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ORIGINAL ARTICLE

Synthesis of hybrid nanoflowers using extract of *Ascoseira mirabilis*, a large brown parenchymatous macroalga endemic to the Antarctic Ocean, as the organic component and evaluation of their antimicrobial, catalytic, and antioxidant activities

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Abstract

Organic@inorganic hybrid nanoflowers (hNFs), which are widely used in enzyme purification and catalytic activity applications, include both organic and inorganic components. In this study, hNFs were synthesized with the combination of *Ascoseira mirabilis* extract and Cu in phosphate-buffered saline (PBS) while altering the concentration and medium pH instead of using expensive molecules that are difficult to obtain such as enzymes or DNA. According to the obtained FE-SEM images, the morphology of the hNFs was related to the pH of the PBS (synthesis did not occur at pH 5) and the volume of the extract. The presence of Cu and other components was detailed with EDX mapping. The presence of functional groups playing key roles in the synthesis process was evaluated based on FT-IR peaks. The Cu hNFs exhibited peroxidase-like catalytic activity against guaiacol and demonstrated antimicrobial and antioxidant activities. This study is original and innovative in terms of using an *Ascoseira mirabilis* extract for hNF synthesis and evaluating the antioxidant, catalytic, and antimicrobial activities of *Ascoseira mirabilis*-based hNFs. The research sheds light on hNF synthesis and the possibility of biological activity application studies performed with bioextracts instead of biomolecules obtained via expensive and complex processes.

Keywords Copper nanoflower · Nanoparticle · Green synthesis · Catalytic · Antimicrobial · Antioxidant

Introduction

Nanomaterials are synthesized by physical, chemical, and biological methods, and all of these methods have their own advantages and disadvantages (Ghaffari-Moghaddam et al. 2014). Flower-shaped organic@inorganic hybrid nanoflowers (hNFs) exhibit properties of accelerated reaction kinetics and carrier immobility due to their large surface areas in terms of the surface-to-volume ratio (Ghaffari-Moghaddam et al. 2014). In addition, the 3D structure of NFs provides the ability to increase the efficiency of surface reactions (Shende et al. 2018).

hNFs, as interesting forms of nanomaterials, have attracted the attention of researchers due to potential applications in the fields of catalytic activity, optoelectronics, biosensors, solar cells, drug delivery, antioxidants, antimicrobials, purification, and enzyme immobilization, among others (Shende et al. 2018; Kharisov 2008; Yin et al. 2015; Güven et al. 2022; Celik et al. 2018; Cao et al. 2018). Liu et al. (2021) demonstrated the catalytic activity of Cu-based

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hNFs synthesized with the thermophilic lipase enzyme of *Alcaligenes* sp. by hydrolysis of *p*-nitrophenyl caprylate. They reported that the immobilized enzyme could be reused for eight cycles. Gül and Ocsoy (2021) emphasized that laccase-based hNFs still exhibited effective catalytic activity against malachite green dye in the 14th cycle. It has been reported that Cu hNFs synthesized by the coordination of graphene oxide and laccase enzymes are effective in the removal of crystal violet and neutral red dyes from water. Li et al. (2017) suggested that immobilized enzymes exhibit higher levels of activity than free enzymes. In another study, it was suggested that hNFs synthesized with α-chymotrypsin enzyme and calcium coordination could be used as enzyme reactors for the highly efficient digestion of proteins (Yin et al. 2015). It was determined that NFs obtained by combining bovine serum albumin and Zn ions in phosphate-buffered saline (PBS) medium could absorb Cu ions (Zhang et al. 2016). Tran et al. (2021) suggested that DNA-based Cu NFs could be used as sensors for the detection of phenolic compounds. They also reported that the obtained DNA-NFs could catalyze neutral red dye.

However, the use of expensive biomolecules such as enzymes or DNA as organic components in the synthesis of hNFs limits their applications due to both cost and limited supply. In recent years, as an alternative to these molecules, studies have been undertaken to obtain low-cost hNFs by combining bioextracts and various metal ions and to determine their potential applications. Güven et al. (2022) reported that Cu hNFs synthesized with cherry stalk extract exhibited antimicrobial activity against *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Escherichia coli*, and *Enterococcus faecalis* strains. Researchers have noted that hNFs have catalytic and antioxidant activities. Koca et al. (2020) determined that Cu-based hNFs synthesized with allicin extract exhibited peroxidase-like activity against guaiacol. Demirbas (2021) reported that Cu-based hNFs synthesized with an orange peel extract exhibited effective antimicrobial activity against *Yersinia ruckeri*. The antimicrobial effects of Cu NFs designed with lemon peel extract against *Candida albicans*, *Staphylococcus aureus*, and *E. coli* strains were also demonstrated (Demirbas 2020). Photocatalytic activities of Ag hNFs synthesized using a *Kalanchoe daigremontiana* extract against methylene blue dye were reported and antimicrobial activities were demonstrated against *S. aureus* and *E. coli* strains (Molina et al. 2019). Kumar et al. (2021) emphasized the catalytic activities of Au hNFs with *Nephelium lappaceum* extract by reducing 4-nitrophenol to 4-aminophenol. Although there are studies such as these on the use of various plant extracts for hNF synthesis in the literature, no study was identified addressing the usage of algae extracts in synthesis.

Ascoseira mirabilis is a brown macroalga endemic to the Antarctic Ocean. In this study, Cu hNFs were synthesized using an *Ascoseira mirabilis* extract as an alternative to enzymes or DNA. The obtained hNFs were characterized by FE-SEM, EDX, and FT-IR analysis and they were observed to have antioxidant, antimicrobial, and catalytic activities. This study confirms that organic@inorganic hybrid hNFs can be synthesized inexpensively and effectively using bioextracts such as plants, algae, and fungi. These findings may guide future work in nanotechnology and multidisciplinary study areas related to this field in terms of developing ecofriendly and low-cost hNF synthesis approaches and potential applications.

Materials and methods

Synthesis of Cu hNFs

Cu hNFs were synthesized using an algal extract and their antimicrobial activities were evaluated. Dried algal samples (5 g) were held in 50 mL of distilled water at 80 °C for 1 h and the resulting extract was filtered with Whatman No. 1 filter paper and then centrifuged (10,000 rpm, 10 min). For the synthesis of organic@inorganic hNFs, algal extracts at different volumes (0.65, 1, and 1.65 mL) were used with 8×10^{-4} M Cu (aqueous copper sulfate 5-hydrate) in 10 mM PBS buffer (pH 5, 7.4, and 9). The reaction was ensured by vortexing and then the mixtures were incubated at 4 °C for 3 days. The precipitates that formed at the bottoms of the tubes were centrifuged (10,000 rpm, 10 min) and then washed with distilled water (Koca et al. 2020). Characterization of the obtained nanostructures was performed by FE-SEM, EDX mapping, and FT-IR analysis.

Antimicrobial activity of Cu hNFs

The antimicrobial activity of the algae-based Cu hNFs synthesized at pH 7.4 with 1 mL of extract was tested against *Escherichia coli* and *Staphylococcus aureus* strains. In tests performed by broth dilution method, increasing concentrations of Cu NF (0–140 µg/mL) were added to tubes with bacteria (10^8 CFU/mL) (negative control: tubes without bacteria; positive control: tubes without hNFs) and incubated at 37 °C for 24 h. Minimum inhibitory concentrations (MICs) were recorded based on the turbidity observed in the tubes at the end of the incubation process (Güven et al. 2022).

Antioxidant activity of Cu hNFs

In a test based on DPPH oxidation (Güven et al. 2022), the Cu hNFs (synthesized at pH 7.4 with 1 mL of extract) were reacted with DPPH (0.1 mM) at different concentrations (0.15625, 0.3125, 0.625, 1.25, 2.5, 5, and 10 mg/mL) to evaluate their antioxidant activity. After the

mixture was incubated for 30 min in the dark, samples with color changes (purple to orange) were read at a wavelength of 517 nm. DPPH activity was determined by the following formula:

$$\text{Scavenging activity (\%)} = \left(\left[\text{Absorbance of control} - (\text{Absorbance of sample} - \text{Absorbance of blank})\right]/\text{Absorbance of control}\right) \times 100$$

Here, absorbance of control was a sample replaced by an equivalent volume of distilled water and absorbance of blank was the same volume of 99.5% ethanol replacing the DPPH solution.

Catalytic activity of Cu hNFs

The catalytic activity of the Cu hNFs (synthesized at pH 7.4 with 1 mL of extract) was tested by a method based on the oxidation of guaiacol (Koca et al. 2020). The oxidation of guaiacol, which occurred with the reaction of Cu hNFs (3 mg), H_2O_2 (1 mL, 22.5 mM), and guaiacol (1 mL, 45 mM) in PBS buffer (10 mM, pH 6.8, 50 mL), was recorded with a spectrophotometer at 570 nm. A mixture without hNFs was used as a blank solution under the same conditions.

Results and discussion

The morphologies and diameters of Cu hNFs synthesized with an algal extract were evaluated by FE-SEM analysis. The elemental composition and functional groups of the hNFs were detailed by EDX mapping and FT-IR analyses, respectively, while the peroxidase-like catalytic activities of the hNFs were explained by a Fenton-like mechanism.

Characterization of hNFs

According to the findings of characterization tests, the diameters of the petals and hNFs synthesized by the reaction of 1 mL of *Ascoseira mirabilis* extract and 8×10^{-4} M Cu ions in 10 mM PBS buffer (pH 7.4) were 31 µm (Fig. 1a) and 27 nm (mean), respectively (Fig. 1b). With changes in the concentration of the algal extract and the pH of the PBS medium, changes in the morphological structures of the synthesized hNFs and differences in the size distribution were observed (Fig. 2a–d). It was noted that no blue precipitate was formed and synthesis did not occur in the tubes at any extract concentrations with PBS of pH 5. The formation mechanism of NFs has been discussed in detail in previous studies (Güven et al. 2022; Koca et al. 2020; Demirbas 2021). In a mechanism mainly consisting of nucleation, growth, and a finishing phase, the process starts with the formation of primary phosphate crystals as a result of the reaction of Cu ions and amide, hydroxyl, and diol groups of the bioextract (nucleation phase) and it is completed with the arrangement of the petals (Güven et al. 2022; Koca et al. 2020; Demirbas 2021; Baldemir Kilic et al. 2020; Baldemir et al. 2017). Baldemir Kilic et al. (2020) determined the diameters of hNFs synthesized with *Artemisia absinthium*, *A. vulgaris*, and *A. ludoviciana* extracts to be in the range of 2–10 µm. It was also reported that while hNFs were synthesized with the use of 0.1 mg/mL bioextract in the reaction, hNF formation was not observed with the use of 0.5 mg/mL bioextract. These researchers showed that the content and concentration of the bioextract as an organic component had significant effects on the formation and size

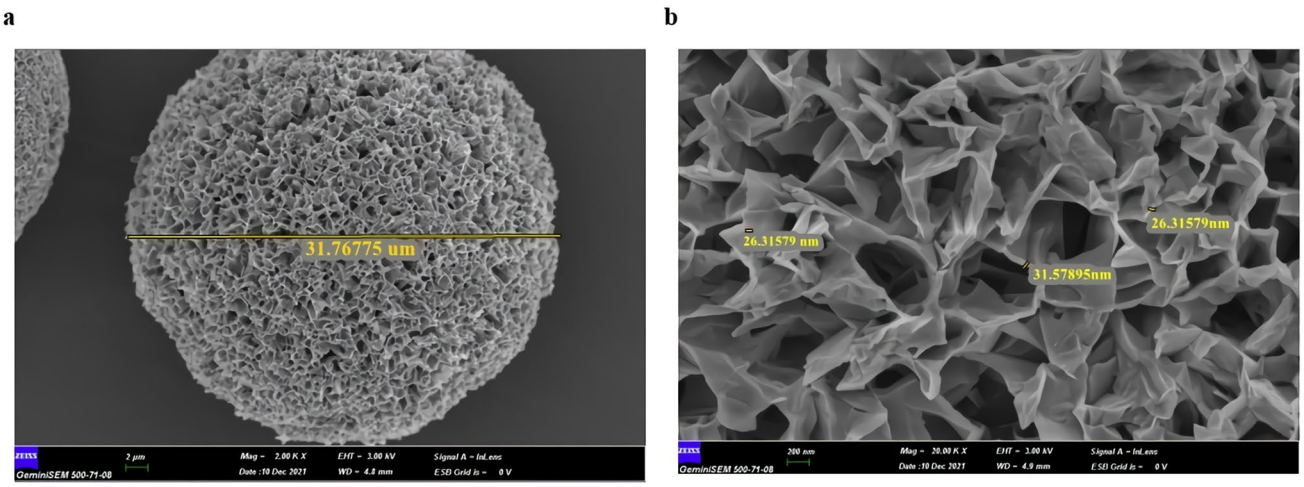


Fig. 1 FE-SEM analysis of hNFs synthesized with PBS (pH 7.4, 1 mL of extract): **a** diameter of hNFs; **b** diameters of petals

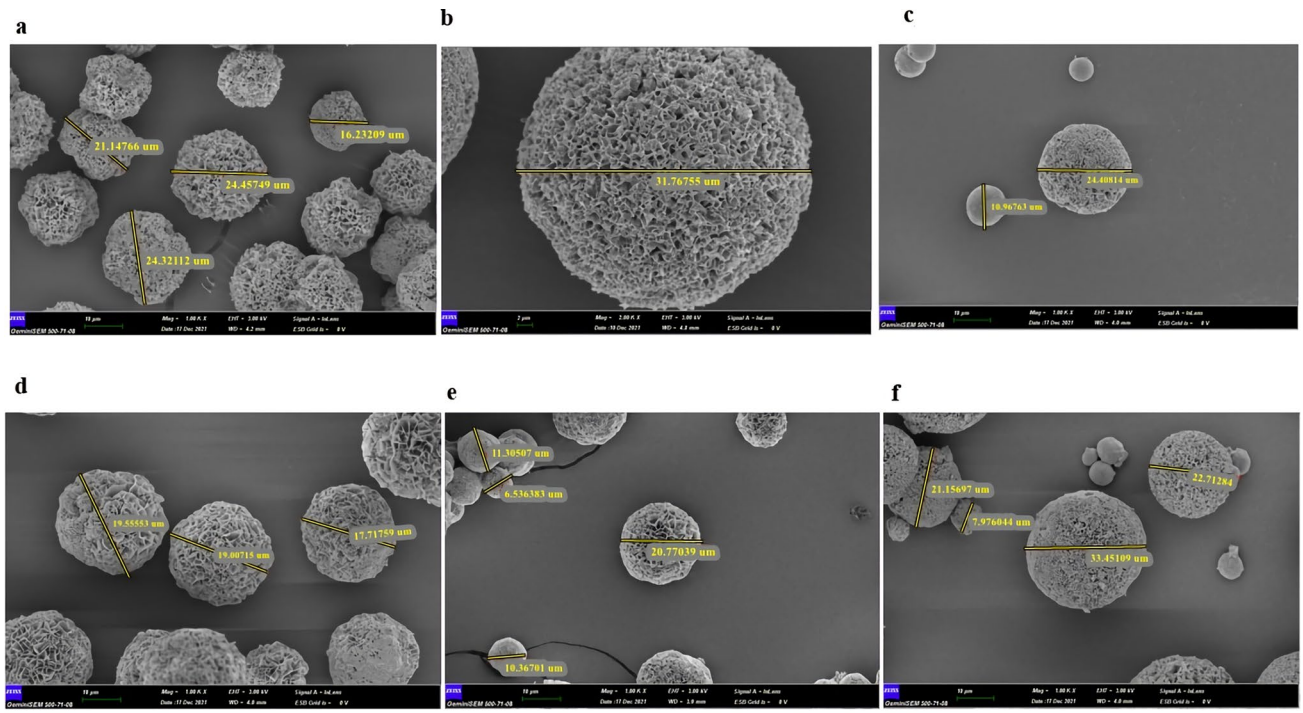


Fig. 2 FE-SEM analysis of hNFs synthesized with PBS of different pH levels: **a** pH 7.4, 0.65 mL extract; **b** pH 7.4, 1 mL extract; **c** pH 7.4, 1.65 mL extract; **d** pH 9, 0.65 mL extract; **e** pH 9, 1 mL extract; **f** pH 9, 1.65 mL extract

of the hNFs. In another study, NF synthesis did not occur at concentrations of *Trigonella foenum-graecum* extract of 0.02, 0.03, or 0.05 mg/mL; in addition, it was emphasized that the encapsulation yields of NFs increased with increasing concentrations of the extract from 0.1 to 0.5 mg/mL (Altınkaynak et al. 2019). Güven et al. (2022) reported that Cu-based hNFs with cherry stalk extract were synthesized in the PBS buffer pH range of 6–9, while hNFs were not synthesized at any other pH levels (Güven et al. 2022). In a study in which urease-based NFs were synthesized in PBS in the pH range of 6–9, the results were explained by the effect of medium pH on the binding affinity of urease molecules and Cu ions (Somturk et al. 2016). Although one interesting study reported that chance is an important factor in the growth of NFs (Virk 2011), on the contrary, we argue that the concentration of the extract and the pH of the PBS significantly affect the size, morphology, and formation of NFs based on the consistency between our findings and literature data.

Inorganic and organic components of the hNFs were determined by EDX (Fig. 3) and FT-IR (Fig. 4) analysis, respectively. The presence of Cu and other components in the structure of the hNFs was demonstrated by the EDX spectrum (Fig. 3a) and EDX mapping (Fig. 3b–f). The weight % of Cu in the hNFs was determined to be 15.45%. The distribution of the four key elements of C (turquoise color), O (green color), P (yellow color), and Cu (red color) in the hNFs was also confirmed with EDX mapping (Fig. 3b). The elements of C (Fig. 3c), O (Fig. 3d), P (Fig. 3e), and Cu (Fig. 3f) in the hNFs were analyzed by mapping and represented with different colors. Functional groups were determined by FT-IR analysis. The presence of C–H (alkane groups) was revealed at wavenumbers of 2916, 2848, and 1453 cm^{-1}. The peaks at 1652 and 1143 cm^{-1} corresponded to amine (–NH) and aliphatic ether (C–O), respectively. The primary phosphate crystals that formed in the PBS buffer were associated with peaks at 1039, 987, 717, 623, and 558 cm^{-1} (Güven et al. 2022; Koca et al. 2020; Koca 2022). Characterization of these peaks confirmed the formation of the organic@inorganic hNFs in PBS buffer together with their morphology.

Catalytic, antimicrobial, and antioxidant activities of hNFs

To determine the catalytic, antimicrobial, and antioxidant activities of hNFs synthesized under different pH and extract concentration conditions, hNFs produced by the reaction of 8×10^{-4} M Cu with 1 mL of algal extract in a PBS medium of pH 7.4 were used. The peroxidase-like catalytic activity of the hNFs was determined by spectrophotometric readings of the oxidation of guaiacol (Fig. 5a). The conversion of guaiacol to 3,3-dimethoxy-4,4-diphenoquinone as a result of the reaction may be

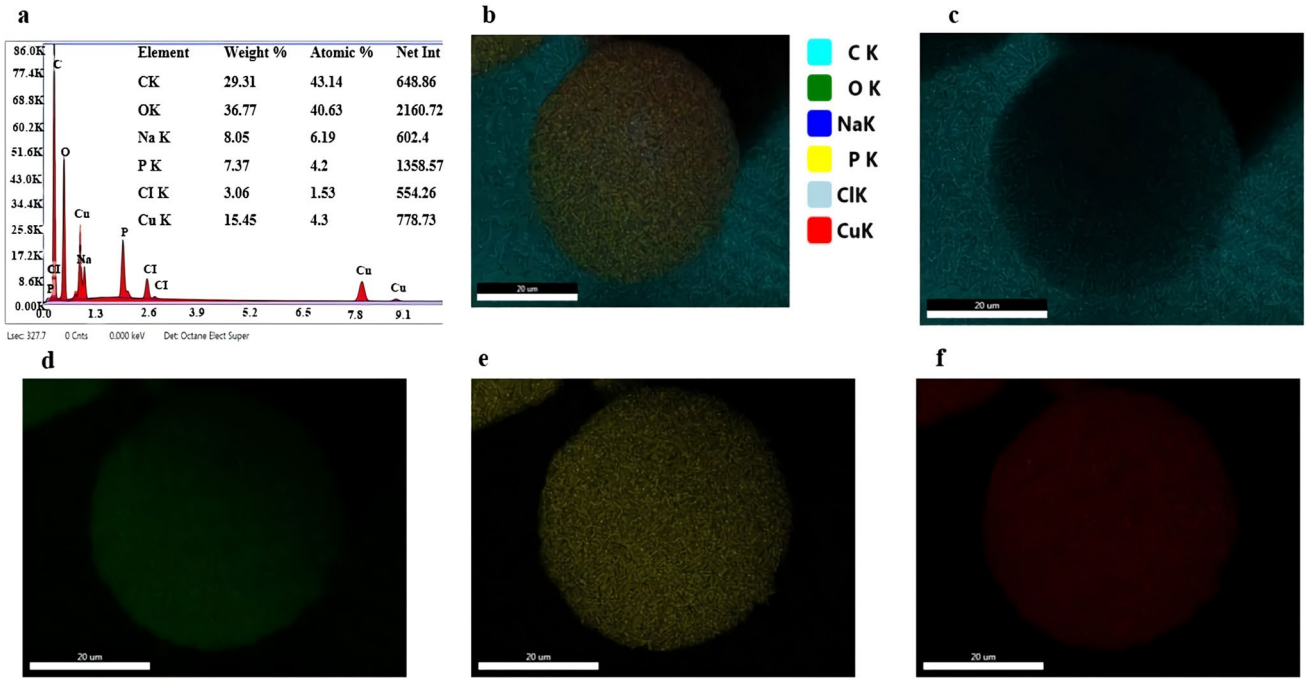


Fig. 3 EDX mapping of Cu hNFs (pH 7.4, 1 mL extract): **a** EDX spectrum; **b** distribution of key elements; **c** presence of C; **d** presence of O; **e** presence of P; **f** presence of Cu

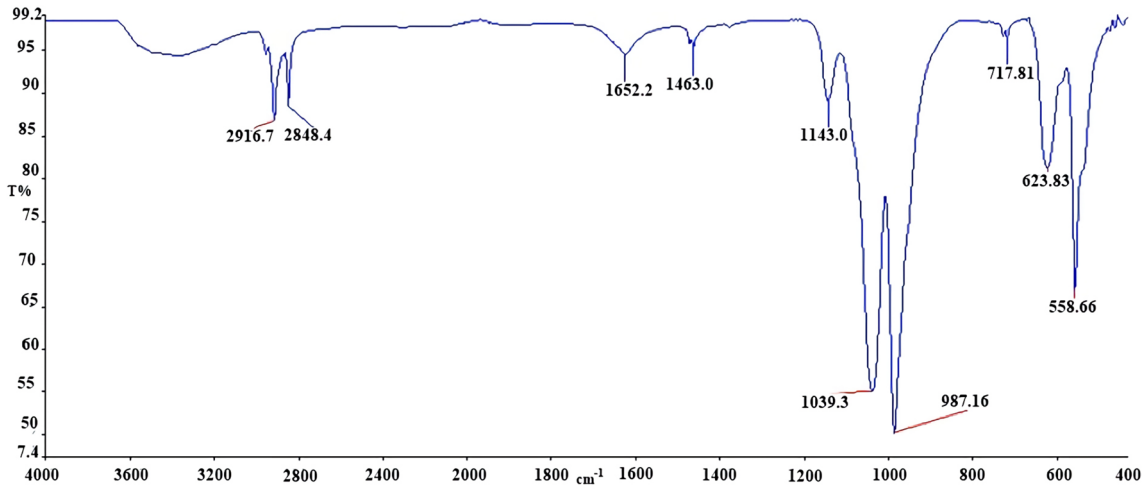
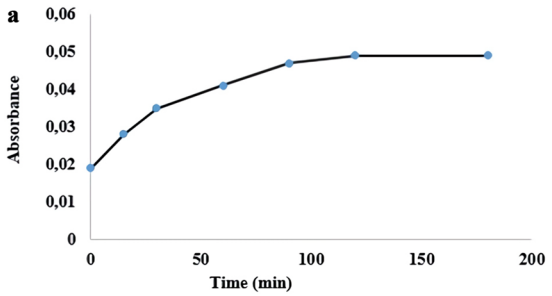


Fig. 4 FT-IR analysis of Cu hNFs (pH 7.4, 1 mL extract)

explained by Fenton's mechanism (Fig. 5b) (Güven et al. 2022; Koca 2022). The free radicals formed by the reaction of Cu^{+1} with H_2O_2, formed by the reaction of H_2O_2 and Cu^{+2} in the reaction medium containing Cu hNFs facilitated the oxidation of the substrate (Fig. 5b). By this mechanism, 3,3-dimethoxy-4,4-diphenoquinone was formed by the oxidation of guaiacol (Koca 2022). Similar to our study, the peroxidase-like catalytic activities of Cu NFs prepared with cherry stalk, thymol, allicin, *Viburnum opulus*, and *Laurocerasus officinalis* against guaiacol were explained by a Fenton-like mechanism (Güven et al. 2022; Koca et al. 2020; Koca 2022; Ildiz et al. 2017; Baldemir et al. 2018). Mei et al. (2022) noted that tetracycline degradation caused by CeO_2 hNFs was mediated by radicals that formed as a result of Fenton's mechanism. Dadi et al. (2020) reported that the peroxidase activities of gallic acid@Cu NFs depended on reaction time, substrate concentration, and NF morphology. In other previous studies,



$$H_2O_2 + Cu^{+2}Cu^{+1} + HOO\cdot + H^+ \quad (1)$$

b

$$H_2O_2 + Cu^{+1}Cu^{+2} + OH^- + \cdot OH \quad (2)$$

Fig. 5 Catalytic activity of Cu hNFs (pH 7.4, 1 mL extract): **a** absorbance change of guaiacol; **b** Fenton reaction

the peroxidase activity of amino acid-based Cu hNFs, which permitted substrate oxidation, was explained by Fenton's mechanism (Wu et al. 2016; Jiang et al. 2021).

In this study, the MICs of the Cu hNFs were found to be 70 and 17.5 µg/mL against *Staphylococcus aureus* and *E. coli*, respectively (Fig. 6). The findings of our study are consistent with those of previous work (Demirbas 2021; Koca et al. 2020; Güven et al. 2022). Celik et al. (2020) emphasized that the morphological structures of hNFs are important parameters affecting their antimicrobial activities. Yilmaz et al. (2022) reported that taurine-based Cu hNFs caused oxidative damage to bacterial membranes as a result of free radicals that occurred after a Fenton-like reaction with the presence of H_2O_2 in the medium, and other studies support that finding (Demirbas 2020, 2021; Koca et al. 2020; Güven et al. 2022).

Free radicals that occur as a result of various bioreactions and cause oxidative damage are detoxified by antioxidants that prevent the oxidation of molecules (Yin et al. 2015; Jiang et al. 2021). The DPPH scavenging activity with

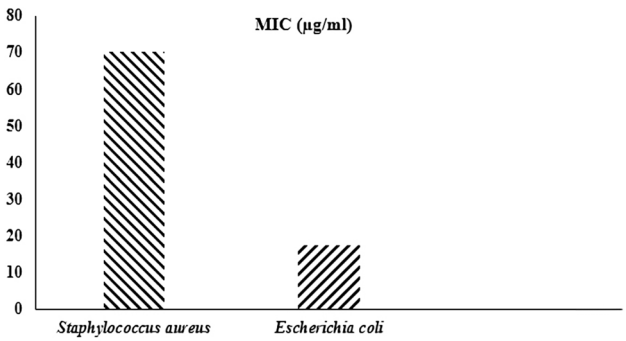


Fig. 6 Antimicrobial activity of Cu hNFs (pH 7.4, 1 mL extract)

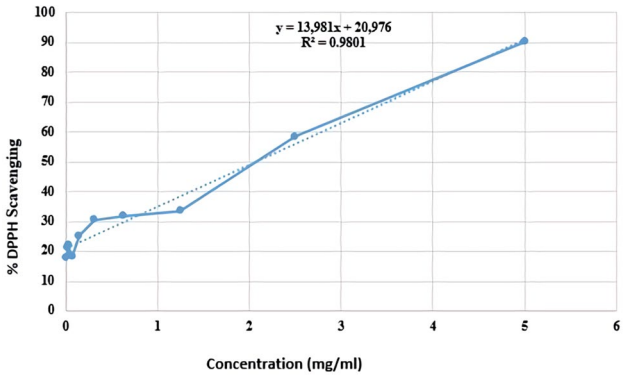


Fig. 7 Antioxidant activity of Cu hNFs (pH 7.4, 1 mL extract)

increasing concentrations of Cu hNFs is shown in Fig. 7. The antioxidant capacity of the algae@Cu hNFs as reflected by the 50% inhibitory concentration (IC_{50}) was calculated at 2.07 mg/mL. In previous studies, it was noted that the free radical scavenging activity increased with the increase in the concentration of the biosynthesized nanomaterials (Jiang et al. 2021; Varadharaj et al. 2020; Öztürk Küp et al. 2020; Jayakumar and Vedhaiyan 2019; Demirbas et al. 2017, 2019). Güven et al. (2022) reported that NFs exhibited enhanced antioxidant activity against DPPH with increasing concentrations (IC_{50}: 1.35 mg/mL). Consistent with previous studies, our findings revealed that the Cu hNFs showed antioxidant properties by exhibiting DPPH scavenging activity with increasing concentrations.

In light of this information, we attribute the peroxidase-like catalytic, antioxidant, and antimicrobial activities of these hNFs synthesized with the incorporation of an algal extract and Cu to the decomposition of guaiacol, DPPH, and bacterial membranes caused by reactive free radicals formed as a result of Fenton's mechanism.

Conclusion

Cu NFs were synthesized under conditions of pH 7.4 and 9 with the incorporation of Cu and *Ascoseira mirabilis* extract at different concentrations. As a result, hNFs with a complete flower morphology were synthesized under optimum conditions of pH 7.4 and 1 mL of extract. These algae@Cu hNFs were characterized by FE-SEM, EDX mapping, and FT-IR analyses and were synthesized using the algal extract cheaply, effectively, and in wide ranges of pH and concentration instead of expensive and difficult-to-obtain molecules. We have demonstrated that the obtained hNFs have antioxidant activity against DPPH and catalytic activity against guaiacol. It is believed that the findings of this study will offer guidance for nanotechnology, biotechnology, biomedical, and environmental applications.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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