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ORIGINAL ARTICLE

Antimicrobial and antioxidant properties of green synthesis CuO nanoparticles using *Salvia officinalis* hydroalcoholic extract

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ABSTRACT

Background and Objectives: Following the emergence of microbial resistance to chemical antimicrobial agents, researchers have recently focused on the effects of nanoparticles and their antimicrobial activity. This research aimed to investigate the antimicrobial and antioxidant activity of copper oxide nanoparticles (CuO-NPs) produced using the hydroalcoholic extract of *Salvia officinalis* (*S. officinalis*).
Materials and Methods: In this study, after preparing the hydroalcoholic extract of *S. officinalis* and synthesizing CuO-NPs using this extract, the synthesized nanoparticles were evaluated. The antimicrobial properties of synthesized CuO-NPs were investigated by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using the microbroth dilution method. The minimum biofilm inhibitory concentration (MBIC) and antioxidant activity of CuO-NPs and the hydroalcoholic extract of *S. officinalis* against *Pseudomonas aeruginosa* (*P. aeruginosa*) were investigated.
Results: CuO-NPs have a size in the range of 40 nm and a monoclinic crystal structure. Antimicrobial activity assessment by well diffusion showed that the synthesized CuO-NP nanoparticles at concentrations below 5 mg/mL had no antimicrobial effect on any of the bacteria studied. The lowest MIC of the synthesized CuO-NPs was observed against *Staphylococcus saprophyticus* (*S. saprophyticus*), and the highest against *P. aeruginosa* and *Bacillus cereus* (*B. cereus*). On the other hand, the highest MBC was related to *B. cereus*. The plant extract exhibited much weaker antimicrobial activity than CuO-NPs. The results obtained showed that the MBIC for CuO-NPs was 312 µg/mL. The half-maximal inhibitory concentration (IC_{50}) obtained from CuO-NPs and the hydroalcoholic extract of *S. officinalis* was 2.79 and 1.97mg/ml, respectively.
Conclusion: Based on the results of this study, the green synthesis of CuO-NPs using hydroalcoholic extract of *S. officinalis* is a suitable candidate for the preparation of antimicrobial and antibiofilm compounds that also have antioxidant properties.

Keywords: Green synthesis; Copper oxide nanoparticles (CuO-NPs); Antimicrobial; Antioxidant; *Salvia officinalis*

INTRODUCTION

Nowadays, due to the excessive use of antibiotics and chemicals, bacterial antibiotic resistance is increasing. Sometimes even the strongest antibiotics have no effect on bacteria and pathogenic agents, and the body cannot resist these pathogens. Therefore, with the increasing resistance of pathogenic agents

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to antimicrobial chemicals, new methods for drug production are necessary. One of the methods is nanotechnology, which today affects people's daily lives due to its wide applications in science and industry. Nanotechnology is a scientific field based on nanoparticles, materials with dimensions of 1 to 100 nanometers that have a three-dimensional structure (1, 2).

The use of plant extracts for nanoparticle synthesis is widely reported in recent research (3). Plant extracts may act as both reducing and capping agents in the synthesis of nanoparticles (4, 5). The biological reduction process for producing metal nanoparticles involves combining biological molecules in plant extracts (enzymes, proteins, amino acids, vitamins, polysaccharides, and organic acids such as citrate). It is well established that, in the production of nanoparticles using plant extracts, the extract readily combines with a metal salt solution at room temperature. Plants have a strong ability to accumulate metal ions and reduce them biologically. For this reason, plants have been proposed as an environmentally friendly route for the biosynthesis of metal nanoparticles and for detoxification applications (6, 7).

Sage (scientific name: *Salvia officinalis* (*S. officinalis*)) is a flowering, seedless, dicotyledonous plant, belonging to the Lamiaceae order, Shahpasand suborder, mint family, and Salvia genus (8). This plant is the most valuable medicinal plant. It is a mint plant. The flowers of this plant are purple-blue and sometimes white, which can be seen between June and July. It has brownish roots and numerous, branched, square, and hairy stems. This plant grows in the Mediterranean and Asian regions. However, it is also cultivated in some European countries. In Iran, this plant is also cultivated in East Azerbaijan. Sage contains bitter substances, catechin group tannins (salvia tannin), flavonoids (epigenin, luteolin), volatile essential oils (cineole, camphor, alpha, and beta thujone), glycosidic substances, tocopherol, rosmarinic acid, and ascorbic acid. Brain tissue is prone to damage due to its high unsaturated fatty acids, high oxygen consumption, and weak antioxidant activity (9).

S. officinalis is used as a seasoning in foods and drinks, and in the production of herbal medicines (10). *S. officinalis* exhibits digestive, diuretic, anticonvulsant, antipyretic, antimicrobial, and glycemic regulatory properties. Also, this plant has beneficial effects in the treatment of gout, chronic rheumatism, Alzheimer's disease, nervous vertigo, headaches of nervous origin, and headaches caused by indigestion, colds, and abdominal pain (11).

In general, plant extracts play an important role in reducing metal ions and subsequently stabilizing them. It seems that the diversity in the composition and concentration of these biologically active molecules among different plants, as well as their interactions with metal ions, is the main reason for the increased diversity of nanoparticle sizes and shapes produced (12). Copper has antibacterial properties that reduce oxidative stress and has antioxidant properties (13). Copper nanoparticles (CuO-NPs) have antibacterial properties that make them a potential option for combating microorganisms and infections (14-16). Therefore, the aim of the current study was the Synthesis and characterization of the Hydroalcoholic extract of *S. officinalis* conjugated CuOP-NPs and the evaluation of its antimicrobial and antioxidant activity.

MATERIALS AND METHODS

Ethical approval. The present study was conducted after approval from Lorestan University of Medical Sciences and receipt of the ethics code (IR.LUMS.REC.1400.020).

Preparation of hydroalcoholic extract and synthesis of CuO-NPs by hydroalcoholic extract of *S. officinalis*. In the first step, the plant material was shade-dried at room temperature and subsequently ground into a powder. Plant extraction was then conducted using the soaking method (17). To synthesize CuO-NPs, 75 mL of freshly prepared extract was combined with 100 mL of freshly prepared 0.01 M copper sulfate (CuSO4) solution and stirred continuously for 24 hours at 60°C. The mixture was then centrifuged at 12,000 rpm for 20 min, and the procedure was repeated twice to remove all impurities. Finally, the color change of the reaction solution indicated the formation of nanoparticles; those produced in the oven at 60°C were dried for subsequent analyses (18).

Characterization of as synthesized CuO-NPs. Physicochemical properties of green synthesized CuO-NPs were evaluated using several spectroscopic and microscopic methods. Firstly, Uv-visible spectroscopy was performed for CuO-NPs and plant extract using an spectrophotometer system (Jenway, model 6505, UK). For this, 1ml of CuO-NPs or plant extract

was poured in a quartzes cuvette and absorbance was recorded at scanning range of 250-750 nm (19).

Fourier Transform Infrared (FTIR) spectrometry analysis was carried out for CuO-NPs and plant extract. FTIR spectra were recorded from KBr-sample powder in 1% pellets by pressing method and then placed on FTIR system (Bruker Tensor 27, Optics GmbH). Scanning range of FTIR spectra was selected at 400-4000 cm^{-1} at a spectral resolution of ±4 cm^{-1}.

Field emission scanning electron microscopy (FE-SEM) was applied for morphological and Size determination of CuO-NPs. Therefore, dried powder of CuO-NPs was prepared and then sputter-coated with gold film using a coater apparatus (SCD005 Model, Canonsburg, PA, USA). After that, the FE-SEM images were prepared by a TESCAN MIRA3 FE-SEM system (Czech Republic).

Particle size distribution studies were performed using the DLS technique, and zeta potential surface charge distribution was measured using a Zetasizer Nano ZS spectrometer (Malvern Instruments Ltd, UK) at a scattering angle of 1731 and the desired temperature, in a polycarbonate chamber cell with Pd electrodes (19).

In-vitro antimicrobial properties of biosynthesized CuO-NPs. The antimicrobial efficacy of CuO-NPs against *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*), *Staphylococcus saprophyticus* (*S. saprophyticus*), *Klebsiella pneumoniae* (*K. pneumoniae*), and *Bacillus cereus* (*B. cereus*) was evaluated using the well diffusion agar technique. This procedure was conducted after preparing a microbial suspension from each strain to a standardized cell density of 1.5×10^8 CFU/ml (0.5 McFarland). After that, 5-mm-diameter wells were created using a sterile punch. Subsequently, various concentrations of the CuO-NPs solution were introduced into the wells. The plates were then incubated at 37°C for 24 hours. The susceptibility of the bacteria was determined by measuring the diameters of the inhibition zones, indicating non-growth halos (20).

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined by the microbroth dilution method (21). To determine the MIC in 96-well microplates, first, 100 mL of Mueller-Hinton broth was poured into 10 wells of the microplate, and then 100 mL of the sample was added to the first well of each row. Then, 100 µl were taken from the first well and added to the next well, and this was repeated until well number 10, after which 100 µl were poured out from well number 10. From the bacterial culture, a uniform suspension equivalent to the standard solution of half McFarland (1.5×10^8 CFU/ml) was prepared in broth culture medium, diluted 100-fold, and 100 µl was added to each well. After 24 h of incubation at 37°C, turbidity indicating bacterial growth or non-growth was observed, and according to the definition, the well with no turbidity was considered equivalent to the MIC. To determine MBC, the MIC concentration and several concentrations above it were used to inoculate Mueller-Hinton agar plates. After incubation, the lowest concentration of nanoparticles at which the bacteria did not grow was considered the MBC. In another method, the well diffusion technique was used to check the antimicrobial properties. In this method, different concentrations of CuO-NPs and hydroalcoholic extract were spread onto wells on Mueller-Hinton agar medium and then cultured on a half-McFarland microbial suspension in Mueller-Hinton broth. After incubation for 24 h at 37°C, the diameters of the inhibitory areas were measured (21).

Antibiofilm properties of synthesized CuO-NPs against *P. aeruginosa*. Antibiofilm effects of CuO-NPs and the hydroalcoholic extract of *S. officinalis* were evaluated against *P. aeruginosa*. For this purpose, *P. aeruginosa* was grown on Muller-Hinton broth after treatment with different concentrations of CuO-NPs and the extract in a 24-well plate. After 72 h of incubation, the biofilms formed in the wells were gently rinsed with phosphate-buffered saline (PBS) to remove nonadherent bacteria. The biofilms attached to the well surfaces were then stained with crystal violet. After careful washing and dissolution of the dye in ethanol, the optical density was measured at 590 nm using a spectrophotometer. The level of light absorption indicates the extent of biofilm formation; the lowest concentration that effectively inhibits biofilm formation is called the minimum biofilm inhibition concentration (22).

Antioxidant activity of synthesized CuO NPs. To measure the antioxidant properties of the extract, the DPPH method was used. In this technique, 0.5 ml of different concentrations of the synthesized extract and

ascorbic acid as a standard antioxidant were poured into the test tube, then 1 ml of methanolic solution of 0.1 mM of DPPH (2-2-diphenyl-1- picrylhydrazy) was added and after mixing and 30 minutes passed, at room temperature and in the dark, it was read with a spectrophotometer at a wavelength of 517 nm. Then, the concentration of nanoparticles with a radical inhibition percentage of 50 (IC_{50}, half maximal inhibitory concentration) was calculated (23).

Statistical analysis. All experiments were performed in triplicate. The results are shown as mean ± SD. A t-test was conducted for statistical analysis at a 95% confidence level and with $p \leq 0.05$).

RESULTS

UV–Vis spectroscopy. In the first step of the biosynthesis of CuO-NPs using *S. officinalis* hydroalcoholic extract, the color change of the reaction sample was used as an indicator to confirm the formation of CuO-NPs. This color change typically shifted from green-blue to deep red-brown, indicating the formation of CuO-NPs (Fig. 1A). To confirm the formation of CuO-NPs, optical absorption spectroscopy in the visible-ultraviolet (UV-Vis) range was used. Nanoparticles typically exhibit a characteristic absorption peak in the wavelength range of 250-750 nm, with a maximum at 376.5 nm, which shifts the surface plasmon resonance. This peak is considered a definitive indication of the initial formation of CuO-NPs. In Fig. 1B, two absorption spectra are observed. The red spectrum of the *S. officinalis* extract, with a peak at 278.7 nm, could confirm the presence of bioactive molecules,

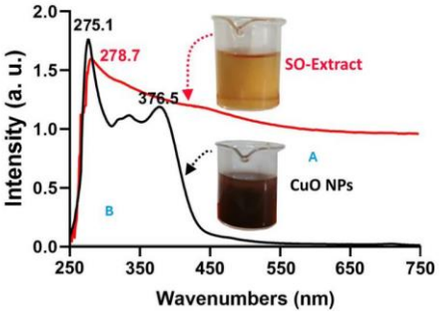

Fig. 1. Formation and verification of CuO-NPs. (A) Color change of the reaction mixture during the synthesis of CuO-NPs. (B) UV-visible spectrum of CuO-NPs biosynthesized using hydroalcoholic extract of *S. officinalis*

such as polyphenols, flavonoids and terpenoids, that act as reducing and stabilizing agents in the synthesis of CuO-NPs. The black spectrum represents the reaction sample after synthesis and shows a prominent absorption peak at 376.5 nm. The literature reports that copper ions, when oxidized to Cu_2O/CuO, exhibit a distinct absorbance in the UV-visible range of 300-400 nm. The stabilizing ligands from the *S. officinalis* hydroalcoholic extract may alter the dielectric properties, thereby quenching the usual Surface plasmon resonance (SPR) bandgap.

SEM. Scanning electron microscopy (SEM, TESCAN MIRA 3 LMU) imaging was used to investigate the morphology and size of CuO-NPs synthesized by the hydroalcoholic extract of S. officinalis. Fig. 2 shows the formed CuNPs at two different scales with magnifications of 3,10 μm. As shown in the images, the CuO-NPs are nearly spherical and, in some areas, aggregated. This aggregation behavior could be due to van der Waals forces between nanoparticles or their surface interactions during the synthesis process. SEM results showed that these nanostructures ranged from 20 to 70 nm, with an average size of about 40 nm.

FTIR spectral analysis. Fig. 3 shows the FTIR spectra of the hydroalcoholic extract of *S. officinalis* (A) and CuO-NPs synthesized (B). As shown in Fig. 3A, certain functional groups are present in the spectrum of *S. officinalis* extract. According to the results, the broadband at 3415 cm^{-1} corresponds to free hydroxyl groups, the band at 1620 cm^{-1} corresponds to the C=C groups of the aromatic ring, and the band observed at 1085 cm^{-1} corresponds to the stretching vibrations of the C-OH groups. These functional groups indicate the bioactive compounds in the plant extract. Fig. 3B shows the spectrum of CuO-NPs. Based on these results, the band positions are similar to those in the spectrum of the *S. officinalis* extract, indicating that the extract compounds are adsorbed on the surface of CuO-NPs via π-electron interactions, thereby stabilizing the nanoparticles (24). One of the important and characteristic peaks in the FT-IR spectrum of CuO-NPs is the peak related to metallic bonds between copper and oxygen, which is observed at the position of 457 cm^{-1}. This peak indicates the formation of a bond between a copper ion (Cu) and oxygen (O) and confirms the formation of CuO-NPs. The presence of this peak indicates the crystalline structure of copper oxide and the formation of high-quality nanoparticles.

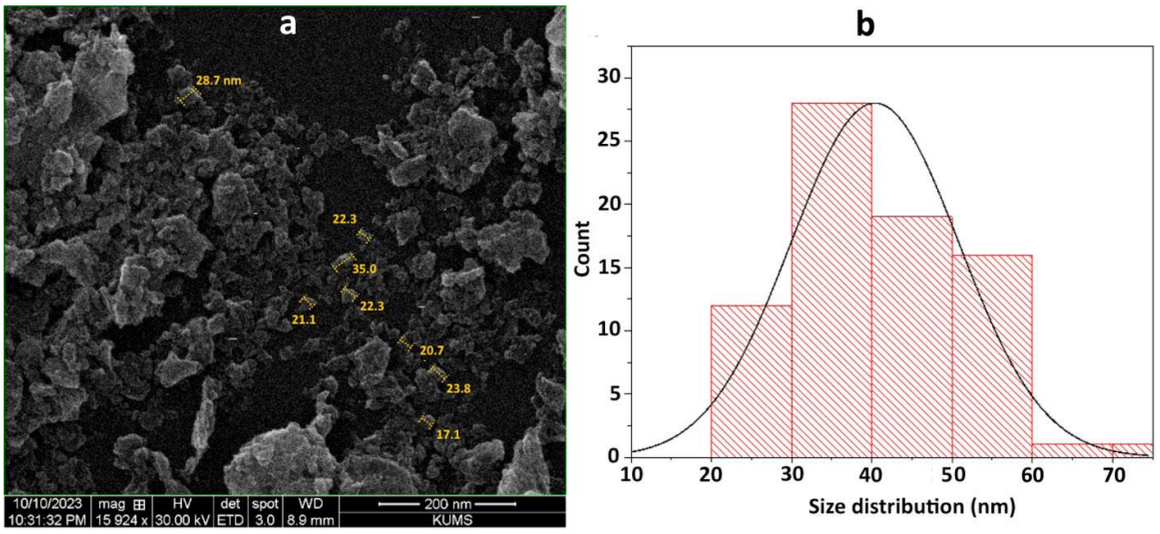

Fig. 2. Scanning electron microscopy (SEM) of CuO-NPs synthesized by the hydroalcoholic extract of *S. officinalis*

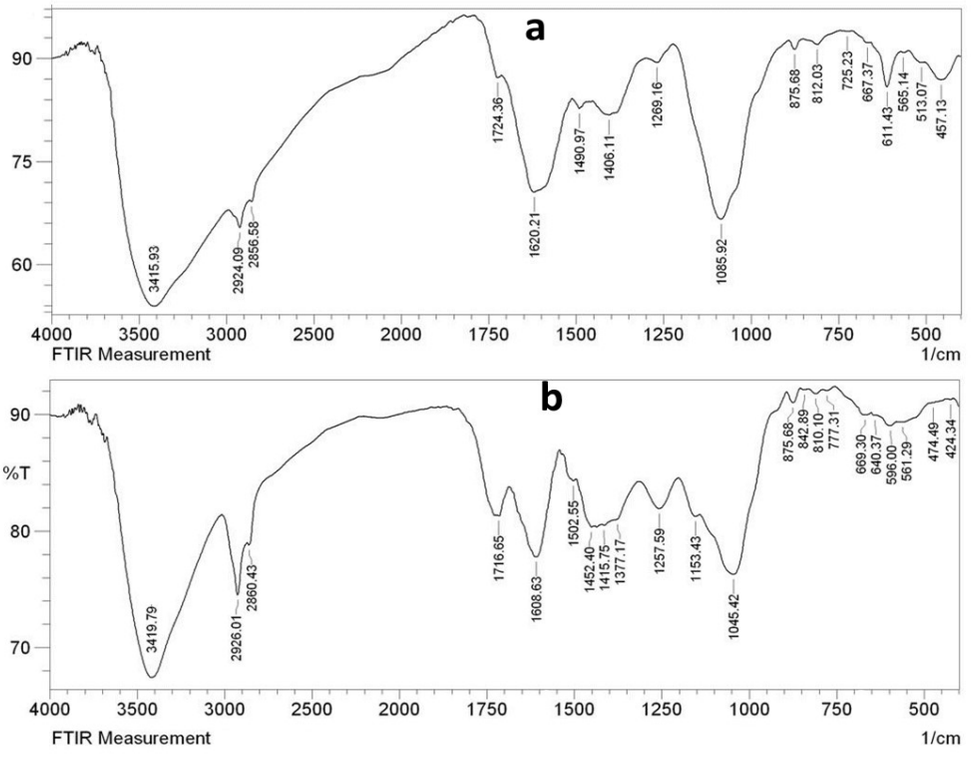

Fig. 3. FTIR spectrum. A: Synthesized CuO-NPs. B: Hydroalcoholic extract of *S. officinalis*

Zeta potential and particle size determination. The hydrodynamic size of the synthesized CuO nanoparticles was approximately 100-300 nm, with a zeta average of 270.2 nm (Fig. 4A). Furthermore, the zeta potential value for CuO nanoparticles was estimated to be -13.0 mV (Fig. 4B). These results indicated that the hydroalcoholic extract of *S. officinalis* is a good source for reducing the fine size of CuO-NPs. The higher negative zeta potential value confirmed that interparticle repulsion counteracted aggregation. This indicates that the synthesized nanoparticles exhibit greater stability in a liquid medium. Furthermore, zeta potential values in the range of -30 have been reported to be more stable in solution. In fact, the higher

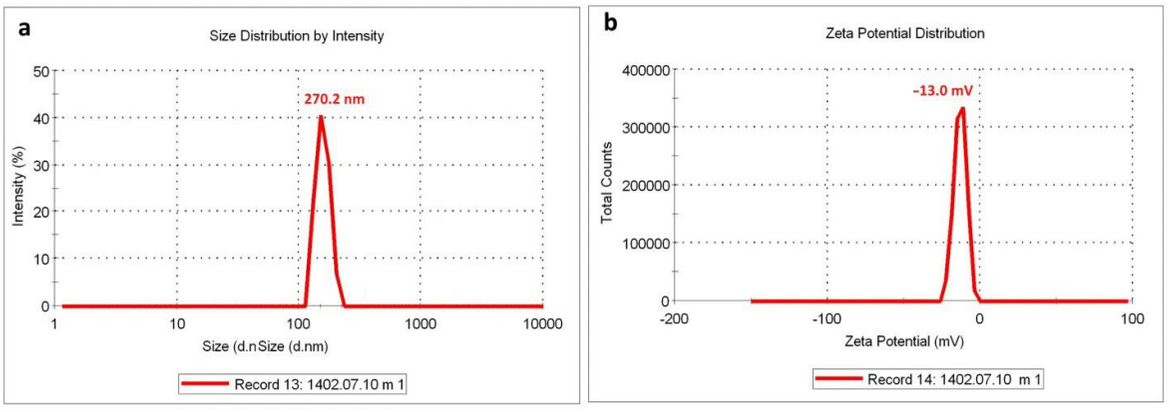

Fig. 4. Zeta potential and particle size determination of CuO-NPs. A: DLS, B: Zeta potential

negative zeta potential (-13.0 mV) indicates a strong interparticle repulsion force leading to enhanced or increased stability (16).

Antimicrobial activity assessment by well diffusion. Figs. 5 and 6 shows the antimicrobial activity of CuO-NPs and the hydroalcoholic extract of *S. officinalis*. The growth inhibition zones obtained from the treatment of different bacteria at different concentrations of CuO-NPs (0.31 -10 mg/mL) clearly show the concentration-dependent growth inhibition pattern. The synthesized CuO-NP nanoparticles at concentrations below 5 mg/mL showed no antimicrobial effect against any of the bacteria studied. On the other hand, the hydroalcoholic extract of *S. officinalis* did not exhibit antimicrobial activity at concentrations of 0.31-40 mg/mL against the bacteria, *P. aeruginosa*, *S. aureus*, *S. saprophyticus*, *K. pneumoniae*, and *B. cereus*. These differences indicate that although Chloramphenicol (positive control) has a greater antimicrobial effect at lower concentrations, CuO-NPs still show potential ability to inhibit bacterial growth at higher concentrations. Additionally, statistical analysis using two-way ANOVA and Tukey's post hoc test showed that, in all strains, there was no significant difference between the group treated with CuO-NPs (10 mg/ml) and the positive control. Besides, the least significant difference was observed between the CuO-NPs (10 mg/ml) group and the hydroalcoholic extract of *S. officinalis* (E).

MIC and MBC. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined for CuO-NPs, the hydroalcoholic extract of *S. officinalis*, and chloramphenicol. The MIC values obtained are summarized in Table 1. Based on the results, the highest MIC was associated with the extract, and the lowest with chloramphenicol. The lowest MIC of the synthesized CuO-NPs was observed against *S. saprophyticus*, and the highest against *P. aeruginosa* and *B. cereus*. On the other hand, the highest MBC was related to *B. cereus*. The antimicrobial properties of the plant's hydroalcoholic extract were also evaluated. The findings indicated that the plant extract exhibited much weaker antimicrobial activity than CuO-NPs.

Antibiofilm activity assessment. In this study, the antibiofilm effects of CuO-NPs and the hydroalcoholic extract of *S. officinalis* against *P. aeruginosa* were investigated using microdilution and crystal violet staining. By analyzing the optical absorption results, the minimum biofilm inhibitory concentration (MBIC) for CuO-NPs and the hydroalcoholic extract of *S. officinalis* was determined. The results showed that the MBIC for CuO-NPs was 312 µg/mL, and for the hydroalcoholic extract of *S. officinalis* was 10 mg/mL. This difference indicated a greater antibiofilm property of CuO-NPs at lower concentrations compared to the hydroalcoholic extract of *S. officinalis*. In addition, qualitative analysis of the formed biofilms was performed using light microscopy on slides and 3D images of the biofilms were obtained. Fig. 7 shows the biofilm density formed in the presence of an MBIC equivalent concentration of CuO-NPs, hydroalcoholic extract of *S. officinalis*. These images clearly demonstrate the biofilm density. In the control group without any antimicrobial treatment, the highest biofilm density was observed. In the groups treated with CuO-NPs and the hydroalcoholic extract of *S. officinalis*, biofilm density was significantly reduced in the hydroalcohol-

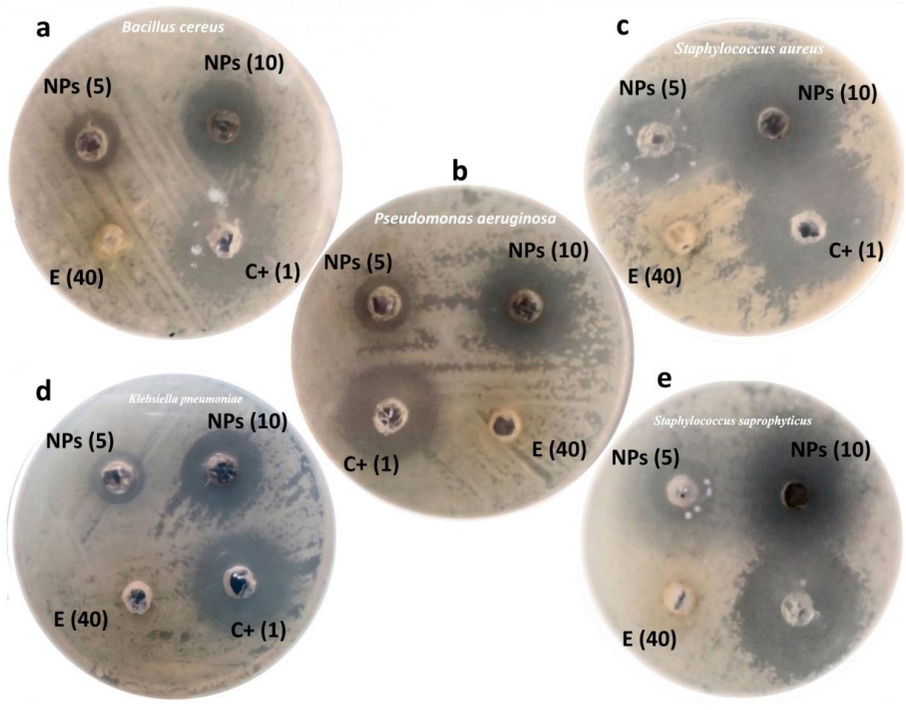

Fig. 5. Antimicrobial effects of CuO-NPs and *S. officinalis* extract (E) based on agar well-diffusion assay against a) *B. cereus*, b) *P. aeruginosa*, c) *S. aureus*, d) *K. pneumoniae*, and e) *S. saprophyticus* at 5 and 10. The concentrations of the Chloramphenicol, positive control (C+) and *S. officinalis* extract were 1 mg/ml and 40 mg/ml, respectively.

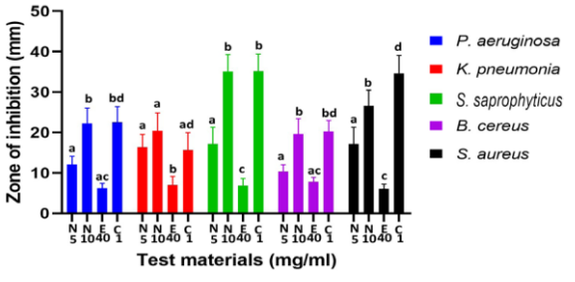

Fig. 6. Statistical analysis of hallo zone inhibition diameters based on well diffusion agar, calculated by Mean ± SD and analyzed with two-way ANOVA and multiple comparison based on Tukey test. The different alphabetical labels indicated a significant difference within treatment groups for each bacterial strain.

ic extract of *S. officinalis*. Among the extracts and nanoparticles studied, CuO-NPs exhibited the lowest biofilm density, indicating a more effective antibiofilm effect than the extract. These results indicate the high potential of CuO-NPs in inhibiting bacterial biofilm formation.

Antioxidant activity. The antioxidant activity of CuO-NPs and the hydroalcoholic extract of *S. officinalis* at different doses (0.31 -10 mg/mL) was evaluated by DPPH free radical scavenging assay, using ascorbic acid as a positive control. The mean percentage inhibition of biosynthesized CuO-NPs and hydroalcoholic extract of *S. officinalis* was (88.6,61.5,42.4,31.4,18.7,7%), (99.7,75.25,50,38.7,20.5,9%), respectively. Consequently, the IC50 obtained from CuO-NPs and the hydroalcoholic extract of *S. officinalis* was 2.79 and 1.97mg/ml, respectively (Fig. 8).

DISCUSSION

Copper oxide nanostructures can be used as antimicrobial and antifungal agents across many industries due to their broad applicability. Copper and copper-based compounds have effective biocidal properties that are commonly used in the formulation of health-related functional compounds. There are various methods for preparing CuO-NPs, among which green synthesis has been developed recently, given that biological synthesis is safe, biocompatible and environmentally friendly (25, 26). CuO-NPs can

Table 1. MIC and MBC of compounds against bacterial strains.

Bacteria	MIC (µg/ml)			MBC (µg/ml)		
	CuO-NPs	Chloramphenicol	Extract	CuO-NPs	Chloramphenicol	Extract
P. aeruginosa	625	39	-	1250	39	-
K. pneumoniae	312	39	-	625	39	-
S. saprophyticus	9.7	19	5000	9.7	19	10000
B. cereus	625	39	-	2500	156	-
S. aureus	19	39	10000	39	78	20000

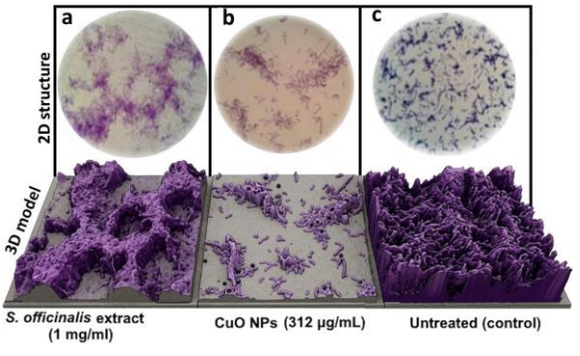

Fig. 7. Antibiofilm activity of a) *S. officinalis* extract, b) CuO nanoparticles plus untreated control on *P. aeruginosa*. The bottom images are 3D models of the above biofilm structure.

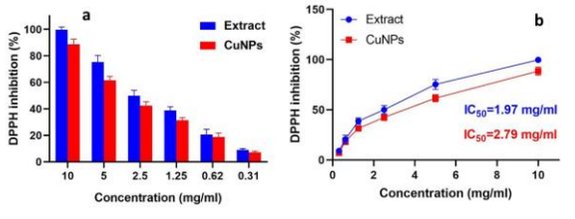

Fig. 8. Antioxidant activity of CuO-NPs and hydroalcoholic extract of *S. officinalis*

be synthesized through a green approach using plant extracts. Green nanoparticles have amazing properties that make them suitable for biomedical applications (27). Therefore, this study investigated the synthesis, characterization, and biological properties of green-synthesized CuO-NPs using *S. officinalis* hydroalcoholic extract.

The CuO-NPs synthesized in our study exhibited a broad absorption band at around 275.1 nm in the hydroalcoholic extract of *S. officinalis.* In contrast, narrow and broad peaks at wavelengths of 278.7 and 376.5 nm were obtained from CuO-NPs. The results were consistent with previous studies, such as the study of Saif et al., which aimed to green synthesis of CuO-NPs mediated by plants to compare the toxicity of engineered and plant-based CuO-NPs in *Daphnia magna* and the study of Udayabhanu et al., which aimed to investigate the photocatalytic, antioxidant and antibacterial properties of green synthesis of CuO-NPs mediated by *Tinospora cordifolia* (28, 29). Our findings are consistent with the results of Noor and Sathiyavimal et al., who reported that SEM micrographs of CuO-NPs showed agglomerates of asymmetric spherical particles (30, 31).

FTIR spectroscopy was used as a key tool to identify the functional groups and surface interactions of CuO-NPs synthesized from the hydroalcoholic extract of *S. officinalis.* The specific functional groups in the spectrum of *S. officinalis* extract included a band at 3415 cm^{-1} (free hydroxyl groups), 1620 cm^{-1} (C=C groups of aromatic rings), and 1085 cm^{-1} (stretching vibrations of C-OH groups). These functional groups indicate the presence of bioactive compounds in the plant extract that are involved in nanoparticle synthesis. The spectrum of CuO-NPs had a band position similar to that of *S. officinalis* extract, indicating that the compounds present in the extract were adsorbed by immobilization on the surface of CuO-NPs. In the FT-IR spectrum of CuO-NPs, the peak at 457 cm^{-1} was related to the metal and the homogeneity. CuO-NPs. FT-IR spectroscopy not only helps identify the functional groups involved in nanoparticle synthesis but also confirms the role of plant metabolites in the stability and degradation of CuO-NPs. Therefore, chemical interactions between the functional groups present in the plant extract and the surface of nanoparticles are considered as a key factor in the successful synthesis of nanoparticles and improving their stability (24). DLS analysis of the synthesized CuO-NPs showed that the average size of CuO-NPs was 270 nm, while zeta poten-

tial measurements showed a negative potential of about -13.0 mV. The negative zeta potential induces a strong repulsive force between molecules, thereby contributing to the stability and acceptable quality of CuO-NPs (16).

In the present study, the in vitro antibacterial properties of CuO-NPs against Gram-negative *P. aeruginosa* and *K. pneumoniae*, and Gram-positive *S. aureus*, *S. saprophyticus*, and *B. cereus*, were evaluated using the agar well diffusion method. In the well diffusion method, CuO-NPs showed a significant zone of bacterial inhibition on all five bacterial strains studied at a concentration of 10 mg/mL. Based on the findings, CuO-NPs may exert antimicrobial effects through several mechanisms. Released copper ions may bind to DNA molecules and form cross-links within and between nucleic acid strands, thereby disrupting the DNA helical structure. In addition, copper ions released from the nanoparticles may bind to the negatively charged bacterial cell wall, leading to membrane rupture, protein denaturation, and cell death (25). The MIC of the biosynthesized nanoparticles was 625 µg/mL against *P. aeruginosa* and *B. cereus*, 312 µg/mL against *K. pneumoniae*, 19 µg/mL against *S. aureus*, and 9.7 µg/mL against *S. saprophyticus*. Thus, the MIC of CuO-NPs ranged from 9.7 to 625 µg/mL. This suggests that the biosynthesized nanoparticles are efficient nanoantimicrobial agents effective across a broad spectrum, especially against *S. aureus* and *S. saprophyticus*. The MBCs of CuO-NPs against *P. aeruginosa*, *S. aureus*, *S. saprophyticus*, *K. pneumoniae* and *B. cereus* were recorded as 1250, 39, 9.7, 625 and 2500 µg/mL, respectively. In the study of Khairy et al., the antibacterial activity of green-synthesized CuO-NPs using ethanolic extracts of *Azadirachta indica* and *Simmondsia chinensis* against *S. aureus*, *E. coli*, *P. aeruginosa*, *Acinetobacter* spp., *K. pneumoniae*, and *Stenotrophomonas maltophilia* was evaluated and based on the results, the MIC of CuO-NPs was between 62.5 and 125 µg/mL (32). This was not consistent with our results. In many studies, the MIC and MBC values of CuO-NPs have been reported to range from 100 to 5000 µg/mL (33-36).

The biosynthesized CuO-NPs exhibited significant antibiofilm activity against *P. aeruginosa* at 312 µg/mL. In the study by Khairy et al., the antibacterial activity of synthesized green CuO-NPs was evaluated against multidrug-resistant (MDR) bacteria. Their results showed that the synthesized CuO-NPs had antibiofilm properties against *Acinetobacter* spp, *E. coli*, *S. aureus*, and *K. pneumoniae* at concentrations of 62.5-125 µg/mL (32). In the study by Agarwala et al., the antibiofilm effects of CuO-NPs against *S. aureus* and *E. coli* were observed at 120 and 160 µg/mL, respectively (37). Studies have reported that nanoparticle inhibition of biofilm may be due to inhibition of exopolysaccharide synthesis (38). In our study, the antioxidant activity of CuO-NPs and the hydroalcoholic extract of *S. officinalis* was assessed at concentrations ranging from 0.31 to 10 mg/mL using the DPPH assay. The maximum DPPH scavenging activity for CuO-NPs at the highest concentration (10 µg/mL) was 88.6%. The IC_{50} obtained from CuO-NPs and the hydroalcoholic extract of *S. officinalis* was 2.79 and 1.97mg/ml, respectively. In a study following the biosynthesis of CuO-NPs mediated by *Echium amoenum* extract, the antioxidant properties were investigated, and the IC_{50} values for CuO-NPs and *Echium amoenum* extract were 35.46 and 95.31 µg/mL, respectively (39). In the study by Javad et al., the antioxidant and anti-inflammatory activity of green-synthesized silver nanoparticles (AgNPs) was evaluated using *S. officinalis* extract, and based on the results, an IC_{50} of approximately 830 µg/mL for DPPH was calculated (40).

In recent studies in Iran, the biological activities of green CuO-NPs synthesized using various plant extracts have been studied. For example, Zamanian et al. (2024) synthesized CuO-NPs using aqueous extracts of medicinal plants, and the results showed that phytochemicals in the extracts, especially phenolic compounds, played an important role in the regeneration and stabilization of the nanoparticles (41). The results of this study also showed that the nanoparticles exhibited significant antibacterial activity against Gram-positive and Gram-negative bacteria, which was attributed to the release of copper ions. These results are consistent with the present study's findings on the role of plant compounds in the synthesis and stability of CuO-NPs. In another study by Barani et al. (2024) in Iran, CuO-NPs were synthesized and evaluated using *Moringa peregrina* extract (42). The results showed that these nanoparticles had stable morphology and significant antibacterial and anticancer activity, and that reducing particle size increased the contact surface area and improved the biological effects. This finding is consistent with the present study's results on the strong antibacterial effects of CuO-NPs and the role of their size and

surface properties. Also, in a study by Ranjbar et al. (2023) in Iran, CuO-NPs synthesized using *Tanacetum parthenium* plant extract were investigated, and the phenolic and flavonoid groups present in the extract were found to play a key role in the reduction and stabilization of the nanoparticles (43). These results are consistent with the FTIR data of the present study, which shows the presence of hydroxyl groups and aromatic compounds. Also, the differences in MIC values reported in these studies relative to the present study can be attributed to variations in the chemical composition of the extracts, nanoparticle size, and synthesis conditions. In summary, studies conducted in Iran show that green-synthesized CuO-NPs using plant extracts exhibit significant biological activity, and differences in their physicochemical properties directly affect their antibacterial and antibiofilm effects. However, the overall consistency between the results of these studies and the present study confirms the high potential of CuO-NPs synthesized using *S. officinalis* extract for biomedical and antibacterial applications.

CONCLUSION

This study reports that the green synthesis of CuO-NPs using the hydroalcoholic extract of *S. officinalis* is a suitable method for preparing antimicrobial and antibiofilm compounds with antioxidant properties. The UV-visible absorption spectrum shows a blue color change as the concentration of the plant extract in the reaction mixture increases during synthesis. SEM images show that the particles are nearly spherical. FTIR and DLS results confirmed the equivalence of the synthesized CuO-NPs. The formed CuO-NPs were highly stable and exhibited a significant antibacterial effect against both classes of Gram-negative bacteria. In addition, CuO-NPs formed had antioxidant) IC_{50} = 2.79 mg/mL, and anti-biofilm properties against *P. aeruginosa* at a concentration of 312 µg/mL. Thus, the antibacterial activity of CuO-NPs synthesized using *S. Officinalis* hydroalcoholic extract makes them promising antimicrobial agents for various applications.

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REFERENCES

1. Patel B, Channiwala M, Chaudhari S, Mandot A. Green synthesis of silver nano-sols by leaf extract of ocimum sanctum and their efficacy against human pathogenic bacterium. *J Green Sci Technol* 2015; 2: 39-44.
2. Saxena A, Tripathi R, Zafar F, Singh P. Green synthesis of silver nanoparticles using aqueous solution of Ficus benghalensis leaf extract and characterization of their antibacterial activity. *Mater Lett* 2012; 67: 91-94.
3. Gour A, Jain NK. Advances in green synthesis of nanoparticles. *Artif Cells Nanomed Biotechnol* 2019; 47: 844-851.
4. Hussain I, Singh N, Singh A, Singh H, Singh S. Green synthesis of nanoparticles and its potential application. *Biotechnol Lett* 2016; 38: 545-560.
5. Villagrán Z, Anaya-Esparza LM, Velázquez-Carriles CA, Silva-Jara JM, Ruvalcaba-Gómez JM, Aurora-Vigo EF, et al. Plant-based extracts as reducing, capping, and stabilizing agents for the green synthesis of inorganic nanoparticles. *Resources* 2024; 13: 70.
6. Jadoun S, Arif R, Jangid NK, Meena R. Green synthesis of nanoparticles using plant extracts: A review. *Environ Chem Lett* 2021; 19: 355-374.
7. Nadaroglu H, GÜNGÖR AA, Selvi İ. Synthesis of nanoparticles by green synthesis method. *Int J Innov Res Rev* 2017; 1: 6-9.
8. Grdiša M, Jug-Dujaković M, Lončarić M, Carović-Stanko K, Ninčević T, Liber Z, et al. Dalmatian sage (*Salvia officinalis* L.): A review of biochemical contents, medical properties and genetic diversity. *Agric Conspec Sci* 2015; 80: 69-78.
9. Lu Y, Foo LY. Polyphenolics of Salvia—a review. *Phytochemistry* 2002 ; 59: 117-140.
10. Walch SG, Kuballa T, Stühlinger W, Lachenmeier D. Determination of the biologically active flavour substances thujone and camphor in foods and medicines containing sage (*Salvia officinalis* L.). *Chem Cent J* 2011; 5: 44.
11. Khakpour S, Khosravi M, Jafari Marandi S, Ahadi Ali M. The effect of hydroalcoholic extract of Salvia officinalis L. on the inflammatory reduction in male mice. *AMU-TMUJ* 2014; 24: 136-142.
12. Singh R, Shedbalkar UU, Wadhwani SA, Chopade B.

Bacteriagenic silver nanoparticles: synthesis, mechanism, and applications. *Appl Microbiol Biotechnol* 2015; 99: 4579-4593.
13. Bogutska K, Sklyarov YP, Prylutskyy Y. Zinc and zinc nanoparticles: biological role and application in biomedicine. *Ukr Bioorg Acta* 2013; 1: 9-16.
14. Eivazihollagh A, Bäckström J, Dahlström C, Carlsson F, Ibrahem I, Lindman B, et al. One-pot synthesis of cellulose-templated copper nanoparticles with antibacterial properties. *Mat Lett* 2017; 187: 170-172.
15. Chatterjee AK, Chakraborty R, Basu T. Mechanism of antibacterial activity of copper nanoparticles. *Nanotechnology* 2014; 25: 135101.
16. Nabila MI, Kannabiran K. Biosynthesis, characterization and antibacterial activity of copper oxide nanoparticles (CuO NPs) from actinomycetes. *Biocatal Agric Biotechnol* 2018; 15: 56-62.
17. Shahmohamadi S, Khosravi M. Effect of hydroalcoholic extract of Salvia officinalis L. on the activity of catalase and superoxide dismutase in an oxidative stress model created by intracerebroventricular STZ injection in male rats. *Physiol Pharmacol* 2013; 17: 176-184.
18. Alnaddaf LM, Almuhammady AK, Salem KF, Alloosh MT, Saleh MM, Al-Khayri JM (2021). Green synthesis of nanoparticles using different plant extracts and their characterizations. In: Nanobiotechnology: Mitigation of Abiotic Stress in Plants. Cham: Springer; pp. 165-199.
19. Kaviya S, Santhanalakshmi J, Viswanathan B, Muthumary J, Srinivasan K. Biosynthesis of silver nanoparticles using Citrus sinensis peel extract and its antibacterial activity. *Spectrochim Acta A Mol Biomol Spectrosc* 2011; 79: 594-598.
20. Zangeneh MM, Joshani Z, Zangeneh A, Miri E. Green synthesis of silver nanoparticles using aqueous extract of Stachys lavandulifolia flower, and their cytotoxicity, antioxidant, antibacterial and cutaneous wound-healing properties. *Appl Organomet Chem* 2019; 33(9): e5016.
21. Govindaraju K, Kiruthiga V, Kumar VG, Singaravelu G. Extracellular synthesis of silver nanoparticles by a marine alga, Sargassum wightii Grevilli and their antibacterial effects. *J Nanosci Nanotechnol* 2009; 9: 5497-5501.
22. Mirzaei SZ, Lashgarian HE, Karkhane M, Shahzamani K, Alhameedawi AK, Marzban A, et al. Bio-inspired silver selenide nano-chalcogens using aqueous extract of *Melilotus officinalis* with biological activities. *Bioresour Bioprocess* 2021; 8: 56.
23. Hosseinimehr SJ, Mahmoudzadeh A, Ahmadi A, Ashrafi SA, Shafaghati N, Hedayati N. The radioprotective effect of *Zataria multiflora* against genotoxicity induced by γ irradiation in human blood lymphocytes. *Cancer Biother Radiopharm* 2011; 26: 325-329.
24. Gawande MB, Goswami A, Felpin FX, Asefa T, Huang X, Silva R, et al. Cu and Cu-based nanoparticles: synthesis and applications in catalysis. *Chem Rev* 2016; 116: 3722-3811.
25. Naika HR, Lingaraju K, Manjunath K, Kumar D, Nagaraju G, Suresh D, et al. Green synthesis of CuO nanoparticles using Gloriosa superba L. extract and their antibacterial activity. *J Taibah Univ Sci* 2015; 9: 7-12.
26. Waris A, Din M, Ali A, Ali M, Afridi S, Baset A, et al. A comprehensive review of green synthesis of copper oxide nanoparticles and their diverse biomedical applications. *Inorg Chem Commun* 2021; 123: 108369.
27. Murugan B, Rahman MZ, Fatimah I, Lett JA, Annaraj J, Kaus NH, et al. Green synthesis of CuO nanoparticles for biological applications. *Inorg Chem Commun* 2023; 155: 111088.
28. Nethravathi P, Kumar MP, Suresh D, Lingaraju K, Rajanaika H, Nagabhushana H, et al. Tinospora cordifolia mediated facile green synthesis of cupric oxide nanoparticles and their photocatalytic, antioxidant and antibacterial properties. *Mater Sci Semicond Process* 2015; 33: 81-88.
29. Saif S, Tahir A, Asim T, Chen Y. Plant mediated green synthesis of CuO nanoparticles: comparison of toxicity of engineered and plant mediated CuO nanoparticles towards Daphnia magna. *Nanomaterials (Basel)* 2016; 6: 205.
30. Noor S, Shah Z, Javed A, Ali A, Hussain SB, Zafar S, et al. A fungal based synthesis method for copper nanoparticles with the determination of anticancer, antidiabetic and antibacterial activities. *J Microbiol Methods* 2020; 174: 105966.
31. Sathiyavimal S, Vasantharaj S, Veeramani V, Saravanan M, Rajalakshmi G, Kaliannan T, et al. Green chemistry route of biosynthesized copper oxide nanoparticles using Psidium guajava leaf extract and their antibacterial activity and effective removal of industrial dyes. *J Environ Chem Eng* 2021; 9: 105033.
32. Khairy T, Amin DH, Salama HM, Elkholy IMA, Elnakib M, Gebreel HM, et al. Antibacterial activity of green synthesized copper oxide nanoparticles against multidrug-resistant bacteria. *Sci Rep* 2024; 14: 25020.
33. Chandrakanth RK, Ashajyothi C, Oli AK, Prabhurajeshwar C. Potential bactericidal effect of silver nanoparticles synthesised from *Enterococcus* species. *Orient J Chem* 2014; 30: 3.
34. Das PE, Abu-Yousef IA, Majdalawieh AF, Narasimhan S, Poltronieri P. Green synthesis of encapsulated copper nanoparticles using a hydroalcoholic extract of *Moringa oleifera* leaves and assessment of their antioxidant and antimicrobial activities. *Molecules* 2020; 25: 555.
35. Rajivgandhi G, Maruthupandy M, Muneeswaran T,

Ramachandran G, Manoharan N, Quero F, et al. Biologically synthesized copper oxide nanoparticles enhanced intracellular damage in ciprofloxacin resistant ESBL producing bacteria. *Microb Pathog* 2019; 127: 267-276.

36. Prabhu Y, Rao KV, Sai VS, Pavani T. A facile biosynthesis of copper nanoparticles: A micro-structural and antibacterial activity investigation. *J Saudi Chem Soc* 2017; 21: 180-185.
37. Agarwala M, Choudhury B, Yadav R. Comparative study of antibiofilm activity of copper oxide and iron oxide nanoparticles against multidrug resistant biofilm forming uropathogens. *Indian J Microbiol* 2014; 54: 365-368.
38. Mohanta YK, Biswas K, Jena SK, Hashem A, Abd Allah EF, Mohanta TK. Anti-biofilm and antibacterial activities of silver nanoparticles synthesized by the reducing activity of phytoconstituents present in the Indian medicinal plants. *Front Microbiol* 2020; 11: 1143.
39. Shakib P, Mirzaei SZ, Sharafi Z, Saki R, Goudarzi GR, Sepeavand A, et al. Biofabrication of copper oxide nanoparticles mediated with Echium amoenum petal extract for evaluation of biological functions. *Biomass Convers Biorefin* 2024; 14: 25651-25661.
40. Baharara J, Ramezani T, Mousavi M, Asadi-Samani M. Antioxidant and anti-inflammatory activity of green synthesized silver nanoparticles using Salvia officinalis extract. *Ann Trop Med Public Health* 2017; 10: 1265-1270.
41. Zamanian Z, Tajbakhsh E, Arbab Soleimani N, Ghasemian A. Aqueous extract-mediated green synthesis of CuO nanoparticles: Potential anti-tuberculosis agents. *Food Sci Nutr* 2024; 12: 5907-5921.
42. Barani M, Mir A, Roostaee M, Sargazi G, Adeli-Sardou M. Green synthesis of copper oxide nanoparticles via Moringa peregrina extract incorporated in graphene oxide: evaluation of antibacterial and anticancer efficacy. *Bioprocess Biosyst Eng* 2024; 47: 1915-1928.
43. Ranjbar M, Khakdan F, Mukherjee A. In vitro analysis of green synthesized CuO nanoparticles using Tanacetum parthenium extract for multifunctional applications. *Environ Sci Pollut Res Int* 2023; 30: 60180-60195.