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Algae-mediated biosynthesis of zinc oxide nanoparticles from *Cystoseira crinite* (Fucales; Sargassaceae) and it's antimicrobial and antioxidant activities

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Abstract

Zinc oxide nanoparticles (ZnO-NPs) have gained attention due to their distinctive properties and applications. In the current study, we developed an eco-friendly, economical, and green route for the synthesis of ZnO-NPs using the aqueous extract of the brown marine alga *Cystoseira crinita* (Fucales; Sargassaceae). ZnO-NPs were characterized by UV-Vis spectroscopy, transmission electron microscope (TEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and dynamic light scattering (DLS). The antimicrobial and antioxidant activities were investigated. The UV absorption peaks at 268 nm and 360 nm confirm the formation of ZnO-NPs., and XRD patterns indicated the crystalline nature of ZnO-NPs. The TEM revealed that the size of ZnO-NPs was ranged between 23 to 200 nm corresponding to DLS, while the shape is multilayered rectangular particles. FTIR analysis revealed that phenolic compounds and proteins were the reducing and capping agents. ZnO-NPs showed antimicrobial activities against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Candida albicans*, and *Aspergillus niger*, furthermore, showed moderate DPPH radical-scavenging activity.

Keywords: Marine algae, *Cystoseira crinita*, Green synthesis, Zinc oxide nanoparticles, Antimicrobial activity, Antioxidant activity.

1. Introduction

Nanotechnology is a modern branch of science and technology and is one of the most revolutionary technologies in the 21st. In recent years, Nanotechnology has received considerable attention due to dealing with particles less than 100 nm in size at one or more of their three dimensions, which give them unique physicochemical properties, these properties lead to significant changes in mechanical properties, melting point, optical absorption, thermal and electrical conductivity, in addition to biological and catalytic activity [1- 3]. Zinc oxide is one of the semiconductors and is characterized by a wide direct bandgap of about 3.37 eV [4] and a high exciton binding energy of 60 meV [5]. ZnO-NPs have gained much attention by many scientists worldwide due to several satisfactory properties such as high electron mobility, good transparency, high room temperature luminescence, chemical, and thermal stability even under harsh processing conditions, in addition to biocompatibility and ease of fabrication [6- 8]. Due to its distinctive properties, ZnO-NPs is broadly used for numerous applications such as drug delivery and destroying of tumor cells [9, 10], antibacterial and antifungal activity [11, 12], Antioxidant [13], medical textile anti-biofilm [14], and anti-diabetic activities [15], and due to strong UV absorption properties of ZnO, it is increasingly used in personal care products, such as cosmetics and sunscreen [16].

Several physical and chemical techniques were developed for the synthesis of ZnO-NPs, such as physical vapor deposition [17], laser pyrolysis [18], high energy ball milling [19], chemical vapor

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synthesis [20], polyol synthesis [21], and sol-gel method [22]. However, these methods are expensive, give low yield, poor size distribution, and use dangerous chemicals. Biosynthesis of nanoparticles is a sustainable eco-friendly, economical, high-yielding, expeditious, and energy-efficient approach [23]. Currently, scientists have focused their attention on the biosynthesis of nanoparticles based on plants [11], bacteria [24], fungi [14], and enzymes [25]. Among all living organisms, algae are called nano-factories because both living and dead-dried biomass were used for the synthesis of various types of nanoparticles including ZnO NPs., Previous studies demonstrated the efficacy of using microalgae [26-29] and macroalgae [30-32]. The use of biological sources such as marine brown macroalgae for the synthesis of ZnO-NPs has numerous benefits for many applications. The genus *Cystoseira* (Fucales; Sargassaceae) contains a wide variety of metabolites such as lipids, protein, carbohydrates, terpenoids, steroids, phlorotannins, phenolic compounds, pigments, and vitamins [33, 34], these biomolecules act as reducing agents, capping agents, and stabilizers for nanoparticles [35].

Due to increasing antibiotic resistance, many attempts to found alternatives approaches for destroying resistant microbes are needed. The higher surface-to-volume ratio of phyco-synthesized ZnO-NPs deserved it for the antimicrobial activities. Many studies have reported, green synthesized ZnO-NPs can be used to restrain antibiotic-resistant bacteria and fungi [13, 32, 36- 40]. ZnO-NPs also play a vital role as antioxidant materials for free radicals produced from the metabolic process [41- 43].

The present study aimed to phyco-synthesis of ZnO-NPs from the brown alga *C. crinita*, as well as the characterization of the synthesized ZnO-NPs, and evaluation of their activity as antimicrobial and antioxidant.

2. Experimental Section

2.1. Materials

Zinc sulfate ($ZnSO_4$) was used as the zinc precursor, NaOH, Mueller Hinton Agar (MHA), sabouraud dextrose agar, Antibiotic discs, and other reagents used were of analytical grade obtained from Sigma-Aldrich. The biomass of marine brown macroalgae *C. crinita* was collected from Hurghada, Red Sea coast, Egypt.

2.2. Algal extract preparation

The thallus of *C. crinita* (Fig. 1A) was washed with tap water followed by distilled water to remove the epiphytes and salt minerals, then dried and ground into fine powder. 10 g of algal powder was mixed with 100 ml of distilled water and heated at 60 °C for 30 min using a magnetic stirrer and left on shaking overnight. The extract was well filtered then cooled and stored for the biosynthesis process.

2.3. Biosynthesis of ZnO-NPs:

ZnO-NPs were prepared according to Lu et al. [44] with modification. Algal extract (5 ml) was added to a 50 ml flask and consequently, 15 ml of zinc precursor ($ZnSO_4$, 0.05 M) was added, the pH of the reaction mixture was adjusted to 6.5 by dropwise of 1M NaOH with continuous stirring for 30 min at temperature 45 °C. The synthesis of ZnO-NPs was inferred by the change of the reaction mixture color into cloudy white. The reaction product was centrifuged at 3000 rpm for 15 min, and the resulting white precipitate was washed with deionized water five times to remove any unreacted solutes and phytochemicals. Finally, the white precipitate was oven-dried at 70 °C for 48 h.

2.4. Characterization of phyco-synthesized nanoparticles

It was characterized by several techniques. The optical property was confirmed by measuring the wavelength of the reaction mixture in the UV-Vis spectrum (UV-2100, UNICO, USA) spectrophotometer (from 200 to 1000 nm). The Morphological characterization and size of the samples were done using transmission electron microscope (TEM) JEOL JEM- 2100 electron microscope, Japan. The size distribution and average size of phyco-synthesized nanoparticles were determined by dynamic light scattering (DLS) using Zeta sizer nano series (Nano ZS, Malvern, UK). X-ray diffraction analysis was performed to determine the crystallinity and elemental composition of the structure of phyco-synthesized nanoparticles using Bruker AXS X-ray diffractometer with Cu-Kα 54056 A˚, radiation source in the 2θ range of 10-80° at 40 kV and 40 mA (D8-ADVANCE, Germany). Finally, Fourier Transform Infrared spectrometer (FTIR) was used to detect the possible functional groups of biomolecules in *C. crinita* extract which is responsible for synthesis and capping of phyco-

synthesized nanoparticles agent. This screening was done in the range of 4000 - 400 cm $^{-1}$ using FTIR spectroscopy (VERTEX 70 Spectroscopy, Japan).,

2.5. Antimicrobial activity of ZnO-NPs

Clinical isolates of both bacteria and fungi were used to evaluate the antimicrobial activity of synthesized ZnO-NPs using the agar well diffusion method. The antimicrobial activity was tested against Gram-positive (*Staphylococcus aureus* and *Bacillus cereus*), Gram-negative (*Escherichia coli* and *Salmonella typhi*), and fungi (*Candida albicans* and *Aspergillus niger*). The microbial strains were swabbed uniformly onto individual Mueller Hinton agar (bacteria) and sabouraud dextrose agar (fungi). In each plate, wells were cut out using a standard cork borer (7 mm diameter). Using a micropipette, 100 µL of ZnO-NPs (10 mg mL-1) was added to each well. After incubation at 37 °C for 24 h for bacteria and 28°C for 72h for fungi. Bacitracin served as a positive (+ve) control for bacteria and Flucytosine for fungi, and sterile distilled water (SDW) was used as a negative (-ve) control, To determine the minimum inhibitory concentration of ZnO-NPs (MIC), the dilutions of phyco-synthesized nanoparticles were prepared at the concentrations of 39.06, 78.12, 156.25, 312.5, 625, 1250, 2500, 5000, and 10000 µg mL^{-1} the diameters of the inhibition zone were measured in mm [45].

2.6. Antioxidant Activity

The free radicals scavenging activity of phyco-synthesized nanoparticles was evaluated by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method [46]. DPPH solution (0.1 mM) was prepared using 95 % ethanol and reacted with different concentrations of ZnO-NPs (3.9, 7.812, 15.625, 31.25, 62.5, 125, 250, 500, 1000 µg mL^{-1}) was prepared and mixed with DPPH solution. The mixtures were shaken and kept at room temperature (~25 °C) for 30 min in darkness. After incubation, centrifugation was carried out for 5 min and absorbance was estimated at 517 nm with the control samples (DPPH solution without sample), and ascorbic acid was used as standard material. Antioxidant activity of ZnO-NPs and standard was calculated as DPPH scavenging activity (%) as follow:

DPPH scavenging activity (%) = (Absorbance of control – Absorbance of the sample)/ (Absorbance of control) ×100

3. Results and discussion

3.1. Synthesis of ZnO nanoparticles

In this study, ZnO-NPs were biosynthesized using the aqueous extract of *C. crinite* (brown color solution) and zinc sulfate solution as a metal precursor (colorless solution) The phyco-synthesis of ZnO nanoparticles was verified visually by the change of previously mentioned colors into cloudy-white color at the end of the reaction (Fig. 1B-D).

Fig. 1. Visual observation of ZnO-NPs.
[(A) Macroalgae *C. crinita*, (B) aqueous extract, (C) Zinc sulfate solution, and (D) Reaction mixture].

3.2. Characterization of phyco-synthesized nanoparticles

3. 2.1. *UV–Vis absorption spectroscopy*

UV-visible spectroscopy is an essential technique to confirm the formation and stability of metal nanoparticles in an aqueous solution [47]. The formation of ZnO-NPs was confirmed by using the UV–Vis spectrum which showed an emission peak at 360 nm while the excitonic absorption peak is found at about 268 nm as shown in Figure (2). Talam et al. [48] was reported that the absorption spectrum of prepared ZnO nanopowder exhibits a strong absorption band at about 355 nm and excitonic absorption peak at about 258 nm due to the ZnO nanoparticles which lie much below the bandgap wavelength of 358 nm. Also, Mekky et al. [49] reported the ZnO-NPs showed strong absorption bands at 265 and 370 nm in the ultraviolet region, indicating the formation of ZnO-NPs. The presence of bands between 230–390 nm is an indication of ZnO-NPs formation as reported by several recent studies [44, 50- 52].

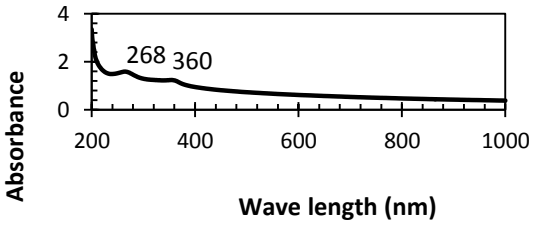

Fig.2. Fig. 2. UV-Vis spectrum of ZnO-NPs synthesized by *C. crinita*.

3. 2.2. *TEM micrographs analysis*

The size and morphological properties of the phyco-synthesized ZnO-NPs were investigated by transmission electron microscope (TEM). The size of ZnO-NPs has ranged between 23 nm to 140 nm, while the shape is multilayered rectangular particles without agglomeration (Fig. 3). As far as we know, ZnO-NPs have never been synthesized in the form that was produced in this study. This might be related to the biochemical properties of *C. crinita*. Aziz et al., [30] reported that aqueous extract of *Sargassum muticum* involved in the formation of ZnO- NPs with hexagonal shape and size ranges from 3 nm to 57 nm with an average size of 42 nm.

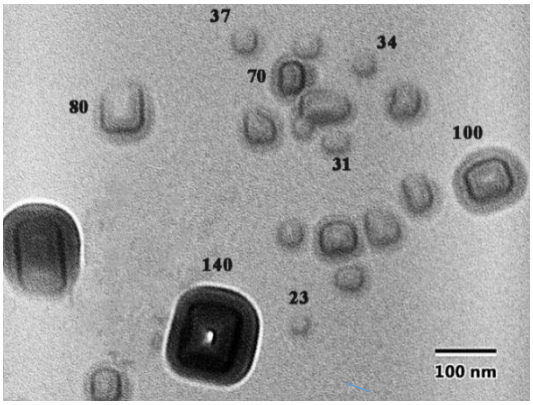

Fig.3. TEM image of *C. crinita*-mediated synthesized ZnO-NPs.

3.2.3. *X-ray diffraction (XRD):*

The principle of XRD is that, X-rays are passed through a nanoparticle-producing pattern which gives information about size and shape [53, 54] The XRD analysis of ZnO-NPs synthesized by *Cystoseira crinita* detected characteristics peaks at 2θ values of 31.78, 34.42, 36.26, 47.6, 56.6, 62. 8 and 68 were assigned to (100), (002), (101), (102), (110), (103), and (112) planes, respectively that also well consistent with the Joint Committee on Powder Diffraction Standards, (JCPDF Card No.00-005-0664), Figure (4).

It is important to note that the peak at 20- 30 2θ could indicate the presence of impurities in the reaction mixture. All the recorded peak intensity profiles were characteristic of the nanoparticles' hexagonal wurtzite structure that gives evidence of the well-crystallized structure of phyco-synthesized ZnO-NPs. According to Debey-Scherrer equation $D = k\lambda / \beta \cos \theta$, where D is particle size (nm), k is a constant equal to 0.94, λ is the wavelength of X-ray source (0.1541 nm), β is the full width at half maximum (FWHM) and θ is the half diffraction angle, Bragg angle (degree). The average particle size of synthesized particles was calculated to be 42.6 nm. Our result is in accordance with numerous studies that have reported similar XRD patterns of phyco-synthesized ZnO-NPs [14, 30, 51, 55].

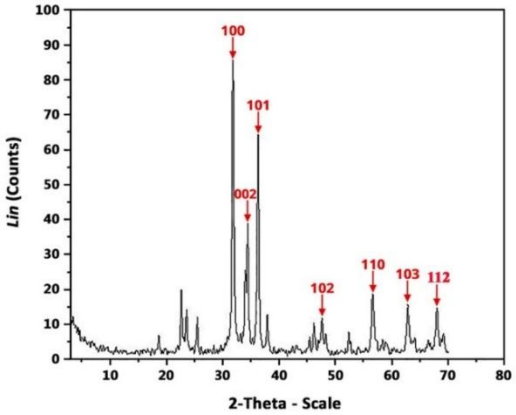

Fig. (4): XRD patterns of C. crinita-mediated synthesized ZnO-NPs.

3.2.4. *Fourier Transform Infra-Red analysis (FTIR):*

FTIR is a significant technique used for the identification and characterization of a substance i.e., the functional group responsible for the reduction and acting as a capping agent in biosynthesis nanoparticles [56]. Concerning *C. crinita* mediated synthesis of ZnO-NPs. The FT-IR analysis detected principal peaks at 3257, 1590, 1382, 1036, 693, 598, and 520, cm^{-1} as shown in Fig. (5). The peak at 3257 cm^{-1} was attributed to the OH stretching H-bonded alcohols and phenols, the band at 1590 cm^{-1} is attributed to the stretching vibration of (NH) C=O group that are characteristic of proteins. A peak at 1382 cm−1 can be attributed to the CH-aliphatic. Another band at 1036 cm^{-1} could be assigned to C-N stretching vibrations of aromatic and aliphatic amines. The peaks lower than 1000 cm^{-1} (693, 598, and 520) were attributed to characteristic absorption peaks of the Zn-O bond. The data of FT-IR analysis exhibit the role of phenolic compounds and proteins in *C. crinita* extract in the reduction, capping, and stabilizations of biosynthesized ZnO-NPs as revealed in Fig. (6). Reported similar investigations have shown that metal oxides give absorption bands in the fingerprint regions below 1000 cm^{-1} arising from inter-atomic vibrations [11, 30].

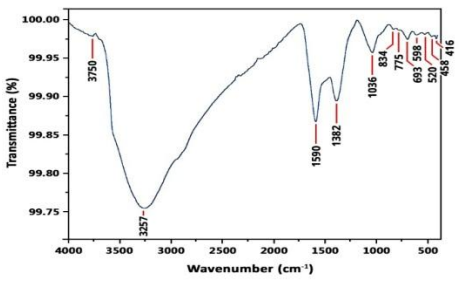

Fig. 5. FTIR spectrum of *C. crinita*-mediated synthesized ZnO-NPs.

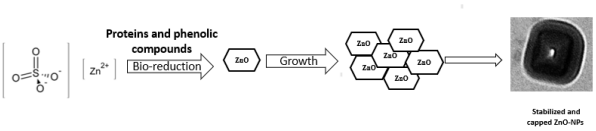

Fig. 6. The The suggested mechanism of *C. crinita*-mediated synthesized ZnO-NPs.

3.2.5. *Dynamic light scattering (DLS), particle size analysis*

DLS is a technique used to determine values of the size, size distribution, and polydispersity index of particles in a colloidal suspension. Figure (6) shows the graphical illustration of the average particle size distribution of ZnO-NPs suspension. The size ranged from 14 to 200 nm. The highest fraction of ZnO-NPs present in the solution was found to be 36.2 nm. From the graph, it was obvious that the ZnO-NPs suspension having various sizes which are in good agreement with the result obtained by TEM analysis. The difference in the size of ZnO-NPs may be attributed to the presence of proteins, enzymes, and carbohydrates that are involved in the formation and capping of nanoparticles using different mechanisms [57].

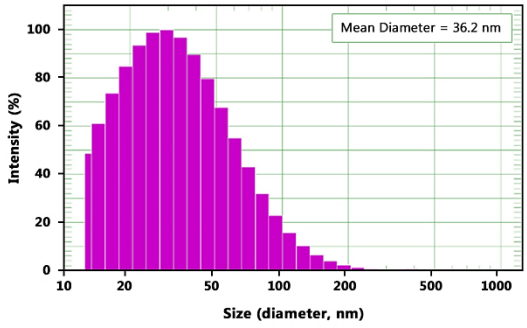

Fig. 7. DLS graph of *C. crinita*-mediated synthesized ZnO-NPs.

3.2. In vitro antimicrobial activity

The antimicrobial activity of the phyco-synthesized ZnO-NPs was examined against two gram-positive bacteria, two gram-negative bacteria, and two fungal strains (Fig. 7). According to the data listed in Table 1, phyco-synthesized ZnO-NPs were exhibited a considerable antimicrobial activity against all tested strains. Generally, the diameter of the inhibition zone was ranged from 22.33±0.33 in the case of *Candida albicans* to 31±0.57mm in the case of *Aspergillus niger*. On the other side, all tested bacteria did not see any susceptibility toward Bacitracin. Flucytosine inhibited the growth of multicellular fungus *Aspergillus niger* by inhibition zone diameter of 29.66±0.66 mm but it was not affected the growth of *Candida albicans*. The different concentrations of ZnO-NPs were confirmed the antimicrobial activity and it was found dose-dependent. The MIC for *Bacillus cereus, Staphylococcus aureus, Escherichia coli, and Candida albicans* was 312.5µg/mL whereas the MIC for *Salmonella typhi* and *Aspergillus niger* was 156.25 and 1250 µg/mL, respectively.

The inhibitory effect of ZnO-NPs synthesized by *C. crinita* may arise from their large surface area to volume ratio [2], or it could be the positive charge of ZnO-NPs which interacts with the negatively charged microbial membrane, which affects cell membrane permeability and intracellular protein leakage, leading to growth inhibition [58- 61]. Moreover, NPs inhibit bacterial growth through inhibition of cell proteins by fusing with thiol (-SH) groups [62]. Also, possibly due to its interaction with the phosphorous group in DNA, which leads to inhibition of DNA replication and thus inhibition of protein synthesis [63, 64]. In general, NPs may affect biochemical processes through the generation of reactive oxygen species (ROS) that lead to membrane destruction, damage to
cellular components such as lipids, DNA, and proteins, and eventually, lead to growth inhibition [65].

Table 1. Zone of inhibition and MIC of ZnO-NPs against pathogenic microbial strains.

	Zone of inhibition (mm)			
Microbial strain	ZnO-NPs	Positive control	Negative control	MIC (µg mL^{1})
Bacillus cereus	26.0 ±0.57	0	0	312.5
Staphylococcus aureus	27.3 ±0.33	0	0	312.5
Escherichia coli	24.3 ±0.33	0	0	312.5
Salmonella typhi	23.6 ±0.88	0	0	156.3
Candida albicans	22.3 ±0.33	0	0	312.5
Aspergillus niger	31.0 ±0.57	29.7 ±0.66	0	1250

3.3. Antioxidant activity

The DPPH method was used to estimate the antioxidant activity of phyco-synthesized ZnO-NPs. As shown in Figure 8, ZnO-NPs exhibited moderate DPPH radical-scavenging activity, inferred from the reduction of purple colour to yellow colour, which was determined by the decreases in absorbance of mixture at 517 nm. ZnO-NPs at a concentration of 1 mg ml^{-1} showed 67.2 % inhibition, and for 3.9 µg ml^{-}

[1] it was 5.77%. Compared to the standard, ascorbic acid at 1 mg ml-1 was found to be 95%, although at 3.9 µg ml-1 it was 21.46%. In general, the ascorbic acid showed higher radical scavenging activity as

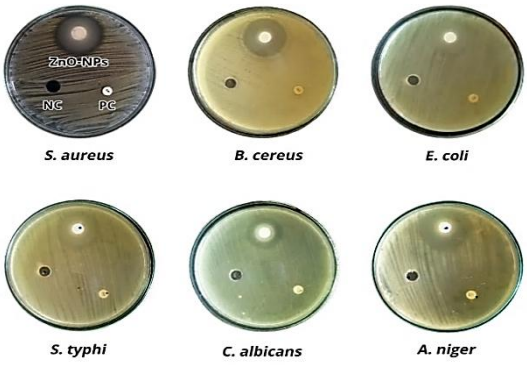

Fig. 8. Antimicrobial assay of phyco-synthesized ZnO nanoparticles, positive control (PC)and negative control (NC)

compared to ZnO-NPs, these results indicate the activity of ZnO-NPs in scavenging free radicals in a dose-dependent manner in agreement with Chandra et al. [13]. The results of phyco-synthesized ZnO-NPs were proved to be potent the DPPH free radical scavenging activity with an effective concentration of antioxidants needed to obtain a half-maximum (EC_{50}) value of 201.45 µg ml^{-1} (Fig. 8). The antioxidant activity of phyco-synthesized ZnO-NPs may be related to the polyphenolic compounds that remained on the surface of the ZnO-NPs., these bioactive components participate in donating hydrogen atoms to convert DPPH into its reduced form [66].

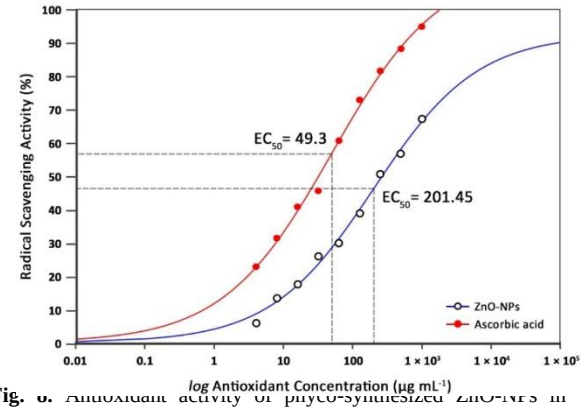

Fig. 9. Antioxidant activity of phyco-synthesized ZnO-NPs in comparison to ascorbic acid (standard).

4. Conclusion

In this study, the green synthesis of ZnO-NPs using an aqueous extract of the brown macro-alga *C. crinite* was investigated. The synthesized ZnO-NPs have explained two excitation peaks at 268 and 360 nm with TEM and XRD patterns which refer to the formation of hexagonal wurtzite structure with an average size of 42.6 nm that confirmed by DLS analysis. The phyco-synthesized ZnO-NPs showed good antimicrobial activity against (*Staphylococcus aureus and Bacillus cereus, Escherichia coli*, and *Salmonella typhi*), and fungi as (*Candida albicans* and *Aspergillus niger*) and showed a moderate DPPH radical-scavenging activity. Based on the preliminary results that we obtained in this study, it is possible to use ZnO-NPs synthesized by *C. crinite* as an alternative to commercial antibiotics, but this needs further studies which concerned with the toxicity of these ZnO-NPs and other experiments that allow their safe use.

Conflicts of interest

There are no conflicts to declare.

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