ORIGINAL ARTICLE

Green synthesis of copper oxide via onion (*Allium cepa*) peel extract and evaluation of its antioxidant activity

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Abstract

Aim: The present work aimed to synthesize copper oxide (CuO) nanoparticles through green approach using waste onion (*Allium cepa*) peel extract and to evaluate the CuO antioxidant properties. **Materials and Methods:** In this study, onion peel extract was used as a natural reducing agent for the reduction of copper sulfate. While antioxidant activity of synthesized compound was evaluated using ABTS and DPPH assay. **Results:** Fourier transform infrared spectroscopy (FTIR) spectroscopy, zeta potential analysis, and XRD analysis were used to characterize the synthesized CuO. The FTIR measurements confirmed presence of plant moieties on CuO particles. From X-ray diffraction analysis data, we found that CuO particles were crystalline in nature with an average grain size of 42.9 nm. The average zeta potential value of synthesised CuO particles was found to be −20 mV, this showed that these particles were stable in nature. Percent antioxidant activity as per ABTS and DPPH assay was calculated to be 55.55% and 33.6%, respectively. **Conclusion:** Results of this study showed that the CuO synthesized using onion peel extract exhibited significant antioxidant activity.

Key words: Green synthesis, Copper oxide, Antioxidant, Onion peel, ABTS, DPPH, Nanoparticles, CuO

INTRODUCTION

Nanotechnology is used in a variety of fields, including physics, materials science, chemistry, biology, computer science, and engineering.[1] Nanotechnology has become increasingly popular in recent technological and scientific revolutions across a variety of fields and disciplines, including environmental, sensing, bioenergy, and agricultural systems. Nanomaterial production must be manageable, practical, and affordable if modern nanotechnology is to be produced. Because of the potentially toxic chemicals utilized and the detrimental byproducts generated, chemical synthesis, for instance, is neither entirely safe nor environmentally beneficial.[2]

Recent breakthroughs in nanoscience and nanotechnology have resulted in a wide range of metal and metal oxide nanoparticle applications across a wide range of fields; copper oxide nanoparticles (CuO NPs) have received more attention than other types of metal oxides due to their distinct properties and applications.[3] The world has been transformed by nanotechnology, from basic food packaging materials to medical medicines. Most of the sciences have used nanoscale items in some capacity. The use of nanotechnology has led to the development of molecules with the desired number of dimensions. Although nanotechnology has given us many benefits, studies by various research groups have also reported that some nanomaterials are hazardous to both human health and the environment.[4] The production of environment friendly and economically viable NPs is still in its infancy because of nanotoxicity, unforeseen health hazards, and scaling issues. Using green synthesis methods to produce NPs, one can lessen these risks. Green technologies have many advantages, including reduced environmental impact, increased biocompatibility, improved stability, lower capital, and operating costs.[5]

Plants are used as natural reducing and capping agents in the production of NPs in the green synthesis

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Received: 03-09-2022
Revised: 18-10-2022
Accepted: 25-10-2022

approach.[6] Synthesis of copper NPs using non-edible and waste parts of plants is considered as a sustainable nanoparticle synthesis approach.[7] Hence, in this study, we have synthesized CuO using onion (*Allium cepa*) dry peel extract through bioreduction approach. *A. cepa*, also known as onion, is a culinary and therapeutic spice from the *Liliaceae* family. Onion contains a variety of biologically active compounds, including phenolic acids, thiosulfinates, and flavonoids.[8] Therefore, it is presumed that onion peel extract might also aid in the reduction of $CuSO_4$ to CuO in this study.

MATERIALS AND METHODS

Materials Used

Dry onion peels, filter paper, and distilled water.

Chemicals Used

Fehling solution A (Lobachemie India), Fehling solution B (Lobachemie India), Methanol (Lobachemie India), Ethanol (Jiangsu, China).

*(*A. cepa* onion plant was authenticated by Dr. Meenu Sood (Botanist) at Y.S Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India. Authentication details of plant: UHF-Herbarium no 13982.

Methods

Preparation of dry onion peel extract

Authenticated dry onion peels were washed with distilled water and left to shade dry. About 2 g of shade dried onion peels were cut into small pieces and added into 200 ml of distilled water. This mixture was boiled for 20 min, cooled, and filtered using a Whatman filter paper (No 1). Filtered onion peel extract was centrifuged and the supernatant was stored at 4°C till further use.

Synthesis of CuO

CuO NPs were synthesized using bioreduction method. Briefly, mixture of Fehling A and Fehling B was taken in a conical flask, and plant extract was added into the mixture at 60°C with continuous stirring till brick red precipitates were obtained. The precipitates were centrifuged at 7000 rpm, formed pellet was washed twice with distilled water and finally with methanol. Washed pellet was left to dry at 60°C for 24 h in oven. Further, the dried sample was crushed using mortar pestle and characterized using UV spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), DLS, and scanning electron microscope (SEM).

Characterization of CuO

The optical absorption of CuO NPs in the wavelength range of 800–200 nm was measured using a double beam UV-visible spectrophotometer (Shimadzu UV 1900). Functional group identification on FTIR, Bruker, USA in the 4000–400 cm^{-1} range was used to determine the nature of the bioactive chemicals involved in the bioreduction of $CuSO_4$. The morphology and size of the produced NPs were determined using SEM-Nova, Nanosem-450, FEI, USA. The crystalline and lattice structure of the NPs was determined using an X-ray diffractometer (Rigaku Corporation) at diffraction angles (2θ) ranging from 20° to 80°. While the zeta potential of synthesized CuO was measured using Malvern Zetasizer at 25°C.

Antioxidant assay

The antioxidant activity of synthesized NPs was carried out as per ABTS and DPPH assay.

DPPH assay

The assay was conducted in accordance with a methodology that was adopted by Shimada *et al.* (1992). In 80% ethanol, a 0.3 mM DPPH solution was prepared. To 2.5 ml of DPPH solution, 500 µL of sample (1 mg/ml) was added and allowed to react with DPPH solution. To evaluate the decrease in absorbance at 517 nm, the reaction mixture was vortexed and incubated at 30°C for 30 min in a dark environment. Plain DPPH reagent was used as control, and the 80% of ethanol served as blank. The following formula was used to calculate the free radical scavenging activity:[9]

$$\text{Scavenging effect (\%)} = 1 - ([\text{Absorbance sample}/\text{Absorbance control}] * 100)$$

ABTS assay

The scavenging activity was estimated according to method described by Pellergini *et al.* (1999). Stock solution of ABTS was mixed with an equal quantity of potassium persulfate (2.45 mM) to prepare ABTS (7 mM in water). This solution was then allowed to sit for 12–16 h at room temperature in dark until it reached a stable oxidative state. ABTS solution was diluted with 80% ethanol, till an absorbance value of 0.80 ± 0.05 at 734 nm was achieved. To 2.9 ml of ABTS solution, 100 µL of sample solution (1 mg/ml) was added and allowed to stand at room temperature for 30 min. in dark. The absorbance of this mixture was measured at 734 nm, and following equation was used to calculate % antioxidant activity.[10]

$$\text{Scavenging effect (\%)} = 1 - ([\text{Absorbance sample}/\text{Absorbance control}] * 100)$$

RESULTS AND DISCUSSION

Synthesis of CuO

CuO NPs were effectively synthesized utilizing aqueous onion peel extract as a bio-reducing agent. Mixture of Fehling

A and Fehling B was used as precursor. Precipitation occurred after adding plant extract to this precursor solution at 50°C. CuO formation was confirmed by the appearance of brick red precipitates. Pictorial representation of bio reduction method used for synthesis of CuO is given in Figure 1, while Table 1 lists all the observations made concerning synthesized NPs generated using green synthesis.

UV Spectroscopy

The result obtained from UV-Visible spectroscopy of CuO-NPs is presented in Figure 2; the biologically synthesized NPs showed an absorption peak near 228 nm in the UV-region which corresponds to CuO. Observed λmax value of synthesized CuO was very close to λmax value reported in the literature in another study.[11] Therefore, from UV-Vis spectroscopy study, it may be suggested that reduction of $CuSO_4$ to CuO occurred successfully; however, this was

Table 1: Detailed observations about obtained CuO NPs

S. No.	Observations	
1.	Color of obtained CuO NPs	Brick Red powder
2.	Weight of obtained CuO NPs	0.701 g
3.	State of obtained CuO NPs	Solid Powder

CuO NPs: Copper oxide nanoparticles

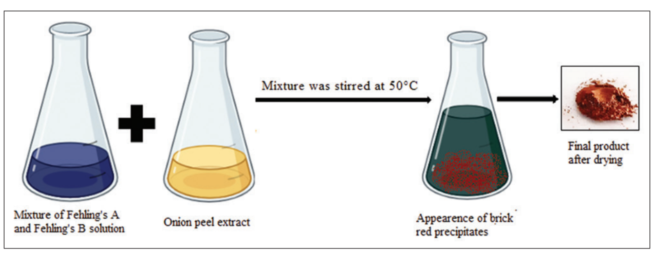

Figure 1: Pictorial representation of bio reduction method used for synthesis of copper oxide

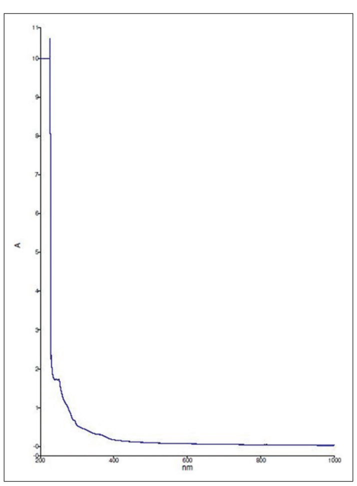

Figure 2: UV spectrum of synthesized copper oxide nanoparticles

preliminary confirmation. For additional confirmation, we utilized various other characterization techniques mentioned below.

FTIR Spectroscopy Study

The FTIR technique was used to identify and estimate the potential biomolecules responsible for capping and stabilizing the CuO NPs synthesized by onion peel extract. The peaks in the spectrum [Figure 3] originate from the various chemical moieties present in onion peel extract that was used to synthesize these particles. The FTIR spectra of plant extract and biologically synthesized NPs are depicted in Figures 3 and 4, respectively. Figure 3 suggested that the presence of hydroxyl groups in plant extract as a broad peak was obtained at 3328 cm^{-1}; this broad peak is suggestive of the presence of various hydroxyl groups present in onion peel extract. The peak at 1637 cm^{-1} may be a result of amide vibrations since the amide I band (between 1600 cm^{-1} and 1700 cm^{-1}) is mostly linked to the C=O stretching vibration. Similarly, FTIR spectrum of CuO [Figure 4] was also obtained, peak at 3462 cm^{-1} and 1637 cm^{-1}, respectively, suggest the presence of hydroxyl groups and amino groups on surface of synthesized sample. Various other peaks obtained below 1000 cm^{-1} such as 923 cm^{-1}, 627 cm^{-1}, 538 cm^{-1}, and 467 cm^{-1} indicate formation of metal oxide from its metallic salt.[12]

XRD

The XRD spectrum [Figure 5] of synthesized CuO NPs demonstrates the orientation and the crystalline nature of CuO NPs. The XRD spectrum showed four diffraction peaks at 29.62°, 36.49°, 42.39°, and 61.49° which authenticated the formation of well crystalline nature of CuO. The Scherrer's equation ($d = 0.89\lambda/\beta\cos\theta$) was used to calculate the average grain size of synthesized CuO particles, this equation is valid only for the measurement of crystallite sizes up to 200 nm.[13] The average grain size of the CuO particles, as per this equation, was calculated to be 42.9 nm, suggesting that CuO particles are in nanorange. Moreover, the sharp peaks

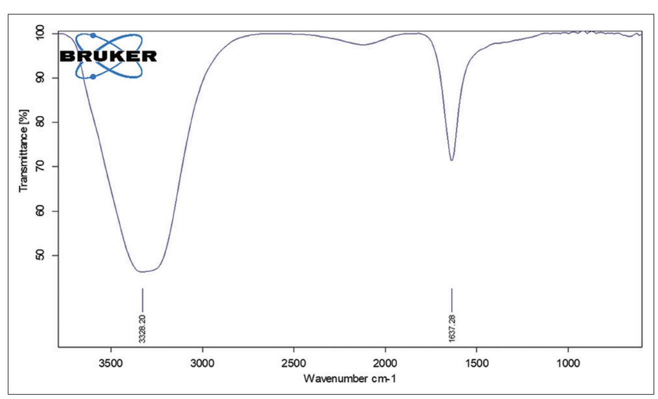

Figure 3: Fourier transform infrared spectroscopy spectrum of onion peel extract

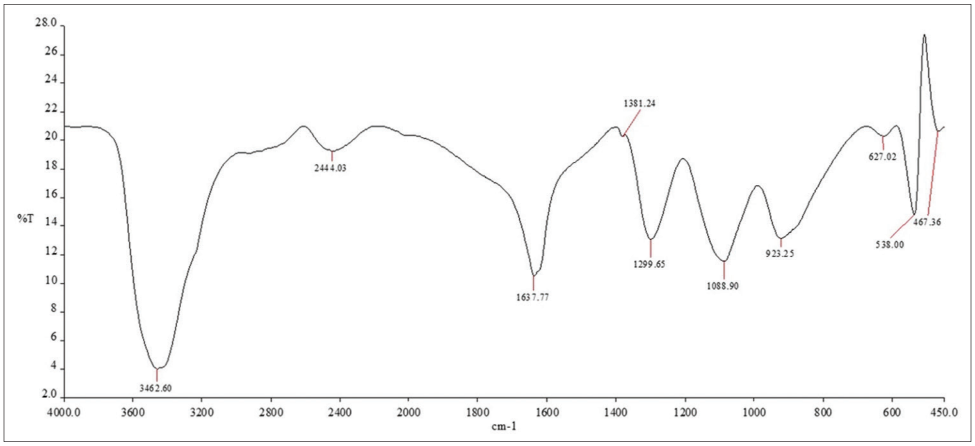

Figure 4: Fourier transform infrared spectroscopy spectrum of synthesized copper oxide nanoparticles

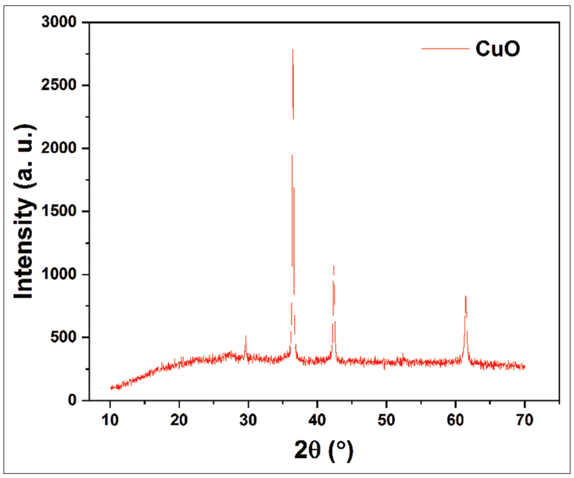

Figure 5: XRD spectrum of synthesized copper oxide

in this figure [Figure 5] validate the crystallinity and higher purity of prepared NPs.

Zeta Potential Distribution of CuO NPs

Zeta-potential is the potential developed at the solid-liquid interface in response to the relative movement of the test particles and the solvent. The stability of NPs depends upon the electrical charge; hence, it is very important parameter to know. The zeta potential with a value of ±30 mV is generally chosen to infer particle stability; the absolute value >30 mV indicates a stable condition, whereas a low zeta potential value of <30 mV indicates a condition toward instability, aggregation, coagulation, or flocculation.[14] To study the zeta potential, the prepared CuO-NPs were dispersed in double distilled water. The zeta potential of prepared NPs was found to be −20 mV, as shown in the Figure 6 which indicated that the synthesized NPs were reasonably stable and well-dispersed.

Antioxidant Activity

The antioxidant activity of CuO NPs synthesized through onion peel extract was carried as per ABTS and DPPH

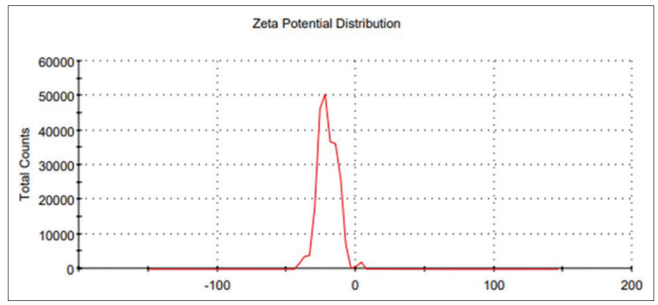

Figure 6: Zeta potential distribution of synthesized copper oxide nanoparticles

Table 2: Antioxidant activity of CuO NPs using ABTS and DPPH assay

S. No.	Antioxidant assay	Percent antioxidant activity
1.	ABTS assay	55.55%
2.	DPPH assay	33.6%

CuO NPs: Copper oxide nanoparticles

assay. The absorbance values recorded at 515 nm (for ABTS assay) and 734 nm (for DPPH assay) were used to calculate percentage antioxidant activity. Results [Table 2] showed that the CuO NPs synthesized using onion peel extract exhibited significant antioxidant activity, as percent antioxidant activity as per ABTS and DPPH assay was calculated to be 55.55% and 33.6%, respectively.

CONCLUSION

The green production of CuO NPs with onion peel extract, their characterization and analysis of their antioxidant activity through DPPH and ABTS assay was given in this paper. UV-VIS spectroscopy, FTIR spectroscopy, XRD, SEM, and seta potential analysis were used to characterize the CuO NPs. The conversion of $CuSO_4$ to CuO was confirmed visually by observing appearance of brick red precipitates and was further confirmed through above mentioned analysis techniques too. The existence of biological moieties responsible for the reduction and stabilization of CuO NPs was confirmed by FTIR analysis. While zeta potential value (−20 mV) of green synthesized CuO particles suggested that these particles were stable in nature. In addition, XRD analysis showed that CuO particles were crystalline in nature with an average grain size of 42.9 nm, as calculated using Scherrer equation. Moreover, evaluation of antioxidant activity of CuO NPs suggested that green synthesized CuO exhibited significant antioxidant activity. However, the mechanism of action behind antioxidant activity of CuO was not deciphered in this study; therefore, it is suggested that this study should be further continued in the future to get in depth insights about the antioxidant action of CuO.

REFERENCES

1. Bayda S, Adeel M, Tuccinardi T, Cordani M, Rizzolio F. The history of nanoscience and nanotechnology: From chemical-physical applications to nanomedicine. Molecules 2019;25:112.
2. Rai PK, Kumar V, Lee S, Raza N, Kim KH, Ok YS, *et al*. Nanoparticle-plant interaction: Implications in energy, environment, and agriculture. Environ Int 2018;119:1-19.
3. Akintelu SA, Folorunso AS, Folorunso FA, Oyebamiji AK. Green synthesis of copper oxide nanoparticles for biomedical application and environmental remediation. Heliyon 2020;6:e04508.
4. Sharma D, Shrivastava R, Bisht GS. Nanomaterial in diverse biological applications. In: Metabolic Engineering for Bioactive Compounds. Singapore: Springer; 2017. p. 293-317.
5. Kumar S, Lather V, Pandita D. Green synthesis of therapeutic nanoparticles: An expanding horizon. Nanomedicine 2015;10:2451-71.
6. Jeevanandam J, Kiew SF, Boakye-Ansah S, Lau SY, Barhoum A, Danquah MK, *et al*. Green approaches for the synthesis of metal and metal oxide nanoparticles using microbial and plant extracts. Nanoscale 2022;14:2534-71.
7. Benassai E, Del Bubba M, Ancillotti C, Colzi I, Gonnelli C, Calisi N, *et al*. Green and cost-effective synthesis of copper nanoparticles by extracts of non-edible and waste plant materials from *Vaccinium* species: Characterization and antimicrobial activity. Mater Sci Eng C Mater Biol Appl 2021;119:111453.
8. Lawande KE. Onion. In: Handbook of Herbs and Spices. Sawston, United Kingdom: Woodhead Publishing; 2012. p. 417-29.
9. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem 1992;40:945-8.
10. Fellegrini N, Ke R, Yang M, Rice-Evans C. [34] Screening of dietary carotenoids and carotenoid-rich fruit extracts for antioxidant activities applying 2, 2’-azinobis (3-ethylenebenzothiazoline-6-sulfonic acid radical cation decolorization assay. Methods Enzymol 1999;299:379-89.
11. Sharma D, Thakur N, Vashistt J, Bisht GS. Antibacterial evaluation of cuprous oxide nanoparticles synthesized using leaf extract of *Callistemon viminalis*. Indian J Pharm Educ Res 2018;52:449-55.
12. Petit T, Puskar L. FTIR spectroscopy of nanodiamonds: Methods and interpretation. Diam Relat Mater 2018;89:52-66.
13. Muniz FT, Miranda MR, dos Santos CM, Sasaki JM. The Scherrer equation and the dynamical theory of X-ray diffraction. Acta Crystallogr A Found Adv 2016;72:385-90.
14. Lin PC, Lin S, Wang PC, Sridhar R. Techniques for physicochemical characterization of nanomaterials. Biotechnol Adv 2014;32:711-26.

Source of Support: Nil. **Conflicts of Interest:** None declared.