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Original Research Article

Synthesis and Characterization of Copper Nanoparticles Utilizing Pomegranate Peel Extract and Its Antibacterial Activity

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ABSTRACT

Nanotechnology is a fascinating research area as a result of producing nanoparticles with different shapes, sizes, chemical composition, dispersity, and their several applications for the human being. Manipulation, creation, and using metallic nanoparticles is greatly important considering reduction of dimensions. Therefore, unique thermal, electronic, and optical characteristics are obtained. The biosynthesis approaches of nanoparticles are prioritized compared to physical and chemical processes as a result of the lower time costs and energy. Green synthesis of nanoparticles is an eco-friendly technique using natural solvents. The current work includes the eco-friendly, and green synthesis of CuNPs utilizing Cu $(NO3)_2$. H_2O solution and Peel of Pomegranate extract. Various bio-components exist in the Pomegranate Peel extract works as a reducing agent for this synthesis. The diminant surface plasmon resonance (SPR) peak achieved at 350 nm in UV-Visible spectra confirmed the formed CuNPs. Based on SEM analysis, the spherical uniformly and morphology sized particles (36.99-55.17 nm) were obtained. The green synthesis of copper nanoparticles mediated by the Pomegranate Peel extract was clearly illustrated by FTIR spectrum. The structural characterization was performed utilizing XRD in line with reflections of the face-centered cubic (fcc) phase of the CuNPs (111, 200, 220, and 400). It was found that biologically synthesized copper nanoparticles effectively controlled the progression of human pathogens, namely Salmonella.

Keywords: Biosynthesis; Pomegranate Peel; Nano-particales; Green Chemistry; Cu NPs

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Introduction

Recently, there has been a huge deal of attention toward nanotechnology from different fields such biotechnology, chemistry, physics, engineering, medicine, and material sciences. Nanoparticles are synthesized by chemical and physical approaches suffering from some disadvantages such as expensive reagents, hazardous reaction circumstances, longer time, and tedious procedure for isolating nanoparticles. Therefore, there is an opportunity to establish novel approaches for synthesizing nanoparticles requiring inexpensive reagent, eco-friendly and less drastic reaction conditions [1-5].

The green synthesis technique is the best technique in comparison to the other methods like chemical reduction, electrochemical reduction, photochemical reduction, and heat evaporation. This green synthesis technique has numerous advantages over other methods such as cost-effectiveness, use of less temperature, simplicity, the usage of less toxic materials. Furthermore, methods for various metal nanoparticles increasingly require eco-friendly features. The metal nanoparticles are mainly focused on studying as a result of their potential applications in various fields like magnetic recording media or catalysis, microelectronics, nano-sensors, optoelectronics, nanoelectronics, and information storage devices. Copper is the most extensively utilized substance in the world as a result of its electrical, catalytic, optical, biomedical, and antifungal/ antibacterial applications among different metal particles like gold, iron, palladium, silver, zinc, and quantum dots [6-10]. Metallic nanoparticles with specific morphologies and sizes can be synthesized readily utilizing physical and chemical approaches. Nevertheless, such approaches employ toxic chemicals as non-biodegradable stabilizing agents, reducing agents, and organic solvents. Therefore, they are potentially dangerous to the biological systems and environment [11-13]. Plant materials for synthesizing nanoparticles could be more beneficial since it requires no elaborate procedures like intracellular synthesis and multiple purification steps or microbial cell culture maintenance. In the present work, we found the synthesis of copper nanoparticles utilizing Pomegranate Peel extract. The color changes confirmed the synthesized nanoparticles and UV-Visible spectroscopy characterized them. Fourier transform infrared (FTIR) spectral measurements were conducted to recognize the potential biomolecules. To observe the nanoparticles' size, SEM (Scanning Electron Microscope) was used. The structural characterization was conducted utilizing XRD (X-Ray Diffraction). Furthermore, efficacy was evaluated to prevent various pathogenic bacterial growth [14-17].

Materials and methods

The utilized plant: The current work includes the synthesis of copper nanoparticles (CuNPs) by the green technique utilizing the Pomegranate Peel extract. Pomegranate was collected from Qom province, Iran. It is one of the most favorite and famous fruit in this area.

Preparing the plant extract

To prepare the plant extract, 20 g of fresh Pomegranate Peel was used gathered from the university campus. To eliminate all the unwanted visible particles and dust, fresh Pomegranate Peel was washed extensively with tap water after a final wash two times with deionized water. The Pomegranate Peel was cut into small pieces and then shade-dried for 3 days (Fig.1).

Figure 1. The shade-dried Pomegranate Peel

Then, the shade-dried small Pomegranate Peel was placed in 100 ml of distilled water and boiled for 15 min on a water bath. After boiling, the red solution was cooled and filtered utilizing Whatman filter paper no.1 to eliminate particulate matter and obtain the exact answer. It was then kept at 4℃ for more utilization [18].

Synthesizing Copper nanoparticles

To produce copper oxide nanoparticles, the plant extract was utilized to decrease copper Cu2+ ions. Over synthesizing Copper nanoparticles, 1 mM copper nitrate solution in 500 ml, and Pomegranate Peel extract were mixed drop-wise, while continuously stirring utilizing a magnetic stirring. Adding extract changes the solution color from straw yellow to light green revealing the Copper nanoparticles (CuNPs) formation (Fig.2).

Figure 2. Synthesis of CuNPs using Pomegranate Peel extract: (a) Test tube containing 1 mM Cu $(NO_3)_2H_2O$. (b) Pomegranate Peel extract (c) CuNPs after 2hrs

Separating the created nanoparticles from the precipitate collected through centrifugation (10 min, 1000 rpm), the nanomaterials were rinsed three times with ethanol and deionized H2O 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 [18-20].

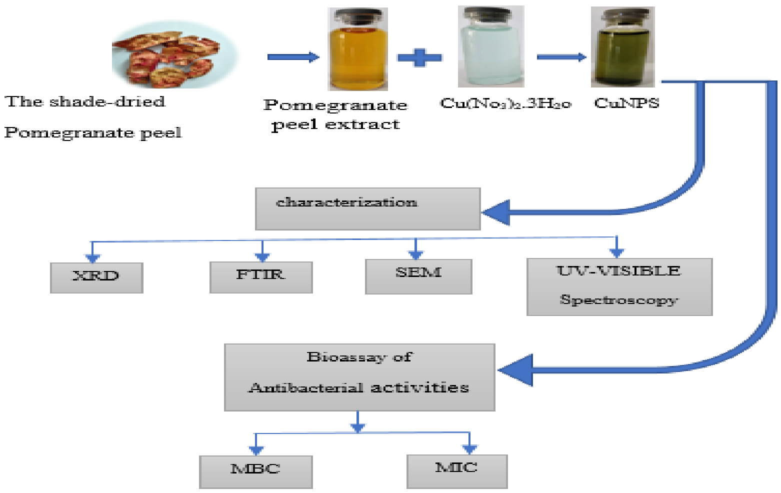


Figure 3: Graphic abstract

Results and Discussions

UV-Vis Spectra: a physic-miniature UVS-2500 spectrophotometer was used to perform UV-Vis spectral analysis. UV-Visible absorption spectrophotometer was utilized with a resolution of 1 nm within 190-1100 nm. The UV-Visible spectra of the CuNPs solution were recorded after 24 h [21-

23]. The high and sharp peak appears in the spectrum at 350 nm, indicating the size and stability of the Copper nanoparticles [19, 20]. The higher absorbance reveals an incremented conversion of cu^{2+} to (fig. 4) Cu as a nanoparticle resulting in the greater concentration of CuNPs [21].

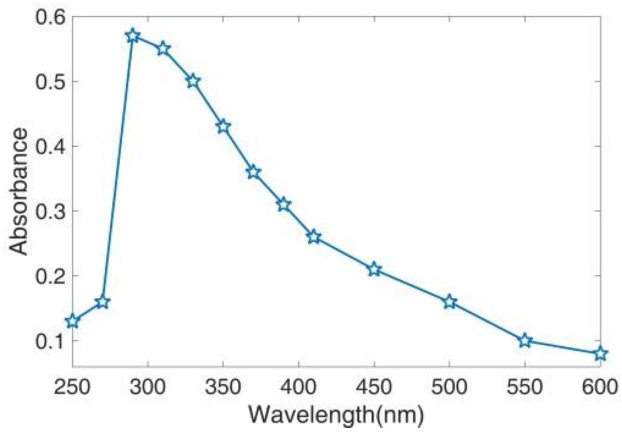
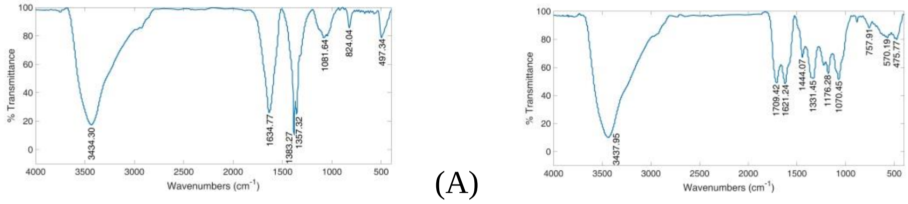


Figure 4. UV-Vis absorption spectra of CuNPs using Pomegranate Peel extract

FT-IR Spectra: To record the FT-IR spectrum, the Avatar Thermo Spectrophotometer system was used. FTIR provides information on entities with their functional group associated with the synthesized Nanoparticles. Based on the FTIR spectra (Fig.5), the eco-friendly green synthesis of Copper nanoparticles mediated by the Pomegranate Peel extract was revealed. In the spectra, the extensive peak at 3437.95 cm -1 is equivalent to O-H or N-H stretching in amino acids, phenols, and alcohols. The peak a 1709.42 cm -1 is related to C=O stretching in Ketones [29].

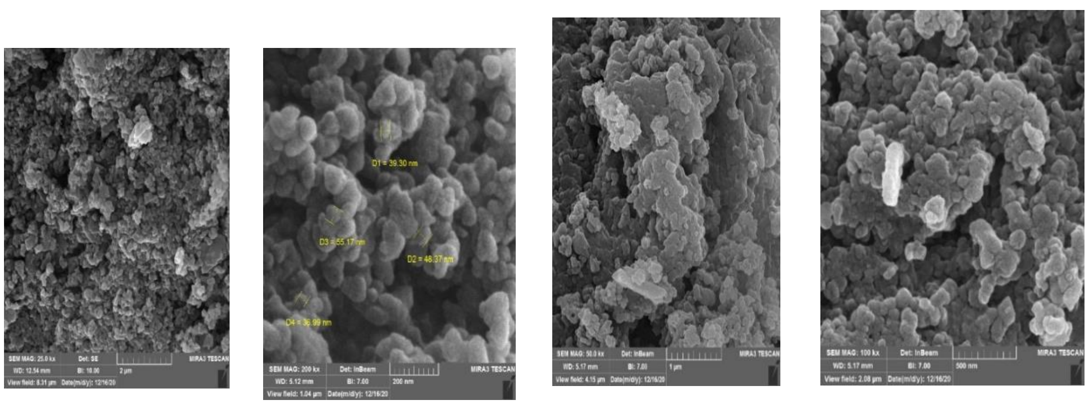
The peak at 1331.45 cm^{-1} to CH_2 & CH_3 deformation. The peak at 1621.24 cm -1 is related to C=C stretching and the peak at 1070.45 cm -1 is related to C-O stretching. The weak peaks within 850-550 cm -1 are related to C-Cl stretching in halo compounds. Thus, the synthesized nanoparticles were surrounded by metabolites and byproteins like terpenoids with functional groups of alcohols, ketones, aldehydes, and carboxylic acids [30]. According to the analysis of FTIR studies, it was found that the phenolic group possesses a stronger capacity for binding metal. It indicates that the phenols could be possibly from the metal nanoparticles (i.e., Cu nanoparticles capping) to avoid agglomeration and thus stabilize the medium. This indicates that the biological molecules could conduct dual functions of stabilization and formation of Cu nanoparticles in the aqueous medium [22, 30].



(A) (B)

Figure 5. (A) FTIR pattern of CuNPs using Pmegranate Peel extract. (B) FTIR pattern of Pomegranate Peel extract

SEM: The structural and morphological features of the Cu $(NO_3)_2$ nanoparticles were found utilizing FE-SEM Tescan Mira3 (Fig.6). The sizes of the synthesized nanoparticles within the nanometer range, and they had non-homogenous structures. According to these images, only some nanoparticles with spherical shapes were synthesized. Although some nanoparticles were well-separated from each other, they mostly existed in the agglomerated form. Therefore, such SEM results revealed the synthesized particles' nanostructure performance. The findings are consistent with former reports [23, 24], however, some slight differences exist owing to the chemical compositions. Fig.5 shows the uniformly sized and spherical morphology particles (36.99-55.17 nm)



A **B** **C** **D**

Figure 6: (A) SEM image of CuO nanoparticles taken at 2 µm, (B) SEM image of CuO nanoparticles taken at 200 µm, (C) SEM image of CuO nanoparticles taken at 1 µm, (D) SEM image of CuO nanoparticles taken at 500 nm.

XRD

The XRD analysis was used to determine the nature and particle size of the synthesized copper nanoparticles at an angle within the range of 20º-80º. The synthesized copper nanoparticles' amorphous nature was confirmed by X-ray diffraction analysis (Fig.7). The peak position is at 2θ of 32.41, 35.61, 38.81, 48.91, 53.32, 58.22, 60.61, and 63.6, which were assigned to (110), (111), (111), (200), (202), (002), (113), (220), and (311) planes, which are in good agreement with those of

CuO-NPs ob- tained from international center of diffraction data (ICDD) card no (801916). This confirms the formation of crystalline monoclinic morphology. Copper oxide nanoparticles show sharp and well-defined reflections on XRD patterns which give the verification of the crystalline nature of CuO nanoparticles [25]. Furthermore, the crystallite size of CuO-NPs was 15 nm, calculated by using the Debye Scherer equation ($D=k\lambda/\beta\cos\theta$), where k, λ, β, and θ are Scherer constant, wavelength of X-rays (1.5418 A˚), peak broadening at half the maximum intensity, and Bragg angle, respectively.

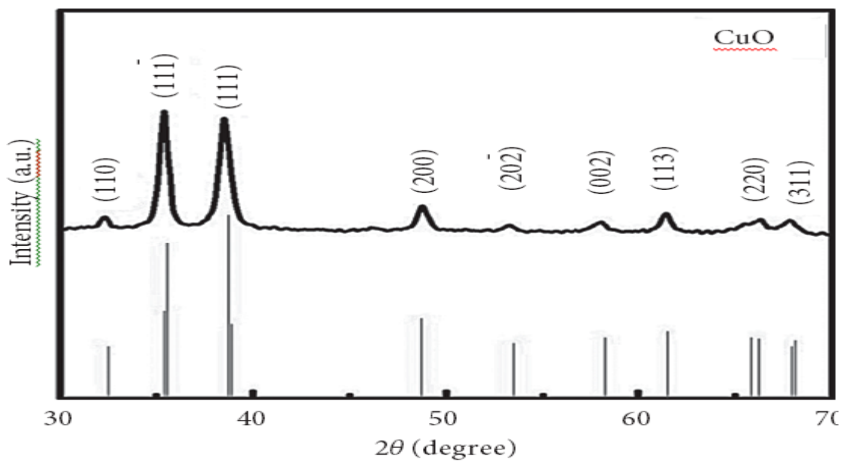


Figure 7. XRD pattern of CuNPs using Pomegranate Peel extract

Antibacterial activity

To measure antibacterial activity of Cu $(NO_3)_2$ nanoparticle against Salmonella (ATCC®9270TM) for the 1-15 mg⁄mlconcentration, MBC and MIC were determined. Preparing a pure culture of bacteria Salmonella (ATCC®9270TM) in the Muller Hinton Broth, it was then centrifuged followed by discarding the supernatant. Then, by adding 20 ml of sterile normal 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 Cu $(NO_3)_2$ nanoparticles 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 performed at 37°C for 24 h. A negative and positive control were incubated, as well. Followed by incubation, observing some test tubes was performed for microbial growth through turbidity. The least concentration was recorded as MIC, in which no visible growth was found.

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 Salmonella (ATCC®9270TM) – (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, thus, the MBC was the zone with the least inhibition growth.

After incubation of the test tubes for 24 hours at 37°C, the turbidity was found in the sample (1 mg/ml) and no turbidity and bacterial growth were considered in more solutions with more concentrations, the zone inhibition of examined solutions were as follow:

Table 1: concentration and zone of inhibition (mm)

Concentration (mg/ml)	Zone inhibition (mm)
	Salmonella (ATCC®9270^{TM})
2.5	0
5	0
10	12.8
15	14.7

These results confirm that the MIC and MBC of CuNPs for Salmonella was found to be 2.5 and 10 mg/ml.

Conclusion

In this work, we established an environmentally safe green, and eco-friendly technique for synthesizing copper nanoparticles from Pomegranate Peel extract with higher speed. The Pomegranate Peel extract was further appropriate for synthesizing the small-sized copper nanoparticles. The changes in color represent the existence of various phytochemicals, approved by FT-IR and UV-Vis spectroscopy. FT-IR indicates that the primary amines of proteins and phenols are in charge of the stabilization, reduction, and capping of copper nanoparticles. This is proved by FT-IR and UV-Vis spectroscopy. FT-IR indicates that the primary amins of proteins and phenols are mostly in charge of the capping and reduction of these nanoparticles to foil accumulation and present the stability to the medium. The nanoparticles have very small sizes within the range of 10-100 nms approved by SEM. The structural characterization was performed utilizing XRD. Furthermore, the antimicrobial studies revealed that the nanoparticles are toxic to various types to dry resistant micro-organisms. It was concluded that the Pomegranate Peel is a perfect substance for the fast copper nanoparticles synthesis and acts as a potential antimicrobial agent.

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