Research Article

Green Synthesize and Characterization of Copper Nanoparticles Using Iranian Propolis Extracts

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The propolis produced by bees is used by them to protect their hives. The cavity inside the hive’s walls is filled in during cold days to reduce entry points and mummify any intruders to ensure their survival. A current focus in nanotechnology and nanoscience is the green biosynthesis of nanoparticles (NPs) using biomaterials. Research on green methods for making metal oxide NPs is gaining momentum to safeguard the environment from the potential dangers associated with toxic chemicals. This study aimed to synthesize copper NPs (CuNPs) via propolis extraction, a novel application of nanoscience. The study was conducted under a range of pH, time conditions, and concentration ratios, and its properties were characterized by UV-Vis absorption spectra, XRD, and FTIR. An FTIR analysis revealed that compounds found in propolis extract could have an effect on the surface modification of the synthesized NPs. The propolis (Khalkhal) extract spectrum exhibited a sharp peak at 3422 cm⁻¹, caused by free hydroxyl groups and their intra/intermolecular hydrogen bonds. There were sharp peaks at 2925, 1637, and 1515 to 1076 cm⁻¹ associated with the C=O and C=C aromatic stretching frequencies. According to UV-Vis spectrophotometry investigation, CuO NPs exhibit a characteristic peak at 385 nm, showing significant surface plasmon resonance (SPR) with propolis (Khalkhal) extract. Furthermore, specific wavelengths of CuO NPs demonstrate peaks at 243, 292, and 350 nm for propolis (Gilan) extract. The green synthesis of CuNPs from Gilan and Khalkhal propolis can be an appropriate candidate for clinical applications such as drug delivery systems, drug formulation, and biomedical applications.

1. Introduction

Nanotechnology is gaining strength because NP and nanometric materials exhibit unique properties that enable their use in many fields [1, 2]. As a result of their antimicrobial and antiviral properties, NPs have recently gained widespread recognition in the medical field, making them an excellent research object for combating infectious diseases. NPs are capable of solving multiple problems in science and technology. Due to their antimicrobial and antiviral properties, NPs have garnered extensive attention in the medical field in recent years. This makes them an excellent focus for research pertaining to the prevention and treatment of infectious diseases [3–7]. Several aspects of the NPs are influenced by the method used to synthesize them, such as their size, shape, and morphology. Due to rapid oxidation, this area of research is challenging. Even though silver and gold have been extensively studied, the studies have been limited due to their high prices. The United States Environmental Protection Agency classifies copper (Cu) as an antimicrobial substance, abundant and relatively inexpensive, compared to other noble metals [1, 4, 8–11]. Copper has recently gained popularity due to the COVID-19 pandemic, which reports that SARS-CoV-2 can decompose faster on copper surfaces than on plastic surfaces (4 hours).
Green synthesis of nanomaterials, which does not use toxic chemicals, has been a significant area of nanoscience study in recent decades. This promotes the development of environmentally friendly processes [12].

There are several methods of synthesis using physical methods, including condensation, evaporation, and laser ablation [13]. As a viable alternative to physical and chemical methods, green synthesis (plant-mediated synthesis of nanoparticles) has been demonstrated to be a viable alternative. As compared with other methods of synthesis, it is a simple, rapid process that uses less toxic materials that are environmentally friendly. In addition to reducing environmental problems such as solar interaction, catalysis, and agricultural production, green synthesis improves renewable energy production. Zhang et al. suggest that plant material often plays a critical role in determining the size and surface morphology of nanoparticles synthesized through green synthesis [14]. Plant extracts are capable of serving both as reducing agents and capping agents. Compared to chemical methods, green synthesis produces larger nanoparticles than those produced by chemical methods. As a result of their high biocompatibility, nanomaterials derived from green sources are highly biofunctional. According to some reports, the green synthetic route produces higher yields of nanoparticles than the chemical route [15]. A green method for synthesizing CuNPs is to use cotton textile fibers and water as a solvent to produce highly stable CuNPs that can be stored for months without oxidation. Based on characterization studies, it has been demonstrated that when nanocomposites with high copper contents are synthesized, the crystallinity index of cellulose is modified based on the reaction conditions, and the chemical structure of cellulose is destroyed. Using UV spectroscopy in the 300–350 nm range, CuO NPs was confirmed to be the by-product of the reaction [12].

Metallic nanoparticles can be synthesized using plant extracts and microorganisms, where the active biological component serves as a capping and reducing agent, reducing costs. Therefore, excessive energy and pressure are unnecessary for experimental studies, resulting in energy-efficient and environmentally friendly processes. To produce NPs, bacteria found in complex eukaryotic plants can be used to produce ionic nanoparticles (green synthesis). Because heat, pressure, and energy are not required, these bacteria can handle higher production levels [16–20]. A variety of adverse reactions can occur due to toxic chemicals adsorbing onto the surfaces of NPs. Green synthesis takes advantage of many of the most important aspects to obtain stable and well-characterized nanoparticles, including optimizing reaction conditions [21]. A plant’s detoxification capacity and capacity to accumulate heavy metals should be considered reaction conditions, such as pH and temperature, to determine which plant is appropriate for green synthesis [22]. Some plants have medicinal properties that have led them to be used to manufacture NPs. Various plants, including Aloevera, *Punica granatum*, and *Allium sativum*, have been used to synthesize CuNPs [23, 24].

In an investigation conducted by Sharmila et al., copper oxide (II) nanoparticles were synthesized from *Cassia Ariculata* leaves with a spherical shape and a size range of 30–35 nm. An important drawback of this method is that the extract must be stored at 4°C for later use, and the mixture of the extract and copper sulfate should be incubated at 37°C for four days [25]. Using *Fusarium oxysporum*, a ubiquitous and anamorphic fungus, Gupta et al. synthesized Cu NPs. The resulting NPs exhibited spherical or elongated shapes with a diameter of 13 nm; however, in this work, the major limitation is the use of fungi as a bio-reducing, stabilizing, and capping agent, since they must shake at 150 rpm for two days [12]. As another method, Arunkumar et al. conducted experiments using sterile distilled water to treat Lantana camara leaf extracts in order to obtain CuO NPs using CuCl2.2H2O as a salt precursor, NaOH, and sterile distilled water as the plant treatment solution. In this method, CuO NPs with sizes between 17 and 21 nm were obtained with seed-spheroid shapes and agglomerated structures; one important disadvantage was the need to titrate with NaOH at the midpoint of the reaction, and high temperatures were required for calcining these materials (500°C/4h) [26].

Propolis consists of waxes, pollen, phenolic acids, flavonoids, and aromatic balsams. The composition of propolis depends on where and how it is made; it is different depending on which species and plants are utilized [27]. Propolis is biologically active because of its flavonoids [27]. Flavonoids possess unique biochemical properties, including attaching to heavy metal ions and biological polymers and their ability to scavenge free radicals and catalyze electron transfer [27]. Propolis inhibits DNA synthesis, inhibits the incorporation of thymidine, leucine, and uridine into tumoral cells, and therefore has antitumor effects [27]. Bees use propolis to seal their hives to prevent microbes from entering. Its antimicrobial properties may be attributed to the synergistic action of its components [27]. In addition to its antibacterial properties, propolis inhibits bacterial enzyme activity and motility by interfering with the integrity of the membranes. As such, propolis can prevent the growth of bacteria resistant to antibiotics [27]. In addition to its wide range of applications, treatments for conditions like urinary tract infections have been found in propolis, periodontal disease, ear infections, cancer, sinus congestion, Parkinson’s disease, gastritis, influenza, intestinal infections, headaches, conjunctivitis, and warts [27]. In this study, copper nanoparticles were synthesized and characterized using Iranian propolis extracts (Khalkhal and Gilan) with biotechnology.

## 2. Methods and Materials

### 2.1. Materials

- Coomassie Brilliant Blue R-250 (BRR-250), CuSO4.5H2O (Merck, 99%), Absolute ethanol, NaOH (Vetec, 98.5%), sodium hydroxide, Direct Red 81 (DR-81), and copper (II) sulfate pentahydrate were purchased from Sigma Aldrich, Germany. All reagents and solutions were analytical grades, and double-distilled water was used.

### 2.2. Propolis Sampling and Preparation of Propolis Extract

Propolis was collected in 2022 in Khalkhal (Ardabil province) and Gilan province. Samples were frozen (−20°C).
Using a tetrafold volume of 70% ethanol, raw propolis samples were extracted for three days (in the dark). To remove less soluble substances and waxes, the suspensions were filtered on Whatman filter paper, no. 1. EEP stands for ethanol extract of propolis. To evaporate the solutions, rotating evaporators were used. The powder was then obtained by freeze-drying the solutions [28, 29].

2.3. Synthesis of Copper Nanoparticles. To synthesize CuNPs, we dissolved 50 mg of dried propolis extract in 100 mL of deionized water, and we adjusted the pH to 8 with NaOH. The extract solution was slowly added to 100 mL of 6 mM copper sulfide solution while being stirred (1,000 rpm). They stirred in the dark for 24 hours at 37°C to 40°C. Centrifugation was done for 15 minutes at 25°C at 13,000 rpm upon obtaining the colored mixture (dark brown). All residues on the pellet were removed by washing it twice with deionized water. Following that, precipitates were lyophilized and analyzed (Figure 1).

2.4. Characterization of the CuNPs

2.4.1. UV-Vis Spectroscopy. Molecular spectroscopy using UV-Visible light is UV-visible spectroscopy. In conjunction with electromagnetic waves, this method measures Plasmon resonance and total oscillations of electron conduction bands [30]. In addition to measuring the absorption of fluids, this method has also been applied to the measurement of absorption of other materials. During UV-visible spectroscopy analysis, a beam of light is split into two halves, with one part analyzing the compounds inside the transparent cell. In contrast, the other part examines the reference molecules [31]. The substance under examination absorbs light of a particular wavelength; this phenomenon is known as surface plasmon resonance (SPR). The surface plasmon resonance for CuO NPs ranges from 200 to 350 nanometers. Under UV-Vis spectroscopy, time was assessed on the formation of CuNPs. The optical properties of metal nanoparticles are sensitive to concentrations, shapes, sizes, and agglomeration states, making UV-Vis spectroscopy an excellent tool for identifying them. This unique peak appears at a specific wavelength of light due to SPR electrons present on NP surfaces.

2.4.2. XRD Analysis. As a radiation source, Cu Kα (λ =1.540 Å) served as a crystallographic analyzer for the CuNPs with a scan speed of 0.4°/min and a 2θ = 10–700 radiation source. By identifying the compound’s crystal structure and chemical composition using XRD analysis, the compound was identified. X-ray energy-dispersive spectroscopy was also used to assess the chemistry of nanoparticles.

2.4.3. FTIR Analysis. FTIR spectrophotometers at long wavelengths are used to identify different functional groups of NPs. The propolis extract containing NPs is found to contain functional groups. Absorption is caused when FTIR light is in contact with a bond or vibration frequency corresponding to a particular resonant frequency. Energy is absorbed by inorganic atoms depending on their mass, molecular energy, and vibrational coupling. Different compounds or molecules have different FTIR spectra that reflect their unique arrangement of atoms. These peaks are prominent in CuO NPs and correspond to O–H, C = O, C–N, C–H, and C = C. It has been attributed to either O–H or N–H of alcohol/phenol for absorption at 3000–3350 cm⁻¹. Aromatic C–H bending can be ascribed to absorbance peaks between 820 and 880 cm⁻¹. This wavelength range exhibited a strong absorption peak. From 1600–1790, there was an absorption band for carbonyl–C = O. The surface modification of the synthesized NPs was examined with FTIR using compounds in propolis extract.

3. Results

3.1. UV-Vis Spectrophotometry. A UV-visible spectrophotometer was used to measure the surface plasmon resonance (SPR) properties of the CuO NPs. The SRP of electrons on the nanoparticle surface is responsible for forming a unique peak at specific wavelengths of light. With propolis (Khalkhal) extract, UV-Vis spectra of CuO NPs show a characteristic peak at 385 nm, exhibiting an SPR. Moreover, specific wavelengths of CuO NPs using propolis (Gilan) extract imply peaks at 243, 292, and 350 nm in UV-Vis spectrophotometry investigation. A black band indicates that metallic copper is present, which implies the presence of CuO NPs (Figures 2 and 3).

3.2. XRD. CuO nanoparticles with prominent peaks can be seen under X-ray diffraction using propolis (Khalkhal) extract. Bragg’s reflection of CuO NPs using propolis (Khalkhal) extract shows diffraction peaks around 2θ = 35.74°, 39.04°, and 49.04°, representing, and crystallographic planes of face-centered cubic (FCC). Also, a reflection of CuO NPs using propolis (Gilan) extract shows diffraction peaks around 2θ = 25.54°, 26.69°, 38.79°, and 48.84°, representing, and crystallographic planes of fcc (Figures 4 and 5).

3.3. FTIR Results. The Fourier transform is used in FTIR spectroscopy to measure molecule vibration frequency. In the frequency range of 4000–400 cm⁻¹, Figures 6 and 7 show CuO NPs’ infra-red spectra using propolis (Khalkhal) extract. A sharp peak of 3422 cm⁻¹ was observed in the propolis (Khalkhal) extract spectrum due to free hydroxyl groups and their intra-/intermolecular H-bonds. C = O and C = C aromatic stretching frequencies were associated with the sharp peaks at 2925, 1637, and 1515 to 1076 cm⁻¹. A 602 cm⁻¹ absorption band was observed in CuO NP monoclinic phase (Figure 6). There is also a peak of 3410 cm⁻¹ in the propolis (Gilan) extract spectrum due to free hydroxyl groups and their intra-/intermolecular H-bonds from polyphenolic compounds. The peaks which appeared at 2920 and 1614 and ranged from 1515 to
1057 cm$^{-1}$ were related to saturated hydrocarbons (Csp3-H) and aromatic stretching frequencies of C=O and C=C. NPs in the monoclinic phase of CuO were observed to have absorption bands 603 cm$^{-1}$ (Figure 7).

4. Discussion

Nanotechnology, a vast field, is receiving considerable attention. Bio nanotech (microbes to higher plants) has gained global attention since chemical synthesizing produces very few NPs and is toxic and unsustainable [32–37]. A study conducted by Barbosa et al. described the use of Brazilian propolis to synthesize silver nanoparticles (AgNP–P) and assess their antimicrobial properties. Maximum absorbance was observed in the UV-Vis spectrum of AgNP–P at 412 nm, indicating that it has a spherical form and was formed spherically. Using dynamic light scattering, a hydrodynamic size of 109 nm was calculated and a polydispersity index of 0.3 was determined, indicating stability and good size distribution. As a result of centrifugation, microscopy analysis

![Figure 1: The synthesized CuO nanoparticles from Khalkhal [1] and Gilan [2] samples.](image1)

![Figure 2: Specific wavelengths of CuO NPs using propolis (Khalkhal) extract in UV-Vis spectrophotometry assessment.](image2)

![Figure 3: Specific wavelengths of CuO NPs using propolis (Gilan) extract in UV-Vis spectrophotometry assessment.](image3)
confirmed the purification procedure and demonstrates the presence of propolis around the silver nanoparticles. Based on XRD patterns, the main planes of the metallic silver crystal structure were determined; at the same time, FTIR revealed the major groups responsible for silver reduction, as indicated by the thermal analysis, representing 22% of AgNP–P. Furthermore, results indicated a synergistic interaction between propolis and silver NPs, which suggests that this may be an effective antimicrobial product for use in infections [38].

In this study, CuNPs were synthesized from propolis extract and optimized and characterized. The compounds contained in the propolis extract from Khalkhal and Gilan during the study. For the production of CuNPs, green biosynthesis offers the main advantage of stabilization. CuNPs are stabilized by the capping agents contained in compounds for more than 30 days, but they oxidize and settle down after 24 hours due to the chemical production of CuNPs and large-size CuNPs produced by the compound [39].

In this study, the key factors are pH, concentration ratio, temperature, and time in synthesizing copper nanoparticles, but these are difficult to determine. Some color changes are observed within 30 minutes. By observing the color change, we can observe that CuNPs are being produced, which was confirmed through UV-Visible studies. The CuNPs obtained from Aloe vera flower extract changed color within 30 minutes. Using UV-Vis in conjunction with the SPR at 578 nm, the formation of NPs was confirmed. Using the UV-Vis spectroscopy method, it was observed that a peak in the absorption range between 500 and 700 nm was observed in the CuNPs synthesized from the extract of Asparagus.
adscendens leaves after 1 h of reaction [40]. *Cissus arnotiana*-mediated biosynthesis of CuNPs is reported in the study of Rajeshkumar et al. Nanotechnology and nanomedicine hold substantial promise for antibacterial and antioxidant activity. The use of green synthesized CuNPs in biomedical applications is predicted to increase exponentially due to their outstanding properties. Due to the thin peptidoglycan layer and electrical interaction between bacterial cells and CuNPs, Gram-negative strains, such as *E. coli*, exhibit potent antibacterial activity [41].

Based on an aqueous extract of *Cymbopogon citratus* leaves, Cherian et al. have demonstrated the feasibility of a simple, eco-friendly method for the synthesis of esters for the functionalization of CuONPs from *Cymbopogon citratus*. *Cymbopogon citratus* esters such as di-propylene glycol diacrylate, α-monoolein, and iso-octyl phthalate demonstrated substantial contributions to Cu$^{2+}$ and Cu$^{3+}$ reduction, as well as surface functionalization in GC-MS analysis of Cymbopogon citratus and *Cymbopogon citratus* CuONPs. An analysis of comparable FTIR spectra reveals the presence of auxiliary bioactive in the form of proteins, sugars, and polyphenols in association with CuONPs in *Cymbopogon citratus*. It is noteworthy that this research provided insight into the efficiency of bioactive esters as capping, reducing, and stabilizing agents at different pH levels (4.0 to 12.0). In this regard, it can be speculated that *Cymbopogon citratus* CuONPs may act as a stable antimicrobial agent at harsh environmental pH levels. It was shown that *Cymbopogon citratus* CuONPs exhibited significant interactions and intracellular uptake in biofilm-producing *E. coli*-336 and MSRA-1 was validated. Data have unequivocally demonstrated that *Cymbopogon citratus*...
CuONPs can effectively combat biofilm infections and multi-drug resistance in biomedical settings [42].

According to Amin et al., *Aerva javanica* leaf extract was used for the synthesis of CuONPs. It was determined from XRD analysis that CuONPs possess a crystalline morphology, and the average crystal size obtained was 15 nm. According to SEM images, the particles are spherical and their size ranges from 15 to 23 nm. Based on the FTIR analysis of the extract, it can be concluded that CuONPs are synthesized primarily by reducing and capping agents present in the extract. The antimicrobial activity of the synthesized CuONPs was evaluated against bacterial and fungal pathogens. As a result, CuONPs showed maximum antimicrobial activity against all selected bacteria and fungi [43].

The morphology of CuONPs with interesting structure has been investigated by Selvanathan et al. using *Muntingia calabura* leaf extracts for phytochemical-assisted NPs synthesis. The unique combination of secondary metabolites in the plant extract, such as flavonoids and polyphenols, is deduced to be an effective capping agent for the manufacturing of NPs with distinctive morphologies similar to those produced by conventional chemical synthesis. CuONPs were confirmed to be monoclinical and crystalline by XRD analysis. Through X-ray photoelectron spectroscopy and Raman spectroscopic analysis, the phase purity and chemical identity of the product have been confirmed. These results indicated that a single-phase CuO has been formed without the presence of any other impurities [44].

Prakash et al. have investigated the synthesis of CuONPs from rhizomes extract of *Picrorhiza kurroa*. The presence of tannins, flavonoids, glycosides, phenols, and flavonoids in biosynthesis NPs was confirmed using FTIR. The biosynthesized NPs were determined to be 20–40 nanometers in size based on SEM and TEM studies. Antimicrobial activity has been demonstrated in these particles against various pathogenic microorganisms [45]. An aqueous extract of Juglans regia green husk was used to study the green biosynthesis of CuNPs by Ayadi Hassan et al. To produce environmentally friendly CuNPs under ambient conditions, dried green Juglan regia husk extract was used. In addition to its considerable antibacterial, antibiofilm, antifungal, antioxidant, and photocatalytic properties, the CuNPs are versatile nanomaterial [46]. Sumitro examined the use of medicinal fruit extract of *Piper retrofractum* Vahl in the green synthesis of CuNPs. By SEM, it has been confirmed that the synthesized CuNPs have spherical shapes and contain a high Cu content (70.3%). In the TEM image, it can be seen that the particle size distribution of the CuNPs exhibits a high degree of uniformity with a size range of approximately 2 to 10 nm in optimum conditions. As revealed by XRD, CuNPs display a 26.4% crystallinity phase. In addition to being relatively stable, the synthesized CuNPs inhibit *E. coli* and *S. aureus*. *Piper retrofractum* Vahl fruit extract was a suitable tool for synthesizing CuNPs with high particle size uniformity in a greener way [47]. Incubation of the *Allium Sativum* CuNPs at room temperature was monitored using a UV-Vis spectrum for 48 hours. After mixing the two solutions, the color changed from straw yellow to lighter green. Compounds present in the plant extract are critical for forming and stabilizing CuNPs. Increased concentration of plant extract will accelerate the reduction of the Cu+2 ions, resulting in a decrease in the CuNPs size [32]. Several techniques were used to characterize CuNPs.

In this study, less separated CuNPs can be observed with broader SPR peaks during shorter wavelengths. Specifically, CuNPs with a peak absorption of 385 nm were observed in Khalkhal, 243, 292, and 350 nm for propolis extract from Gilan, indicating that the particles are well-stabilized with an appropriate diameter [48]. The UV-Vis spectrum of well-distributed and stable CuNPs was measured at 385 nm in propolis from Khalkhal and 243, 292, and 350 nm in propolis extract of Gilan. By obtaining Citron juice (Citrus medica Linn) as a source of CuNP of 20 nm, its peak absorption wavelength was 631 nm [49]. *Asparagus adscendens* was used as a biogenic source of CuNPs, resulting in an average CuNP size of 40–100 nm and an SPR of between 500 and 700 nm [32]. To determine the purity of the NPs, we used XRD to determine their peak intensity, location, and width [50]. In this study, the peaks confirm the formation of CuNPs. It was found that the powders were pure from impurities, and standards of guidelines confirmed it.

5. Conclusion

As propolis has not previously been used to synthesize CuNPs, this study presents a novel method of synthesizing CuNPs by extracellular biogenesis from propolis extract. The process of green biosynthesis was successful due to the use of a non-toxic, easy-to-use, cost-effective, and eco-friendly approach. Tools for characterizing CuNPs provide insight into their stability in future applications. In the future, CuNPs are likely to be used in drug delivery systems, drug formulations, and biomedicine applications since they are greenly biosynthesized from natural products. However, the precise antimicrobial and the synergistic effects of the propolis combined with CuONPs might be clarified. Clinical studies must be designed to evaluate the function of green biosynthesized CuONPs using propolis to determine the long-term efficacy of this method and propolis extract. Also, in this study, there was the limitation of different species of propolis distributed around the world to find the ideal propolis for use in the CuONPs green biosynthesis.

Data Availability

The data generated or analyzed during this study are included within the published article and available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest in this study.

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