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Antibacterial properties of copper nanoparticles synthesized by aqueous extract of *Oliveria decumbens* flowers

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ABSTRACT

Introduction: The production of nanoparticles by biological methods is less hazardous than traditional chemical methods and is also cost-effective. The aim of this study was to synthesize copper nanoparticles using the aqueous extract of *Oliveria decumbens* flowers and to investigate their antibacterial properties.

Materials & Methods: The soaking method was used for extraction. Nanoparticle synthesis was carried out by reducing copper sulfate with the aqueous extract of *O. decumbens* flowers. The resulting nanoparticles were characterized using spectrophotometric analysis, infrared spectroscopy, and scanning electron microscopy. The antibacterial effects of the extract and the synthesized nanoparticles on *Staphylococcus aureus, Bacillus cereus, Escherichia coli,* and *Klebsiella pneumoniae* were investigated using the disk method, and their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were determined.

Results: The aqueous extract of *O. decumbens* flowers was found to reduce copper sulfate. The produced nanoparticles were spherical, with an average size of 36.93 nm. Although the aqueous extract had no effect on the tested bacteria, the synthesized nanoparticles at concentrations of 2000 and 4000 µg/disk exhibited antibacterial activity against all these bacteria. The MIC and MBC values were determined to be 1000 µg/ml for *S. aureus* and *B. cereus* and 2000 µg/ml for *K. pneumoniae* and *E. coli*.

Conclusion: The results showed that the aqueous extract of *O. decumbens* flowers acts as a reducing and stabilizing agent for copper sulfate. The synthesized copper nanoparticles exhibited acceptable antibacterial activity against the studied Gram-positive and Gram-negative bacteria.

Keywords: green synthesis, infrared spectroscopy, scanning electron microscopy, microdilution, nanoparticles

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Introduction

Antibiotic resistance, while posing a major threat to global health, complicates treatment and leads to high morbidity and mortality. Mechanisms of microbial resistance include the transfer of resistance-carrying plasmids, enzymatic degradation, and genetic mutations. Innovative therapeutic strategies are being developed to address these challenges, and nanoparticles have potential applications in the fight against antibiotic-resistant pathogens (1).

Nanoparticles are compounds with different chemical and physical properties, and their size ranges from 1 to 100 nm (2). Various methods are used to synthesize metal nanoparticles, such as chemical-mechanical methods, gas-phase evaporation, chemical reduction, hydrothermal irradiation, plasma, vacuum vapor deposition, electron beam irradiation, electrolysis, photocatalytic decomposition, sol-gel, inverse microemulsion, and ultrasonication. The use of a chemical reducing agent increases the toxicity of copper nanoparticles to living organisms. The other methods mentioned are also technically complex and require specialized equipment; therefore, they are not economically viable (3). As a result, there is an increasing trend towards the use of green synthesis-based methods.

In green chemistry, biological systems such as plants and microorganisms are used to synthesize metal nanoparticles, including copper metal, in an environmentally friendly manner. This approach highlights their role in important aspects, including environmental health, sustainability, and cost-effectiveness, as well as antimicrobial and anticancer applications (4). Among them, the use of plants has received more attention due to their availability and low risk. Today, many reports are available on the synthesis of copper nanoparticles using extracts of various plants, including damiana leaves, henna flowers (5), and thyme leaves (6).

Due to their very small dimensions, effective surface area, and high surface-to-volume ratio, nanoparticles can bind to the bacterial membrane surface and release metal ions to destroy the bacterial membrane, leading to cell lysis (7). Compared to other metal nanoparticles, copper nanoparticles have higher catalytic activity. In addition to their antimicrobial properties, these nanoparticles have other useful properties, including environmental compatibility, non-stimulant and non-sensitizing properties, non-resistance to microorganisms, resistance to heat, and high stability. The antimicrobial properties of these particles have led to their widespread use in wound dressings, bandages, and all devices that play a role in the wound healing process. The use of these nanoparticles in biosensors for the diagnosis and treatment of diseases such as cancer is also very valuable (2, 3, and 8).

Oliveria decumbens belongs to the Apiaceae family, which is a native plant of the Iranian flora and grows in the tropical regions of southern and southwestern Iran. This plant is also known by the local names Denak, Den, and Moshkorak (9).

Mountain agarwood is an annual, erect, aromatic plant with a height of 20-45 cm. Leaves: ovate with short divisions; flowers: pink-purple, fragrant. This plant is used in traditional medicine to treat fever, diarrhea, and abdominal pain. Thymol, gamma-terpinene, and paracetamol are the main compounds of mountain agarwood extract, all of which are antioxidants. It has been found that the essential oil of mountain agarwood, which contains a high amount of thymol and carvacrol, can be an effective source as an antibacterial, anti-Helicobacter pylori, and cytotoxic agent (10).

Since our studies showed that the synthesis of copper nanoparticles (CuNP) with the flowers of the aforementioned plant has not been performed so far, the aim of this study was to evaluate the feasibility of synthesizing copper nanoparticles using the aqueous extract of the flowers of this plant in the complete stage and to evaluate the antibacterial activity of the produced nanoparticles against some pathogenic bacteria.

Materials and methods

Preparation of aqueous extract of Oliveria decumbens plant

Oliveria decumbens plant samples were collected in late May from areas around Kohdasht city in Lorestan province and, after identification, were registered with the herbarium code SBU-1404-317. The samples were washed with distilled water, and their flowers were separated and dried in the shade. For extraction, the soaking method was used, in which 20 grams of powdered flowers were added to 200 ml of deionized water and kept at 50 °C for 48 hours. Then, the resulting extract was filtered with Whatman filter paper and centrifuged for 10 minutes at 12,000 RPM to remove impurities (Thermo Fisher Scientific, model: Sorvall ST 40).

Bacterial strains studied

The bacteria used in this study included Staphylococcus aureus (PTCC1112), Bacillus cereus (PTCC1556), Escherichia coli (PTCC1330), and Klebsiella pneumoniae (PTCC1053), which were obtained from the Microbiology Laboratory of Lorestan University of Medical Sciences.

Copper Nanoparticle Synthesis

In order to optimize the best extract concentration for the synthesis of copper nanoparticles, different volumetric ratios of the freshly obtained extract were made with 0.01 M copper sulfate $CuSO_4(5H_2O)$ solution. These ratios included 3:6, 4.5:6, 6:6 and 9:6 (copper sulfate extract) and were stirred continuously on a magnetic stirrer (pH 7); They were kept at 60 °C for 24 hours and then centrifuged at 12,000 RPM for 20 minutes. Finally, the synthesized nanoparticles were dried in an oven at 60 °C for further analysis (11).

Identification and characterization of the synthesized nanoparticles

In order to confirm the formation of copper nanoparticles and also to determine the characteristics of these nanoparticles, spectrophotometric, FT-IR, and SEM analyses were performed. In this study, the reduction of copper ions to copper nanoparticles was investigated using a UV-Vis spectrophotometer (JENWAY 6405); in this way, 300 μl of the nanoparticles obtained from each dilution were diluted with 3 ml of distilled water, and their spectra were measured in the range of 350-700 nm (12). In order to identify the functional groups present in the copper nanoparticles, FT-IR spectrum analysis was performed. Here, nanoparticles obtained from the extract and KBr grains were mixed together in a ratio of 1:100, and after pulverization, analysis was measured using FT-IR spectroscopy in the range of 400-4000 with a resolution of 4 cm^{-1} (11). The size and morphological characteristics of the synthesized nanoparticles were also examined using a 15 kV scanning electron microscope with a magnification of x 10 and a resolution of 1 nm (Mira3, Czech) (13).

Investigation of antibacterial effects

First, a concentrated stock of nanoparticles was prepared with a concentration of 50 mg/mL of deionized distilled water. In order to investigate the antibacterial effects of the synthesized nanoparticles, the disk diffusion method (DDM), determination of the minimum inhibitory concentration (MIC), and determination of the minimum bactericidal concentration (MBC) were used. To determine the diameter of the

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growth inhibition zone, a turbidity equivalent to half McFarland (1.5×10^6 CFU/mL) was prepared from the 24-hour culture of each bacterium in normal saline solution, and 50 µL of it was cultured uniformly on Mueller Hinton agar plates. From the concentrated stock of nanoparticles and after vortexing, respectively, values of 10, 20, 40, and 80 µl were placed on sterile raw discs and dried at room temperature (25°C) under sterile conditions. These coated discs (with different concentrations of 500, 1000, 2000, and 4000 µg/disc of copper nanoparticles) were then placed on the medium containing bacteria. In order to compare the effect of the extract with the nanoparticles, a series of discs coated with 4000 µg/disc of extract was also placed as a control (as the highest concentration of nanoparticles). After 24 hours of storage at 37°C, the diameter of the zone of inhibition was measured. This test was repeated three times, and the average obtained was reported as the result (14). To determine the MIC values of the produced copper nanoparticles, the highest concentration used was 4000 µg/ml of culture medium, which was investigated by diluting by a factor of 2 according to the CLSI standard dilution method A8-M07. This test was performed as microdilution was performed in Mueller Hinton broth. Microbial growth was monitored by adding triphenyltetrazolium chloride (TTC) at a concentration of 5 mg/ml. After 24 hours of incubation at 37°C, the wells were visually inspected for turbidity, and the lowest concentration of the extract at which no bacterial growth occurred was considered the minimum inhibitory concentration.

To determine the MBC, wells without turbidity (relevant to the determination of MIC) were cultured in Mueller Hinton agar. The media were incubated for 24 hours at 37°C, and the lowest concentration of the substance that killed at least 99.9% of the bacteria was reported as MBC (15). The above steps were repeated 3 times for each bacterium.

Statistical comparison of means using two-way analysis of variance (two-way ANOVA) at a confidence level of 95% was used (SPSS ver. 18).

Results

Results of synthesis of copper (II) nanoparticles from aqueous extract: The aqueous extract obtained from the flowers of the *Oliveria decumbens* was a pale purple solution. With the addition of copper sulfate to the aqueous extract, the color of the extract changed from pale purple to yellowish green, and with the passage of time, brown particles began to precipitate, which was evidence of the synthesis of the desired nanoparticles. Also, the peaks obtained from UV-VS spectrophotometric analysis were further evidence of the formation of copper nanoparticles. One of the most interesting features of metal nanoparticles is their optical properties, which change in proportion to the shape and size of the nanoparticles. In metal nanoparticles, surface plasmon resonance (SPR) is responsible for their unique optical properties. Considering that copper nanoparticles have maximum optical absorption in two regions of about 400 and 500–600 nm and the solution obtained from the ratio 9:6 (copper sulfate: extract) showed maximum absorption (576 nm), this ratio was selected as the optimal concentration of the extract and used in subsequent experiments (Figure 1). Figure 1 shows that the characteristic surface plasmon resonance band occurred at a wavelength of 576 nm for copper nanoparticles. After centrifugation and drying, the weight of the produced nanoparticles was measured to be 950 mg.

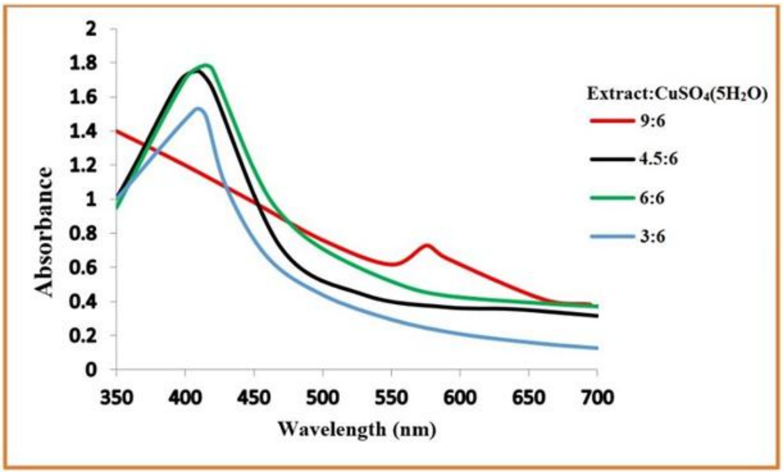

Figure 1. Spectrophotometric absorption spectrum of copper nanoparticles synthesized using different ratios of *Oliveria decumbens* aqueous extract.

FT-IR spectrum analysis

The FT-IR spectroscopy results of the plant extract and synthesized nanoparticles are shown in Figure 3. In Figure A.2, the broad and extended peak observed in the region of 2900 to 3600 (centered at 1 cm, 3431.01) could indicate the presence of OH groups in alcohols or N–H groups in amines and amides. The 1-cm peak at 2941.74 could be related to the CH stretching in CH_3. The 1-cm peaks at 1633.11, 1577.45, and 1528.22 could indicate vibrational activities in aromatic rings, especially double bonds (C=C). In plants, many phenolic compounds, such as tannins, phenols, and catechins, have this type of vibrational activity in this region. The peaks at 1481.06 and 1419.24 cm^{-1} can indicate vibrational activities in aromatic rings and skidding activities related to different states in benzenes and aromatic compounds and can indicate different types of phenolic groups, catechins, or other natural aromatic compounds. The broad and short peak in the 1-cm^{-1} region from 980 to 1240 cm^{-1} centered at 1098.76 cm^{-1} can indicate the presence of C–O groups, which are common in esters, alcohols, and polysaccharides. The peak at 1 cm, 914.67, may indicate vibrational activity in aromatic rings, especially in the region of skidding activities, and is related to vibrational motions in C–H bonds in aromatic rings, which are often seen in phenolic compounds, catechins, and other plant phytochemicals. The peak at around 1 cm, 797.33, may be related to vibrational activities in aromatic rings or in the structures of cluster activities related to benzene rings. It may also indicate the presence of phenolic groups and aromatic compounds in this plant extract (16).

Together, these peaks indicate that the aqueous extract of *Oliveria decumbens* flowers is rich in polyphenols, alkaloids, terpenoids, and fatty acids. Considering that copper nanoparticles also have absorption peaks in the regions of 3246, 2587, 1715, 1327, and 1170 cm^{-1} (Figure B.2), it can be said that the biomolecules of the aqueous extract of *Oliveria decumbens* flowers act as both a reducing agent and a coating for copper nanoparticles; therefore, they can protect copper nanoparticles against oxidation and conversion to copper oxide.

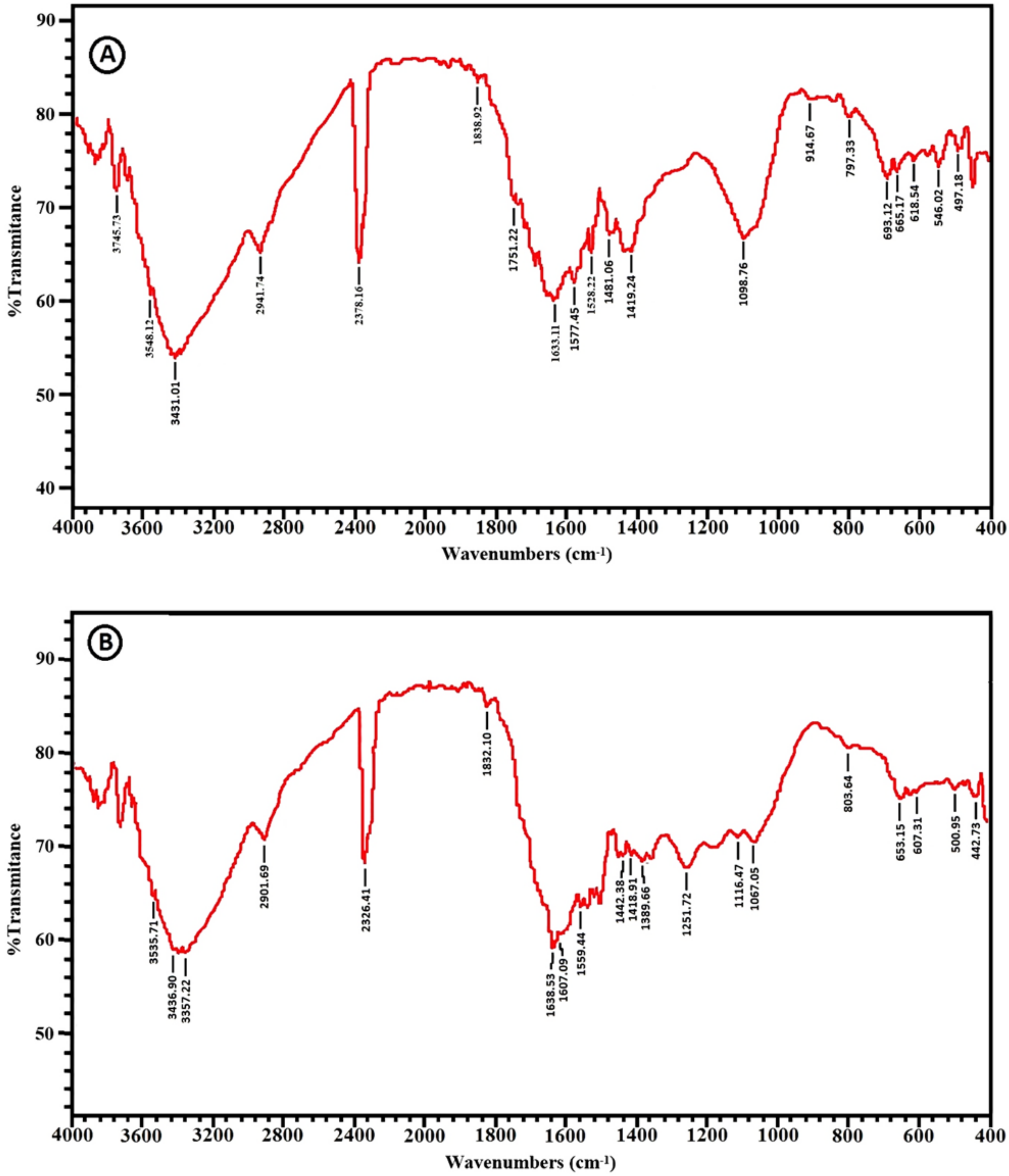

Figure 2. Spectra obtained from FT-IR analysis. A: Aqueous extract of *Oliveria decumbens* plant and B: Synthesized copper nanoparticles.

Based on the results of scanning electron microscopy, the synthesized copper nanoparticles had a spherical morphology, and the particle size was determined to be between 32.57 and 39.82 nm with an average of 36.93 nm (Figure 3).

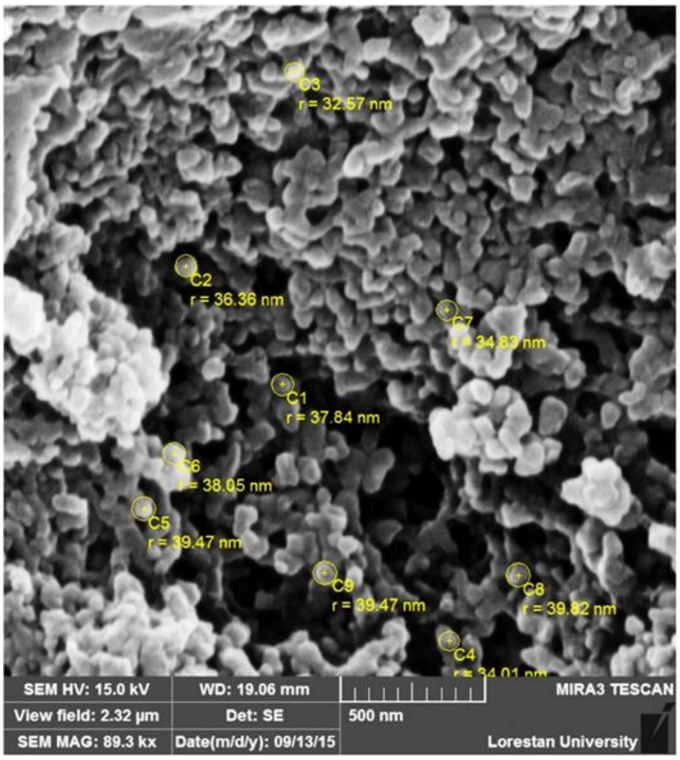

Figure 3. Scanning electron microscope image of synthesized copper nanoparticles, showing the shape and diameter of the synthesized nanoparticles.

DDT results showed that the aqueous extract of *Oliveria decumbens* at the concentrations used had no effect on the growth of the studied bacteria. The synthesized nanoparticles also had no effect on the growth of the studied bacteria at concentrations of 500 and 1000 µg/disk but had antibacterial effects at concentrations of 2000 and 4000 µg/disk (Figure 4). The results of statistical analysis showed that the effect of nanoparticles on gram-positive bacteria was greater than the effect on the gram-negative bacteria studied (Table 1). As shown in this table, statistically, the effect of 2000 µg/disk of nanoparticles on gram-positive bacteria *Bacillus cereus* and *Staphylococcus aureus* is the same as the effect of 4000 µg/disk on *Klebsiella pneumoniae* and *Escherichia coli*.

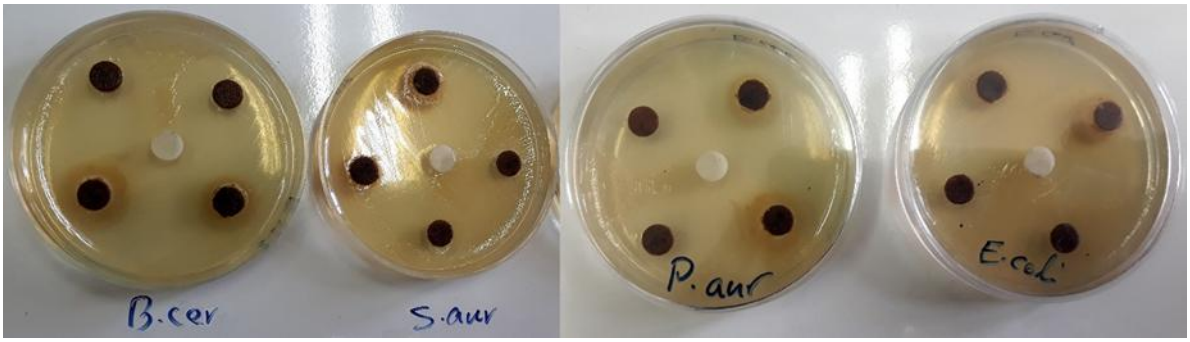

Figure 4. Results of the diameter of the growth inhibition zone obtained from the aqueous extract of *Oliveria decumbens* flowers and concentrations of 500, 1000, 2000, and 4000 µg/disk of synthesized nanoparticles against the studied bacteria.

Results of determining the diameter of the growth inhibition zone caused by synthesized copper nanoparticles using the aqueous extract of *Oliveria decumbens* flowers on the studied bacteria (values shown in the same color are not statistically significantly different from each other at a significance level of 5%) (Table 1).

Table 1. Effect of Different Concentrations of Copper Nanoparticles Synthesized Using *Oliveria decumbens* Flower Extract on the Growth Inhibition of Selected Bacterial Strains.

Nanoparticle Concentration (μg/disc)	*Escherichia coli* (Growth Inhibition Zone Diameter, mm)	*Klebsiella pneumoniae* (mm)	*Staphylococcus aureus* (mm)	*Bacillus cereus* (mm)
2000	12 ± 15	19 ± 12	81 ± 17	96 ± 18
4000	24 ± 21	35 ± 19	93 ± 25	77 ± 27

The results of determining the minimum growth inhibitory concentration and minimum lethal concentration of the synthesized nanoparticles against the studied bacteria showed that these values were equal. As shown in Table 2, Gram-negative bacteria had higher resistance to these nanoparticles.

Table 2. Results of determining the minimum inhibitory concentration and minimum lethal concentration of synthesized copper nanoparticles on the studied bacteria.

Bacterial Strains	MIC (μg/mL)	MBC (μg/mL)
Staphylococcus aureus	1000	1000
Bacillus cereus	1000	1000
Klebsiella pneumoniae	2000	2000
Escherichia coli	2000	2000

Discussion

Despite the discovery and successful use of antibiotics in the 20th century, unfortunately, over time, bacteria have developed widespread resistance to these agents. This resistance has not only made the treatment of common infections more difficult but has also led to an increase in the complexity and cost of treatment protocols. Today, extensive efforts are being made all over the world to overcome such a problem. In the meantime, the use of medicinal plants and natural compounds as an alternative or supplement to antibiotics is a low-risk solution to reduce dependence on antibiotics. In recent years, the synthesis of metal nanoparticles with the help of biological agents, especially plants, has attracted the attention of researchers (17). The aim of this study was to produce copper nanoparticles by green synthesis using the aqueous extract of mountain rose flowers and to investigate the characteristics of the produced nanoparticles. In the next step, the antibacterial effects of these nanoparticles against some gram-positive and gram-negative pathogenic bacteria were also investigated. In general, nanoparticles are new tools to combat resistant bacteria and can play an effective role in the prevention and treatment of bacterial infections. The production of active ingredients in the form of nanoparticles increases their solubility and bioavailability (17).

In the present study, in addition to the fact that the addition of copper (II) sulfate caused a change in the color of the aqueous extract of mountain rose flowers and was a reason for the formation of nanoparticles, considering that copper nanoparticles can have maximum light absorption in two regions of about 400 and between 500 and 600 nm (18), the peaks formed from UV-Vis spectrophotometric analysis in these regions showed that copper nanoparticles were formed. In various studies, the maximum light absorption of copper nanoparticles using the spectrophotometric method has been obtained in different ranges. For example, the group of Ekpanodo and Olabmeyob (2024) obtained an absorption maximum in the range of 440-480 nm for copper nanoparticles synthesized using Echinochloa pyramidalis extract (19). Also, Gopinath et al. (2014) obtained an absorption maximum for the aqueous extract of Nerium oleander leaves at a wavelength of 350 nm (20). Other researchers have also been able to synthesize copper nanoparticles using a green method using plant extracts. For example, Frasha et al. (21) produced copper nanoparticles from the aqueous extract of Phragmites australis leaves. Also, Shaik et al. (22) produced copper nanoparticles from the extract of Clitoria ternatea leaves using a green method. The group of Fazeli et al. (23) also synthesized copper nanoparticles from Artemisia biennis extract using a green method.

In the present study, the copper sulfate reduction method was used to prepare copper nanoparticles. The presence of functional groups in the aqueous extract of the mountain garnet plant, including phenols and flavonoids, was confirmed by FT-IR spectrum analysis. The FT-IR spectrum results of copper nanoparticles synthesized with the extract of mountain garnet flowers also showed an absorption peak similar to the extract but with a lower shift and intensity, which indicated that the biomolecules present in this extract are the agents of copper ion reduction. That is, the functional groups present in the extract play a role in the formation of these nanoparticles (24).

Since the oxidized form of copper is more stable than pure copper, in the absence of suitable protective agents, copper nanoparticles are oxidized and form aggregates. The formation of such aggregates reduces the efficiency of the nanoparticles. The use of some plant extracts as reducing agents surrounds Cu^{2+} ions and prevents the oxidation of copper nanoparticles (22). In the present study, the results of FT-IR spectrum analysis showed that the bioactive molecules in the aqueous extract of mountain rose flowers played a role as both a reducing agent and a coating for copper nanoparticles and thus were able to protect copper nanoparticles against oxidation and conversion to copper oxide and thus enable the production of copper nanoparticles.

The antibacterial effect of metal nanoparticles is associated with their small size and high surface area-to-volume ratio. This feature allows the nanoparticles to have more contact and interaction with the bacterial membrane and disrupt its integrity (25). In the present study, the shape of the produced nanoparticles was uniform and spherical, and their average size was determined to be 36.93 nm. The shapes and sizes of copper nanoparticles produced by green chemistry methods by other researchers have been diverse. For example, Al-Khalifi et al. (26) produced spherical copper nanoparticles with an average size of 38.5 nm from Artemisia extract. In contrast, Parvatalo et al. (27) obtained irregularly shaped copper nanoparticles with dimensions ranging from 500 to 1500 nm using the extract of Tinospora Cordifolia. Nasi et al. (2) suggested that nanoparticles that are larger and less uniform may exhibit different reactivity characteristics. In general, copper compounds show antibacterial activity against both Gram-positive and Gram-negative bacteria, and these effects vary depending on the size of these compounds. The smaller the size of the nanoparticle, the greater its antibacterial potency due to the greater its binding to the bacterial surface (2). In the study of Nasi et al. (3), the lowest MIC and MBC values for synthesized copper nanoparticles against Staphylococcus aureus were recorded as 3125 µg/mL and 6250 µg/mL, respectively. Whereas these values for the copper nanoparticles synthesized in the present study were only 1000 µg/mL. One of the reasons for this higher potency could be the much smaller size of the nanoparticles produced in the present study. Copper ions can damage various cellular functions and cause cytotoxicity and are therefore an effective microbial inhibitor. In general, copper ions damage microbial cells by producing reactive oxygen species (ROS) and replacing or binding with various cofactors in metalloproteins. Compared with other nanoparticles, copper-containing nanoparticles can dissolve faster in solutions, release more metal ions, and exert a stronger antimicrobial effect (28).

Copper nanoparticles and the ions released from them are positively charged, and the surface of gram-positive and gram-negative bacteria is negatively charged. Therefore, through electrostatic interaction, these metal nanoparticles are adsorbed to the surface of bacteria and form strong bonds to disrupt the cell wall. This process increases the cell permeability and allows the metal nanoparticles to enter the cell easily (29).

Copper ions are capable of damaging cell membranes, DNA, RNA, and other molecules (30). They can also damage microbial proteins and, in addition, interfere with the ribosomal function of translating mRNA into protein, thereby activating cytochrome b (31). The negative charge in the bacterial membrane is also easily attacked by positively charged nanoparticles. This process disrupts cellular structures and activities, leading to cell death (32). The antibacterial potency of nanoparticles varies due to differences in the type of nanoparticles, their concentration, how they are manufactured, and also differences in the bacterial strains studied. Several factors influence the susceptibility of different microorganisms to heavy metal nanoparticles, including the structure of the bacterial cell wall and the rate of bacterial growth (6).

Considering that in the present study, both Gram-positive and Gram-negative bacteria were sensitive to the produced nanoparticles, and considering the mechanisms by which copper nanoparticles affect bacteria, which were mentioned above, it is likely that the produced nanoparticles were able to exert their antibacterial effects on the studied bacteria through various mechanisms such as the production of reactive oxygen species, the effect on the cell wall and membrane, damage to DNA, RNA, and proteins, and disruption of ribosomal function. In the present study, the examination of the MIC and MBC values obtained showed that the antibacterial power of the produced copper nanoparticles against Gram-positive bacteria was greater than that against Gram-negative bacteria, which was consistent with the results obtained by Emami Karvani and Chehrazi (33). It has been suggested that Gram-positive bacteria are more sensitive to nanoparticles compared to Gram-negative bacteria due to the simpler structure of the cell wall (34). The higher sensitivity of Gram-positive bacteria could also be related to differences in cell wall structure, cell physiology, metabolism, or degree of contact (35). Compared to lipids, copper ions have a higher affinity for proteins, so Gram-positive bacteria with higher levels of peptidoglycan and protein content in the cell wall are more easily killed by copper-containing nanoparticles (29). Therefore, one of the reasons for the higher sensitivity of the Gram-positive bacteria studied to the produced nanoparticles (and consequently the difference in the MIC and

MBC values obtained) could be for this reason (i.e., the higher affinity of copper nanoparticles to proteins). However, the results of the present study were inconsistent with those of Ferra et al. (36). This difference in results could be due to the difference in the type of bacteria studied as well as the difference in the type and method of synthesis of the nanoparticles studied. The MBC/MIC ratio provides important information about the bactericidal/bacteriostatic nature of the antibiotic. In general, if the MBC/MIC ratio is low, usually around 4:1 or less, an antimicrobial agent is said to be both inhibitory and bactericidal. The MBC of an antimicrobial agent is usually somewhat higher than its MIC, because higher concentrations of an antimicrobial agent are required to kill microorganisms compared to the concentration required to inhibit their growth. However, if the antimicrobial agent is very potent, it has the ability to kill microorganisms, and therefore the MIC and MBC values can be equal or close to each other (37). Based on what has been said, the equality of MIC/MBC values for the bacteria studied in the present study indicates the strong antibacterial properties of the produced copper nanoparticles. In the studies of Goodarzi et al. (2017), it was also found that the MIC and MBC values of the supernatant obtained from some different strains of Lactobacillus were equal against some different strains of Proteus (38). Also, in the study of Soleimanzadeh et al. (2024), the MIC and MBC values of the supernatant of Lactobacillus acidophilus against E. coli were equal (39). In the studies of Rodríguez-Melcón et al., in which twelve different antimicrobial substances, including a number of biocides and antibiotics, were investigated on eight strains of Listeria monocytogenes, it was found that the MIC and MBC values of some of these substances were equal (40).

Finally, it is necessary to mention that in the present study, copper nanoparticles were made for the first time by using the reduction of copper sulfate by the aqueous extract of *Oliveria decumbens* flowers. These nanoparticles were spherical in shape, very small in size, and had good uniformity, so it can be said that they have high quality and capability in terms of antibacterial properties. Comparison of the results obtained from determining the MIC and MBC values obtained in the present study showed that the MIC and MBC values of these nanoparticles against the bacteria studied were equal, indicating their very high antibacterial power.

Conclusion

In this study, copper nanoparticles were prepared for the first time by using a simple, fast, cheap, and environmentally friendly method using copper sulfate reduction with the aqueous extract of *Oliveria decumbens*. The functional groups in the extract of *Oliveria decumbens*, which were confirmed by quantitative and qualitative methods, were responsible for the reduction of copper metal ions to copper nanoparticles. Therefore, the biomolecules in the extract not only reduced copper ions but also protected them from oxidant agents after being prepared. The nanoparticles synthesized by this method were spherical in shape, and their average size was 36.93 nm. The results of this study showed that the synthesized copper nanoparticles have the ability to fight some pathogenic microorganisms, and this ability was greater against gram-positive bacteria than gram-negative bacteria. Based on the results obtained from this study, it can be stated that the quality of the nanoparticles produced in the present study and their antibacterial effects are appropriate. Therefore, the aqueous extract of *Oliveria decumbens* flowers can be used for the synthesis of copper nanoparticles on a large scale.

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CRediT authorship contribution statement

Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Resources, Software, Data Curation, Writing–Original Draft Preparation, Writing–Review & Editing, Visualization, Supervision, Project Administration: KE, AK.

Conflict of interest

The authors declare no conflict of interest.

Ethical considerations

Due to the lack of animal testing, there was no need to obtain an ethics code. The authors avoided data fabrication, falsification, plagiarism, and misconduct.

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References

1. Chilanga F, Kasozi KI, Mazeri S, et al. A systematic review of antimicrobial resistance transmission inferences at the human-livestock interface in Africa. NPJ Antimicrob Resist. 2025; 3: 58. doi: 10.1038/s44259-025-00126-y.
2. Nkosi N. C, Basson AK, Ntombela ZG, Dlamini NG, Pullabhotla RVSR. Green synthesis of copper nanoparticles using a bioflocculant from Proteus mirabilis AB 932526.1 for wastewater treatment and antimicrobial applications. App Nano. 2025; 6: 5. doi: 10.3390/applnano6010005.
3. Pricop A, Negrea A, Pascu B, Nemeş NS, Ciopec M, et al. Copper nanoparticles synthesized by chemical reduction with medical applications. Int J Mol Sci. 2025; 26: 1628. doi: 10.3390/ijms26041628.
4. Gebreslassie YT, Gebremeskel FG. Green and cost-effective biofabrication of copper oxide nanoparticles: Exploring antimicrobial and anticancer applications. Biotech Rep. 2024; 41: e00828. doi: 10.1016/j.btre. 2024.e00828.
5. Kirubakaran D, Selvam K, Dhaneeshram M, Shivakumar M. S, Rajkumar M, et al. Biogenic synthesis of copper nanoparticle using Impatiens chinensis L: Insights into antimicrobial antioxidant and anticancer activity. J Mol Struct. 2024; 1317: 138991. doi: 10.1016/j.molstruc.2024.138991.
6. Ebrahimi K, Shiravand S, Mohammedi AA, Nabi-Afjadi M, Zalpoor H, et al. Biosynthesis of copper nanoparticles using aqueous Thymus daenensis (Celak) flora and investigation of its antifungal activity. J Med Microbiol Infect Dis. 2022; 10: 98-103. doi: 10.52547/JoMMID.10.3.98.
7. Fangfang Y, Yalong Li, Bin Mu, Aiqin Wang, Yameng Song, et al. Insight into the synergistic antibacterial mechanism of bio ZnO nanoparticles synthesized from Sophora japonica flower buds extract. Inorg Chem Communic. 2024; doi: 10.1016/j.inoche.2024.112341.
8. Afonso IS, Cardoso B, Nobrega G, Minas G, Ribeiro J. E, et al. Green synthesis of nanoparticles from olive oil waste for environmental and health applications: A review. J Env Chem Engin. 2024; 12: 114022. doi: 10.1016/j.jece.2024.114022.
9. Mirahmad A, Ghoran SH, Alipour P, Taktaz F, Hassan S, et al. *Oliveria decumbens* Vent. (Apiaceae): Biological screening and chemical compositions. J Ethnopharm. 2024; 318: 117053. doi: 10.1016/j.jep.2023.117053.
10. Shiryanpour S, Nouri L, Azizi M, et al. Active chitosan film containing single and double nanoemulsions of *Oliveria decumbens* Vent essential oil/anthocyanin of eggplant for chicken preservation. Food Measur. 2025; doi: 10.1007/s11694-025-03477-2.
11. Mali SC, Dhaka A, Githala CK, Trivedi R. Green synthesis of copper nanoparticles using Celastrus paniculatus Willd. leaf extract and their photocatalytic and antifungal properties. Biotech Rep. 2020; 27: e00518. doi: 10.1016/j.btre. 2020.e00518.
12. Ansari MA, Ahmad I, Naeem S, Husain D, Patil AB, et al. Green synthesis and characterization of crystalline copper nanoparticles via sodium borohydride reduction towards enhanced gas sensing application. J Ind Chem Soc. 2024; 101: 101157. doi: 10.1016/j.jics.2024.101157.
13. Alraae A, Moussadik A, Benzaouak A, Kacimi M, Dahhou M, et al. Eco-friendly synthesis and catalytic activity of Cu nanoparticles deposited on expanded muscovite to reduce organic pollutants. Resul Surf and Interface. 2024; 17: 100343. doi: 10.1016/j.rsurfi.2024.100343.
14. Wong TTY, Lee CH, Luk HWS, Tse CWS, Ho PL. Predictive value of direct disc diffusion testing from positive blood cultures for detection of antimicrobial nonsusceptibility. Microorg. 2025; 13: 398. doi: 10.3390/microorganisms13020398.
15. Parvekar P, Palaskar J, Metgud S, Maria R, Dutta S. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles against Staphylococcus aureus. Biomat Invest Dentist. 2020; 7: 105-109. doi: 10.1080/26415275.2020.1796674.
16. Sewell M, Farley C, Portal EAR, Lindsay D, Ricci ML, et al. Broth microdilution protocol for determining antimicrobial susceptibility of Legionella pneumophila to clinically relevant antimicrobials. J Microbiol Methods. 2025; 228: 107071. doi: 10.1016/j.mimet.2024.107071.
17. Jiao M, Kong W, Liu W, Dong Z, Yang J, et al. Boosting the antibacterial potency of natural products through nanotechnologies. Int J Pharmaceutics. 2025; 674: 125437. doi: 10.1016/j.ijpharm.2025.125437.
18. Nguyen PA, Nguyen AVP, Dang-Bao T, et al. Green synthesis of copper nanoparticles using cocoa pod extract and its catalytic activity in deep oxidation of aromatic hydrocarbons. SN App Sci. 2020; 2: 1795. doi: 10.1007/s42452-020-03539-8.
19. Akpanudo NW, Olabemiwob OM. Green synthesis and characterization of copper nanoparticles (CuNPs) and composites (CuC) using the Echinochloa pyramidalis extract and their application in the

remediation of PAHsin water. Water Practic Technol. 2024; 19: 324. doi: 10.2166/wpt.2024.011.
20. Gopinath M, Subbaiya R, Selvam MM, Suresh D. Synthesis of copper nanoparticles from Nerium oleander leaf aqueous extract and its antibacterial activity. Int J Curr Microbiol App Sci. 2014; 3: 814-8. doi: 10.1245/fvd.2014.011.
21. Frahtia A, Derouiche S, Niemann J. Novel and facile green synthesis of copper nanoparticles using aqueous extract of Phragmites australis leaves and evaluation of their antioxidant antihemolytic anti-inflammatory and anticancer effects. J App Biotech Rep 2025; 12: 1545-53. doi: 10.30491/jabr.2024.450158.1710.
22. Shaik R, Buggana A, Thalari V, Sandhya Rano Kedharnath B, Golla N. Green synthesis characterization and biological activities of copper nanoparticles using Clitoria ternatea leaf extract. Int J Nano Dimen. 2025; 16: 1-12. doi: 10.57647/j.ijnd.2025.1601.05.
23. Fazeli S, Rafiee F, Ferdousi A. Biosynthesis of copper nanoparticles using Artemisia biennis Willd plant extract for antibacterial and anti-biofilm activities. Int Electr Microbiol. 2025; 11: 63-75. doi: 10.61186/iem.11.1.63.
24. Amaliyah S, Pangesti D. P, Masruri M, Sabarudin A, Sumitro SB. Green synthesis and characterization of copper nanoparticles using Piper retrofractum Vahl extract as bioreductor and capping agent. Heliyon. 2020; 6. doi: 10.1016/j.heliyon. 2020.e04345.
25. Ebrahimi K, Shiravand S, Mahmoudvand H. Biosynthesis of copper nanoparticles using aqueous extract of Capparis spinosa fruit and investigation of its antibacterial activity. Marmara Pharmaceut J. 2017; 21: 866-71. doi: 10.12991/mpj.2017.31.
26. Al-Khafaji MAA, Al-Refai'a RAK, Al-Zamely OMY. Green synthesis of copper nanoparticles using artemisia plant extract. Mater Today Proceed. 2022; 49: 2831-35. doi: 10.1016/j.matpr.2021.10.067.
27. Parvathalu K, Rajitha K, Chandrashekar B, Sathvik K, Pranay Bhasker K, et al. Biomimetic synthesis of copper nanoparticles using Tinospora cordifolia plant leaf extract for photocatalytic activity applications. Plasmon. 2024; 19: 825-834. doi: 10.1007/s11468-023-02037-y.
28. Wang L, Hu C, Shao L. The antimicrobial activity of nanoparticles: present situation and prospects for the future. Int J Nanomed. 2017; 12: 1227-49. doi: 10.2147/IJN.S121956.
29. Ma X, Zhou S, Xu X, Du Q. Copper-containing nanoparticles: Mechanism of antimicrobial effect and application in dentistry-a narrative review. Front Surg. 2022; 5: 905892. doi: 10.3389/fsurg.2022.905892.
30. Baraiya DH, Pappuswamy M, Antony PU, Ganesh S, Pragatheesh A, et al. Copper nanoparticles: A review on synthesis characterization and applications. Asian Pacific J Canc Biol. 2020; 5: 201-210. doi: 10.31557/APJCB.2020.5.4.201.
31. Gheidar H, Haddadi A, Sadeghi Kalani B, Amirmozafari N. The Effect of Nanoparticles on Antimicrobial Resistant Bacteria and Expression Level of the Genes Involved in Biofilm of S. aureus. J Med Bacteriol. 2018; 7: 30-41.
32. Abbaszadegan A, Ghahramani Y, Gholami A, Hemmateenejad B, Dorostkar S, et al. The effect of charge at the surface of silver nanoparticles on antimicrobial activity against gram-positive and gram-negative bacteria: A preliminary study (Article No. 53). BioMed Res Int. 2015; 2015: 1-8. doi: 10.1155/2015/72065.
33. Emami-Karvani Z, Chehrazi P. (2011). Antibacterial activity of ZnO nanoparticle on gram-positive and gram-negative bacteria. African J Microbiol Res. 2011; 1368-73. doi: 10.5897/AJMR10.159.
34. Azam A, Ahmed AS, Oves M, Khan MS, Habib SS, et al. Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: a comparative study. Int J Nanomed. 2012; 7: 6003-9. doi: 10.2147/IJN.S35347.
35. Girma A, Mebratie G, Mekuye B, Abera B, Bekele T, et al. Antibacterial capabilities of metallic nanoparticles and influencing factors. Nano Economics. Advan Online Pub. 2024. doi: 10.1002/nano.202400049.
36. Fera F, Habibah W, Ode S, Rizki A, Ivansyah A, et al. Green synthesis of copper ions nanoparticles functionalized with rhamnolipid as potential antibacterial agent for pathogenic bacteria. Heliyon. 2024; 10. doi: 10.1016/j.heliyon. 2024.e24242.
37. Batista S, Fernández-Pittol M, San Nicolás L, Martínez D, Narváez S, et al. Design and Validation of a Simplified Method to Determine Minimum Bactericidal Concentration in Nontuberculous Mycobacteria. Antibiotic. 2025; 14: 381. doi: 10.3390/antibiotics14040381.
38. Goudarzi L, Kasra Kermanshahi R, Mousavinezhad Z, Soltan Dallal MM. Evaluation of Antimicrobial Activity of Probiotic Lactobacillus Strains against Growth and Urease Activity of Proteus spp. J Med Bacteriol. 2017; 6: pp.31-43. doi: 10.2017/jmb140815.
39. Soleymanzadeh SM, Minaeian S, Majidpour A, Adabi M, Hosseini Doust R. The Lactobacillus acidophilus Supernatant: An Effective and Safe Alternative to Antibiotics. IJT. 2024; 18: 52-60. doi: 10.61186/IJT.18.1.52.

40. Rodríguez-Melcón C, Alonso-Calleja C, García-Fernández C, Carballo J, Capita R. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for Twelve Antimicrobials (Biocides and Antibiotics) in Eight Strains of Listeria monocytogenes. Biology (Basel). 2021; 2911: 46. doi: 10.3390/biology11010046.

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