Research Article

Nano Biomed Eng

Biogenic Synthesis of Copper Oxide Nanoparticles Using *Falcaria vulgaris* L. Leaf Extract and Assessment of Its Antibacterial Activity against *Salmonella* Paratyphi-A

Hamed Mirhadi[1], Alireza Momeni[2], Mohammad Hadi Meshkatalsadat[1]

[1] Department of Chemistry, Qom University of Technology, Qom 1519-37195, Iran
[2] Department of Chemistry, University of North Texas, TX 75201, USA

Corresponding author. E-mail: meshkatalsadat.m@qut.ac.ir

Received: Mar. 22, 2024; **Revised:** Apr. 20, 2024; **Accepted:** May 21, 2024

Citation: H. Mirhadi, A. Momeni, M.H. Meshkatalsadat. Biogenic synthesis of copper oxide nanoparticles using *falcaria vulgaris* l. leaf extract and assessment of its antibacterial activity against *salmonella* paratyphi-a. *Nano Biomedicine and Engineering*, 2024, 16(3): 473−483.
http://doi.org/10.26599/NBE.2024.9290089

Abstract

The copper oxide nanoparticle has attracted increasing attention among all metal oxides due to its distinctive properties and applications. Chemical and physical methods of synthesizing copper oxide nanoparticles are challenged by high reagent costs, equipment limitations, and environmental hazards. Following green chemistry principles, copper oxide nanoparticles were synthesized using *Falcaria vulgaris* leaf extract as a bio-reducing agent and copper nitrate as a source of copper in this study. As a result, copper oxide nanoparticles can be produced without the use of organic chemicals, and this method is non-toxic, economically viable, easily scaled up, and takes less time to produce. The formation of copper oxide nanoparticles was confirmed by the dominant surface plasmon resonance (SPR) peak at 350 nm. Based on scanning electron microscope (SEM) images, the particles were spherical and morphologically sized between 58–200 nm. Energy dispersive X-ray spectroscopy (EDX) analysis confirmed the presence of Cu and O in the pyhto-synthesized nanoparticles. Fourier transform infrared (FTIR) spectroscopy as well showed a distinct peak of Cu–O stretching vibration positioned at 609.36 cm^{-1}. The structural characterization was performed utilizing X-ray diffraction (XRD) in line with reflections of the face-centered cubic (fcc) phase of the copper oxide nanoparticles. The average particle size of nanoparticles was found to be 82.9 nm by using XRD analysis and Scherrer equation. In addition, since *Salmonella* Paratyphi-A is less studied than many other bacteria, we examined the antibacterial properties of copper oxide nanoparticles, against this relatively resistant bacterium. By checking the inhibition zone and minimum concentration of phyto-synthesized nanoparticles, it was determined that they effectively inhibited bacteria growth.

Keywords: antibacterial; biosynthesis; green nanotechnology; pharmaceutical science; plant extract

Introduction

"Nano", measured as a billionth of a meter, is also a key term in nanotechnology and nanoparticles. In the interdisciplinary field of "nano", nano-scaled objects' fundamental properties are investigated [1]. An important aspect of nanotechnology is to understand, manipulate, and control matter on the atomic and molecular level [2]. Nanotechnology has generated much excitement worldwide, but it has been slowed

down by a lack of understanding of its risks and fewer policies to manage. Researchers, however, continue to move ahead, conquering challenges ranging from managing to producing, funding, regulatory, and technical aspects. As one of the branches of green technology, green nanotechnology revolves around the principles of green chemistry and green engineering, with the word "green" referring to the use of biological materials [3]. Based on these principles and green nanotechnology, many metal nanoparticles, such as silver, copper, gold, and zinc, have been produced to date. It is well known that these nanoparticles have a variety of biomedical and environmental applications. Copper oxide (CuO) nanoparticles (NPs) can also be used in a wide variety of applications, including antibacterial, agricultural, gas sensor, battery, and high-temperature superconductors in addition to solar energy conversion [4–6].

CuO NPs and other metal oxide nanoparticles can be prepared by various methods, including sol-gel, quick precipitation, sonochemistry, electrochemistry, solid-state reaction, alcohol synthesis, microwave irradiation, and liquid–liquid interface techniques [7–9]. There are disadvantages associated with most physicochemical methods, as these methods rely on toxic chemicals. Approaches involving physicochemical reactions are highly cost and energy intensive. It is not feasible to produce nanoparticles on a large scale and in a stable manner by physical methods. Chemical processes take a long time to process. Furthermore, the thermal decomposition method produces nanoparticles with poor dispersion in water [10]. Alternatively, green chemistry and its principles produce the cost-effective, non-toxic, and environmentally friendly product without hazardous organic solvents. Green synthesis does not require special equipment, and it takes less energy to synthesize nanoparticles [11].

Green synthesis is carried out through the use of fungi, algae, bacteria, and plants. However, plant constituents, such as leaves, fruits, roots, stems, and seeds, have been widely employed to synthesize various natural products [12]. It is believed that medicinal plants and their parts contain many beneficial components, including bioactive compounds [13]. Herbal plant extracts contain a variety of phytochemicals, particularly secondary metabolites, such as alkaloids, tannins, terpenoids, and flavonoids, which reduce and stabilize metal oxides. Their low cost, recyclability, and abundance make them an excellent source of green synthesis [14]. Compared to fungi and algae, plants are easier to cultivate, non-pathogenic, convenient for preparing the extract for the reaction, and easy to maintain [15]. There have been several plant sources used in the biosynthesis of CuO NPs, such as *Rumex nepalensis* [16], *Magnolia champaca* [17], *Seriphidium oliverianum*, [18], and *Malva sylvestris* [19] leaves extract. The genus *Falcaria*, which belongs to the Apiaceae family, contains two endemic species to Iran, namely *Falcaria vulgaris* and *Falcaria facades*. In addition to flavonoids, coumarin, saponins, and tannins, this plant extract which has been used relatively less in nanoparticle synthesis, also contains significant amounts of carvacrol and spathulenol with antioxidant and antibacterial properties [20]. Furthermore, germacrene B is the most commonly discovered essential oil in leaf extracts [21]. It was hypothesized that with the help of these phytochemicals, which each have unique biomedical properties, nanoparticles with stronger antibacterial properties could be produced. *F. vulgaris* is usually an annual or biennial herbaceous plant in the Apiaceae family. It is upright with ribbed, branching stems and reaches one or two feet in height. Central Asia, Europe, and the Mediterranean region are the native areas of this plant. There are numerous valuable and important compounds present in the plant leaf, including spathulenol, carvacrol, limonene, tannins, and saponins [22]. The extract of *F. vulgaris* has been used to biosynthesis nanoparticles of copper, cerium, and silver [23–25].

Researchers have shown that CuO NPs possess antimicrobial properties against *Escherichia coli*, *Bacillus subtilis*, *Vibria cholera*, *Pseudomonas aeruginosa*, *Clostridium difficile*, *Syphillis typhus*, and *Staphylococcus aureus* [26]. As an antimicrobial, CuO NPs are used most commonly in hospitals. In the right dosage, their antimicrobial properties kill more than 99.9% of Gram-positive and Gram-negative bacteria within a few hours after exposure [27]. It is important to note that although research has focused on some famous bacteria, many other bacteria are more dangerous and less susceptible to antibiotics. Studying antibacterial substances is therefore of great importance to medical and pharmaceutical science. Among them is *Salmonella* Paratyphi-A, which is a Gram-negative bacillus resistant to common

antibiotics. In light of *Salmonella*'s resistance to physical and chemical factors, some of its members are used to evaluate the effectiveness of disinfectants [28]. There are many complicated mechanisms involved in the pathogenic mechanism of this bacterium. It is believed that Salmonella enters the digestive tract through contaminated foods, plants, and water. It attaches to the intestinal mucosa, and spreads throughout the body, including the lymphatic system. Humans, domestic animals, birds, reptiles, quadrupeds, rodents, poultry, reptiles, birds, and plants can all carry salmonella. Nevertheless, there are differences between serotypes. In contrast to the typhoidal serovars (Typhi and Paratyphi A) that cause enteric fever only in humans, the non-typhoidal *Salmonella serovars* (Typhimurium and Enteritidis) are broadly distributed and predominantly cause gastroenteritis [29]. In humans, serotypes adapted are able to remain in the gallbladder for a long time, making humans chronic carriers [30]. In addition, plants are susceptible to Salmonella infection due to its ability to attach to surfaces and attach to organs before actively infecting the plants' interiors, causing colonization of organs and suppression of immune defenses. The virulence of *Salmonella* originating from plants also extends to humans. So, plants are an alternative host for *Salmonella* pathogens, and play a role in their transmission [21, 22]. A study by Gupta et al. [31] found that more than 300 000 Indians are infected with enteric fever every year. Even though *Salmonella* Paratyphi-A infections are lower than *Salmonella* Typhi, they still infect a significant number of people. Therefore, it remains necessary to develop effective, easy-to-use, and economically viable antibacterial agents to prevent the pathogenicity of this bacterium and its other serotypes. Further, because this species infects all organisms, including plants consumed by humans and animals, it is essential to design and produce materials that are not harmful to the food chain when used to treat infected plants.

The objective of this study is to use plant extracts to create environment-friendly nanoparticles by adopting an uncomplicated approach, which is based on the principles of green chemistry. Our goal is not only to phyto-synthesize economical and green metal nanoparticles but also to develop antimicrobial agents that are safe and effective in inhibiting bacteria that are resistant to antibiotics, such as fluoroquinolones. In the past years, a few studies have been conducted on the effects of CuO NPs on other bacteria species, such as *Salmonella typhimurium* [32] and *Salmonella* Typhi [33]. Since *Salmonella* Paratyphi-A (ATCC 9150) is one of the lesser studied *Salmonella* species, and its response to CuO NPs has not been well documented, the study was conducted on *Salmonella* Paratyphi (ATCC 9150). Through the production and investigation of eco-friendly CuO NPs that can inhibit the growth of this bacterium, we aim to contribute to nanotechnology science advancement by expanding the use of nanomaterials as green antibacterial agents. In the present study, well diffusion and minimum inhibitory concentrations (MIC) assays were used to examine the antibacterial activity of CuO NPs. In addition, several different methods, including X-ray diffraction (XRD) and scanning electron microscope (SEM), were employed to investigate the morphological properties of these nanoparticles.

Experimental

Material and methods

Falcaria vulgaris plant was collected from the foothills of Tafresh County, in Markazi province, Iran. Tafresh is approximately 1 912 meters above sea level and has a continental and semi-arid climate with 270 mm of annual rainfall. Leaves were collected from wild species found in nature, and we avoided using plants located near farming lands due to agricultural pesticides or chemical fertilizers applied. The Copper (II) nitrate trihydrate was purchased from Merck®. To adhere to green chemistry principles, hazardous reagents were removed from the synthesis, and only water was used in the process.

Preparing plant extract

The leaves were transferred to the laboratory for use immediately upon collection and washed with tap water. To remove organic substances or impurities from the leaves surface, they were washed twice with distilled water at room temperature. After washing the leaves, they were dried at room temperature away from the sunlight. To prepare the extract, 20 g of dried leaves were boiled in 500 mL of distilled water at 80 °C for 30 min. With the use of Whatman grade No. 1 filter paper, the aqueous leaf extract was filtered and the dark yellow hue of pure *F. vulgaris* extract was observed. For further analysis, the filtered extract was centrifuged at 7 500 r/min for 5 min to remove any particles detached from the leaves.

Phyto-synthesis of CuO NPs

CuO NPs were prepared by mixing 30 mL of the desired extract with 100 mL of deionized water, heated to 80 °C. Following that, 100 mL of deionized water containing 10 mmol/L copper (II) nitrate trihydrate was added to the extract solution. To achieve an ideal state of synthesis, after examining the size of nanoparticles at different pH levels, it was determined that smaller nanoparticles are synthesized at pH 12. Therefore, pH 12 was used as a medium for the biosynthesis reaction. In green synthesis, the pH of the mixture plays a significant role in changing the electrical charge of biomolecules. Studies have demonstrated, for instance, that larger nanoparticles formed at acids (pH 2–4) compared to basic pH. At basic pH, metallic nanoparticles with smaller size at basic pH levels can produce a greater yield [34, 35]. The color change of the solution from deep blue to dark green indicated that CuO NPs have been produced after 30 min [23]. After washing with double-distilled water and standard ethanol, samples were centrifuged at 8 000 r/min for 15 min. The nanoparticles were dried in a hot air oven at 100 °C for 1 h in order to evaporate the water trapped within their pores.

Characterization of green synthesized CuO NPs

Shimadzu UV-160 A series ultraviolet–visible (UV–Vis) spectrophotometers were used to characterize green synthesized CuO NPs and leaf extract at 200–800 nm. The Fourier transform infrared (FTIR) spectrum was measured using a Jasco 6300 spectrometer. The identification of phytochemicals responsible for reducing CuO NPs and forming Cu–O bonds was accomplished using FTIR spectroscopy. Using a Philips-PW1730 advanced diffractometer operating at 40 kV and 40 mA, an XRD pattern was obtained to examine the structural properties of synthesized nanoparticles. The Debye Scherer's equation is used to estimate the average scale of the CuO NPs [35]:

$$D = \frac{k\lambda}{\beta\cos\theta} \quad (1)$$

SEM (TESCAN MIRA3) was used to analyze the particle size and morphology of the sample surfaces. In addition, to gain a deeper understanding of the characteristics of phyto-synthesized CuO NPs, an analysis of the sample was conducted using energy dispersive X-ray (EDX) spectroscopy (TESCAN MIRA II).

Minimal inhibitory concentration determination

As recommended by the Clinical and Laboratory Standards Institute, the MIC of CuO NPs were determined by broth microdilution [36]. *Salmonella* Paratyphi-A serotype (ATCC 5702) strain, was purchased from Kashan University, Iran. The MIC identifies the susceptibility or resistance of specific bacterial strains to a particular antibiotic when tested *in vitro*. A MIC value is defined as the lowest concentration of an antibacterial agent that completely inhibits bacterial growth [37]. The assessment was conducted with tetracycline as a positive control. A concentration of 0.031–2 mg/mL of CuO NPs was used in the experiment. In order to test the MIC, one isolate colony was suspended in 100 μL of nutrient broth. Following 24 h of growth in suspensions, the inoculums were diluted in distilled water in order to achieve final inoculums of 5×10^5 colony-forming units per milliliter (CFU/mL). We examined colony morphology and Gram staining to ensure that isolates were pure throughout the study. Nine plates containing nutrient broth were prepared using twofold serial dilutions of CuO NPs solutions, starting with a stock solution of 2 mg/mL. As a final step, an equal volume of bacterial inoculum was added to the wells of the microtiter plate containing 0.05 mL of serial compound dilutions. The enzyme-linked immunosorbent assay reader was used to determine the MIC after 24 h of incubation at 37 °C.

Well diffusion assay

In order to determine the antimicrobial activity of CuO NPs, agar well diffusion was used. We autoclaved all reagents, glassware, and media at 120 °C for 20 min. In order to prepare the bacterial suspension, a single colony was grown overnight in nutrient broth and the turbidity was adjusted to 0.5 McFarland standards. The suspension of bacteria was inoculated onto Mueller–Hinton agar plates, and 0.5 mg/mL of CuO NPs were added to the center 6 mm well. Since the MIC result displayed the minimum effective concentration of nanoparticles, the test was conducted using the MIC result. The plates were incubated at 37 °C for bacterial growth for 24 h. Moreover, tetracycline (50 mg/well) were utilized as positive controls. Using the zone of inhibition (ZOI) diameter, antimicrobial activity was

assessed and reproducibility was determined by repeating the assay twice.

Results and Discussion

Formation mechanism of CuO NPs

It is a complex process to synthesize nanoparticles from plant extracts since they contain a diverse collection of biomolecules, few of which are responsible for nanoparticle synthesis. Moreover, it is difficult to predict the exact mechanism for phytosynthesis. Plant metabolites such as alkaloids, alcohol, carbohydrates, flavonoids, phenols, and proteins contain amines, carbonyls, hydroxyls, and methoxides that are essential for nanoparticle reduction and stabilization [38]. A possible explanation for this is that the α-pinene (terpene), spathulenol (tricyclic sesquiterpene alcohol), carvacrol (monoterpenoid phenol) and limonene (cyclic monoterpene) present in the leaf extract of *F. vulgaris* [20] bind to Cu^{2+} ions released from $CuNO_3$ solution and form metal complexes that are reduced to CuO particles. In the initial stages Cu–O grains form, then the grains accumulate and after nucleation, became CuO NPs. In Fig. 1, the synthesis mechanism of CuO NPs is outlined graphically.

Characterization of the phyto-synthesized CuO NPs

It was confirmed by using UV–Vis spectra showing a massive peak at 350 nm that copper ions were reduced to CuO NPs (Fig. 2). Increased absorbance indicates a higher conversion of Cu^{2+} to CuO NPs, resulting in a greater concentration of CuO NPs [39]. Three peaks appear in the UV–Vis spectrum of *F. vulgaris* leaf extract at 216, 269, and 338 nm. Strong absorption at 216 nm is attributed to the π–π^* transitions between C=C bonds in aromatic rings. The absorption at 269 and 338 nm is attributed to n–π^* transitions of C=O and O–H bonds, respectively [40].

FTIR provides information on entities with their functional group associated with the synthesized nanoparticles [14]. In the plant extract FTIR spectra, the extensive peak at 3 442.06 cm^{-1} is equivalent to O–H or N–H stretching in amino acids, phenols, and alcohols. Also, in the FTIR spectra of CuO NPs, there is an extensive peak at 3 408.1 cm^{-1} that is probably a stretching bond between O and H, indicating biomolecules attached to CuO NPs. The peak at 2 919.34 cm^{-1} regards to C–H bond, and the band at 609.36 cm^{-1} can be attributed to vibrations of Cu–O, confirming the formation of CuO NPs [32]. In addition, distinct peak seen at 1 384 cm^{-1} is attributed to the stretching bond of C–O. Moreover, two peaks in both FTIR spectra of leaf extract and CuO NPs at 1 632.95 and 1 625.19 cm^{-1} can be attributed to C=O stretch and N–H bending.

According to the FTIR studies (Fig. 3), the phenolic group binds metals strongly. It indicates that the phenols may be capped by metal nanoparticles (such as CuO NPs) to avoid agglomeration and stabilize the medium. As a result, biological molecules can act as both stabilizers and producers of CuO NPs in the aqueous medium [39, 41].

The XRD pattern of phyto-synthesized CuO NPs presents the formation of nanocrystalline phases by well-defined broad peaks at high intensities (Fig. 4). XRD is used for structural and crystallinity analysis of metal oxide nanoparticles. According to the XRD pattern, the four noticeable peaks appeared at 28.23° (111), 39.09° (200), 47.38° (220), and 75.5° (400) that are in line with face-centered cubic (fcc) phase of the CuO NPs which is in agreement with JCPD card No.

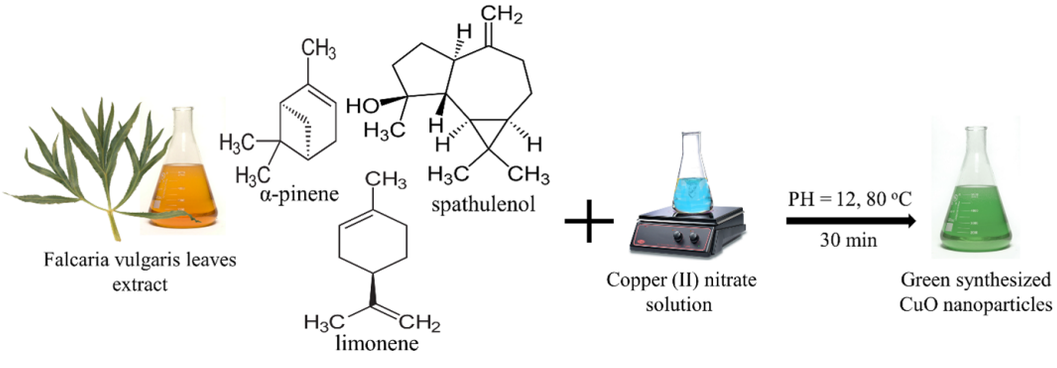


Fig. 1 Schematic of CuO NPs formation using *Falcaria vulgaris* leaf extract.

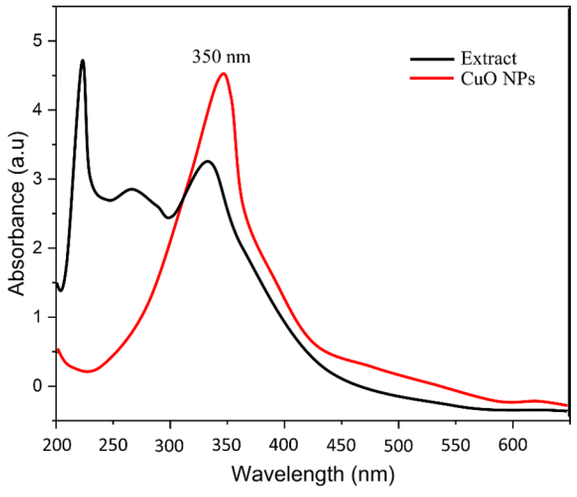


Fig. 2 UV–Vis spectra of CuO NPs (red) and *Falcaria vulgaris* leaf extract (black).

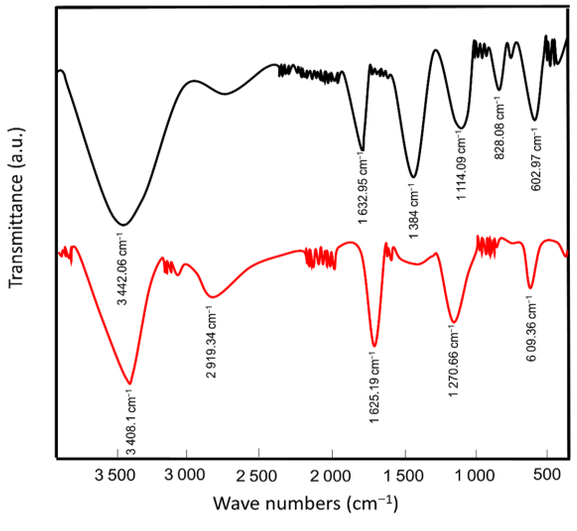


Fig. 3 The FTIR spectrum of *F. vulgaris* leaf extract (black) and biosynthesized CuO NPs (red).

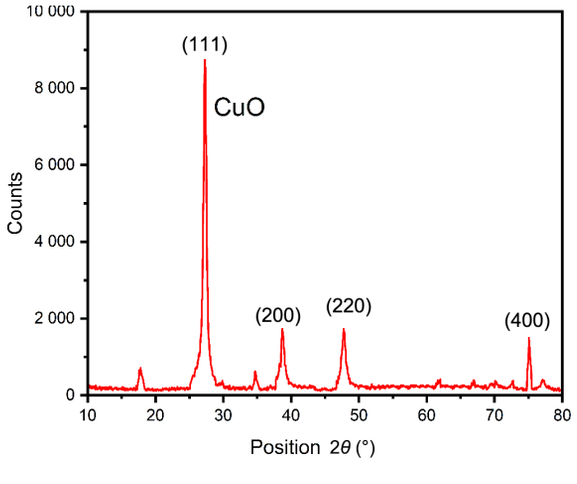


Fig. 4 XRD crystallography of biosynthesized CuO NPs.

48–1548. The average crystallite size of CuO NPs is estimated using Debye–Scherrer formula, which is 82.9 nm.

As shown in Fig. 5, the SEM image shows CuO NPs size and external morphology. According to the SEM image, the CuO NPs are broadly spherical. It indicates a range of particle sizes between (58.00 ± 2.4) and (200 ± 2.4) nm. As it is clear from the figure, the synthesized nanoparticles adhere to each other during formation. This issue can be argued by ecological factors that affect the stability and agglomeration of nanoparticles [42].

The elemental composition of CuO NPs can be determined qualitatively and quantitatively using EDX [43]. The intensities of peaks are used to determine the concentration of specific elements in the CuO NPs, while the positions of peaks are used to identify their composition [44]. As the EDX spectrum reveals, the samples contain the required phases of Cu and O. According to the diagram, Cu and O should contain 71.23 wt.% and 28.77 wt.%, respectively. The EDX analysis of CuO NPs with specific peaks of O and Cu is demonstrated in Fig. 6.

As a result of all these analyses, it is concluded that the leaf extract of *F. vulgaris* can phyto-synthesize the desired nanoparticles with unique properties in a rapid and simplified manner. An overview of the morphological properties of other CuO NPs phyto-synthesized by different plants is provided in Table 1.

According to the attributes of the nanoparticles listed in Table 1 and the analysis of other related studies, it can be concluded that copper nanoparticles produced by phyto-synthesis are mostly spherical-shaped and have a diameter ranging between 10 and 40 nm. It must be noted, however, that the use of different plant extracts may result in changes in nanoparticle size. Also, accumulating biomolecules around the nanoparticle surface may increase the nanoparticle's dimensions resulting in significantly higher dimensions than other nanoparticles. Although this increase in size will change the performance of synthesized nanoparticles, the presence of more biomolecules and phytochemicals around the nanoparticles may result in unique properties. This is because these biomolecules and phytochemicals exhibit different properties, such as therapeutic properties [54].

Antibacterial activity of CuO NPs against *Salmonella* Paratyphi-A

In vitro antibacterial assay was performed using MIC and well diffusion technique. It demonstrates that

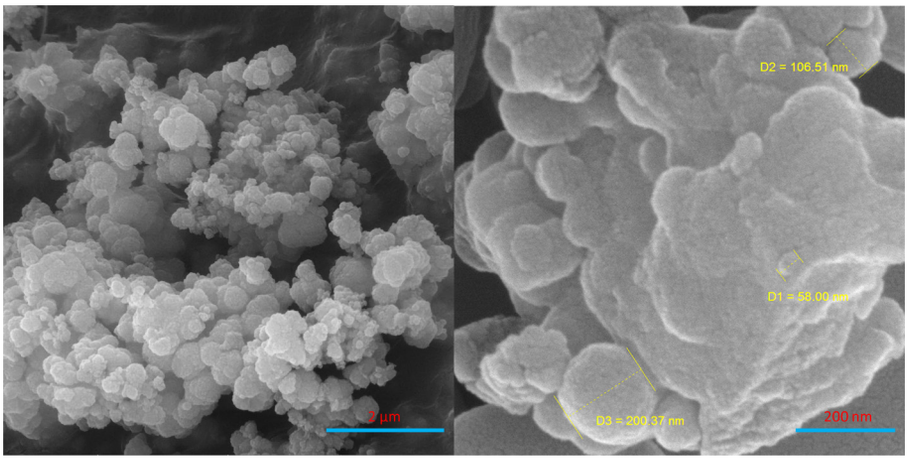


Fig. 5 SEM image of phyto-synthesized CuO NPs.

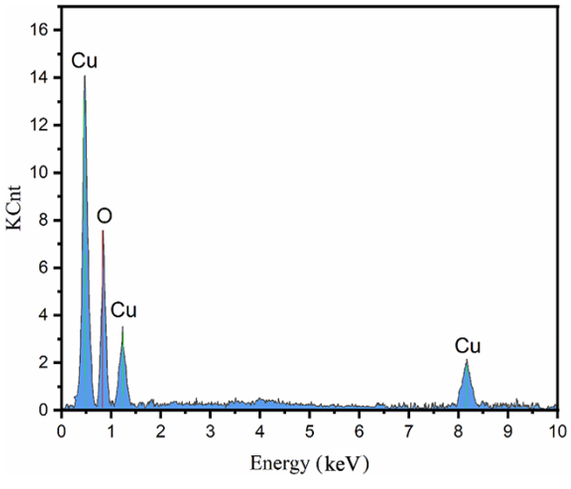


Fig. 6 EDX analysis of CuO NPs.

CuO NPs synthesized by *F. vulgaris* leaf extract prevent bacteria growth. As shown in Fig. 7 and Table 2, CuO NPs inhibit *Salmonella* Paratyphi-A growth by up to 18 mm, and tetracycline (TC), as a positive control, inhibits growth by 15 mm. Also, the leaf extract used in this test, although it is one of the known medicinal herbs, did not show any specific antibacterial effect against bacteria. Additionally, the MIC of CuO NPs was 0.5 mg/mL (Table 2).

A study on the synthesis of CuO NPs by Punica granatum *L.* found that the nanoparticles left a 29.24 mm zone of inhibition against *Salmonella* Paratyphi-A [55]. The nanoparticles designed in the study by Asay et al. were 5−20 nm in dimension, which probably contributes to their improved performance compared to the nanoparticles synthesized in the study by Azam et al. [56]. It appears that the size of these nanoparticles determines how effective they are as antibacterial agents.

F. vulgaris leaf extract can function as a capping agent which contributes to CuO NPs' antimicrobial efficacy. By reducing particle size, it enhances antimicrobial activity. This is due to smaller particles

Table 1 Morphological comparison of CuO NPs phyto-synthesized from plant leaf extracts

Plant name	Nanoparticle size (nm)	Nanoparticle shape	Ref.
Gloriosa superba L.	5–10	Spherical	[45]
Aloe vera	20–30	Spherical	[46]
Eucalyptus globoulus	38–143	Quasi-spherical	[47]
Capsicum frutescens	20–40	Spherical and rectangular-rod	[48]
Phyllanthus amarus	22	Quasi-spherical	[49]
Plectranthus amboinicus	5–40	Spherical and circular	[50]
Juglans regia	80	Spherical	[51]
Aerva javanica	15–23	Spherical	[52]
Malva sylvestris	19–26	Spherical	[19]
Phoenix dactylifera	22–28	Spherical	[53]
Falcaria vulgaris L.	58–200	Spherical	Present study

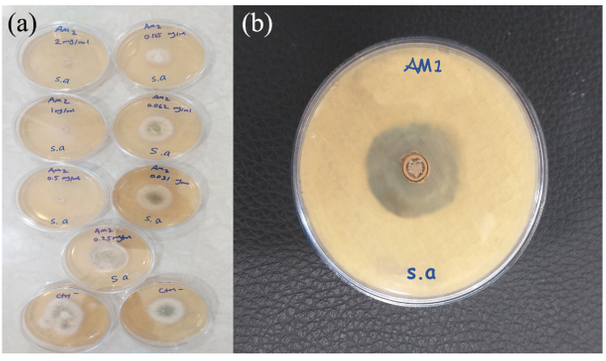


Fig. 7 Minimum inhibition concentration (a) and disc diffusion assay of CuO NPs (b).

Table 2 The antibacterial activity of phyto-synthesized CuO NPs against *Salmonella* Paratyphi-A

Bacterial strain	ZOI (mm)			MIC (mg/mL)
	CuO NPs	*F. vulgaris* leaf extract	Positive control (mm)	
S. Paratyphi-A	18 ± 0.4	—	15 ± 0.4	0.5

typically having a higher surface to volume ratio. As a result, they are more effective antibacterial agents [57, 58]. In addition, it has been suggested that CuO NPs provide bactericidal properties by releasing Cu^{2+} ions in the solution and binding to negatively charged cell walls, causing the cell walls to rupture [59]. An additional effective antibacterial mechanism is the ability of CuO NPs to produce reactive oxygen species (ROS). Due to their ability to generate ROS, they are highly cytotoxic. In response to ROS forming on metal oxide nanoparticle surfaces, bacteria experience oxidative stress, which ruptures their membranes. Bacteria die when they come into contact with ROS that can destroy DNA, RNA, and oxidize lipids and proteins [60]. Fig. 8 illustrates all of these processes schematically for a better understanding of how these nanoparticles exert their antibacterial properties.

In order to improve the antibacterial properties of these nanoparticles, the factors that contribute to their synthesis need to be further investigated. As phyto-synthesized CuO NPs are effective against *Salmonella* Paratyphi-A, they will have a greater chance of competing with classic and well-known antibacterials by investigating the critical factors that affect nanoparticle morphology, such as synthesis temperature, extract concentration, and pH.

A study done by Azam et al. [55] reported that the antimicrobial properties of nanoparticles smaller in size and synthesized at lower temperatures were

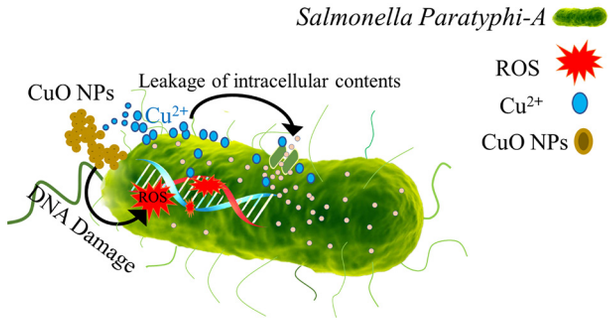


Fig. 8 Schematic of CuO NPs' antibacterial activity against *Salmonella* Paratyphi-A.

higher. Another research by Padil et al. [61] showed the same observation that nanoparticles synthesized at lower temperatures had better antibacterial activity and the zone of bacteria inhibition increased remarkably. It is therefore expected that by creating synthetic conditions at a lower temperature, and making the average size of nanoparticles smaller than those in current study, the antibacterial activity against these resistant bacteria can possibly improve. Furthermore, it is possible to understand the effects of biomolecules and phytochemicals present in plant extracts as well as attached to nanoparticles more accurately by conducting more studies on them. It is also expected that in the future, more practical nanoparticles can be synthesized by selecting the most appropriate phytochemicals.

Conclusion

Using only deionized water as a solvent and copper (II) nitrate trihydrate as a starting material, we were able to phyto-synthesize environmentally friendly CuO NPs from *F. vulgaris* leaf extracts. This method demonstrates how green chemistry principles can be applied to nanoparticle preparation and result in cost-effective nanomaterials. The method also has the advantages of simplicity and stability, allowing further practical scaling up of the production process. The XRD results show that all calcined CuO NPs have fcc phase, indicating that single-phase CuO has been formed during the biosynthesis process. As calculated based on Scherer's equation, the average diameter of nanoparticles was 82.9 nm, and as observed from SEM images, these particles were spherical in shape. Consequently, the synthesized nanoparticles showed good antibacterial properties against a Gram-negative, relatively resistant bacterium. That can be related to a number of factors,

such as the average size below 100 nm, the shape of nanoparticles, the property of creating more ions, and biomolecules attached to the surface of the synthesized nanoparticles. According to the findings of this study, *F. vulgaris* leaf extract alone does not possess antibacterial properties against *Salmonella* Paratyphi-A. The synthesized nanoparticles, however, were able to leave an 18 mm zone of inhibition and prevent bacteria from growing. These nanoparticles inhibit bacteria by producing copper ions and reactive oxygen species. Therefore, they can be applied to a wide range of bacteria based on their inhibition mechanism.

CRediT Author Statement

Hamed Mirhadi: phyto-synthesis, investigation, data, and experiments curation; **Alireza Momeni**: methodology, software, visualization, and writing original draft; **Mohammad Hadi Meshkatalsadat**: validation, writing, reviewing, and editing the final manuscript.

Acknowledgements

Authors are thankful to the Department of Chemistry and deputy of research of Qom University of Technology for their technical support in this project.

Conflict of Interests

All authors declare that there is no conflict of interests.

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