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Original Research Article



Studying the Biosynthesis, Characterization, and Antibacterial Activity of Copper Oxide Nanoparticles

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ABSTRACT

This study reports a facile, fast, eco-friendly, and one-pot approach for the synthesis of copper oxide nanoparticles (CuO NPs) using safe Pistacia vera peel extract. The extract is used as a stabilizing and reducing agent. Different analytical technique including FT-IR spectroscopy for determination of the Pistacia vera peel extract functional groups in the reduction and capping process of copper oxide NPs, UV-Vis absorption spectroscopy for affirmation of CuO presence, energy dispersive spectroscopy (EDS), scanning electron microscopy (SEM), and Xray diffraction (XRD) was used in the present study. In these analyses, a sharp peak at 283 nm in UV-vis tests, and a specific Ft-IR peak at 601.59 cm-1 prove that this synthesis was completed using green chemistry principles. In addition, the average size of the biosynthesized nanoparticles was 32.97 nanometers according to the Scherrer equation. CuO NPs antibacterial activity was examined against Bacillus subtilis and Streptococcus pyogenes wherein CuO NPs, exhibited remarkable antibacterial activity with minimum bactericidal concentrations (MBCs) within the range of 125-1000 μ g/mL. Additionally, it showed better performance than classical antibacterials in the zone of inhibition assay against Bacillus subtilis. Generally, this study demonstrated that copper nanoparticles synthesized with plant mediators are completely competitive with other chemicals, such as drugs.

Keywords: Antibacterial activity; Bacillus subtilis; Biosynthesis; Copper oxide nanoparticles; Pistacia vera; Streptococcus pyogenes

Introduction

Nanotechnology has a great effect on improving the design and efficiency of products in areas as various as biomedical devices, food, cosmetics, electronics, and agriculture, and energy. Recently, the synthesis of nanoparticles has attracted considerable attention due to their electrical, optical, and catalytic properties [1, 2]. Metallic nanoparticles can be obtained using different physical and chemical approaches. However, those approaches need expensive and highly complicated equipment, high temperature and pressure, harsh reaction condition, and hard purification procedures that could produce harmful byproducts and derivatives. Most nanotechnology researchers are interested in replacing green nanotechnology to manage the limitations of other common physical and chemical methods [3]. The green approaches are free from chemical pollution, environmentally safe, non-toxic, less costly, and can be employed in biological applications in contrast to the common chemical procedure where many poisonous and permanent chemical reagents are applied [4, 5]. In general, plant extracts, plant tissue, seeds, stems, gum, fruit, leaves, and other parts of living plants are used to synthesize metal nanoparticles. Copper oxide nanoparticles (NPs) have possessed notable attention because of their broad applications such as catalytic, electrical, sensors, optical, solar energy transformation, high-Tc superconductors, etc. [6]. In addition, it can be used as an anti-fouling, anti-biotic, antifungal, and antimicrobial agent when incorporated in plastics, coatings, textiles, etc. [7].

There are various procedures through which copper oxide NPs can be synthesized, like solgel technique, microwave irradiation, electrochemical methods, sonochemical, alkoxide-based route, precipitation–pyrolysis, one-step solid-state reaction, thermal decomposition of precursor, the method at room temperature, etc. [8]. In the chemical procedures, noxious chemicals such as ethylene glycol, sodium borohydride, dimethylformamide, hydrazine hydrate, etc are highly reactive lead to main biological and environmental problems [9]. Recently, the green synthesis of copper oxide NPs is gaining emphasis due to its inexpensive, eco-friendly, and simplicity [10]. The biosynthesis of copper oxide NPs by different plants such as Calotropis gigantea, Allium sativum, Phyllanthus Amarus, Aloe vera, Ziziphus mauritiana L., etc. have been reported [11, 12]. A wide literature study revealed that there are no reports on the biosynthesis of copper oxide NPs using the Pistacia peel extract.

Pistacia Vera is a member of Anacardiacea family and obtained abundantly in the Zagros Mountains, and especially in northern and western Iran, northern and eastern Iraq, northern Syria, and southern Turkey (Figure 1). Pistacia Vera has anti-inflammatory, antibacterial, antigout, antimicrobial, antiviral, antioxidant, and antiasthmatic effects and is employed in the treatment of diseases such as skin diseases, dysentery, diarrhea, vomiting, fever, respiratory ailments, psoriasis, kidney stones, throat infections, asthma, and stomach ache. The phytochemistry and chromatographic fingerprints investigation of this plant demonstration that it may have flavonoids, fatty acids, phenolic compounds (such as tetragalloylquinic acid, gallic acid, trigalloylglucose acid, and quinic acid) and terpinolene, α -pinene, starch, and triterpenoids [13, 14].



Figure 1. Pistacia vera soft skin

Here we report an inexpensive, rapid, non-toxic, simple, and eco-friendly acceptable procedure for the green synthesis of copper oxide NPs, using Pistacia vera peel extract as a reductant, stabilizer, and capping agent. The synthesized nanoparticles were investigated for antibacterial activity against two species of bacteria, Bacillus subtilis (ATCC6633) and Streptococcus pyogenes (ATCC19615). By comparing CuO NP inhibition zones with control samples, the antibacterial activity of CuO NPs was assessed.

2. Experimental

2.1 Materials and methods

Pistacia skin were collected from Qom province, Iran. The chemicals Copper (II) nitrate trihydrate and NaOH were analytical grade and purchased from Merck®. The synthesis was conducted using only water in compliance with green chemistry principles and without hazardous reagents.

2.2. Preparing plant extract

To prepare the plant extract, 20 g of fresh Pistacia vera skin was used gathered from the Qom pistachio garden. Following a final wash with tap water twice, fresh pistachio peel was extensively washed with deionized water to remove all the visible particles and dust. The Pistacia skin were cut into small pieces and then shade-dried for three days. Then, the shade-dried small Pistachio skin were placed in 100 ml of diazonid water and boiled for 20 min on a water bath. To eliminate particulate matter and obtain a precise result, the straw yellow solution was filtered using Whatman filter paper No. 1. It was then kept at 4°C for more utilization.

2.3. Biosynthesis of CuO NPs

To produce copper oxide nanoparticles, the plant extract was utilized to decrease copper $Cu2^+$ ions. A 1 mM copper nitrate salt was dissolved in 500 ml deionized water, and Pistachio skin extract was added drop-by-drop to the solution. A magnet and a magnetic stirrer were used to stir the solution for 30 minutes at optimum PH (12) and temperature (60°). Copper oxide nanoparticles are created after the extract and copper salt solution are mixed and the reduction and stabilizing reaction is performed (Figure 2).



Figure 2. Mechanism of CuO nanoparticles biosynthesis

Separating the created nanoparticles from the sediment collected through centrifugation (10 min, 10000 rpm), the nanomaterials were rinsed three times with ethanol and deionized water to eliminate loosely bound substances. Hence, centrifugation was performed, and the powdered form of the sample is kept in a sterile, airtight glass bottle. It is then utilized for bacterial growth, XRD, FTIR, and SEM analysis.

2.4. Characterization of green synthesized CuO NPs

The FT-IR spectrum was measured using a Jasco 6300 spectrometer. In order to examine the structural properties of synthesized nanoparticles, an X-ray powder diffraction (XRD) pattern was obtained using a Philips-PW1730 advanced diffractometer operating at 40 KV and 40 mA. Scanning electron microscopy (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 spectroscopy (EDXS) TESCAN MIRA II.

2.5. Antibacterial activity assay

To measure the antibacterial activity of CuO NPs against Bacillus subtilis (ATCC6633) and Streptococcus pyogenes (ATCC19615), MBC and zone of inhibition (ZOI) was measured. Preparing a pure culture of bacteria in the Muller Hinton Broth, it was then centrifuged, followed by discarding the supernatant. Then, by adding 20 ml of sterile standard saline solution, the concentration was set to an optical density of 0.1 at 625 nm (108 CFU/ml, 0.5 Mcfarland) by an appropriate Spectrophotometer. Serial solutions of CuO NPs were prepared in MHB, including 1-15 mg/ml, and a volume of the bacterial suspension was inserted into each sterilized sample solution, which brought the bacterial concentration to 100,000 bacteria/ml. Incubation of the sample solution was achieved at 37°C for 24 h. The dilution demonstrating the MIC and the higher concentration of the solution (comprising 2.5-10.15 mg/ml) was utilized for determining the MBC via disc diffusion technique in the Muller Hinton agar medium inoculated by standardized bacterial suspension (108 CFU/ml, 0.5 Mcfarland). After incubating the plates at 37°C for 24 h, the diameter of the inhibition zone for the sample solutions was determined and compared with the least concentration. In the result, the MBC was the zone with the least inhibition of growth.

3. Results and discussion

3.1. Characterization of the biosynthesized CuO NPs

Observing the spectrum at 283 nm (figure 3), a high peak appears, indicating the stability of CuO NPs. The higher absorbance reveals an incremented conversion of $cu2^+$ to Cu as a nanoparticle resulting in the greater concentration of CuO NPs [15, 16]. In addition, comparing the spectra of nanoparticles and extract reveals that the nanoparticles were successfully synthesized, and the differences in peaks are caused by metal oxide surface plasmon absorption.



Figure 3. UV-VIS spectra of CuO NPs

Based on the FT-IR spectra (Figure 4), the eco-friendly synthesized CuO NPs mediated by the Pistacia vera peel extract shows a specific peak at 601.59 cm-1 associated to Cu-O bond. In the CuO NPs FT-IR spectra, the extensive peak at 3433.87 cm-1 is equivalent to O-H or N-H stretching in amino acids, phenols, and alcohols. The peak a 2003.74 cm-1 is associated with C=O stretching. The peak at 1561.27 cm-1 is associate to N-H bending. The peak at 1246.57 cm-1 is related to stretching bond of C-O. In the peel extract spectra as well, massive peak at 3409.25 cm-1 belongs to O-H stretching and sharp peaks at 1699.1, 1429.12, 1324.00, 1176.54

cm-1 are associated with C=O stretch and N-H bending, CH2, CH3 and C-O stretching, respectively.



Figure 4. The FT-IR spectrum of P. vera peel extract (black) and biosynthesized CuO NPs (red)

CuO NPs were characterized by SEM in terms of their structural and morphological characteristics (figure 5). The sizes of the synthesized nanoparticles within the nanometer range. According to these images, most of the nanoparticles are with spherical shapes. In spite of some well-separated nanoparticles, the majority were agglomerated. This accumulation and adhesion can be due to the presence of biomolecules on the surface of nanoparticles or their high surface to volume ratio [17]. According to the image with a scale of 200 nm, the synthesized nanoparticles range in size from 36.99 to 55.17 nm.

As the Energy Dispersive X-Ray spectroscopy (EDXS) spectrum reveals (figure 6), the samples contain the required phases of Cu and O. According to the diagram, Cu and O should

contain 40.1 and 20.7 weight%, respectively. Other peaks belong to Mg (0.5%), N (17.7%) and S (21%) correspond to the phenols, flavonoids and other biomolecules.



Figure 5. Scanning electron microscopy of CuO NPs synthesized by P. vera peel extract



Figure 6. EDXS diagram of CuO NPs

By analyzing the X-ray diffraction patterns, the unique nature of the synthesized CuO NPs was confirmed (figure 7). The three peaks in the diffraction pattern at angles of 22.65°, 43.74°,

56.3° and 83.48° are related to the JCPDS standard copper numbered 89-2838 [18]. Four Observable peaks at 83.48°, 56.3°, 43.74°, and 22.65° (XRD pattern of biosynthesized CuO NPs) associated to (400), (311), (200), and (111) reflecting the face-centered cubic phase (fcc) of CuO NPs. By analyzing the spectrum using X-ray diffraction (XRD), only one spectral disturbance can be found. Using the Debye Scherrer formula [19], the average crystallite size of CuO NPs is estimated, which is 32.97 nm.



Figure 7. Figure 7. XRD pattern of biosynthesized CuO NPs

3.2 Antibacterial activity of CuO nanoparticles

According to the test results, CuO NPs have significant antibacterial properties and can compete with well-known antibacterials such as Rifampin and Gentamicin. MBC results, which determine the lowest concentration at which antimicrobial agents will kill microorganisms, vary between 125-1000 μ g/mL. Furthermore, zone of inhibition tests indicates that CuO NPs are highly effective antibacterial agents, especially against gram-positive bacteria Bacillus subtilis (figure 8). Invitro test reveals that CuO NPs inhibits B. subtilis more than S. pyogenes, possibly because S. pyogenes' cell wall is more resistant to CuO (table 1).

Copper nanoparticles' ability to bind to bacteria is logically correlated with their surface area. Compared to large-sized particles, nanoparticles exhibit enhanced bactericidal efficacy; therefore, they may confer cytotoxicity on bacteria [20]. A copper ion, which has a strong reduction ability, kills bacteria by destroying their cell walls and membranes, releasing the cytoplasm of the bacteria and oxidizing the nucleus of the bacteria. Since copper nanoparticles have a high affinity for reacting with molecules containing phosphorus, sulfur, and desoxyribonucleic acid (DNA), they are believed to induce damage inside bacteria by interacting with these compounds. As Cu2⁺ ions combine electrostatically with plasma membranes, they can penetrate cell membranes through channels created or closed by the membrane. As a result, intracellular ions and low-molecular-weight metabolites are leaked through the membrane of the cell. Additionally, it is thought that nanoparticles accumulate on envelope proteins, causing the outer cell wall to collapse and deplete intracellular ATP levels [21, 22].



Figure 8. Antibacterial assay of CuO NPs

Table 1.	. Zone	of inhibition	analysis
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Samples	Zone of inhibition (mm)	
	Bacillus subtilis	Streptococcus pyogenes
CuO NPs	21	11
Rifampin	10	21
Gentamicin	9	31

When copper nanoparticles are concentrated to a sufficient level, $Cu2^+$ ions released from nanoparticles are absorbed by bacteria. This mechanism has been attributed to copper nanoparticles' antibacterial properties. By solidifying protein structures or altering enzyme functions, copper ions damage bacterial cell membranes [23]. Upon exposure to copper

nanoparticles in growth medium, bacterial cells are immobilized and inactivated, which leads to a reduction in their replication capacity, resulting in cell death [24]. Microorganisms are less likely to develop resistance to copper due to a variety of mechanisms acting simultaneously [25]. In short, the antibacterial properties of copper nanoparticles and their superior effectiveness than classical antibacterials make them a reliable and effective option. It is particularly advantageous when green synthesis is used because harmful substances are removed from the synthesis process and nanoparticles are produced in an economical and safe manner.

Conclusion

By combining nanotechnology and green synthesis, we can create functional, economical and safe nano-sized materials. Using a bioreduction method based on P. vera peel extract as the reduction agent, this study reports a simple biological and low-cost approach for creating stable copper oxide nanoparticles. The structural characteristics and morphology of the obtained nanoparticles were studied using the UV-Vis, FT-IR, XRD, SEM and EDXS techniques. The result has confirmed the reduction of copper nitrate to copper oxide Nanoparticles with high stability. The peak in the absorption spectrum has confirmed the formation of copper oxide nanoparticles. FT-IR indicates that the primary amines of proteins and phenols are in charge of the stabilization, reduction, and capping. According to XRD results, the synthesized nanoparticles have face-centered cubic phase and a crystal size of 32.97 nm. Moreover, SEM images revealed that the nanoparticles are spherical and range from 36.99 to 55.17 nanometers in size. Moreover, EDS analysis indicates that, in addition to confirming the presence of copper nanoparticles, the elements found in biomolecules of the extract were transferred to the nanoparticles as well. Finally, copper nanoparticles which showed a significant zone of inhibition in the antibacterial assay demonstrated their ability to inhibit Gram-positive bacteria that are relatively resistant.

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Conflicts of Interest

All authors declare that there is no conflict of interest.

Author Contributions

The manuscript was written through contributions of all authors, and the final version of the manuscript has been approved by the authors.

Competing Interests

There is no relevant financial or non-financial interest to disclose by the authors.

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