

Article

Eco-Friendly Synthesis of Copper Oxide Nanoparticles via Pistachio Seed Coat Extract for Antimicrobial, Antioxidant, and Catalytic Applications

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Abstract

This study investigates the biogenic synthesis of copper oxide nanoparticles (CuO NPs) using the aqueous extract of pistachio seed coat as a stabilizing and reducing agent, and a better alternative to the traditional physicochemical approach. The CuO NPs were synthesized by a sustainable approach and characterized using XRD, UV–visible, FTIR, FESEM-EDX, and HRTEM-SAED, and are emphasized for their significance in comprehending how morphology, size, and surface chemistry affect the performance of CuO NPs. The UV–visible spectrophotometry showed an absorption at 273 nm, demonstrating the formation of CuO NPs. SEM and TEM provided a spherical shape with a size range of 50–100 nm. CuO NPs' antifungal activity was examined against the fungal strains *A. niger* and *P. chrysogenum*, and their antibacterial efficacy was evaluated using the agar well disc diffusion method against *E. coli* and *S. aureus*. *Staphylococcus aureus* had the largest inhibitory zone, measuring 18 mm at 100 µg/mL, while *Aspergillus niger* had the smallest, measuring 8 mm at 25 mg. The IC₅₀ value of the sample was 319.55 ± 3.21 µg/mL. The IC₅₀ value indicates that the CuO NPs exhibit moderate antioxidant potential. The synthesized CuO NPs exhibited excellent catalytic efficiency in the cyclization reaction of chalcones. The findings of the present study highlight CuO NPs synthesis by an eco-friendly, cost-effective and sustainable method from pistachio shell extract. The synthesized CuO NPs demonstrated notable biological and catalytic activity that underscores their future need in various fields such as pharmaceutical, biomedical and industrial catalysis.

Keywords: plant extract; biogenic CuO NPs; catalyst; antibacterial; antioxidant



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1. Introduction

Nanotechnology has emerged in recent years as a multidisciplinary branch of scientific and industrial study that focuses on developing new techniques to synthesize NPs with suitable sizes, usually up to 100 nm in diameter, and precise shapes [1]. Nanoscale materials exhibit unique dimensions and surface-to-volume ratios, which significantly improve their catalytic activity, mechanical properties, thermal and electrical conductivity, and melting temperatures [2,3]. Metal oxide nanoparticles are currently widely used in a

variety of industries, such as photovoltaics, batteries, photocatalysis, agriculture, biosensors, medicine, drug delivery, food packaging, automotive, and environmental remediation, because of their unique characteristics [4]. These metal oxide nanoparticles, such as CuO, Fe₂O₃, Fe₃O₄, MgO, TiO₂, and ZnO, show notable biological activity even at very low levels because of their distinct physicochemical characteristics [5]. Among these, scientists working on biodiesel generation, cells, supercapacitors, catalysis, water remediation, and antibacterial and anticancer applications have shown a great deal of interest in CuO NPs. Additionally, they possess anti-inflammatory, antioxidant, anti-diabetic, antifouling, and antifungal properties [6]. This is primarily due to the fact that they provide better thermal stability, biocompatibility, a range of nanoscale structures, ease of synthesis, a large surface area, high conductivity, increased oxygen adsorption capability, abundance, and affordability when compared to other noble metals [7].

Metal nanoparticles are widely used due to their large surface area, adjustable particle size, and significant surface reactivity. These unique characteristics make them valuable across numerous fields, including chemical processing, medical and pharmaceutical applications, electronics, targeted drug and gene transport, biosensing technologies, and environmental cleanup [8,9]. In the human body, copper is an essential trace element that supports the flexibility and structural integrity of bone, skin, and other organs [10,11]. In the past few years, the synthesis of CuO NPs has been reported in the literature using various methods and has been shown to be a catalyst for the removal of industrial effluents from the environment, photodegradation, environmental remediation or adsorptive removal of hazardous chemicals [12,13], sensors for the identification of biomolecules and gases, and a catalyst for accelerating the rate of oxidation reaction [14,15].

CuO NPs have been produced by several techniques, including lithography [16], laser ablation [17], chemical precipitation [18], electrochemical [19], hydrothermal [20], chemical bath deposition [21], and biological procedures, as shown in Figure 1. However, the majority of traditional CuO NP synthesis techniques are complicated, expensive, time-consuming, and require hazardous chemicals, which restricts their practical application [22]. Because of this, green synthesis techniques have been developed to produce non-toxic, eco-friendly products and byproducts, use safe reagents, and require less energy, as shown in Table 1 [23]. CuO NPs have recently been synthesized from a variety of biological sources, including yeast, bacteria, plants, fungi, and algae. Because of their stability, accessibility, and abundance of metabolites, plant extracts are preferred over bacterial or fungal methods for their speed and ease of use [24]. According to the literature reviewed, no reports have been found of biogenic synthesis of CuO NPs using pistachio seed coat extract. The extract from the pistachio seed coat has oxidizing, stabilizing, reducing, and capping properties. However, the synthesis of CuO NPs by using different green resources, such as *Pithecellobium dulce* seed pods [25], *Brassica juncea* (L.) Czern Seed Extract [26], has been reported in a few recent publications. This study presents the eco-friendly synthesis of CuO NPs using pistachio seed coat extract. The synthesized CuO NPs were characterized using techniques such as FTIR, UV-visible, SEM-EDX, XRD, and HRTEM. Antibacterial properties of CuO NPs were studied against *E. coli* and *P. aeruginosa*, and the antifungal activity was studied against *A. niger* and *P. chrysogenum*. It was found that the synthesized CuO NPs were more affordable, safer, and more environmentally friendly.

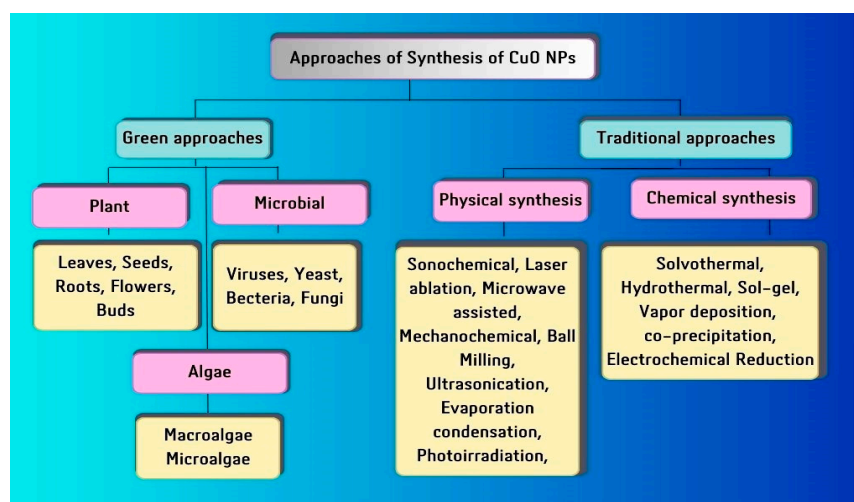


Figure 1. Scheme of CuO NPs.

Table 1. Advantages and disadvantages of CuO NPs produced by chemical, physical, and green methods.

S. No.	Method Used	Advantages	Disadvantages	References
1.	Green	- Eco-friendly and cost-effective synthesis route, reduced toxicity, energy-efficient processing, good biocompatibility - Effective adsorbent material due to small particle size and high surface area	Difficulties in achieving precise control on nanoparticle morphology and size distribution shows lower stability under some conditions	[27–29]
2.	Chemical	- Controlled size - High purity and quality - NPs are considerably stable	- Involves hazardous chemicals and organic reagents - Possibility of toxic contamination - Requires stabilizing and capping materials to prevent aggregation and maintain particle size - Certain stabilizers may themselves be harmful - Can contribute to long-term environmental pollution	[30]
3.	Physical	- No use of toxic ingredients - High purity and quality - Uniform size and shape - High production rates - High-speed method	- Demands substantial energy input - Expensive operational setup - May still result in environmental concerns. Nanoparticles can exhibit reduced stability	[31,32]

2. Materials and Methods

2.1. Preparation of the Extract

The collected pistachio seed coats were washed with double-distilled water (DDW), air-dried, and then ground into a fine powder. In a 250 mL round-bottom flask, 10 g of powdered pistachio seed coat was refluxed in 100 mL of DDW to produce a 10% (w/v)

aqueous extract. The mixture was heated to 60 °C for 3 h. After the reflux process, the solution was cooled to room temperature. The extract was filtered to remove particulate matter, and the filtrate was stored for subsequent use.

2.2. Biosynthesis of CuO NPs from the Seed Coat of Pistachio

After preparing 250 mL of double-distilled water containing 3 mM copper acetate ($\text{Cu}(\text{OAc})_2$), 20 mL of pistachio seed coat was gradually added dropwise to the solution while vigorously stirred with a magnetic stirrer at room temperature for 2 h. The reaction mixture was then heated to 80 °C for 3 h. Two NaOH plates were added to 5 mL of DW during the reaction, and the mixture was heated again until it turned black, indicating the formation of CuO NPs. It was then centrifuged for 10 min at 10,000 rpm. After discarding the transparent solution, the viscous CuO NP layer was obtained and dried in an oven at 50 °C.

2.3. Characterization of CuO NPs

A Shimadzu UV-vis spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) was used to record the UV-vis spectrum over 200–800 nm. TEM (TECNAI G-20, FEI, Hillsboro, OR, USA) and SEM (Nova nano FE-SEM 450 FE, FEI, Hillsboro, OR, USA) were used to measure the shape and size of CuO NPs. Fourier transform infrared (FTIR, model no IR-AFFINITI, Shimadzu, Kyoto, Japan) spectra were obtained using the KBr pellet technique in the range of 4000–400 cm^{-1} . The presence of CuO NPs was also verified by spectroscopic examination using Energy Dispersive X-Ray (EDX, Oxford Instruments, Oxford, UK).

2.4. Antioxidant Activity

The antioxidant potential of the pistachio seed coat extracts obtained was evaluated using the DPPH free radical scavenging assay. Antioxidants are bioactive compounds that inhibit or reduce oxidative damage in cells by neutralizing free radicals, highly reactive and unstable molecules generated in response to various environmental and physiological stresses. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was used to assess the capacity of the extracted materials to scavenge DPPH radicals. 3.9 mL of 0.1 mM DPPH (methanolic) solution was used to combine 100 μL aliquots of substances in varying doses (20–100 $\mu\text{g}/\text{mL}$ in DMSO). After that, the mixture was centrifuged and left in the dark for 30 min. Using methanol as a blank, its optical density (OD) was determined at 517 nm [8]. The free radical scavenging efficiency of both the plant extracts and the synthesized nanoparticles was determined by calculating the percentage inhibition using the following equation:

$$\% \text{ of DPPH free radical scavenging} = (\text{A}_{\text{control}} - \text{A}_{\text{sample}}) / \text{A}_{\text{control}} \times 100$$

where A_{sample} represents the absorbance of the DPPH solution with the sample and $\text{A}_{\text{control}}$ displays the absorbance of the DPPH solution.

IC_{50} values were calculated by fitting a linear regression to the concentration–percentage inhibition relationship. Using an inhibition curve, the antioxidant activity of each sample was expressed as IC_{50} , the concentration required to inhibit the formation of DPPH radicals by 50%.

2.5. Antimicrobial Activity

For the antimicrobial assays, different bacterial species, viz., *E. coli* (MTCC119), *S. aureus* (MTCC3103), and fungal species, viz., *Aspergillus niger* (MTCC13111) and *Penicillium chrysogenum* (MTCC1996), were selected. The above-mentioned strains were obtained from IMTECH, Chandigarh.

The antimicrobial activity of the synthesized samples was evaluated against selected bacterial and fungal strains using the agar diffusion method, while the minimum inhibitory concentration (MIC) was determined by the broth dilution method. For antibacterial studies, Nutrient Agar (NA) was used, whereas Potato Dextrose Agar (PDA) was used for antifungal analysis. Different concentrations of the samples were prepared in dimethyl sulphoxide (DMSO). Sterile agar plates were inoculated with freshly prepared microbial cultures and evenly spread using a sterile spreader. After allowing the cultures to absorb for 30 min, 6 mm diameter wells were aseptically punched into the agar medium. Subsequently, 100 μ L of the sample solutions was introduced into the respective wells. Erythromycin and ketoconazole were used as standard antibacterial and antifungal drugs, respectively. The diameter of the inhibition zones (IZ) surrounding each well was measured to determine the antibacterial efficiency after the inoculation plates were incubated under appropriate conditions. The difference between the sample's inhibition zone and that of the standard drug was used to calculate the Activity Index (AI).

The broth dilution method was used to determine the samples' minimum inhibitory concentrations (MICs). The values ranging from 100 to 6.25 μ g/mL were obtained using a two-fold serial dilution. Different concentrations of the sample solutions were applied to test tubes filled with nutrient broth after microbial culture was added. Absorbance was determined at 600 nm using a spectrophotometer following a 24 h incubation period. The % inhibition, IC_{50} , and MIC values were computed using the absorbance measurements. To ensure accuracy, every experiment was carried out in triplicate [5].

2.6. Catalytic Activity

After adding 5–10 mg of CuO NPs and phenyl hydrazine (0.013 mol) to chalcone (0.01 mol) in 15 mL of acetic acid, the reaction mixture was refluxed at 80 $^{\circ}$ C with continuous stirring for 4 h. Once the reaction was completed, the mixture was put into ice-cold water, and the solid precipitate was filtered, cleaned with water, and recrystallised from ethanol to produce pale yellow crystalline plates [29].

3. Results and Discussion

3.1. UV-Visible Spectroscopy

Using UV-visible spectroscopy, a clear peak in the 200–800 nm region indicates the stability and biogenesis of CuO NPs, as shown in Figure 2. The synthesized CuO nanoparticles showed a significant absorption peak seen below 273 nm [33], which confirms the formation of CuO NPs. This finding also demonstrates their optical characteristics. The synthesis of relatively pure CuO nanoparticles is confirmed by the broad, smooth absorption pattern [34], which lacks sharp peaks and other noticeable features.

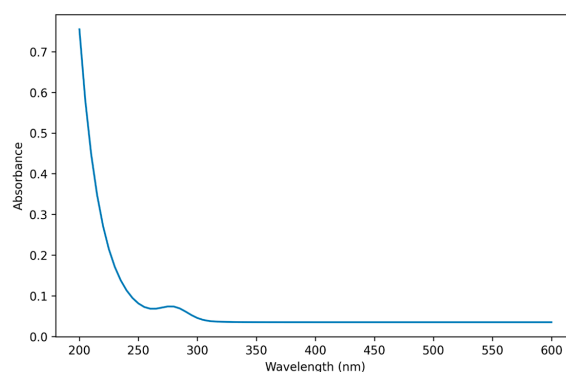


Figure 2. UV-vis absorption spectrum of CuO NPs synthesized using the extract of the seed coat of pistachio.

3.2. FTIR Analysis

FTIR analysis of the pistachio seed coat extract and manufactured CuO NPs was measured in the 4000–400 cm^{-1} wavelength range (Figure 3a,b). FTIR spectra of the plant extract and the synthesized CuO nanoparticles showed clear variations in functional groups before and after nanoparticle formation. The spectrum of the plant extract displayed a broad band in the region of 3333 cm^{-1} , which is commonly associated with O-H stretching vibrations of phenolic and alcoholic compounds [35]. Absorption peaks around 1636 cm^{-1} were related to carbonyl (C=O) groups [36] of proteins, flavonoids, and other naturally occurring biomolecules. Additional bands in the region of 1000–1400 cm^{-1} indicated the presence of C-O and C-N functional groups. After the formation of CuO NPs, slight shifts and reductions in the intensity at 2361 cm^{-1} were due to the involvement of phytochemicals in converting copper ions into CuO nanoparticles, and this peak indicates the stretching vibration of the aliphatic $-\text{CH}_2$ group. Therefore, the pistachio seed coat extract's various functional groups served as stabilizing and reducing agents. Aromatic C=C bond stretching vibrations are responsible for the peaks around 1647 and 1698 cm^{-1} , and a weak peak appears at 1747 cm^{-1} for the keto groups shown in Figure 3b. The peak observed at 548 cm^{-1} is due to the Cu-O stretching modes of vibration [37].

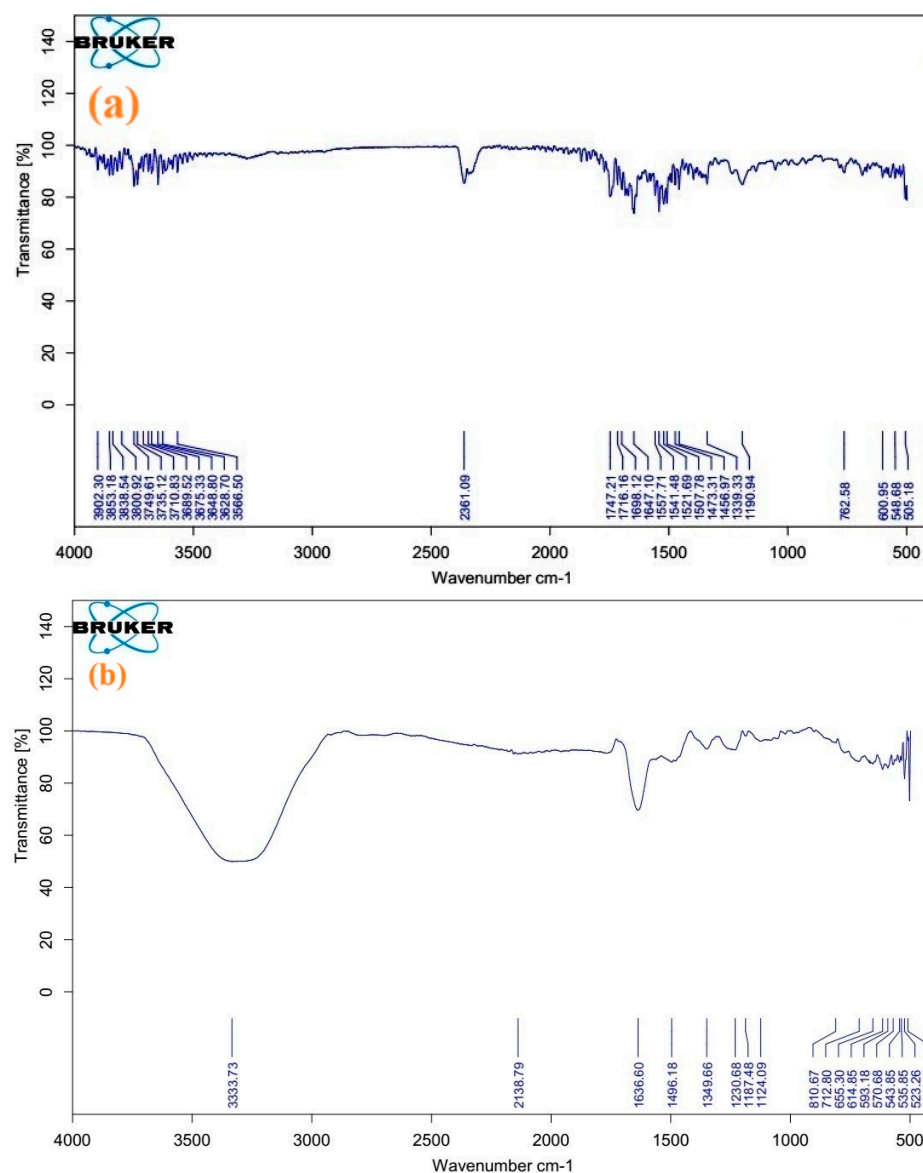


Figure 3. FTIR Spectrum of (a) CuO NPs, (b) extract of pistachio seed coat.

3.3. X-Ray Diffraction Interpretation

XRD is a characterization technique for the analysis of the crystallographic structure of materials, as depicted in Figure 4, which exhibits a broad diffraction pattern with low-intensity peaks, indicating the formation of poorly crystalline CuO NPs, as previously reported [38]. Because Cu atoms are arranged irregularly as disordered chains, the synthesized CuO NPs are found to be amorphous. The XRD spectrum of CuO nanoparticles shows broad as well as sharp peaks, which suggests a mixed phase of amorphous and crystalline states. The diffraction peaks at $2\theta = 13.87, 33.85, 35.28^\circ$, and 38.52° are assigned to (012), (110), (111), (200), (202), (113), and (311) crystal planes of orthorhombic phase CuO NPs, and they match with JCPDS No. 77-1898 [39]. The XRD pattern showed broad diffraction peaks, confirming the nanoscale formation of copper oxide nanoparticles with low crystallinity as well as the presence of residual phytochemicals from the pistachio seed coat extract. Lattice strain and biomolecule capping may cause peak broadening in the diffraction pattern, but the successful synthesis of relatively pure CuO nanoparticles is indicated by the lack of impurity peaks.

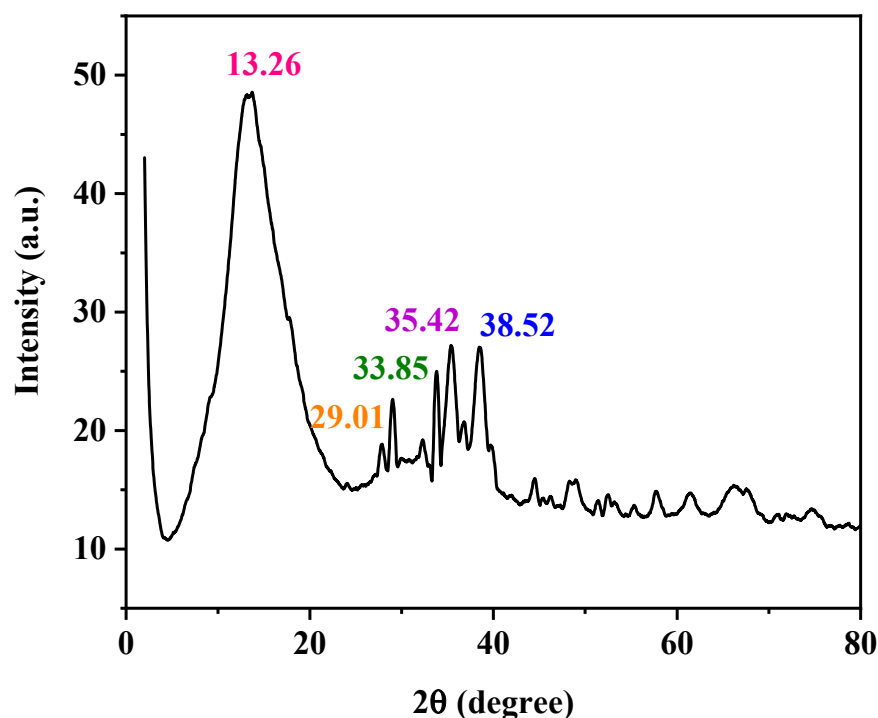


Figure 4. X-ray diffraction pattern of CuO NPs.

3.4. SEM and EDX Analysis

The morphology of the biosynthesised CuO nanoparticles revealed a well-dispersed morphology with particles evenly distributed across the surface, as shown in Figure 5a. The SEM micrograph exhibited the cluster form of CuO NPs due to agglomeration. EDX determines the elemental composition of CuO NPs, which show strong Cu and O peaks, indicating the formation of CuO NPs. The observed oxygen and copper values in CuO NPs are 59.8% and 40.2%, respectively, as shown in Figure 5b [40]. The EDX result reveals a low amount of copper and a high amount of oxygen.

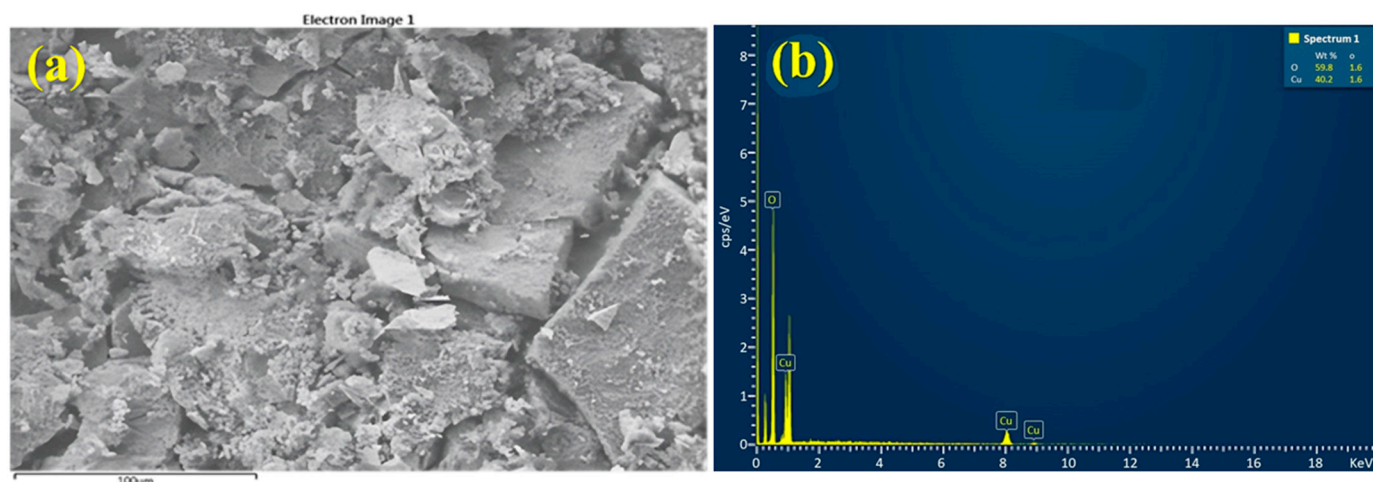


Figure 5. (a) FESEM of CuO NPs; (b) EDS of CuO NPs.

3.5. TEM and SAED Analysis

TEM is an important characterization technique used to obtain direct information about particle size distribution, average particle size, and nanoscale dimensions. The TEM analysis (Figure 6a) revealed that CuO NPs have a quasi-spherical morphology and slight agglomeration, with an average particle size of 34.42 nm, as shown in Figure 6a. The agglomeration is associated with the interparticle attraction and the presence of a phytochemical capping agent from pistachio seed coat extract. The crystallographic morphology of the synthesized nanoparticles was examined using HR-TEM, while the selected area electron diffraction (SAED) pattern was also recorded; see Figure 6b. The HR-TEM images showed the presence of mildly agglomerated cluster-shaped nanoparticles [41]. The SAED pattern shown in Figure 6b exhibits a diffuse bright halo centre with weak discrete diffraction spots distributed in concentric rings, indicating the presence of nanocrystalline CuO with an amorphous nature, and the incomplete rings suggest poor crystallinity [39,42]. These results also resemble the XRD.

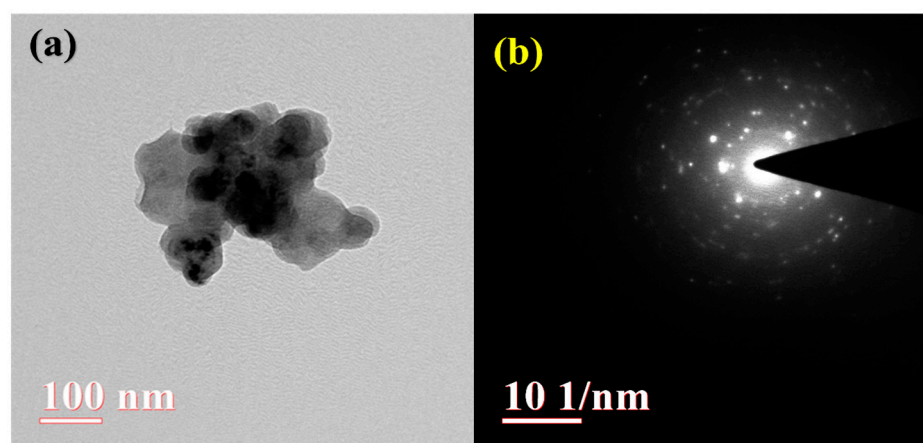


Figure 6. (a) HRTEM image of CuO NPs and (b) SAED pattern of CuO NPs.

4. Antimicrobial Activity

The antimicrobial activity of green-synthesized CuO NPs was assessed by using the agar well diffusion technique against the fungal strains *A. niger* and *P. chrysogenum*, as well as bacterial strains *S. aureus* and *E. coli*. Furthermore, the AI was used to compare the nanoparticles' effectiveness with that of conventional antibacterial treatments. The synthesized CuO NPs exhibited concentration-dependent antimicrobial activity against both

bacterial and fungal strains, as shown in Table 2. In the antibacterial study, *E. coli* showed IZ of 9, 11, 12, and 14 mm at concentrations of 25, 50, 75, and 100 mg/mL, respectively, while the standard antibiotic erythromycin produced inhibition zones ranging from 24 to 32 mm.

Table 2. Inhibition zone diameters (IZ) and Activity Index (AI) of CuO nanoparticles against selected bacterial and fungal strains.

S. No.	Strains	Standard Drug (mg/mL)	Conc. (mg/mL)	Inhibition Zone Diameter (IZ) (mm)	Standard IZ (mm)	Activity Index (AI)
1.	<i>E. coli</i> (Bacteria)	Erythromycin	25	9	24	0.37
			50	11	30	0.36
			75	12	31	0.38
			100	14	32	0.43
2.	<i>S. aureus</i> (Bacteria)	Erythromycin	25	12	31	0.38
			50	14	35	0.4
			75	16	36	0.44
			100	18	38	0.47
3.	<i>A. niger</i> (Fungi)	Ketoconazole	25	9	8	1.12
			50	10	18	0.55
			75	11	21	0.52
			100	12	22	0.54
4.	<i>P. chrysogenum</i> (Fungi)	Ketoconazole	25	8	20	0.4
			50	10	21	0.47
			75	11	23	0.47
			100	14	24	0.58

The related Activity Index (AI) values showed moderate antibacterial effectiveness against the Gram-negative bacteria, ranging from 0.36 to 0.43. *Staphylococcus aureus*, on the other hand, had a higher susceptibility to CuO nanoparticles, with inhibitory zones growing from 12 mm at 25 mg/mL to 18 mm at 100 mg/mL. Inhibition zones of 31–38 mm were observed with the usual medication, and AI values varied from 0.38 to 0.47.

The comparatively higher activity against *S. aureus* suggests enhanced interaction of CuO nanoparticles with Gram-positive bacterial cell walls. The antifungal analysis also revealed dose-dependent inhibition by CuO nanoparticles. Against *Aspergillus niger*, inhibition zones increased from 9 mm to 12 mm as the concentration increased from 25 to 100 mg/mL, whereas ketoconazole produced zones of inhibition between 8 and 22 mm, as shown in Figure 7.

The AI values ranged from 0.52 to 1.12, indicating measurable antifungal effectiveness. Similarly, *Penicillium chrysogenum* exhibited IZ of 8, 10, 11, and 14 mm at increasing concentrations, while the standard antifungal drug showed inhibition zones ranging from 20 to 24 mm. The corresponding AI values ranged from 0.40 to 0.58, indicating a gradual increase in fungal growth inhibition with increasing nanoparticle concentration, as shown in Figure 8. Overall, the findings show that green-synthesized CuO nanoparticles have broad-spectrum antimicrobial action, with moderate but steady antifungal activity and enhanced antibacterial activity against Gram-positive bacteria [5].

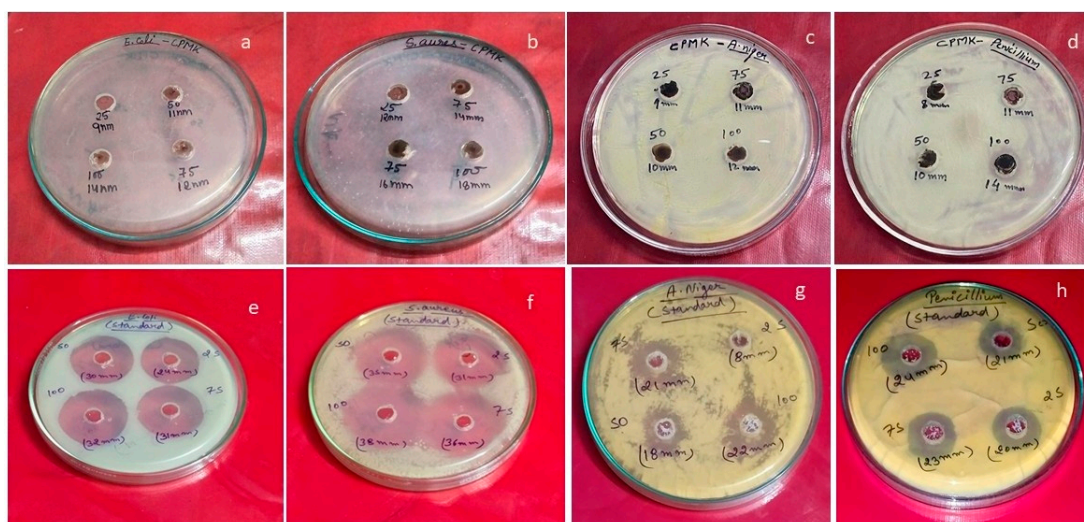


Figure 7. The green-synthesized CuO NPs against selected bacterial and fungal pathogens. Panel (a) illustrates the inhibition zones against *Escherichia coli*, while panel (b) shows activity against *Staphylococcus aureus*. Panel (c) represents antifungal efficacy against *Aspergillus niger*, and (d) represents antifungal efficacy against *Penicillium* at varying concentrations of 25, 50, 75, and 100 mg/mL of the nanocomposites. Panels (e–h) depict the standard antibiotics used as positive controls, with erythromycin applied against *E. coli* and *S. aureus*, and ketoconazole against *A. niger* and *Penicillium*.

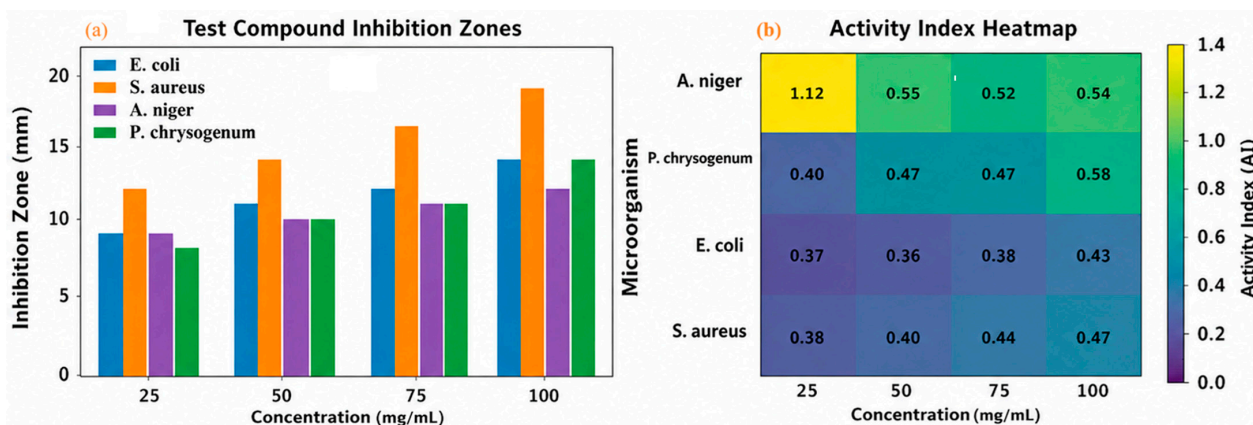


Figure 8. Quantitative evaluation of the CuO nanoparticle's antibacterial activity. (a) Comparison of microorganisms' inhibitory zones. (b) The improved antifungal reaction against *A. niger* is highlighted by a heat map of AI values as a function of concentration.

The produced CuO NPs had significant antibacterial activity against species of bacteria and fungi, and their inhibitory activity increased with concentration. Differences in microbial cell wall susceptibility were shown by the nanoparticles' much higher efficacy against Gram-positive bacteria compared to Gram-negative bacteria. Overall, the findings support CuO nanoparticles' broad-spectrum antimicrobial capability with reliable antifungal and antibacterial activities.

Mechanism of Antimicrobial Activity

The mechanism for the antimicrobial activity of pistachio seed coat-mediated CuO NPs may involve multiple synergistic mechanisms, including Membrane damage and ROS generation, as shown in Figure 9. The nanoparticles are associated with their strong interaction with microbial cells and can damage the cell membrane and promote the formation of reactive oxygen species, causing oxidative stress inside the microorganisms. In addition, the released copper ions may disturb important cellular functions by affecting proteins, en-

zymes, and genetic material, leading to microbial cell death. The small particle size and high surface area of the nanoparticles enhance their interaction with the microbial surface. Moreover, bioactive compounds present in the pistachio seed coat extract may further support the overall antimicrobial effect.

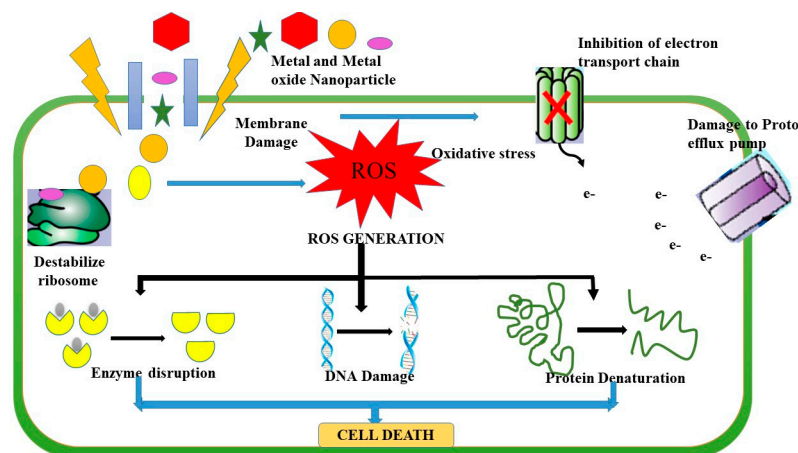


Figure 9. Proposed mechanism of antimicrobial interactions of nanoparticles.

5. Antioxidant Activity

The sample's antioxidant activity during the DPPH assay is shown by the noticeable colour change in the test tubes. Because stable DPPH free radicals are present, the control solution maintains its dark violet colour. The purple colour intensity steadily decreases as the quantity of CuO nanoparticles rises, suggesting that the nanoparticles successfully interact with free radicals and reduce them by donating electrons or hydrogen. The colour of the solution lightens when DPPH radicals transform into their stable nonradical state. As seen in Table 3 and Figure 10, the decrease in purple intensity thus demonstrates the synthesized CuO nanoparticles' capacity to scavenge free radicals; more colour loss indicates stronger antioxidant activity [8].

Table 3. The scavenging capacity of CuO nanoparticles for 1-Diphenyl-2-picrylhydrazyl (DPPH) radicals.

S. No.	Concentration	% Scavenging
1.	20 µg/mL	15.82 ± 0.15
2.	40 µg/mL	20.7 ± 0.12
3.	60 µg/mL	24.84 ± 0.14
4.	80 µg/mL	28.18 ± 0.25
5.	100 µg/mL	33.83 ± 0.17
6.	200 µg/mL	34.68 ± 0.21
7.	IC ₅₀	319.55 ± 3.21

The sample's capacity to neutralize reactive species in a dose-dependent manner was demonstrated by the antioxidant assay, which showed progressive free radical scavenging activity with increasing concentration. The scavenging percentage showed better antioxidant efficacy at increasing dosages, rising progressively from 15.82 ± 0.15% at 20 µg/mL to 34.68 ± 0.21% at 200 µg/mL. The sample exhibited comparatively modest scavenging activity (15.82 ± 0.15%) at the starting concentration of 20 µg/mL, which progressively increased to 20.7 ± 0.12% at 40 µg/mL. Antioxidant potential was further enhanced at 60 and 80 µg/mL, with scavenging activities of 24.84 ± 0.14% and 28.18 ± 0.25%, respectively. The antioxidant capacity improved with increasing concentration, as evidenced by the maxi-

imum radical-scavenging action at 200 $\mu\text{g/mL}$ ($34.68 \pm 0.21\%$). The sample's IC_{50} value was $319.55 \pm 3.21 \mu\text{g/mL}$, which represents the concentration needed to inhibit free radicals by 50%.

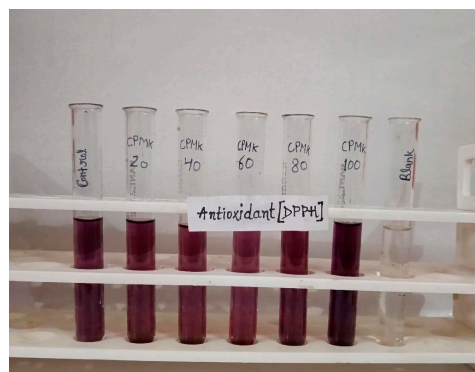


Figure 10. DPPH radical scavenging activity of CuO nanoparticles.

The curve's steadily rising trend shows that as the concentration of NPs increased, antioxidant activity steadily increased. Because there were fewer active sites to interact with DPPH radicals at lower concentrations, the scavenging efficiency remained modest. At the maximum concentration measured, maximum activity is attained. An essential metric for assessing antioxidant strength is the IC_{50} value, which shows the concentration needed to block 50% of DPPH radicals. CuO NPs act by donating electrons to neutralize free radicals, which contributes to antioxidant protection against oxidative stress, as shown in Figure 11. The findings suggest their possible application in pharmaceutical, biomedical, and food fields for combating oxidative stress and related disorders.

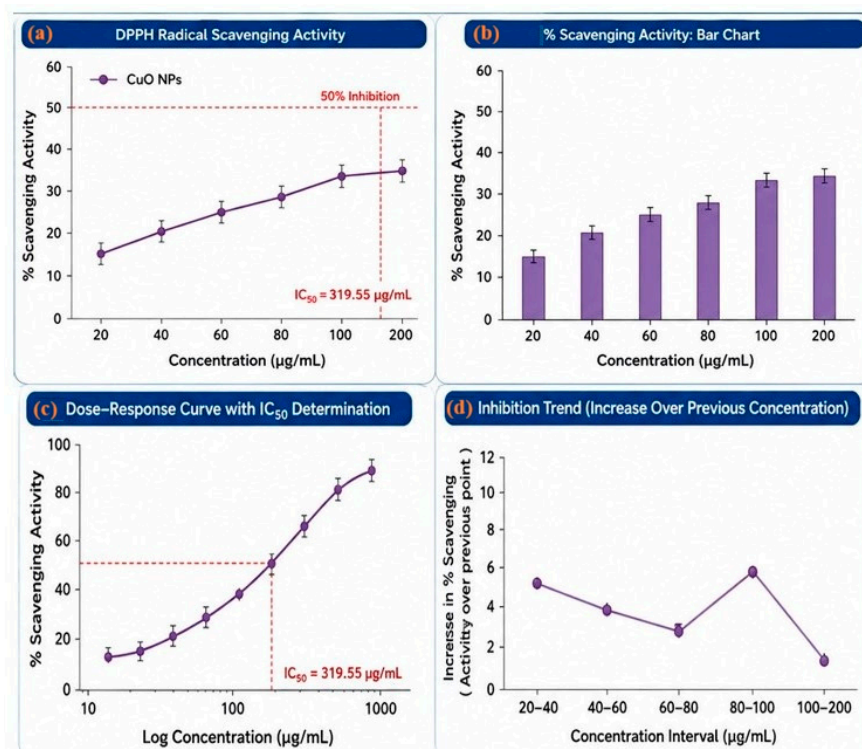
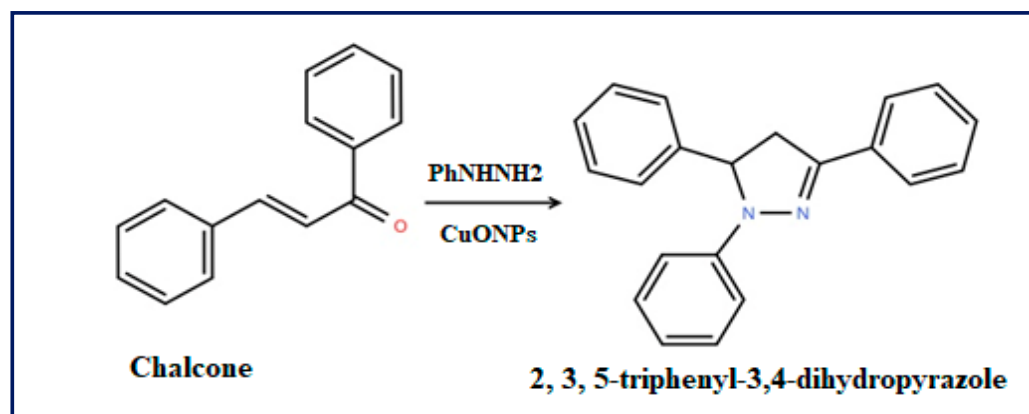


Figure 11. (a) Shows how CuO nanoparticles' DPPH scavenging activity increases with concentration. (b) A comparison of the % scavenging activity at various CuO nanoparticle concentrations. (c) Describes the relationship between dose and response as well as the calculation of CuO nanoparticles' IC_{50} value. (d) Shows the trend of inhibition between CuO nanoparticle concentration intervals.

6. CuO NPs as a Catalyst for the Synthesis of 2,3,5-Triphenyl-3,4-dihydropyrazole

The conversion of a chalcone to a pyrazoline derivative using phenyl hydrazine, with CuO NPs as a catalyst, is shown in Scheme 1. The conversion of a chalcone to a pyrazoline derivative using phenyl hydrazine, with CuO NPs as a catalyst, is shown in Scheme 1, yielding 2,3,5-triphenyl-3,4-dihydropyrazole (215 mg; 75%), which has a melting point of 136–137 °C (literature value: 139–141 °C) [43]. The FTIR and ^1H NMR confirm the formation of 2,3,5-triphenyl-3,4-dihydropyrazole in the presence of CuO NPs.



Scheme 1. Synthesis of 2,3,5-triphenyl-3,4-dihydropyrazole.

The FTIR spectrum of 2,3,5-triphenyl-3,4-dihydropyrazole occurs in the range 400–4000 cm^{-1} , as shown in Figure 12. In the spectrum, two main peaks, 747 and 685 cm^{-1} , are due to aromatic C-H out-of-plane bending vibration, attributed to the presence of a substituted benzene ring. The characteristic absorption in the region of $\sim 3100\text{--}3000\text{ cm}^{-1}$ corresponds to aromatic C-H stretching vibrations, confirming the presence of phenyl substituents in the molecule. In addition, the bands observed at 1323 cm^{-1} are due to N-N stretching vibration, and the C-N stretching vibration is observed at 1395 cm^{-1} . The peak observed at 1590 cm^{-1} is due to C=N stretching vibration, along with the C=C vibration peak at 1489 cm^{-1} , which confirms the cyclization and formation of the pyrazoline ring structure. A weak band region at 2828 cm^{-1} corresponds to the aliphatic C-H stretching vibration of the dihydropyrazole ring. There is no specific peak observed in the range 1650–1750 cm^{-1} of the carbonyl and aldehyde groups, indicating the complete transformation to the product [29]. The FTIR of Chalcone displayed typical bands at 1708–1712 cm^{-1} for the conjugated C=O and 1612–1622 cm^{-1} for the olefinic C=C vibration. In the spectra of the cyclized compound with phenyl hydrazine, the carbonyl vibration was absent, indicating the transformation into the product. In addition, the band of aromatic C-H vibration (3100–3000 cm^{-1}) did not change with the appearance of characteristic peaks C=N, C-N, and N-N; the vibration provides strong evidence for the complete conversion of chalcone into the corresponding 2,3,5-triphenyl-3,4-dihydropyrazoline derivative [44].

^1H -NMR (400 MHz, CDCl_3): δ 7.72 (d, J = 7.0 Hz, 2H), 7.38 (t, J = 7.3 Hz, 2H), 7.33 (d, J = 3.8 Hz, 5H), 7.25 (s, 3H), 7.18 (m, 2H), 7.18 (m, 2H), 7.07 (d, J = 7.7 Hz, 1H), 6.78 (t, J = 7.2 Hz, 1H), 5.27 (dd, J = 12.4, 7.3 Hz, 1H), 3.85 (dd, J = 17.1, 12.4 Hz, 1H), 3.14 (dd, J = 17.1, 7.3 Hz, 1H) (Figure 13).

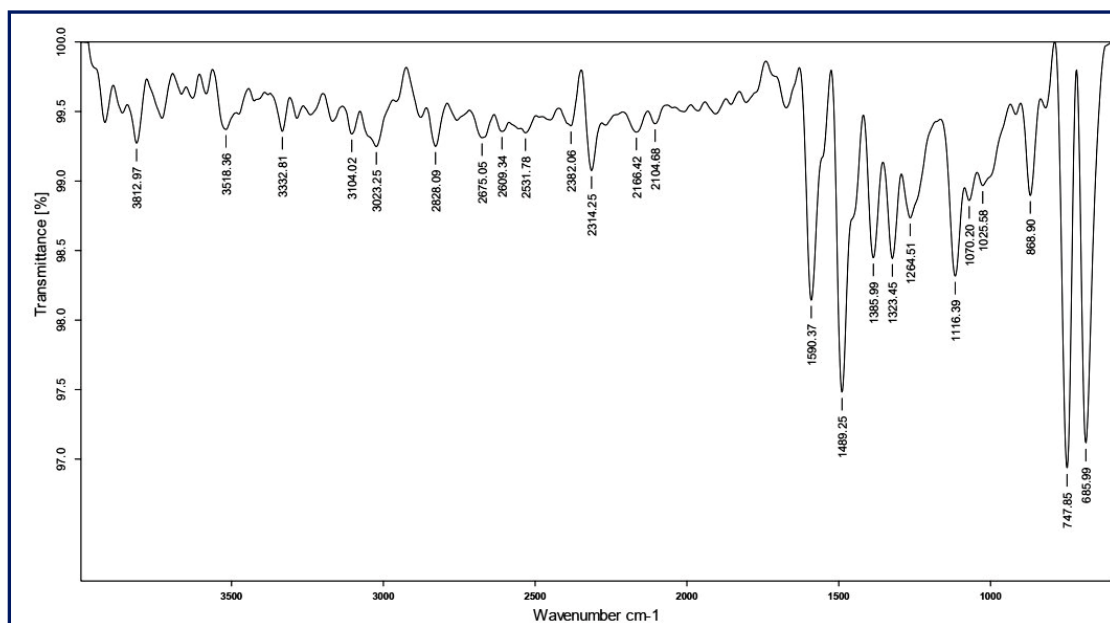


Figure 12. FTIR of 2,3,5-triphenyl-3,4-dihydropyrazole.

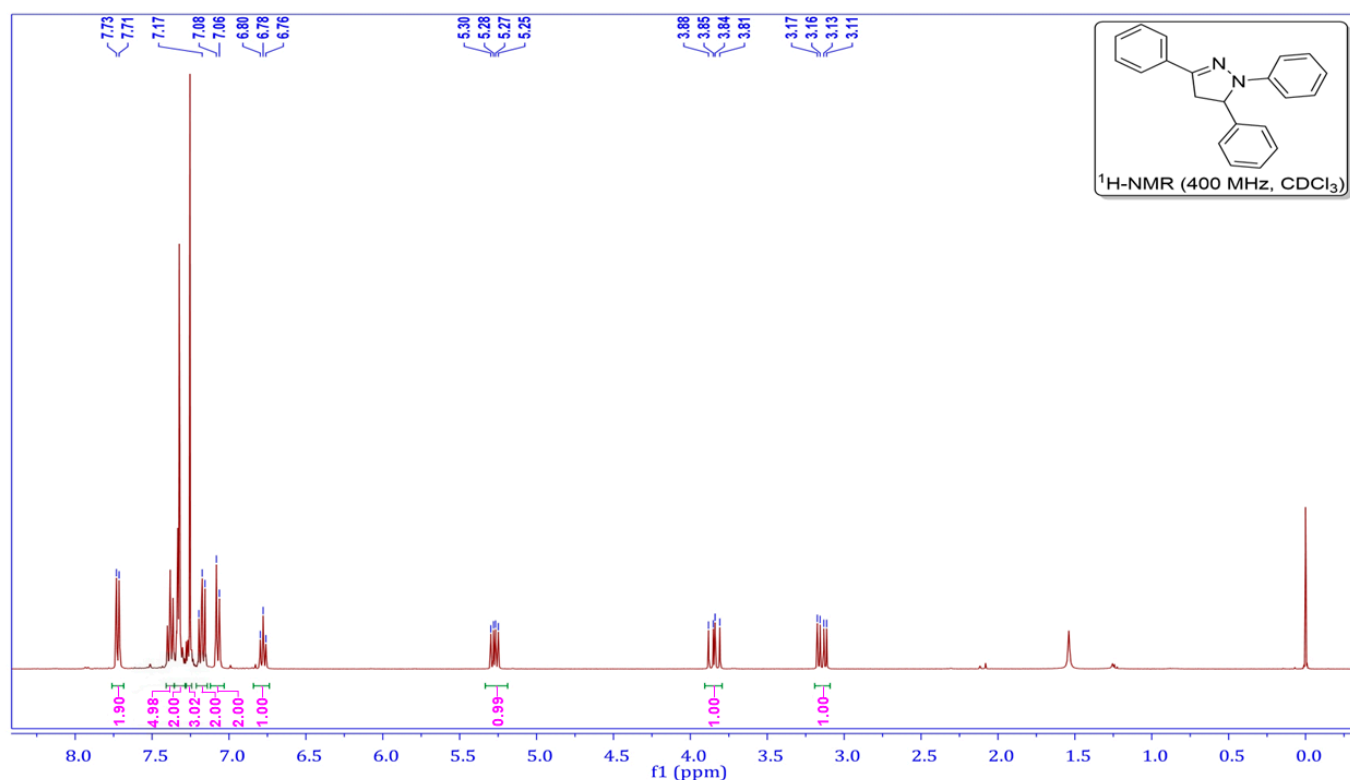


Figure 13. ¹H-NMR of 2,3,5-triphenyl-3,4-dihydropyrazole.

7. Conclusions

The current study presented an innovative, cost-effective, and eco-friendly synthesis approach for the design of CuO NPs using an extract from the pistachio seed coat. The Synthesized CuO NPs exhibit a characteristic UV–visible absorption peak at 273 nm. FTIR analysis revealed prominent absorption at 548 nm due to Cu–O stretching, confirming the formation of CuO NPs. The average size of CuO NPs is 22–23 nm from the XRD pattern, and SEM analysis shows the cluster form of CuO NPs due to agglomeration. EDAX confirms the elemental composition with strong signals of copper and oxygen, showing

Cu (40.2%) and O (59.8%), confirming the purity of synthesized CuO NPs. The synthesized nanoparticles exhibited promising antimicrobial, antioxidant, and catalytic properties. Antimicrobial activity evaluated by the agar well diffusion method showed inhibition zones ranging from 8 to 18 mm, with stronger activity against fungal strains than bacterial strains. In the antioxidant study using the DPPH assay, free radical scavenging activity increased from $15.82 \pm 0.15\%$ to $34.68 \pm 0.21\%$ with increasing concentration, and the IC_{50} value was $319.55 \pm 3.21 \mu\text{g/mL}$, indicating moderate antioxidant potential. Free radical scavenging activity increases from $15.82 \pm 0.15\%$ to $34.68 \pm 0.21\%$ with increasing concentration in the antioxidant research utilizing the DPPH assay, and the IC_{50} value was $319.55 \pm 3.21 \mu\text{g/mL}$, showing moderate antioxidant potential. Additionally, the nanoparticles acted as efficient catalysts in organic reactions, enhancing reaction rates and product yields under mild conditions. Overall, the results demonstrate the potential of the NPs for biomedical and industrial applications. The CuO NPs also served as an efficient catalyst for the synthesis of 2,3,5-triphenyl-3,4-dihydropyrazole, enhancing reaction efficiency under mild conditions. The peak in FTIR at 1489 cm^{-1} and $^1\text{HNMR}$ Value at 5.25–5.30 δ indicate the pyrazoline ring formation and cyclization. Owing to their eco-friendly synthesis approach and significant biological and catalytic performance, these CuO NPs may serve as promising materials for future applications in the pharmaceutical, biomedical, environmental, and industrial sectors.

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