standard size of fungal spore suspension (3×10^3 CFU/mL). The controls were MHB and SDB tubes inoculated with tested microorganisms. All tubes were incubated for a day at 37 °C and 5 days at 28 °C, respectively. The MIC values were recorded as the lowest concentration of antibacterial agents that prevented the growth of bacteria or fungi (Ashour et al., 2020; El-Saadony et al., 2021a).

2.7. Antioxidant assay

2.7.1. ABTS assay

The ABTS* radical scavenging activity (RSA) was determined by the ability of antioxidant agents to eliminate the ABTS* radical. The scavenging activities of *Spirulina* extracts and SeNPs were estimated (Gil et al., 2002) with some modifications. 3 mL of 0.1 mM ABTS* was added to 1 mL of *Spirulina* extracts (2.5, 5, and 10 mg/mL) and SeNPs concentrations (100, 200, 300, 400, and 500 $\mu\text{g/mL}$). The tubes were left for 30 min, then the absorbance was read at 745 nm by spectrophotometer. The control was ABTS* solution, and Tret-Butyl hydroquinone (TBHQ) was used as an antioxidant reference. The ABTS*RSA (%) of *Spirulina* extracts and SeNPs was calculated as the following equation:

$$\text{ABTS radical scavenging ability (\%)} = \frac{\text{Abs.control} - \text{Abs.sample}}{\text{Abs.control}} \times 100$$

2.7.2. DPPH assay

The antioxidant activity of *Spirulina* extracts and SeNPs concentrations was estimated by the scavenging DPPH radical as compared to a positive control (TBHQ). 2 mL ethanolic DPPH was added to 1 mL of each concentration and incubated in the dark for 30 min. The absorbance was estimated at 517 nm. DPPH* reagent was a control (Hassanin et al., 2020). TBHQ was used as an antioxidant reference, and DPPH scavenging activity (%) was measured as the following:

$$\text{DPPH radical scavenging ability (\%)} = \frac{\text{Abs.control} - \text{Abs.sample}}{\text{Abs.control}} \times 100$$

2.8. Statistical analysis

All experiments were performed in triplicate, and data were recorded and analyzed with SPSS package (v 20, SPSS Inc., Chigaco, IL, USA). The Two-way ANOVA test was used to examine the effect of solvent type and concentration on the total phenolic component

of *Spirulina platensis* and its antimicrobial and antifungal activities. One-way ANOVA was performed to analyze the rest of the parameters and to compare the concentrations of *Spirulina platensis* within different solvents with the positive control. LSD test was used to compare the statistically significant differences among mean at $P < 0.05$.

3. Results

3.1. Isolation, screening, and identification of Se-resistant isolate

Thirty-three bacterial isolates were obtained from soil samples at PCA plates supplemented with sodium selenite (1 mM). Only five bacterial isolates were recovered at a 2 mM concentration, and coded as (AL17, AL26, AL39, AL43, and AL51), one isolate was tolerated with sodium selenite (5 mM), and called AL43, and it was considered Se-resistant bacteria. Based on the biochemical tests in the Bergy manual, the screened isolate was gram-negative, motile, short rod, and non-spore-forming under a light microscope and aerobic conditions, and it was identified as *Bacillus subtilis* AL43. The obtained findings showed a maximum similarity of 99% to several *Bacillus* spp., predominantly *Bacillus subtilis*. Thus, the local screened bacterial isolate (*Bacillus subtilis* AL43) was similar to *Bacillus subtilis* spp *subtilis* DSM 10 T DSM.

3.2. SeNPs characterization

The biological SeNPs were fabricated by homogenizing sodium selenite with selected isolate *B. subtilis* AL43 under optimal conditions. After incubation, the appearance of red color in the culture flask suggested the formation of SeNPs. The maximum UV-visible absorption of SeNPs was found at 300 nm (Fig. 1). The TEM observed that the mean diameter of the produced SeNPs was 45–80 nm, indicating that *B. subtilis* AL43 could synthesize intracellular SeNPs (Fig. 2). XRD showed that the biosynthesized nanoparticles are crystalline with a spherical structure. The crystalline size of SeNPs was in the range of 32–86 nm. Zeta seizer and Zeta potential results indicated that the average SeNPs size was 65.24 nm and the Zeta potential was -22.7 mV (Fig. 3). FTIR spectrum showed that the bands at 3242.71 cm^{-1} and 3390.09 cm^{-1} matched the O–H and N–H stretching vibration.

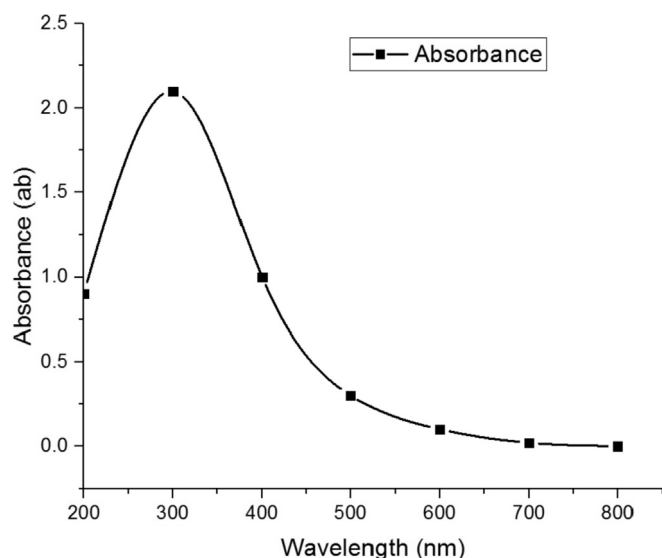


Fig. 1. UV-Vis spectra of SeNPs synthesized by *B. subtilis* AL43.

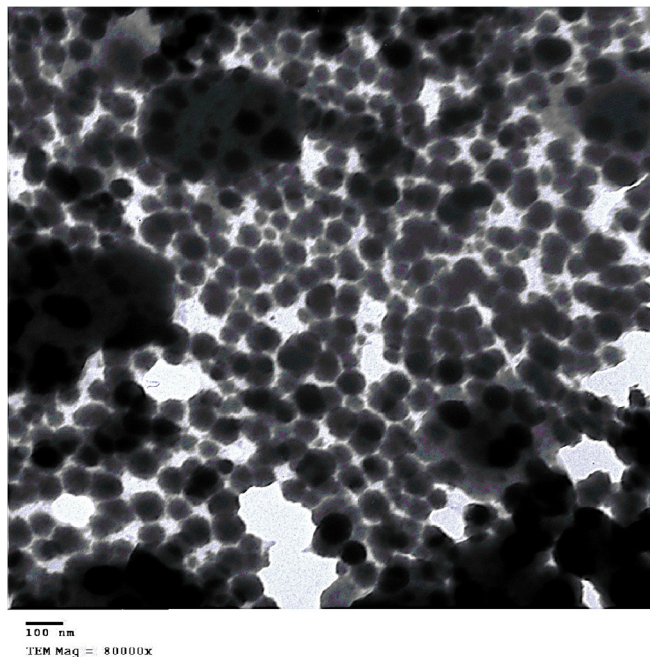


Fig. 2. TEM image of biogenic selenium nanoparticles (SeNPs) synthesized by *Bacillus subtilis* AL43.

The hydrogen-bonded SH stretching vibration appears at 2424.62 cm^{-1} . The band at 1653.93 cm^{-1} is related to C=O. The bands around 1076.81 cm^{-1} are probably correlated to the C–O stretching vibrations. 887.69 cm^{-1} is probably the vibration absorption peak due to the C–O–C. The bands at 540.09 cm^{-1} and 616.63 cm^{-1} indicated the stretching vibration of C–S bond. The increase in depth and width of peaks may result from the C–O stretching vibrations of phenolic compounds attached to SeNPs. These results confirmed the presence of functional biomolecules (protein, phenols, and polysaccharides) attached to the SeNPs surface (Fig. 4).

3.3. Chemical studies

The values of total phenolic content in *Spirulina* extracts showed significant differences, as shown in Table 1. Methanol extract exhibited higher values for all the tested levels, followed by acetone extract and then hexane extract. Table 2 presents the values of the total phenolic content of the different concentrations of SeNPs, which increased in a concentration-dependent manner.

3.4. Microbial studies

3.4.1. Antimicrobial activity of Spirulina

Tables 3 and 4 show the inhibition zones diameter (IZD) of *spirulina* extracts (methanol, acetone, and hexane) against tested bacteria and fungi compared to ciprofloxacin and diniconazole, respectively. The IZD values increased in a concentration-dependent manner. Gram-positive bacteria (*B. cereus*, *S. aureus*, and *L. monocytogenes*) showed higher sensitivity to *Spirulina* extracts than Gram-negative bacteria. Our results showed that the antimicrobial effect against all tested bacteria and fungi was varied among the different extracts. *Spirulina* methanolic extract had higher antimicrobial activity than other extracts, with inhibition zones ranging from 17 to 22 mm for 10 mg/mL concentration. Results of MIC confirmed these results (Fig. 5) where *Spirulina* methanolic extract exhibited the lower MIC (1–2 mg/mL) against tested microorganisms compared to other extracts. On the other hand, the methanolic extract of *Spirulina* had higher antifungal

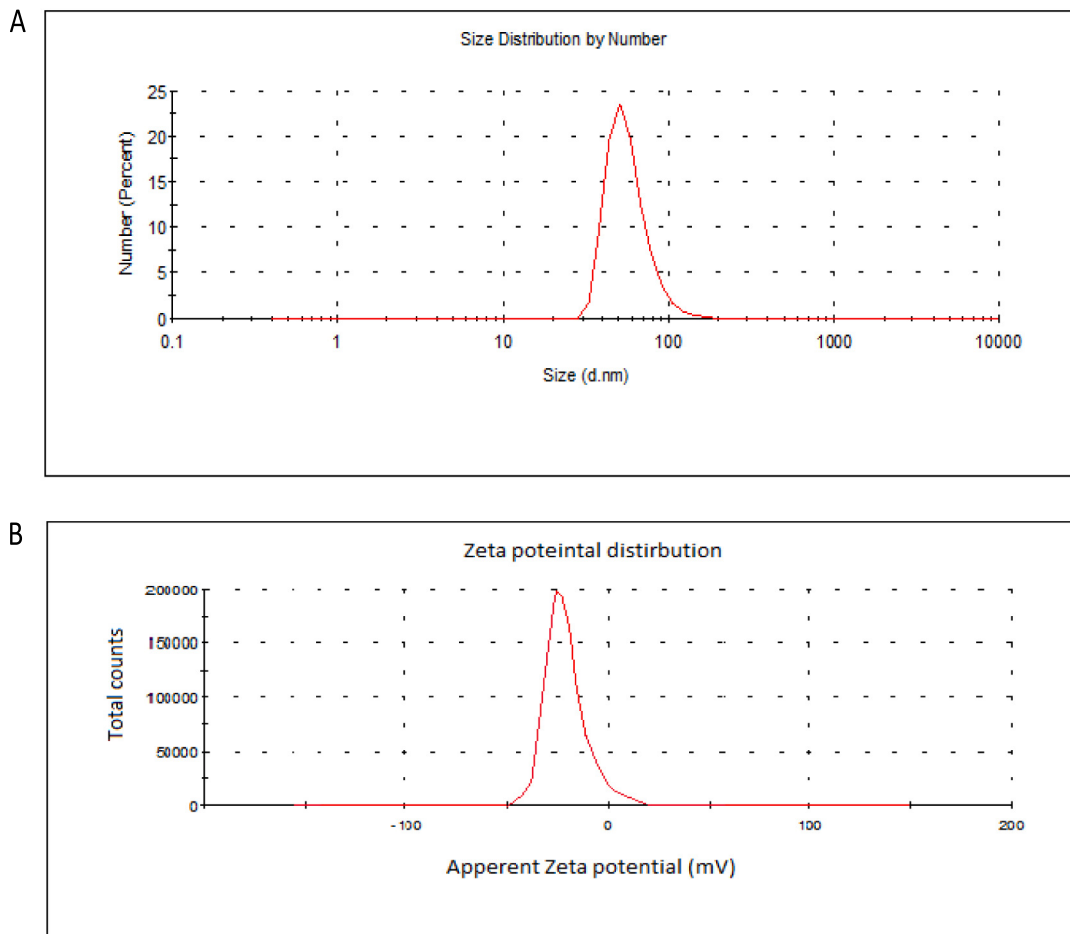


Fig. 3. Size distribution (A) and zeta potential (B) of SeNPs synthesized by *B. subtilis* AL43.

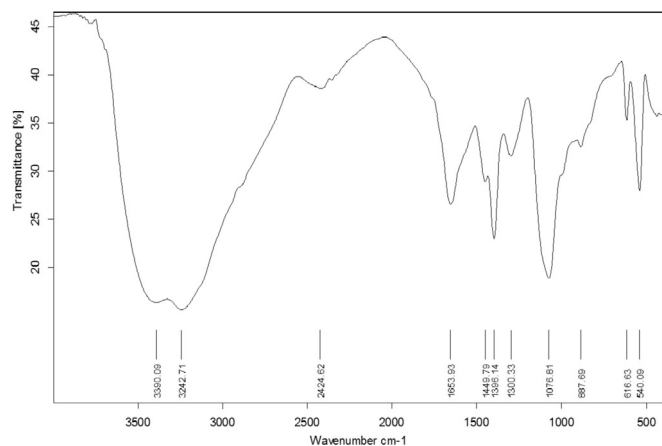


Fig. 4. FT-IR spectrum of SeNPs synthesized by *B. subtilis* AL43.

activity than other extracts with IZDs ranged from 15 to 21 mm as compared to diniconazole. Disc diffusion and MIC results showed that *Candida* sp. were more resistant to *spirulina* extracts than *Aspergillus* sp.

3.4.2. Antimicrobial activity of SeNPs

Tables 5 and 6 present the antimicrobial activity of SeNPs against three gram-positive bacteria and three gram-negative bac-

teria and six fungal isolates. The results showed that the values of IZD increased gradually in a concentration-dependent manner. *B. cereus* exhibited a higher sensitivity to the SeNPs, followed by *L. monocytogenes* and then *S. aureus*. Gram-negative bacteria showed higher resistance than gram-positive bacteria, with the lowest value of IZD for *S. typhi* (13.3–22.4 mm). Concerning the antifungal activity, SeNPs showed higher potential against *Aspergillus* spp. compared to *Candida* spp. The results of IZD showed that *A. niger* had a higher value (27.5 mm), and *C. glabrata* had a lower value (9.9 mm). Further analysis using the MIC test (Fig. 6A) showed significant differences among the tested microbes. The lower MIC value (35 µg/mL) was observed with *B. cereus*, while the higher value (70 µg/mL) was detected with *S. typhi*. A significant difference was found in the MIC level of SeNPs between the fungal isolates. As shown in Fig. 6B, the MIC value of SeNPs against *A. niger* (55 µg/mL) was lower than that detected against other strains, while the higher value (90 µg/mL) was observed against *C. glabrata*.

3.5. Antioxidant activity

3.5.1. Antioxidant activity of Spirulina

The scavenging activity of *Spirulina* extracts (methanol, hexane, and acetone) was evaluated by the antioxidant assays: ABTS• and DPPH• methods (Fig. 7A). All *Spirulina* extracts showed considerable ABTS and DPPH radical scavenging activities. Among *Spirulina* extracts, the methanolic extract exhibited higher ABTS and DPPH radical inhibition (93% and 90%, respectively), followed by acetone

Table 3
Antibacterial activity of *Spirulina* extracts expressed as inhibition zones diameters (mm).

Solvents	Conc. (mg/mL)	<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>	<i>Listeria monocytogenes</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>	<i>Klebsiella pneumonia</i>
Methanol	2.5	19.0 ^{de}	18.3 ^{cd}	16.7 ^{de}	15.2 ^{de}	16.6 ^{de}	17.0 ^c
	5	20.0 ^{cd}	19.0 ^{bc}	18.0 ^{bc}	16.4 ^c	18.0 ^c	16.6 ^c
	10	22.0 ^b	20.3 ^b	19.0 ^b	18.7 ^b	19.7 ^b	19.0 ^b
Acetone	2.5	14.7 ^h	13.3 ^h	12.3 ^g	10.6 ^h	12.0 ⁱ	11.7 ^g
	5	15.6 ^h	14.0 ^{gh}	13.3 ^{fg}	12.0 ^g	13.4 ^h	12.6 ^{fg}
	10	17.0 ^g	15.0 ^{fg}	14.4 ^f	13.0 ^f	14.3 ^{gh}	13.5 ^{ef}
Hexane	2.5	17.7 ^{fg}	16.3 ^{ef}	15.6 ^e	13.7 ^f	14.6 ^{fg}	14.2 ^{de}
	5	18.7 ^{ef}	17.3 ^{de}	16.4 ^e	14.6 ^e	15.7 ^{ef}	15.0 ^d
	10	20.3 ^c	18.7 ^{cd}	17.6 ^{cd}	16.0 ^{cd}	17.0 ^{cd}	16.3 ^c
Positive control (Ciprofloxacin)	10	30.0 ^a	28.0 ^a	25.0 ^a	22.7 ^a	24.0 ^a	20.3 ^a
SEM		0.447	0.465	0.427	0.457	0.456	0.446
P-values							
Solvent		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Concentration		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Solvent × Concentration		0.927	0.966	0.939	0.151	0.852	0.503
Treatments vs. Positive control		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

SEM, standard error of means, means in the same column with different letters are significantly different

Table 4
Antifungal activity of *Spirulina* extracts expressed as inhibition zones diameters (mm).

Solvents	Conc. (mg/mL)	<i>Candida tropicalis</i>	<i>Candida albicans</i>	<i>Candida glabrata</i>	<i>Aspergillus flavus</i>	<i>Aspergillus fumigatus</i>	<i>Aspergillus niger</i>
Methanol	2.5	15.0 ^{cd}	17.0 ^{cd}	16.0 ^{de}	18.0 ^{cd}	19.0 ^{cd}	17.0 ^{cd}
	5	16.1 ^{bc}	18.0 ^{bc}	18.0 ^{bc}	19.0 ^{bc}	20.0 ^{bc}	18.0 ^{bc}
	10	17.0 ^b	19.2 ^b	18.8 ^b	20.0 ^b	21.0 ^b	19.0 ^b
Acetone	2.5	11.0 ^g	13.1 ^g	13.0 ^g	14.0 ^g	15.0 ^g	13.0 ^g
	5	12.3 ^{fg}	14.3 ^{fg}	14.5 ^{fg}	15.4 ^{fg}	16.3 ^{fg}	14.3 ^{fg}
	10	13.2 ^{ef}	15.6 ^{ef}	15.0 ^{ef}	16.0 ^{ef}	17.0 ^{ef}	15.0 ^{ef}
Hexane	2.5	13.1 ^{ef}	15.2 ^{ef}	15.4 ^{ef}	16.0 ^{ef}	17.0 ^{ef}	15.0 ^{ef}
	5	14.4 ^{de}	16.4 ^{de}	16.3 ^{de}	17.3 ^{de}	18.3 ^{de}	16.3 ^{de}
	10	15.0 ^{cd}	17.0 ^{cd}	17.7 ^{cd}	18.0 ^{cd}	19.0 ^{cd}	17.0 ^{cd}
Positive control (Diniconazole)	10	20.3 ^a	22.3 ^a	23.4 ^a	25.3 ^a	28.3 ^a	23.3 ^a
SEM		0.381	0.273	0.136	0.564	0.431	0.412
P-values							
Solvent		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Concentration		0.001	0.001	<0.001	0.001	0.001	0.001
Solvent × Concentration		0.996	0.996	0.871	0.996	0.802	0.374
Treatments vs. Positive control		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

SEM, standard error of means, means in the same column with different letters are significantly different.

extract (88% and 82%, respectively), and hexane extract (60% and 55%, respectively), compared to the standard compound (96% and 94%, respectively).

3.5.2. Antioxidant activity of SeNPs

ABTS* and DPPH* methods were applied to evaluate the antioxidant activity of SeNPs concentrations (100, 200, 300, 400, and 500 µg/mL) (Fig. 7B). SeNPs significantly scavenged the ABTS and DPPH radicals. The results showed that the antioxidant activity of SeNPs increased in a concentration-dependent manner. The standard compound showed the highest scavenging activity against ABTS and DPPH radicals (96% and 93%, respectively). The level of 500 µg/mL showed higher ABTS and DPPH radical inhibition (92% and 89%, respectively) compared to the other tested concentrations.

4. Discussion

4.1. Antimicrobial activity of *Spirulina*

Spirulina has long been used as a functional additive in a number of animal feeds and health food. Therefore, the commercial production of *Spirulina* has gained importance worldwide due to

its multiple benefits. The antimicrobial activity of *Spirulina* has long been a question of great interest in a wide range of fields during the last decades. *Spirulina* can suppress several microorganisms' growth due to its rich content of bioactive ingredients with antimicrobial activity. An objective of this study was to investigate the antimicrobial activity of *Spirulina* against three gram-positive, three gram-negative bacteria, and three strains from both *Candida* spp. and *Aspergillus* spp. The present study results revealed that *Spirulina* extracts had a higher potential to inhibit gram-positive bacteria's growth than gram-negative bacteria. This effect may be attributed to the complicated structure of the cell wall (the outer membrane) of gram-negative bacteria (Breijyeh et al., 2020).

Elshouny et al. reported that *Spirulina* had stronger antimicrobial activity against *S. aureus*, *E. coli*, *P. aeruginosa*, *Salmonella* spp., and *Shigella* spp. than *Chlorella vulgaris*, *Saragassum wightii*, and *Saragassum latifolium* (Elshouny et al., 2021). Additionally, *Spirulina* methanolic extract was the most effective against tested microorganisms (Gheda and Ismail, 2020). The strong antimicrobial activity of methanolic extract may be attributed to its high total phenolic content. It has been reported that pathogens colonize humans and animals gut with the same mechanism of adhesion and invasion, and the antimicrobial activity of *Spirulina* might be attributed to its potential to disrupt attachment and invasion,

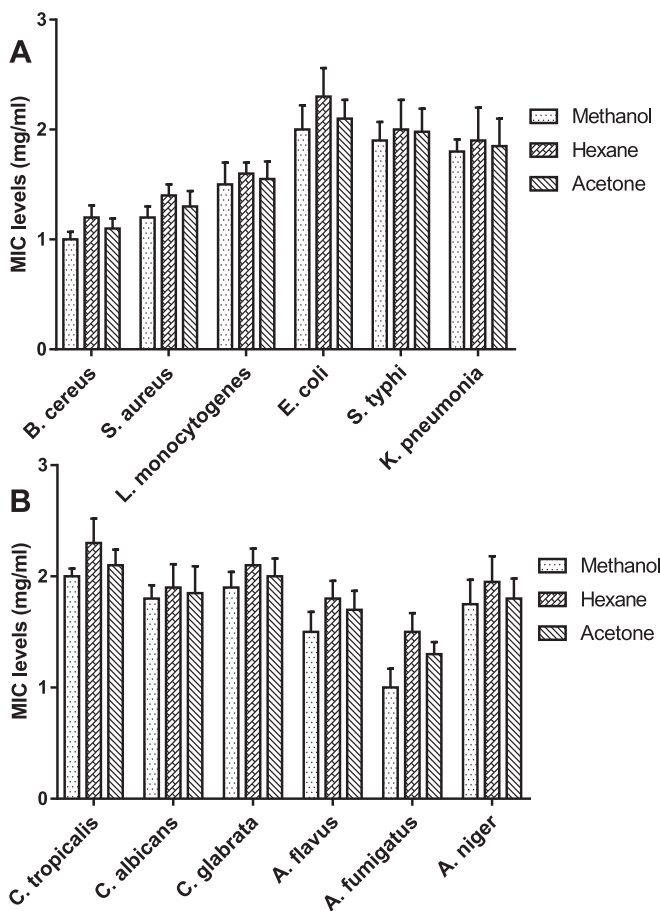


Fig. 5. MIC levels of *Spirulina* extracts (methanol, hexane, and acetone) against (A) tested bacteria, and (B) tested fungi, data are presented as mean ± SE.

motility, biofilm formation, and quorum sensing of pathogens (Abd El-Hack et al., 2019; Abd El-Hack et al., 2020c; Abd El-Hack et al., 2020d; Abdel-Moneim et al., 2020b; Abou-Kassem et al., 2021a; Saleh et al., 2021). The bioactive compounds in *Spirulina* can impair bacterial cell integrity and increase cell permeability, which leads to cytoplasmic content leakage. Cultures of *Campylobacter jejuni* treated with some plant-derived compounds showed a reduction in the activity of the autoinducer AI-2, swarm motility and biofilm formation (90% and 35–75%, respectively) (Castillo et al., 2014).

4.2. Antimicrobial activity of SeNPs

The application of metal-based antimicrobial strategies and nanoparticles presents one of the extremely promising approaches to prevent diseases caused by antibiotic-resistant microbes (Chudobova et al., 2014). Our results showed that SeNPs synthesized by the strain *B. subtilis* AL43 exhibited antimicrobial effect towards both gram-positive and gram-negative bacteria, and even antifungal activity against both *Candida* spp. and *Aspergillus* spp. The antimicrobial activity of biologically and chemically synthesized SeNPs was evaluated before, but with different methodologies and particle sizes (Cremonini et al., 2016; Tran and Webster, 2011; Zonaro et al., 2015). Nevertheless, the biogenic SeNPs showed stronger antimicrobial activity than the chemically synthesized SeNPs (Cremonini et al., 2016). Furthermore, SeNPs were found to have twice as much IZD against *Staphylococcus aureus* as silver nanoparticles (7 and 3 mm, respectively) (Chudobova et al., 2014). It has been reported that the antimicrobial effect of SeNPs exhibits size-dependent responses (Zonaro et al., 2015). The small size of nanoparticles results in increasing surface-to-volume ratio, which improves the biological reactivity of the nanoparticles.

These results suggest a probable mechanism of antimicrobial activity of SeNPs involves the generation of reactive oxygen species (ROS) (Galić et al., 2020; Tiwari et al., 2018), penetration of the nanoparticles into the cell, and disruption of cell survival pathways. Nanomaterial-induced ROS plays a fundamental role in cellular toxicity and apoptosis. SeNPs could interact with DNA and

Table 5
Antibacterial activity of biogenic selenium nanoparticles (SeNPs) expressed as inhibition zones diameters (mm).

SeNPs (µg/mL)	<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>	<i>Listeria monocytogenes</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>	<i>Klebsiella pneumonia</i>
100	20.1 ^f	18.2 ^{ef}	19.8 ^f	15.2 ^{de}	13.3 ^e	16.8 ^{de}
200	23.4 ^e	20.4 ^e	22.3 ^e	17.5 ^d	16.8 ^d	19.6 ^d
300	25.7 ^d	23.5 ^{cd}	24.3 ^d	20.0 ^c	19.4 ^c	21.5 ^c
400	28.1 ^c	25.0 ^c	27.5 ^c	21.2 ^c	20.3 ^c	22.7 ^c
500	31.5 ^b	27.6 ^b	30.8 ^b	23.7 ^b	22.4 ^b	24.2 ^b
Positive control (Ciprofloxacin, 20 mg/mL)	37.2 ^a	33.1 ^a	35.4 ^a	28.3 ^a	26.0 ^a	29.2 ^a
SEM	0.532	0.325	0.223	0.323	0.411	0.316
P-values	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

SEM, standard error of means, means in the same column with different letters are significantly different.

Table 6
Antifungal activity of biogenic selenium nanoparticles (SeNPs) expressed as inhibition zones diameters (mm).

SeNPs (µg/mL)	<i>Candidatropicalis</i>	<i>Candida albicans</i>	<i>Candida glabrata</i>	<i>Aspergillus flavus</i>	<i>Aspergillusfumigatus</i>	<i>Aspergillusniger</i>
100	11.8 ^f	13.1 ^f	9.9 ^f	14.4 ^f	12.8 ^e	15.6 ^f
200	13.6 ^e	15.5 ^e	11.6 ^{de}	16.7 ^e	15.9 ^{de}	18.3 ^e
300	15.4 ^d	17.2 ^d	13.2 ^d	18.5 ^d	17.7 ^d	20.8 ^d
400	18.3 ^c	19.5 ^{bc}	17.8 ^{bc}	21.2 ^c	19.9 ^c	23.5 ^c
500	21.4 ^b	20.9 ^b	19.3 ^b	23.2 ^b	22.7 ^b	27.5 ^b
Positive control (Diniconazole, 20 mg/mL)	28.8 ^a	27.9 ^a	25.1 ^a	29.1 ^a	27.4 ^a	33.6 ^a
SEM	0.257	0.316	0.389	0.276	0.311	0.226
P-values	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

SEM, standard error of means, means in the same column with different letters are significantly different.

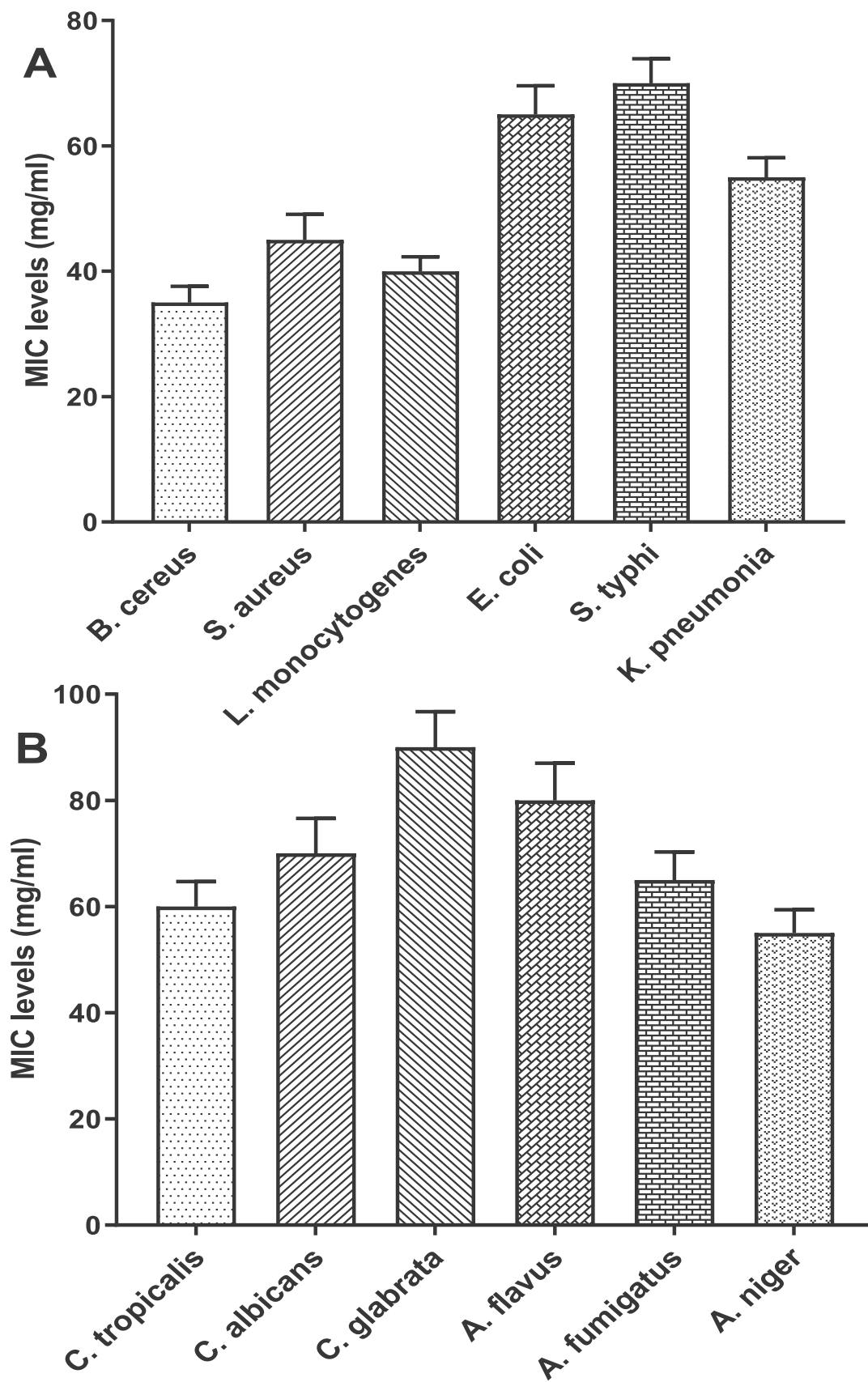


Fig. 6. MIC levels of biogenic selenium nanoparticles (SeNPs) against (A) tested bacteria, and (B) tested fungi, data are presented as mean ± SE.

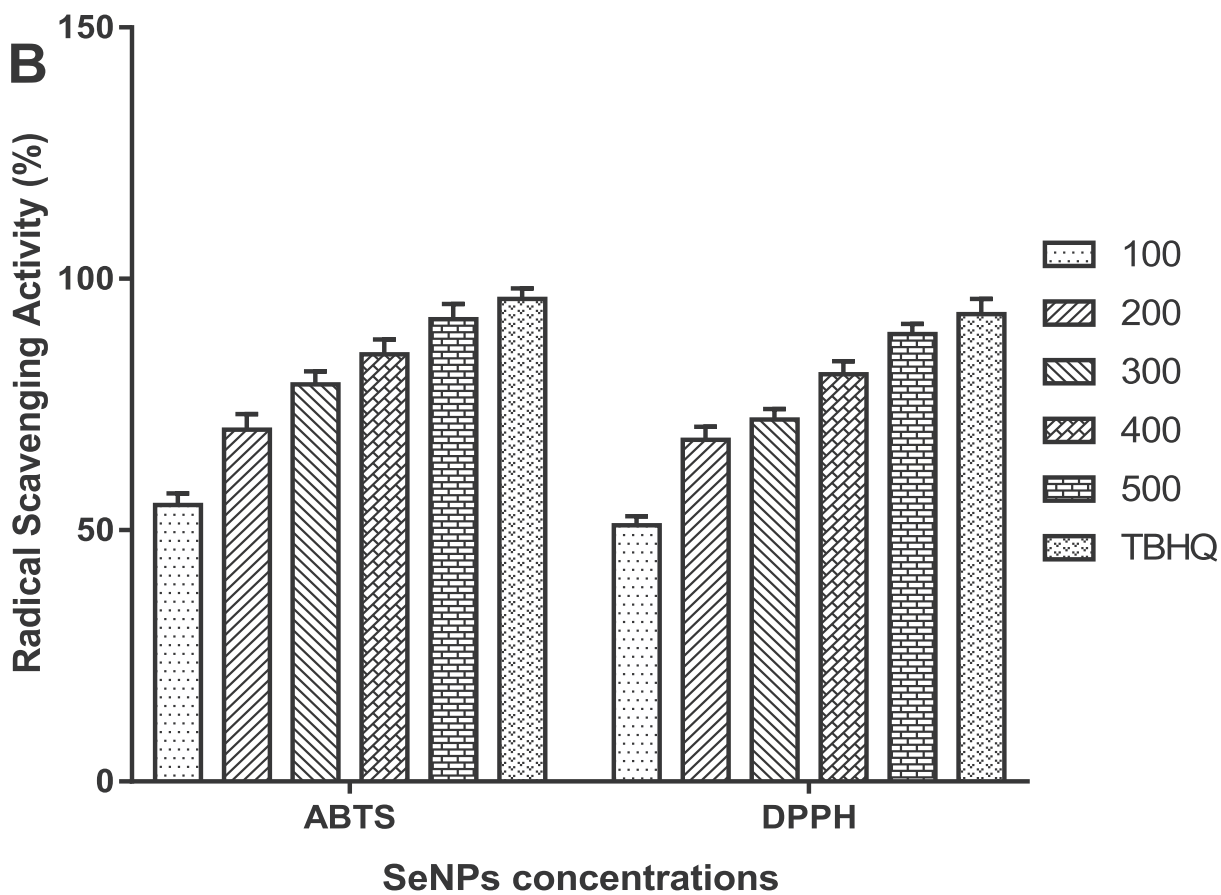
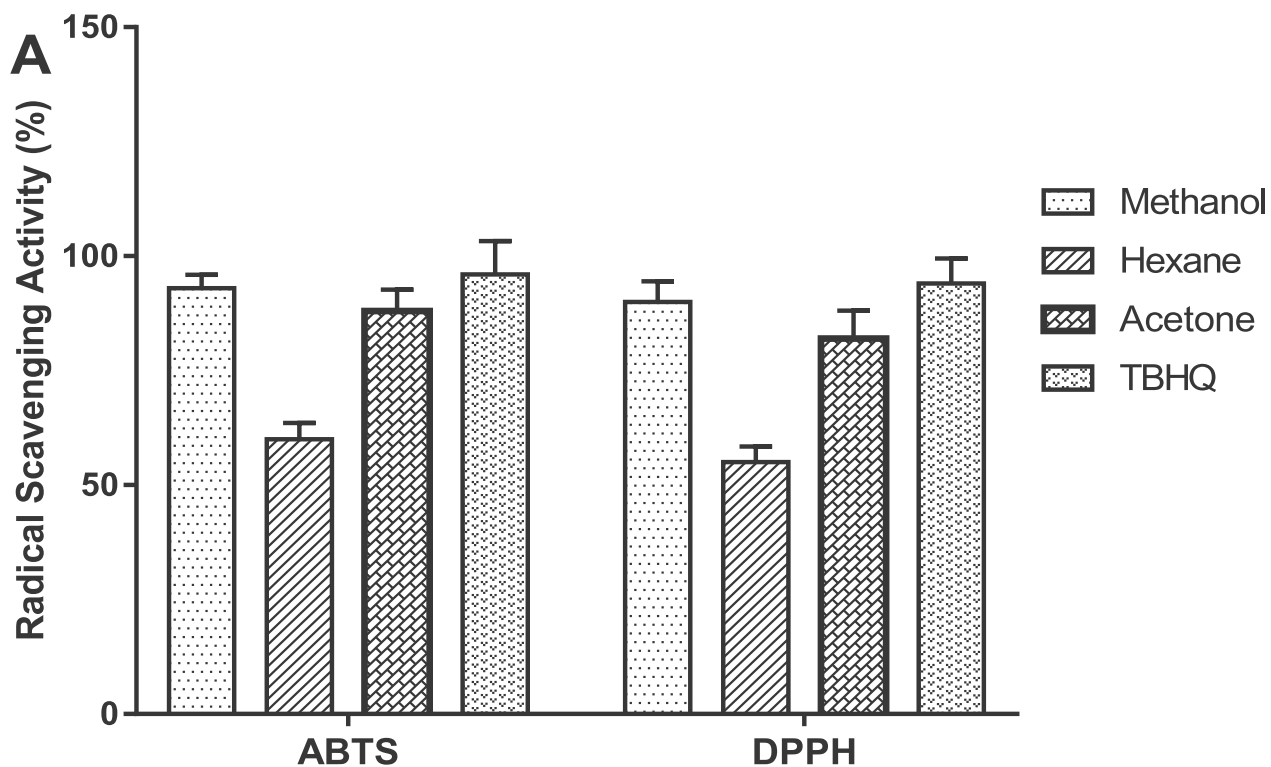


Fig. 7. Scavenging activity of (A) *Spirulina* extracts and (B) biogenic selenium nanoparticles (SeNPs, µg/mL) against ABTS* and DPPH* radicals at room temperature, data are presented as mean ± SE.

impair zntR gene amplified from bacteria (Chudobova et al., 2014). However, the low cytotoxic effect of biogenic SeNPs has been reported (Abbas et al., 2021; Forootanfar et al., 2014). Generation of ROS elevates oxidative DNA damage and membrane lipid peroxidation and subsequently increases cytoplasmic content leakage and damaging cell wall. Estevez et al. found that SeNPs exhibited antimicrobial activity against *Mycobacterium tuberculosis* via impairing their cell envelope integrity (Estevez et al., 2020). Furthermore, the antimicrobial activity of SeNPs appears to be linked to the nanoparticles and the organic cap surrounding biogenic nanoparticles (Cremonini et al., 2016). Several studies demonstrated that the protein could bind to the SeNPs surface, either through free cysteine or amine group in protein, and act as a capping agent for stabilization (El-Saadony et al., 2020a). Similar results reported that bioactive compounds, e.g., phenolics, can attach to the surface of metal nanoparticles (Cheng et al., 2017; Xu et al., 2018).

4.3. Antioxidant activity of *Spirulina*

Besides the high nutritional value of *Spirulina*, several studies provided robust evidence for its potential therapeutic applications. *Spirulina* contains distinctive natural antioxidants, such as polyphenols, carotenoids, and phycocyanin (Abdel-Moneim et al., 2021b; Estrada et al., 2001; Park et al., 2018). In the current study, the antioxidant activity of different *Spirulina* extracts (methanol, hexane, and acetone) was evaluated by ABTS and DPPH assays. Results of the current study revealed that all *spirulina* extracts have a strong antioxidant effect against ABTS and DDPH radicals. This scavenging activity of *Spirulina* is associated with the rich content of bioactive compounds with multiple biological activities. The values of the total phenolic content of *Spirulina* extracts were associated with the relative polarity of each extract. The more polar extract (methanol) had higher total phenolic content, followed by acetone and then hexane (Akkari et al., 2016). A previous study observed a positive association between the bioactive substances content and DPPH and ABTS radical scavenging activity (Park et al., 2018). These bioactive compounds have the potential to scavenge single oxygen, superoxide, and hydroxyl radicals (Abd El-Hack et al., 2019; Abo Ghanima et al., 2020; Aladaileh et al., 2020; Attia et al., 2020; Elbaz et al., 2021; Mesalam et al., 2021; Naiel et al., 2020; Yaqoob et al., 2021). *Spirulina* methanol extract exhibited the highest antioxidant activity compared to other tested extracts, which may be attributed to the high concentration of the bioactive substances in the methanolic extract. A recent study demonstrated that *Spirulina* methanolic extract had stronger antioxidant activity compared to ethyl acetate, hexane extracts (Gheda and Ismail, 2020). The strong antioxidant effect of methanolic extract appears to be associated with its high total phenolic content. Several previous studies (Abd El-Hack et al., 2019; Abd El-Hack et al., 2020a; Abd El-Moneim and Sabic, 2019; Abd El-Moneim et al., 2019; Abdel-Moneim et al., 2020c; Elbaz et al., 2021) proposed synergetic effect between the bioactive compounds, particularly polyphenols, flavonoids, pigments, polysaccharides, chlorophyll, and polyunsaturated fatty acids. Despite the strong antioxidant activity of polyphenols and flavonoids, the total carotenoid content showed a higher positive correlation with the antioxidant activity than the total phenolic and flavonoid content in some extracts of *Spirulina* products (Park et al., 2018). Besides, *Spirulina* act as a probiotic (Abdel-Moneim et al., 2021b; Bhowmik et al., 2009), and the antioxidant effect of probiotics has been well-documented (Abd El-Moneim and Sabic, 2019; Abd El-Hack et al., 2020d; Abdel-Moneim et al., 2020a; Abdel-Moneim et al., 2020b; Amaretti et al., 2013). Some bioactive metabolites from *Spirulina* have been reported to mediate the

activity of the antioxidant enzymes in a cell line model (Bermejo-Bescós et al., 2008). Furthermore, evidence from *in vivo* studies reported a positive correlation between the antioxidant activity of *Spirulina* and the anti-inflammatory effect (Abdel-Daim et al., 2016). These findings are important insights for aggregating the practical and functional importance of these natural products.

4.4. Antioxidant activity of SeNPs

Recently, there has been renewed interest in the biosynthesis of nanoparticles owing to the prospect of using them in the future to make nanomedicine. Several studies have aimed to develop new, functional, and cost-effective antioxidants with lower toxicity (Forootanfar et al., 2014). Biogenic synthesis of selenium nanoparticles by microorganisms holds significant potential to be used as an antioxidant agent due to its eco-friendly, low cytotoxicity, low cost, and does not involve organic solvents (Xu et al., 2018). In the current study, the antioxidant activity of SeNPs was investigated by ABTS and DDPH radical scavenging assays. Our results showed that SeNPs exhibited dose-dependent antioxidant activity against ABTS and DDPH radicals. It has been demonstrated that SeNPs synthesized by *Lactobacillus casei* ATCC 393 (Xu et al., 2018) and *Bacillus paralicheniformis* SR14 (Cheng et al., 2017) for *in vivo* use decreased lipid peroxidation and improved the activity of antioxidant enzymes. Besides, SeNPs synthesized by lactic acid bacteria could attenuate H₂O₂-induced oxidative injury and apoptosis of human normal epithelial cells (NCM460) (Xu et al., 2018). It was also reported that SeNPs synthesized by *Bacillus* mitigated H₂O₂-induced oxidative damage in porcine jejunum epithelial (IPEC-J2) (Cheng et al., 2017). The organic cap surrounding biogenic nanoparticles was found to play a crucial role in the potency of SeNPs to scavenge the free radicals (Cheng et al., 2017; Sheiha et al., 2020; Xu et al., 2018). Consistent with the literature, this research confirmed that enhanced antioxidant activity of SeNPs is correlated to the concentration of the total phenolic content (Akkari et al., 2016; Sheiha et al., 2020; Xu et al., 2018).

5. Conclusion

The obtained results showed that all tested *Spirulina* extracts and SeNPs concentration exhibited antimicrobial and antioxidant activities, which increased in a concentration-dependent manner. Furthermore, antimicrobial and antioxidant activities of *Spirulina* methanolic extract were observed to be the most potent. This potency of methanolic extract may be attributed to its high total phenolic content. We conclude that *Spirulina* and SeNPs may act as promising antimicrobial agents as well as natural antioxidant substitutes. Therefore, they can be utilized as alternatives to antibiotics and traditional chemical drugs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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