

Article

Antibacterial, Antioxidant and Physicochemical Properties of *Pipper nigrum* Aided Copper Oxide Nanoparticles

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Abstract: The *Pipper nigrum* (*P. nigrum*) leaf extract was used for the biosynthesis of copper oxide nanoparticles (CuO NPs) and the successful formation of the resultant product was confirmed through several physicochemical techniques. The chemical structure and the elemental composition were analysed through Fourier transform infrared (FTIR) and energy dispersive X-ray (EDX) spectroscopies, respectively. The crystalline structure and crystallite size were investigated through an X-ray diffractometer (XRD) and a monoclinic crystallite with a size of 40.68 nm was reported. Even-distributed particles with an average particle size of 49.75 nm were seen in the scanning electron micrograph (SEM), whereas the thermal stability was checked during the thermogravimetric analysis (TGA). The ultra-violet and visible (UV-Visible) spectroscopy was operated to study the light absorbance phenomena and to determine the band gap energy from the absorption edge, which was found to be 1.47 eV. The CuO NPs were used as antibacterial agents against gram-negative bacteria (GNB) and gram-positive bacteria (GPB), and greater inhibition zones were seen against the former one. The antioxidant test was also carried out against 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radicals and the antioxidant potential of CuO NPs was found to be higher than ascorbic acid.

Keywords: green synthesis; copper oxide; antioxidant; bacteria; microscopy



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1. Introduction

Microbial resistance against regularly used drugs is a worldwide problem arising due to the excessive and inappropriate utilization of antibiotics, which emphasises the formulation of new antimicrobial agents to counter this issue [1]. In this regard, metal oxide nanoparticles attract more attention due to their enhanced antimicrobial activity against resistant strains. Apart from microbial resistance, several free radicals were identified in many diseases, including cancer and cardiovascular disorder, which destroy the target molecule by extracting an electron to get stable [2]. By scavenging free radicals, the harmful effects of free radical species, such as cancer, heart disease, and neurological disorders can be minimized [3]. Thus, an antioxidant is required to neutralize these free radicals and control their destruction. The metal/metal oxide nanoparticles have shown high antioxidant activity as compared to other antioxidants used previously [4].

Many attempts have been made in the recent years to develop a biological potent material that has the capability to encounter both drug resistant bacteria and free radicals.

The ZnO NPs used in 3–10 mM concentration can inhibit 100% bacterial growth. It is also reported that nanoparticles of CaO and MgO are used to kill different bacterial species, such as *Escherichia coli*, *Bacillus cereus*, and *Bacillus globigii* [5]. Silver NPs have now been used as commercial mediums for antibiotic delivery. It is used to treat many bacterial infections as it has the ability to overcome bacterial resistance against antibiotics [6]. Recently, it has been stated that NPs prepared from plant extract as Ag NPs improved the antioxidant activity of the specific compound in the extract. It is reported that Ag NPs prepared from the leaf extract of some plant shows higher antioxidant and antimicrobial activity [7]. Ag NPs have been extensively used in food packaging, preservation, cosmetics, and medicine due to their antifungal, antibacterial, and antitumor activities. Fe NPs can be used as an antibacterial against different digestive problems. Pd NPs prepared by using the *Filicium decipiens* plant are used for antibacterial efficacy [8]. Au NPs show a great role in the treatment of hyperglycaemia, which leads to oxidative stress that has not been already revealed and acts as a good antioxidative agent [9].

CuO has attained as much importance as antibacterial, fungicides, purifiers, algacides, and antifouling agents [10,11]. CuO NPs have been favored over other NPs in recent decades due to their simplicity and ability to display a variety of potentially valuable physical characteristics, which are highly dependent on their form, size, and composition. They might be used to offer antimicrobial characteristics in a variety of materials, including dye-sensitized solar cells (DSSCs), filters, paints, polymers, and textiles [12,13]. CuO NPs have gotten a lot of interest in the medical profession because they have a minimal risk of microbial resistance. Due to their excellent biocidal characteristics, copper and copper-based compounds are now widely employed in pesticide formulations, and various health-related uses are being studied and/or applied [14]. CuO NPs are very attractive because of their latent applications in many fields, such as antimicrobial, antioxidants, heterogeneous catalysts, imaging agents, and drug delivery agents in the field of biomedicine [15–17]. The CuO NPs were synthesized via an electrochemical method, precipitation-pyrolysis, thermal decomposition of precursor, sonochemical, microwave irradiations, sol-gel technique, and self-catalytic mechanism [18]. However, these methods are complicated, expensive, and have adverse effects on the environment by using an expensive reagent, toxic organic solvents, adverse reaction conditions, and longer reaction completion time [19]. Biogenic synthesis is an alternative to the chemical and physical methods due to the use of low-cost materials, and eco and user-friendly nature. In the past, *Moringa oleifera*, *Phyllanthus amarus*, *Tamarindus indica*, *Hibiscus rosasinensis*, *Azadirachta indica*, *Centella asiatica*, and *Murraya koenigii* plants were used for the green synthesis of CuO NPs [3,19–24]. However, the CuO NPs synthesized by using these plants are either limited only to physico-chemical studies or focus only on antibacterial activity or photocatalytic activity. None of the above-cited articles report the antioxidant activity against ABTS free radicals. Moreover, no literature was found on the synthesis of CuO NPs using *P. nigrum* leaves extract. Thus, this was planned to explore the dynamic multifunctional nature of CuO NPs by performing both antibacterial activity (against both GPB and GNB) and antioxidant activity against ABTS free radicals.

The selection of *P. nigrum* as a green source for the synthesis of CuO NPs was purely based on its easy availability and the nutritional, medicinal, and phytochemical composition based on the literature. Here we report the green synthesis of CuO NPs with *P. nigrum* leaf extract for the first time, and physicochemical properties were studied through XRD, SEM, EDX, TGA, UV-Visible, and FTIR spectroscopy. The antibacterial and antioxidant efficacy of the synthesized CuO NPs was evaluated.

2. Materials and Methods

2.1. Chemical Used

The extremely pure chemicals, including copper (II) acetate monohydrate (98%), sodium hydroxide (99%), dimethylsulphoxide (99.9%), nutrient agar, ABTs, potassium persulphate (98.5%), and ascorbic acid were purchased from Sigma-Aldrich (St. Louis,

MO, USA) and were used without any further purification. Deionized water was used throughout the research work.

2.2. Preparation of Extract

The selection of *P. nigrum* leaves was made on the basis of the local potential medicinal uses of *P. nigrum*. The *P. nigrum* leaves were washed with deionized water thrice and were then dried by placing them in the shade for one day. The leaf extract was prepared by following standard protocol. 40 g of the leaves were transferred into a 1000 mL beaker containing 600 mL deionized water and heated at 50 °C for 7 h. The crude extract obtained was chilled at room temperature and filtered through Whatmann filter paper 42. The clear extract was transferred into an airtight bottle and stored in the refrigerator for further use.

2.3. Synthesis of CuO NPs

For the preparation of CuO NPs, copper acetate monohydrate salt was used. 1% (*w/v*) of CuO NPs were prepared by dissolving 1 g of copper acetate monohydrate $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ in 100 mL deionized water and stirred for about 20 min and 20 mL *P. nigrum* leaves extract with constant heating and stirring. The pH of the reaction mixture was adjusted at 10 by the dropwise addition of NaOH solution (0.2 M). The precipitate formed after 30 min was aged for 12 h, and the upper water layer was removed via pipette and washed the wet precipitates three times with deionized water and dried in an oven at 150 °C. This process was repeated several times to obtain a sufficient amount of CuO NPs and stored in a polyethylene bottle.

2.4. Characterization

The Panalytical X-pert pro (manufactured by CAE, Montreal, QC, Canada) was run between 20° to 80°, to study the crystalline phase, where the crystallite size was calculated the Debye-Scherrer equation (Equation (1) where *K* is Scherrer constant, *D* is particle size in nm, β is full length at half maxima and λ is the wavelength of X-ray). The surface structure was examined through SEM model 5910 (Tokyo, Japan) whereas the EDX model INCA 200 (Abingdon, UK) fixed SEM was used for elemental analysis. The thermogram was recorded by Perkin Elmer Model 6300 TGA analyzer (Hillsborough, NJ, USA) by heating a known amount of CuO NPs was heated up to 900 °C with a 10 °C rise per min. The light absorption phenomena were observed in the UV-Visible spectrophotometer model Shimadzu (UV-800) in the range 200 to 800 nm provided by Thermo Fisher Scientific Waltham, MA, USA. The KBr pellet containing CuO NPs was examined using an FTIR model Nicolet 6700 (Waltham, MA, USA) in the 4000 to 400 cm^{-1} range to determine the chemical composition.

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

2.5. Antibacterial Assay

The antibacterial test was performed against both types of bacterial species by using the Agar well diffusion method. The overnight bacterial culture growth on agar nutrient media was dissolved in newly produced agar medium, then placed into sterile Petri plates, and allowed to solidify. The micropipette tip was used to bored 5 mm wells in agar media, and this procedure was carried out at room temperature in laminar flow. The CuO NPs stock suspension (5 mg/50 mL) was prepared by irradiation under ultrasonic waves. The Petri plates were incubated at 37 °C for 24 h after the wells were loaded with 5, 10, 20, 50, and 100 μL , and the diameter of the inhibition was measured as the activity of CuO NPs [25].

2.6. Antioxidant Assay

The antioxidant test was carried out via the reported procedure with slight modifications. This activity is used to specify the ABTS^{•+} radical cation scavenging activity by

P. nigrum CuO NPs. The $\text{ABTS}^{\bullet+}$ was prepared by mixing ABTS (7 mM) and potassium persulphate (2.5 mM) and incubating it for 16 h at room temperature in dark. The $\text{ABTS}^{\bullet+}$ solution was diluted with distilled water, and the absorbance maxima were observed at 405 nm when analyzed via a UV-Visible spectrophotometer. Then different concentrations of CuO NPs (20, 40, 60, 80, 100 $\mu\text{g/mL}$) were added to $\text{ABTS}^{\bullet+}$ solution, and the mixture was then incubated for 10 min at 37 °C, and their absorbance was measured at 405 nm by using a UV-Visible spectrophotometer. The amount of radical scavenging activity (%RSA) for different concentrations was calculated by using the following Equation (2), where A_o and A_i is the absorbance of reference and the sample respectively.

$$\% \text{ RSA} = \left[\frac{A_o - A_i}{A_o} \right] \times 100 \quad (2)$$

3. Results

The leaves of *P. nigrum* have been found to contain a variety of chemical compounds, including alkaloids, flavonoids, phenolic acids, and volatile oils [26]. In terms of the formation of CuO NPs from an aqueous extract of *P. nigrum* leaves, there are likely several factors at play. Some of the compounds found in the leaves, such as phenolic acids, have been shown to have antioxidant properties and could play a role in reducing copper ions in the solution to form CuO NPs. Additionally, the presence of flavonoids could also play a role in the formation of the NPs by stabilizing the particles and inhibiting their aggregation. It is important to note that the exact mechanisms of NP formation from plant extracts are still not fully understood, and further research is needed to determine the specific factors that contribute to the formation of CuO NPs in this particular system [27].

3.1. XRD Analysis

The characteristic diffraction bands appeared in the X-ray diffractogram shown in Figure 1 at the 2-theta position with the corresponding miller indices are 32.64(110), 35.70 (002), 39.01 (200), 48.92 (−202), 53.69 (020), 58.55 (202), 61.80 (−113), 66.46 (−311), 68.19 (220), 72.59 (311) and 57.49 (222). All of these peaks are attributed to the monoclinic geometry of CuO NPs with a space group of $C2/c$ and are in good agreement with the peaks mentioned in reference card No 00-041-0254. The crystalline nature of the CuO NPs is confirmed by the narrow and strong peaks, and the average crystallite size obtained from the FWHM values is 40.68 nm, with 0.245 percent imperfection observed in the crystal.

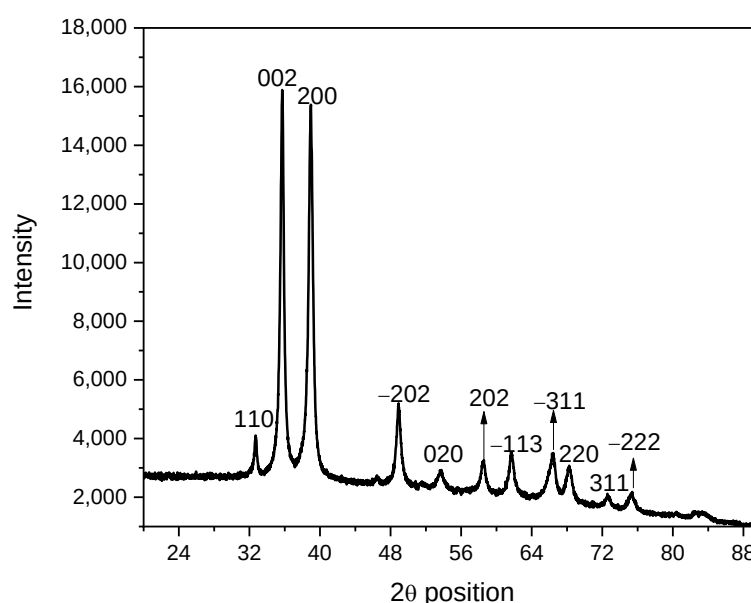


Figure 1. X-ray diffractogram of CuO NPs.

3.2. SEM Analysis

The surface morphology of CuO NPs was examined through SEM, and the obtained micrograph is given in Figure 2. The low-magnified micrograph shows a high degree of agglomeration that led to the formation larger solid structure, where small size particles are randomly distributed on the surface. Some cavities are also seen in the images, which are due to the lopsided arrangement of the particles. This shows a high precipitation rate of the synthesized particles at higher pH. Nearly similar situations are seen in the high magnification image, the complex structure is formed and the irregular arrangement of particles in several cavities with different sizes is formed. Few nearly spherical shapes and some poly headed particles are seen in the image. However, most of the individual particles are varied in size and shape. The particles size calculated through ImageJ software from the SEM image (b) are ranging from 37 nm to 54 nm with an average particle size of 49.75 nm.

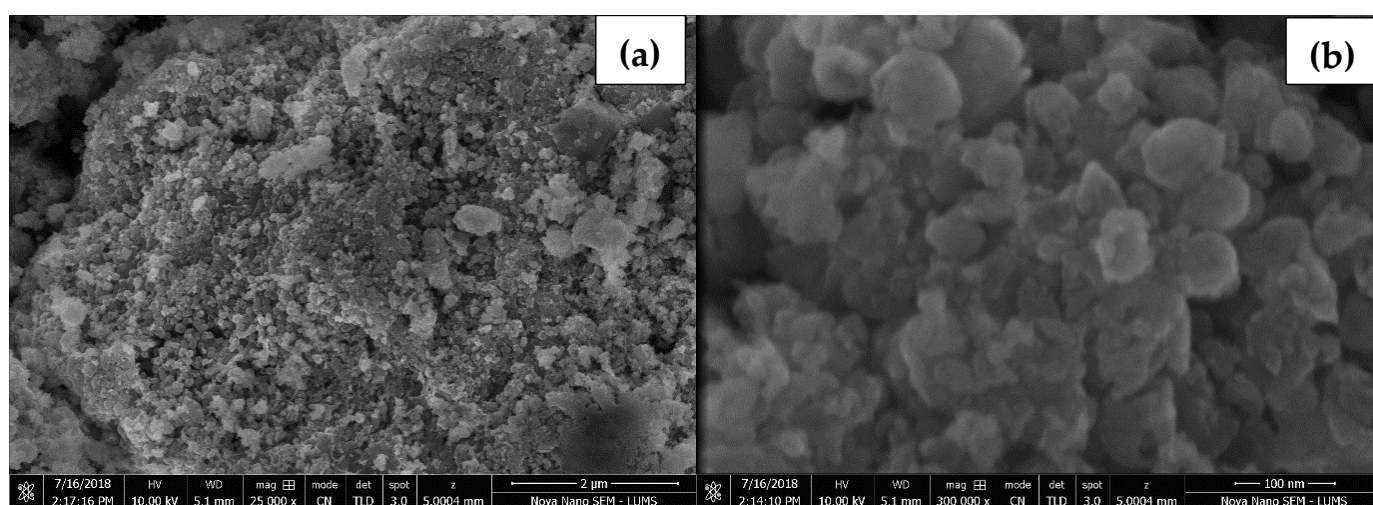


Figure 2. (a) Low and (b) high magnification SEM micrographs of CuO NPs.

3.3. EDX Analysis

The elemental analysis and percentage purity of CuO NPs were determined by EDX and the resulting spectrum is shown in Figure 3. The tiny peak at 0.25 keV is attributed to the presence of oxygen atoms in the CuO sample, whereas the other three peaks at 0.9, 8.00, and 8.90 keV are attributed to the copper. According to the EDX analysis, the weight percent along with atomic percent in parenthesis of copper and oxygen in the sample is 79.1 (48.8) and 20.9 (51.2), respectively. The two other peaks at 6.9 and 7.4 keV are not matched with any metallic impurity. It might be due to the presence of some phytochemicals deposited on the surface of the CuO sample.

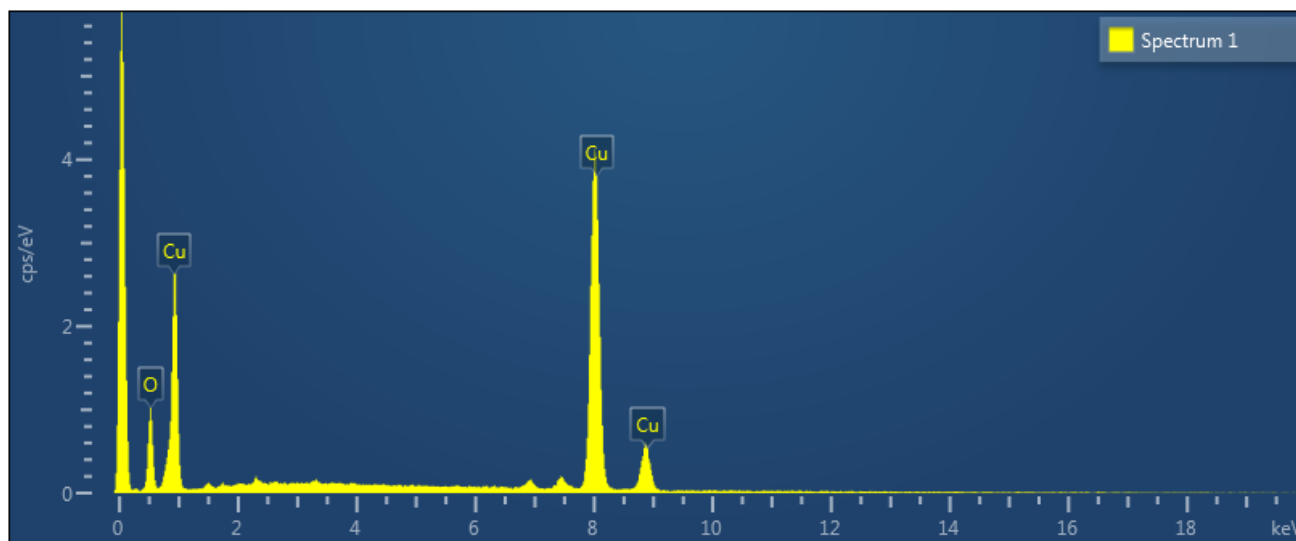


Figure 3. EDX spectrum of CuO NPs.

3.4. TGA Analysis

The TG thermogram of CuO NPs shown in Figure 4, shows 9.99% weight loss occurred in two stages. The initial gradual weight loss occurred in the 40–350 °C temperature range, which was caused by the loss of physically adsorbed water and highly volatile organic compounds. The second weight loss was seen at temperatures ranging from 360 to 750 °C, which was attributed to water evaporation associated with crystal lattice along with the breakdown of the coordinated bioorganic compounds, which is present in the sample due to the use of plant leaf extract in the synthesis of CuO NPs [28].

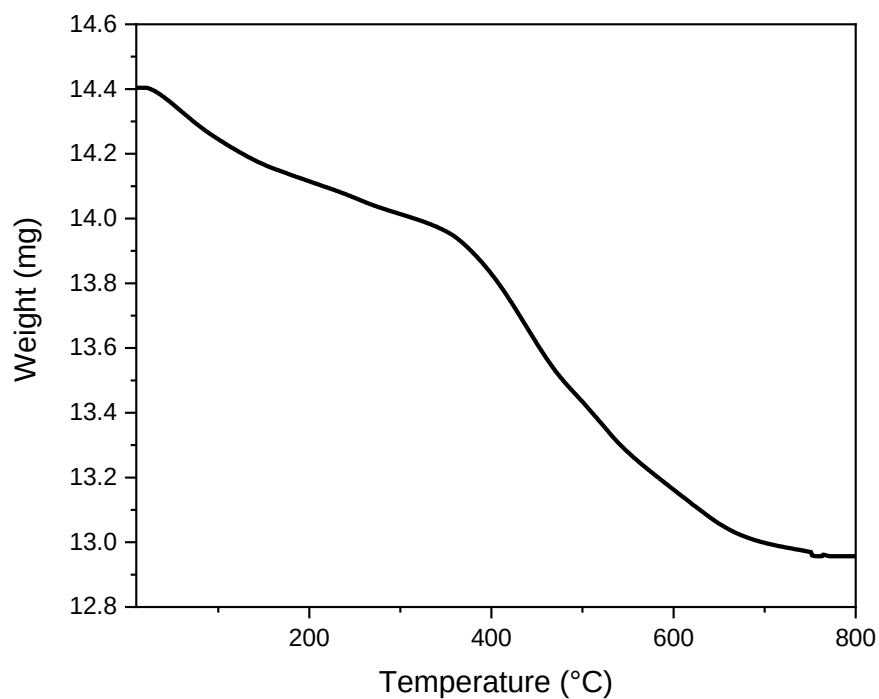


Figure 4. TG thermogram of CuO NPs.

3.5. UV-Visible Analysis

The UV-Visible spectrum exhibits a wide wave band center at 306.42 nm confirming the synthesis of CuO NPs as shown in Figure 5 and is attributed to the oscillation of the excited electrons due to the absorption of electromagnetic radiation [23]. A short absorption band at 395.71 nm and the two other broad absorption peaks centered at 448 and 652 nm might be due to the conversion of non-oxidized Cu-NPs to copper colloid [29]. As the surface Plasmon resonance (SPR) is very sensitive to the nature of particles, inter-particles distance, their size, and shape [30]. This shows that the sample absorbs both UV and Visible light and wide wave bands attributed to the wide size distribution of CuO NPs [31]. The band gap energy is the amount of energy required to excite electrons from the valance band to the conduction band. According to earlier findings, the wavelength of the absorption edge was determined by aligning the steep rising part of the UV-Vis curve with the x-axis of the UV-Visible spectrum. On the basis of the absorption edge, the band gap energy was calculated (band gap = $1240/\text{absorbance edge (nm)}$) to be 1.48 eV, which is lower than the reported band gap values (1.63 and 1.75 eV) for CuO NPs synthesized by a conventional method, suggest that plant-mediated CuO NPs have high conductivity [32].

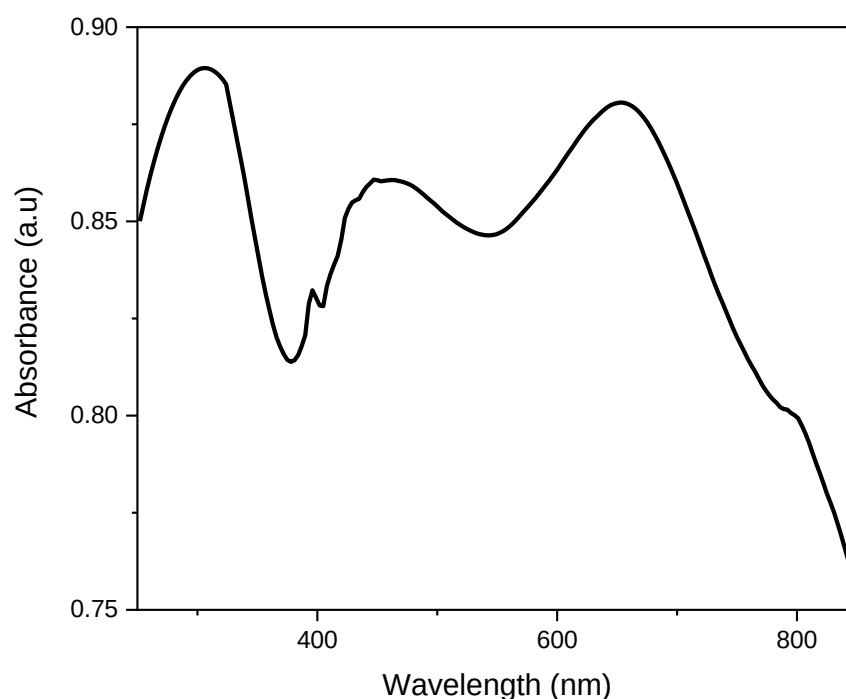


Figure 5. UV-Visible spectrum of CuO NPs.

3.6. FTIR Analysis

The broad band centered at 3440.12 cm^{-1} in the FTIR spectrum of CuO NPs (shown in Figure 6) is assigned to the stretching vibration of the O–H group [33]. The wave band at 1629.48 and 1102.24 cm^{-1} is due to bending vibrations of the hydroxyl group [34]. The peak at 1389.05 cm^{-1} is due to the organic moieties, which may be due to the use of plant material [35]. The synthesis of CuO NPs with monoclinic geometry was confirmed by the absorbance bands at 505.07 cm^{-1} [36].

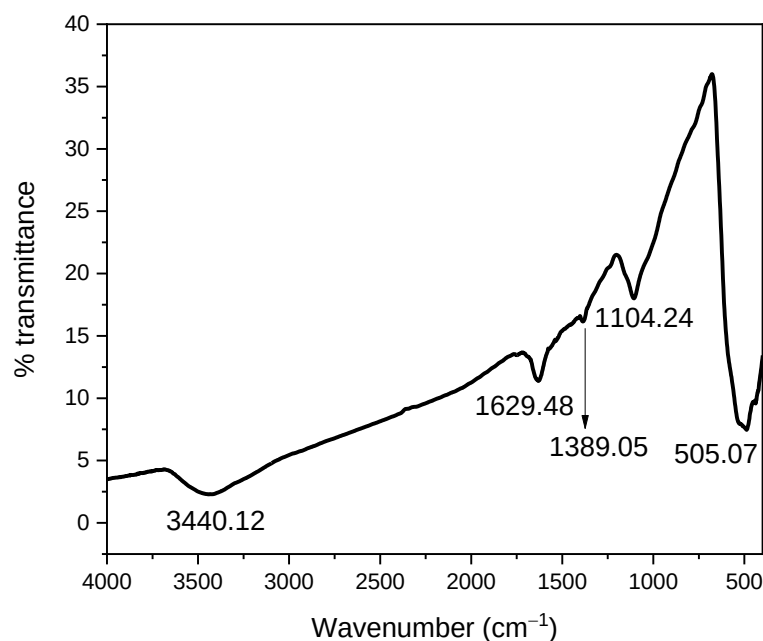


Figure 6. FTIR spectrum of CuO NPs.

3.7. Antibacterial Assay

The antibacterial potential of CuO NPs prepared by green synthesis was examined against GPB (*S. pyogens*, *S. aureus*, *S. epidermis*) and GNB (*E. coli*, *P. aeruginosa*, *K. pneumonia*, *S. marcescom*) and the obtained results are shown in Figure 7. Table 1 shows the clear zone of inhibition measured in millimeters (mm) for the activity of CuO NPs and conventional antibiotics. CuO NPs' dose-dependent activity against the chosen species was tested, and it was discovered that the concentration of CuO NPs in wells increased with increasing concentration of CuO NPs. However, the activity of CuO NPs was found to be lower than that of clindamycin phosphate, which was employed as a control. CuO NPs had substantially more efficacy against Gram-positive bacteria than Gram-negative bacteria. Among Gram-positive bacteria, *S. aureus* was shown to have the highest activity, with a zone of inhibition of 24.00 at 100 mg/mL concentration. The differential in antibacterial activity of CuO NPs against GPB and GNB is related to differences in the composition of the cell wall and the outer protective layer. The increased activity of CuO NPs against Gram-positive bacteria might be attributed to the lack of a protective layer outside the cell wall. The protective layer present in GNB provides extra strength against incoming penetrating agents [37,38]. Cu cation, superoxide radical anions, and hydroxyl radicals are produced in aqueous suspensions of CuO NPs. Cu ions are released by the CuO NPs, which bind with the thiol group of a key bacterial enzyme, resulting in the inactivation and eventual death of microorganisms [27]. The light interaction with the surface of CuO NPs causes electron excitation, which produces oxygen ions upon reaction with absorbed oxygen, resulting in the production of H₂O₂ when joining with an H₂O molecule. Thus, by entering the bacterial cell, H₂O₂ disrupts cytoplasmic processes and becomes deadly to the germs [39,40].

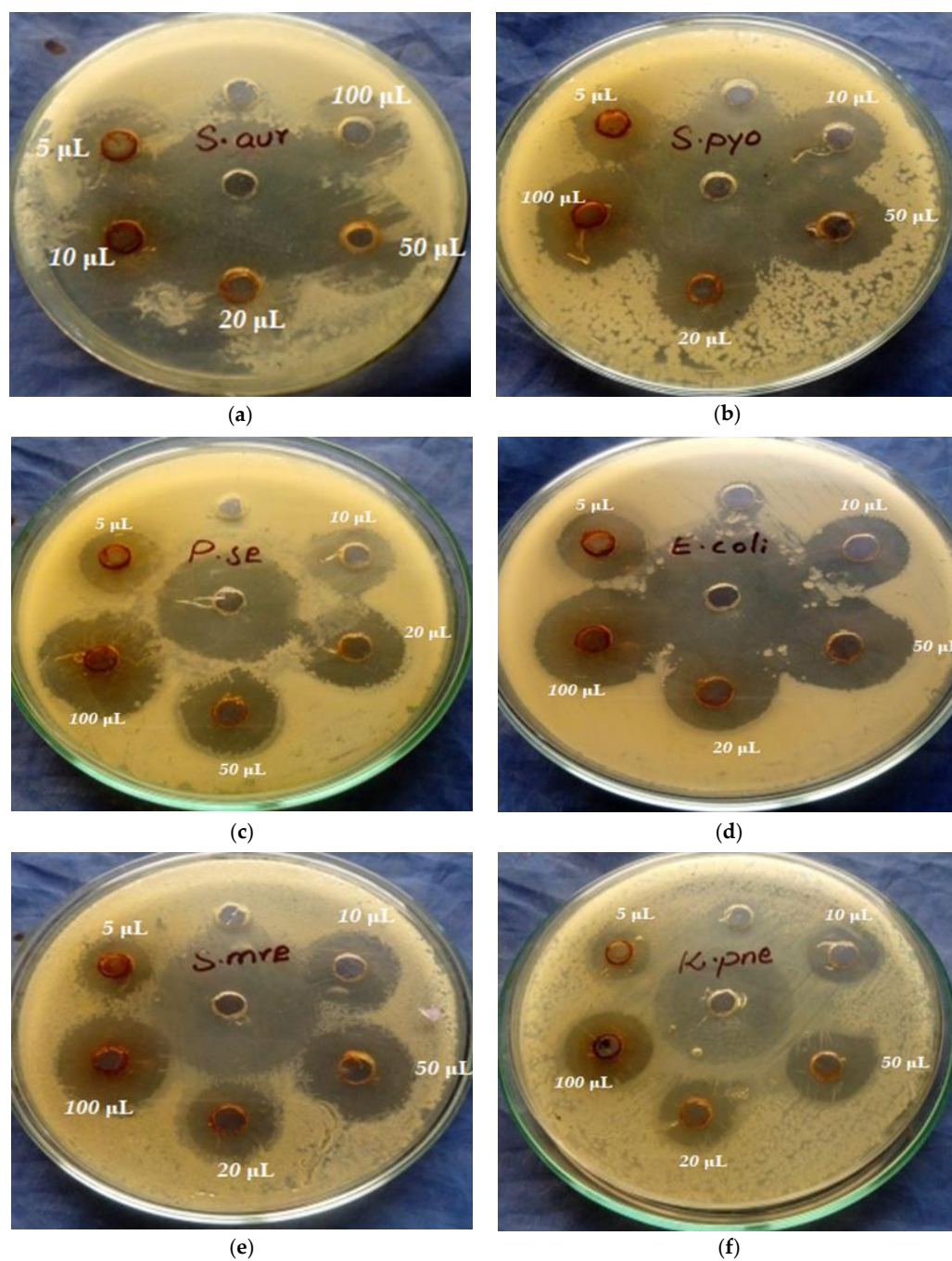


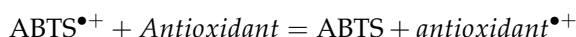
Figure 7. Pictorial representation of antibacterial activity of CuO NPs; where (a) *S. aureus*, (b) *S. pyogenes*, (c) *S. epidermidis*, (d) *E. coli*, (e) *S. marcescens* and (f) *K. pneumoniae*.

Table 1. Antibacterial activity of CuO NPs and antibiotic, the zone of inhibition was measured in millimeter (mm).

Bacteria	Antibiotics	CuO NPs Concentrations (5 mg/50 mL)				
		05	10	20	50	100
<i>S. aureus</i>	29.00 ± 01	18.6 ± 3.51	18.6 ± 1.52	20.00 ± 01	22.5 ± 2.51	24.00 ± 04
<i>S. epidermis</i>	24.3 ± 2.08	19.3 ± 2.51	19.6 ± 1.52	19.6 ± 0.57	21.6 ± 2.08	23.6 ± 1.52
<i>S. pyogens</i>	25.3 ± 5.03	17.3 ± 2.51	19 ± 2.64	20.00 ± 02	22.3 ± 1.52	21.3 ± 2.51
<i>E. coli</i>	27.6 ± 1.52	14.3 ± 4.03	18.6 ± 1.52	18.3 ± 0.57	20.3 ± 0.57	23.00 ± 01
<i>S. marcescom</i>	22.3 ± 7.50	15.00 ± 01	16.3 ± 4.50	17.00 ± 3.60	20.00 ± 01	24.3 ± 1.52
<i>K. pneumonia</i>	25.6 ± 4.04	14.6 ± 1.52	16.6 ± 0.52	16.3 ± 1.52	18.6 ± 1.52	20.3 ± 2.08

3.8. Antioxidant Assay

The antioxidant potential of CuO NPs was determined with the use of ABTS^{•+} and results were compared with the standard ascorbic acid as shown in Table 2. The ABTS^{•+} radical is converted into nonradical ABTS by reacting with an antioxidant agent. As equation is as follows:



Different concentrations of CuO NPs were used to find out antioxidant activity against ABTS free radicals. As the concentration of CuO NPs increases from 20 to 100 µL, the scavenging potential of ABTS free radicals also increased from 43 to 97%, respectively. This shows the presence of a larger number of CuO provides a larger number of surface/binding sites for the free radicals to attach, resulting in an increase in the scavenging potential of ABTS^{•+}. A similar increasing pattern was also seen in the activity of ascorbic acid was used as a standard. According to the results, at the concentration of 80 µL, ascorbic acid showed 58.5%, and CuO NPs showed 83.4% ABTS^{•+} radical scavenging activity. It has been shown that CuO NPs better show ABTS^{•+} radical scavenging activity than the ascorbic acid used as standard [41,42]. The low IC₅₀ value and correlation value also clarify the enhanced antioxidant potential of the CuO NPs as compared to the standard. The results were also compared to the antioxidant activity of *P. nigrum* leaves aqueous extract against ABTS free radicals, which was reported to be 39.62 (0.02) percent with an IC₅₀ value of 144.1 (2.2) g/mL [26,43].

Table 2. Percent ABTS radical scavenging activity of ascorbic acid and CuO NPs.

Samples	Concentration (µg/mL)	%RSA	IC ₅₀ (µg/mL)	Variance (S ²)	STD Deviation (S)	Correlation between Dose and Obtained Result
CuO NPs	20	43	26	3.70	1.93	0.044
	40	62				
	60	73				
	80	83				
	100	97				
Ascorbic acid	20	26	66	4.38	2.10	0.067
	40	38				
	60	46				
	80	58				
	100	65				

3.9. Comparison with Literature

The antibacterial and antioxidant activities of CuO NPs reported in this study are compared with the other plant-mediated CuO NPs reported in the literature (cited in Table 3), and the extracted data is tabulated in Table 3 [44–50]. It is clear from the literature that other studies conducted in the same field are only limited to two to four bacterial

species, where no antioxidant activity was performed. These findings clearly show that the present study reported larger zones of inhibition than the literature, highlighting the efficacy of the *P. nigrum* leaf extract-mediated CuO NPs. Similarly, the antioxidant activity, which was not frequently performed by other researchers, had higher IC₅₀ values than that found in our work, showing the high potential of CuO NPs as an antioxidant [48–50].

Table 3. Comparison of antibacterial and antioxidant activities of CuO NPs synthesized by different plant species with CuO NPs synthesized in the present study.

Plant Source	Antibacterial Activity against Different Bacterial Strains (mm)		Antioxidant Activity	IC ₅₀ Value	References
<i>Piper nigrum</i> leaves extract	<i>S. aureus</i>	24	83.4%	26 µg/mL	Current study
	<i>S. epidermis</i>	23.6			
	<i>S. pyo</i>	21.3			
	<i>E. coli</i>	23			
	<i>S. marcescom</i>	24.3			
	<i>K. pne</i>	20.3			
<i>Bougainvillea</i> leaves extract	<i>E. coli</i>	20			[44]
	<i>S. aureus</i>	16			
<i>Catha edulis</i> leaves extract	<i>E. coli</i>	22			[45]
	<i>S. aureus</i>	17			
	<i>S. pyo</i>	29			
	<i>K. pne</i>	16			
<i>Achillea millefolium</i> leaves extract	<i>E. coli</i>	28			[46]
	<i>S. pyo</i>	17			
	<i>S. aureus</i>	26			
	<i>K. pne</i>	29			
<i>Cedrus deodara</i> aqueous extract	<i>E. coli</i>	29			[47]
<i>Allium sativum</i> extract	<i>E. coli</i>	8	96.09%	40.52 µg/mL	[48]
	<i>S. aureus</i>	7			
	<i>S. pyo</i>	8			
	<i>K. pne</i>	8			
Chitosan neem seed biocomposites	<i>S. aureus</i>	17	56%	91.05 µg/mL	[49]
	<i>E. coli</i>	16			
<i>Rubia cordifolia</i> bark extract			70.88%		[50]

4. Conclusions

A fast, eco-friendly, one-pot synthesis, the simple, clean, energy-saving, and economically feasible route was followed for the synthesis of well-crystalline monoclinic-shaped CuO NPs and was confirmed through XRD analysis. Plant consumption (reducing and capping agents) has played an important role in the synthesis of CuO NPs, resulting in a biocompatible and safer process for the synthesis of nanomaterials that is simple to operate, cost-effective, and reduces the use of toxic reducing agents. The SEM analysis shows the formation of agglomerated samples where the visible boundaries between the particles are not observed, and the peaks in the FTIR spectrum below 600 cm^{−1} ascribed to the metal-oxygen vibration confirm the successful formation of the CuO NPs. However, the antibacterial activity was found to be less than the standard drug. However, significant growth inhibition activity was observed against both types of pathogens. The ABTS free radical scavenging activity of CuO NPs was significantly higher than that of ascorbic acid. The improved antibacterial and antioxidant activities at higher concentrations suggest that quantity plays a significant role in the activity of CuO NPs. The comparison of the present work with the literature concludes that the synthesized CuO NPs are more efficient, compatible, and suitable for biological and environmental applications.

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