Int. J. Nano Dimens., 13 (2): 214-226, Spring 2022

ORIGINAL ARTICLE

Green synthesis, characterization and antimicrobial activity of nanosized Cuprous Oxide fabricated using aqueous extracts of *Allium Cepa* and *Raphanus Sativus*

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Received 30 November 2021; revised 24 December 2021; accepted 07 January 2022; available online 09 January 2022

Abstract

Green synthesis of metal nanoparticles (MNPs) is attracting the attention of chemists as it is cost effective and environment benign technique. Hence it is preferred to methods which use toxic reagents in the synthesis of MNPs. In. In this study, copper oxide nanoparticles (Cu_2ONPs) were synthesized using *Allium cepa* (AC) and *Raphanus sativus* (RS) aqueous extracts. The Cu_2ONPs were investigated using UV–visible spectroscopy (UV–Vis), Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD) scanning electron microscope (SEM) and energy dispersive X-ray spectroscopy (EDX). Peak positions (2θ values) in the XRD pattern of Cu_2O NPs revealed (1 1 0), (1 1 1), (2 0 0), (2 2 0), (3 1 1) and (2 2 2) planes of face-centered cubic (FCC) crystalline structure. The SEM images indicate spherical shape of Cu_2ONPs having low particle size (12-30 nm). The present nanoparticles demonstrated substantial antimicrobial activity against harmful bacteria viz. *S. aureus*, and E. coli and also against C. albicans fungal species. The current study reveals efficacy of *Allium cepa* (Onion) and *Raphanus sativus* (Radish) aqueous extracts as reducing and capping agent for the green synthesis of Cu_2O NPs. The copper nanoparticles derived from *Raphanus sativus* (RS) showed justifiably low grain size and comparable to recently studied copper nanoparticles prepared using plant extracts.

Keywords: *Allium Cepa; Antimicrobial Activity; Copper Nanoparticles; Green Synthesis; Raphanus Sativus.*

How to cite this article
Kumar Bale V., Reddy Katreddi H. Green synthesis, characterization and antimicrobial activity of nanosized Cuprous Oxide fabricated using aqueous extracts of Allium Cepa and Raphanus Sativus. Int. J. Nano Dimens., 2022; 13(2): 214-226.

INTRODUCTION

Metal nanoparticles (MNPs) have been investigated during the past decade due to their distinctive physical, chemical, spectral, mechanical, thermal and biological features [1]. These interesting properties are mainly due to their low dimension and high surface area. MNPs have been studied for the success of future technological developments in biology, medicine and industry. In various applications, particularly, in sensing and catalysis [2] metal oxide nanoparticles are gaining importance. Variety of methods [3-8] has been used to synthesize nano-sized copper particles. However, in all these methods toxic chemicals and reagents are used. The usage of toxic substances limits the application of MNPs in the field of medicine. Therefore, the development of green synthetic methods for the production of MNPs is gaining paramount importance in recent times. Now the strategy is shifted to green synthesis which focuses the benign, cost-effective and bio-compatible reactants for the production of metal oxide nanoparticles (MNPs).

Several green methods for the synthesis of MNPs are reported [9-11] in the literature. In those methods, various sources from animals, plants and microorganisms were employed. Different plant extracts were used for the production of metal nanoparticles (MNPs). Copper nanoparticles derived from using plant extracts showed antimicrobial activity, catalytic and photocatalytic

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activity [12-18]. For example, copper nanoparticles have been prepared using several plant extracts viz., *Aloevera*, *Bifurcaria bifurcate*, *Magnolia kobu*, *Tabernaemontana et, Terminalia arjuna* [19-23]. Likewise, silver nanoparticles were prepared from leaf extracts of *J. curcas* and their antibacterial activity was determined [24]. Synthetic methods based on plant extracts [25-28] have more advantages over the microbial methods. This is due to the high cost of microorganisms' isolation and their culture maintenance. Iron, copper, zinc and silver nanoparticles have been prepared using biological sources and studied their antimicrobial and photocatalytic activity [29-36]. In this research paper, we report green synthesis, characterization and antimicrobial activity of Cu_2O NPs using *Allium cepa* (AC) and *Raphanus sativus* (RS) aqueous extracts for the first time.

MATERIALS AND METHODS

Collection of plant materials

Allium cepa (Onion) and *Raphanus sativus* (Radish) were collected from local market of Anantapuramu city in Andhra Pradesh State, India.

Chemicals

Copper sulphate ($CuSO_4.5H_2O$) and sodium hydroxide (NaOH) were procured S. D. Fine Chem Ltd., and used in the present study. De-ionized water was used in all experiments.

Preparation of Allium cepa (AC) and Raphanus sativus (RS) aqueous extract

Allium cepa (Onion) and *Raphanus sativus* (Radish) were first washed with tap water and de-ionized water and finally dried. About 10 g of cleaned vegetable (Onion or Radish) was chopped finely and taken in a cleaned 250-mL beaker containing 100 mL of de-ionized water. The beaker was kept on a hot plate and heated at 80°C for 1 hour. The contents of beaker were cooled to room temperature and filtered. The volume of extract is reduced to 50 mL by further heating it in a rota-evaporator. The extracts were stored in a refrigerator.

Synthesis of Cu_2O nanoparticles

In a 100-mL beaker, a 2.5 g of $CuSO_4.5H_2O$ was taken and dissolved in 20 mL of de-ionized water A 25 mL of vegetable (*Allium cepa* or *Raphinus sativus*) aqueous extract was added to copper sulphate solution and the pH of reaction mixture was adjusted 9.5 using 2M NaOH. The beaker was kept on a hot plate and the contents were heated at 80 °C for half an hour. During the course of reaction, the colour of reaction mixture has been changed from blue to green and finally to reddish-brown indicating the formation of copper nanoparticles. After cooling to room temperature, the particles were collected, washed with water and dried in an oven at 100 °C for 30 minutes. The yields of AC-Cu_2ONPs (derived from *Allium cepa)* and RS-Cu_2ONPs (derived from *Raphinus sativus)* are 60% and 64% respectively. Colour changes during the formation of RS-Cu_2ONPs are shown in Fig.1 (a-d).

CHARACTERIZATION OF NANOPARTICLES (AC-Cu_2ONPs and RS-Cu_2ONPs)

UV-Visible spectroscopic analysis

Electronic spectra of Cu_2ONPs were recorded by using ELICO SL-210 multipurpose UV-Visible spectrophotometer.

FT-IR spectroscopic analysis

FT-IR spectra were recorded in potassium bromide (KBr) discs using Perkin Elmer Spectrum 100 instrument in 4000–400 cm^{-1} region.

Powder X-ray diffraction analysis

The Cu_2ONPs were analyzed using X-ray diffractometer (Japan Smart Lab SE) in the scan range from 5–100 ° with a scan speed of 10 degree per minute. The instrument was operated at 40 kV voltage and 30mA current.

Scanning electron microscopy (SEM) and Energy dispersive X-ray spectroscopic (EDX) analysis

The SEM and EDX analyses were done by employing JOEL instrument operating at a voltage of 20kV. The Cu_2ONPs were placed on the carbon tape and images were captured. The magnification and size details were indicated on the SEM image.

Antimicrobial Assay

Antibacterial activity of copper oxide nanoparticles (Cu_2ONPs) were investigated by using agar disc diffusion method. The Cu_2ONPs were tested against two bacterial Viz. *Staphylococcus aureus* (*S. aureus,* Gram-positive bacteria), Escherichia coli (E. coli, Gram-negative bacteria) and one fungal species Viz. *Candida albicans (C. albicans)*. A 20 mL of nutrient agar

Fig. 1. Colour changes during the formation of RS-Cu_2ONPs: a)- $CuSO_4$ Solution; b)- RS extract; c)- Mixture of $CuSO_4$ Solution and RS extract and d)-After heating at 80 °C for 1 hour (Reddish-Brown colour indicated the formation of copper nanoparticles.

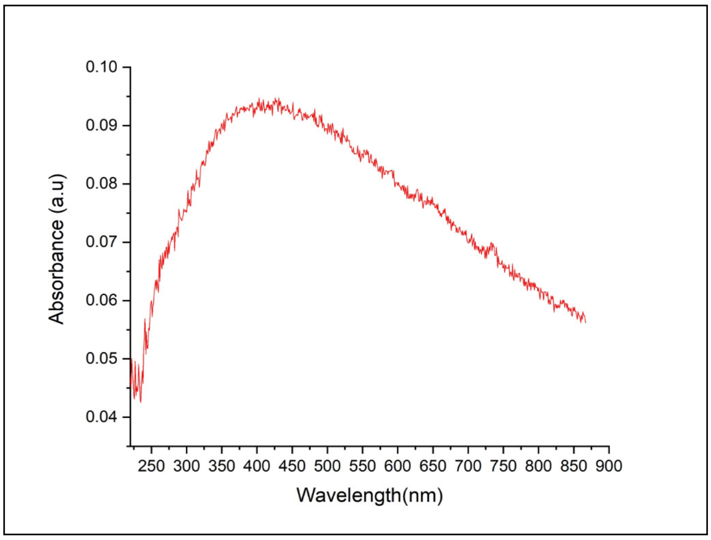


Fig. 2a. UV–Visible spectrum of AC-Cu_2ONPs.

medium was transferred to previously sterilized petri plates. Cultured bacteria and fungi were spread on petri plates. The sterilized 6 mm paper disks were soaked in suspension of nanoparticles in DMSO with different amounts (100, 200, 300 & 500 mcg/mL) of Cu_2ONPs and were dried in an oven. The soaked disks were placed on petri plates. Ciprofloxacin and Flucanazole were taken as positive control for bacterial and fungal species respectively and 10% DMSO was taken as negative control (blank). The petri plates were incubated at 37 °C for 24 hours and their zones of inhibition were measured and recorded in millimeters (mm).

RESULTS AND DISCUSSION

UV-Visible spectroscopic analysis

The copper oxide nanoparticles were dispersed in methanol and electronic spectra were recorded. According to Mie's hypothesis [37], the shape and position of plasmon absorption of nanoparticle very much depends on particle size and surface of adsorbed material. The AC-Cu_2ONPs have been showed (Fig. 2a) a single surface plasmon resonance (SPR) peak at 370 nm indicating

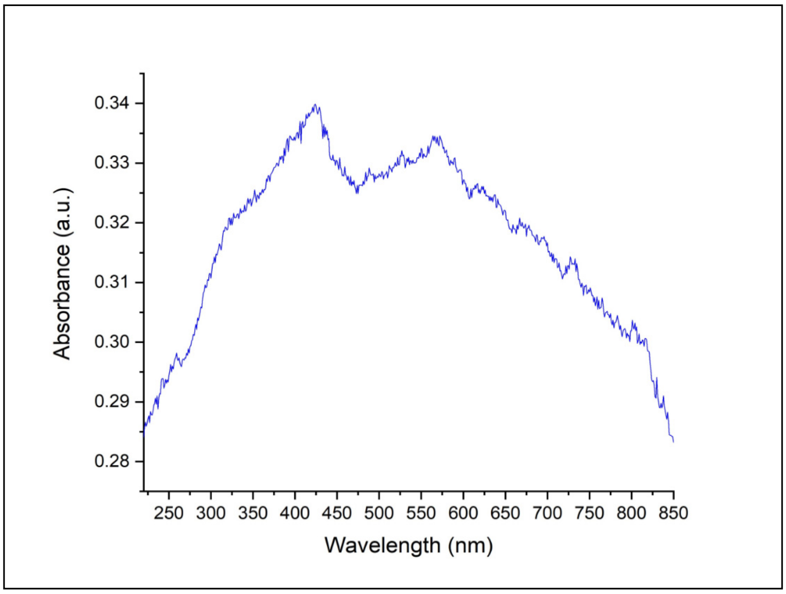


Fig. 2b. UV–Visible spectrum of RS-Cu_2ONPs.

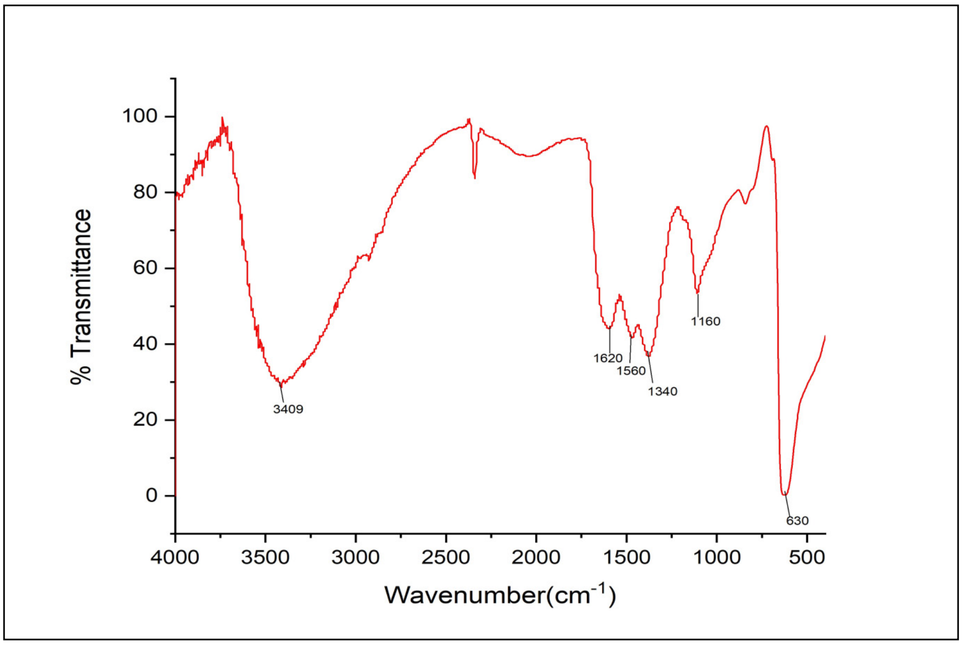


Fig. 3a. FT-IR spectrum of AC-Cu_2ONPs.

isotropic shape of nanoparticle. The spectrum of RS-Cu_2ONPs have been showed (Fig. 2b) two SPR peaks revealing anisotropic appearance of nanoparticles.

FT-IR spectroscopic analysis

The IR spectrum of AC-Cu_2ONPs (Fig. 3a) revealed absorption bands at 3409, 1620, 1560, 1165 and 1340 cm^{-1} corresponding to O-H, > C=O, > C=C < , C–O and > C=S stretching vibrations. The band at 630 cm^{-1} is assigned to Cu-O stretching vibration. In the IR spectrum of RS-Cu_2ONPs (Fig. 3b), bands appeared at 3397, 1602, 1530, 1100 and 1352 cm^{-1} were assigned to O-H, > C=O, > C=C <, C–O and > C=S stretching vibrations respectively. A peak at 600 cm^{-1} was assigned to Cu-O stretching

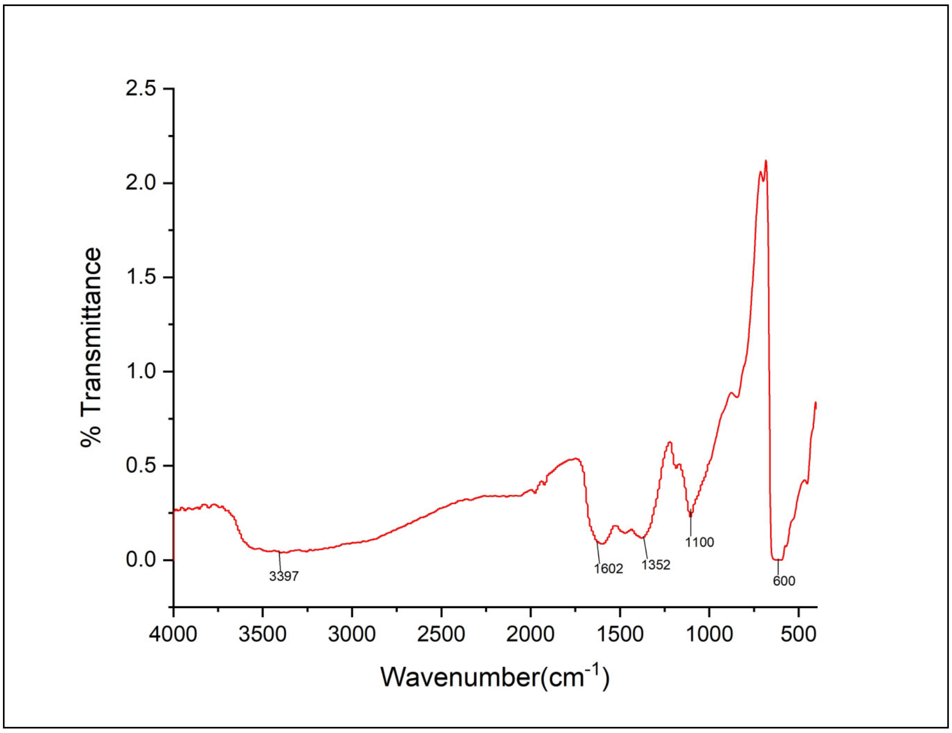


Fig. 3b. FT-IR spectrum of AC-Cu_2ONPs.

vibration. IR data of Cu_2ONPs revealed the presence of certain active biomolecules on the surface of copper nanoparticles.

Powder X-ray diffraction (PXRD) analysis

Powder X-Ray diffractograms of Cu_2ONPs were recorded between 20 to 100°. The PXRD pattern of copper oxide nanoparticles is shown in Fig. 4. The two theta peaks were observed at 29.72, 36.64, 42.52, 61.60, 73.82, and 77.74 in diffractogram of AC-Cu_2ONPs corresponding to (1 1 0), (1 1 1), (2 0 0), (2 2 0), (3 1 1) and (2 2 2) Miller indices respectively. The 2θ peaks for RS-Cu_2ONPs are noticed at 29.79, 36.64, 42.55, 61.62, 73.79, and 77.68 were assigned to (1 1 0), (1 1 1), (2 0 0), (2 2 0), (3 1 1) and (2 2 2) lattice planes. The two theta peaks of AC-Cu_2ONPs and RS-Cu_2ONPs were matched with Standard JCPDS-05-0667 (Cu_2O). The results suggested that both the copper oxide nanoparticles had face centered cubic (FCC) lattice phase. The average grain size of copper oxide nanoparticles was calculated by using Debye-Scherrer equation.

$$D = \frac{0.9\,\lambda}{\beta\, Cos\,\theta} \quad (1)$$

Where, λ = X-ray wave length (0.1541 nm); β = Full width half maximum (rad) and θ =Diffraction angle.

On substituting the values in the equation (1) and on calculation, the average grain size of AC-Cu_2ONPs and RS-Cu_2ONPs is found in the 15-30 and 12-25 nm range respectively. The grain size values are given in Table 1.

Scanning electron microscopy

Morphology of copper oxide nanoparticles is uncovered by performing SEM experiments. The SEM micrographs of AC-Cu_2ONPs and RS-Cu_2ONPs are shown in Fig. 5a & 5b. The SEM images revealed the formation of spherical shaped nanoparticles. The sizes of AC-Cu_2ONPs and RS-Cu_2ONPs are found in the range 25-35 nm and 20-30 nm respectively. The particle size data from SEM are in close agreement with data obtained from PXRD (Table 1).

Results on copper oxide nanoparticles derived using plant extracts [38-56] are summarized in Table 2.

Energy dispersive X-ray spectrometry

The chemical composition of copper nanoparticles is manifested by EDX analysis. The EDX spectra of AC-Cu_2ONPs and RS-Cu_2ONPs are

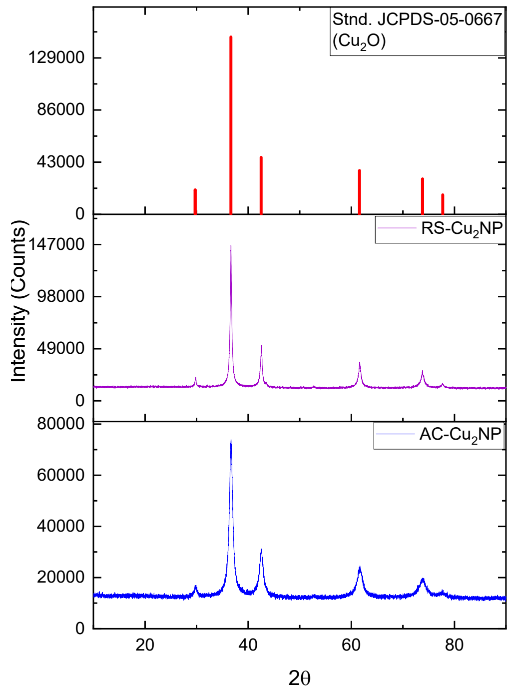
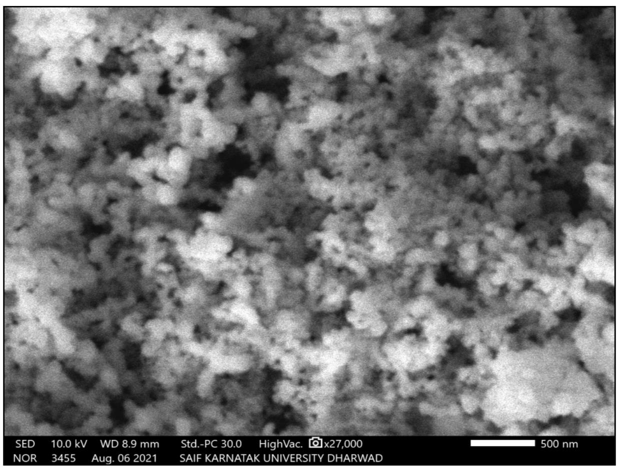


Fig. 4. PXRD patterns of standard JCPDS-05-0667, AC-Cu_2ONPs and RS- Cu_2ONPs.

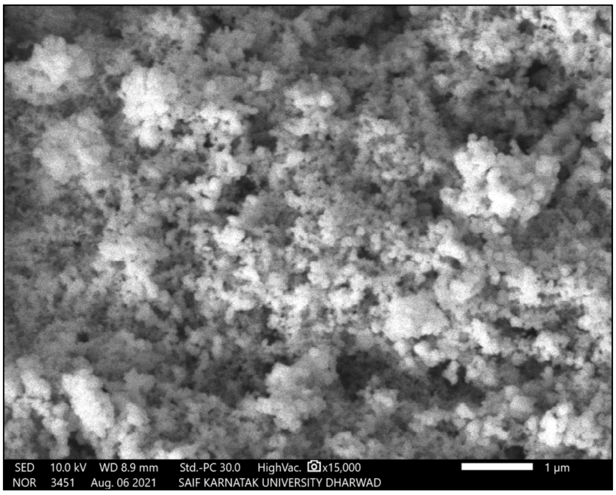
Table 1. PXRD and grain size data of copper oxide nanoparticles.

Peak Position(2θ)				Particle size (nm)	
JCPDS-05-0667	AC-Cu₂ONPs	RS- Cu₂ONPs	h k l	AC-Cu₂ONPs	RS- Cu₂ONPs
29.73	29.72	29.79	1 1 0	14.86	13.50
36.62	36.64	36.64	1 1 1	18.30	11.62
42.53	42.52	42.55	2 0 0	21.25	12.98
61.62	61.60	61.62	2 2 0	30.80	20.26
73.78	73.82	73.79	3 1 1	26.91	16.54
77.68	77.74	77.68	2 2 2	28.87	24.68



a)

Fig. 5. a) The SEM micrograph for AC-Cu_2ONPs, b) The SEM micrograph for RS-Cu_2ONPs.



b)

Continiued Fig. 5. a) The SEM micrograph for AC-Cu_2ONPs, b) The SEM micrograph for RS-Cu_2ONPs.

Table 2. Comparison of copper oxide nanoparticles derived from plant extracts.

S.No.	Plant name	Parts	Nanoparticle	Size(nm)	Shape	Pharmacological applications	Ref.
1.	Allium sativum	Leaves	Cu	100	Spherical	Antimicrobial	[38]
2.	Medicago sativa	Whole plant	CuO	30-100	Triangular, circular, Hexagonal	Antimicrobial	[39]
3.	Camellia sinesis	Leaves	Cu/CuO	67-99	Spherical	Antibiotics	[40]
4.	Citrus	Peel of fruit	Cu/CuO	48-76	Globular	Antibacterial	[41]
5.	Cassia occidentalis	Leaves	Cu	30-65	Spherical, oval	Antimicrobial, antioxidant	[42]
6.	Eucalyptus globules	Leaves	Cu/CuO	12-68	Cuboidal, spherical, oval	Antioxidant, cytotoxicity	[43]
7.	Allium sarvalicum	Leaves	Cu/CuO	45-50	Spherical	Antimicrobial, antioxidant	[44]
8.	Clerodendrum seratum	Flower	Cu/CuO	25-50	Spherical	Antilarvicidal	[45]
9.	Annona muricata	Leaves	Cu/CuO	30-40	Spherical and cubical	Photocatalyst	[46]
10.	Allium sativum	Bulb	CuO	20-40	Spherical and oval	Antimicrobial	[47]
11.	Azadirachta indica	Leaves	Cu/CuO	36	Spherical	Anticancer	[48]
12.	Alliuim eriophyllum	Leaves	Cu/CuO	30-35 nm	Spherical	Anticancer	[49]
13.	Gloriosa superb	Leaves	CuO	20-30	Spherical	Catalytic	[50]
14.	Enteromorpha flexuosa	Sea weed	CuO	5-27	Spherical	Antimicrobial	[51]
15.	Abutilon indicum	Leaves	CuO	10-20	Spherical	Antimicrobial	[52]
16.	Tridax procumbens	Leaves	CuO	12-18	Spherical	Antimicrobial	[53]
17.	Hydrastis canadensis	Whole plant	CuO	12-18	Spherical	Antioxidant	[54]
18.	Gareinia mangostana	Flower	CuO	5-27	Spherical	Antimicrobial	[55]
19.	Hibiscus rosasinesis	Leaves	Cu/CuO	12	Spherical	Antioxidant, anticancer	[56]
20.	Allium cepa	Bulb	Cu_2O	15-30	Spherical	Antimicrobial	Present work
21.	Raphanus sativus	Bulb	Cu_2O	14-25	Spherical	Antimicrobial	Present work

shown in Fig. 6a & 6b.Analytical data obtained from EDX studies are given in Table 3. Data denote high copper content of copper oxide nanoparticles (Cu_2ONPs).

Antimicrobial studies

The copper oxide nanoparticles were tested for their antibacterial activity against Gram +ve *S. aureus*, Gram–ve *E. coli* and fungal species *C. albicans* using Agar disc diffusion method. The bacteria isolates were sub cultured on nutrient agar plates and incubated for 24 hours at 37 °C. After incubation, the culture was diluted with fresh media and was poured on to the plate and spread into agar lawn using a sterile glass spreader. Experiments indicated increased antimicrobial activity of nanoparticles with an increase in quantity of nanoparticles. The results also indicate that higher CuNPs concentrations are more effective against *C. albicans* than *S. aureus* and *E. coli.*

The present copper oxide nanoparticles showed significant inhibition activity towards fungi viz. *C. albicans.* Figs. 7a & 7b exhibit photographs of inhibition zones. The zone inhibition data are summarized in Table 4. For the sake of quick comparison, the zone inhibition data are represented by a bar-graph (Fig. 8).

The antibacterial mechanism action of MNPs has been given in recent literature [57-61]. The

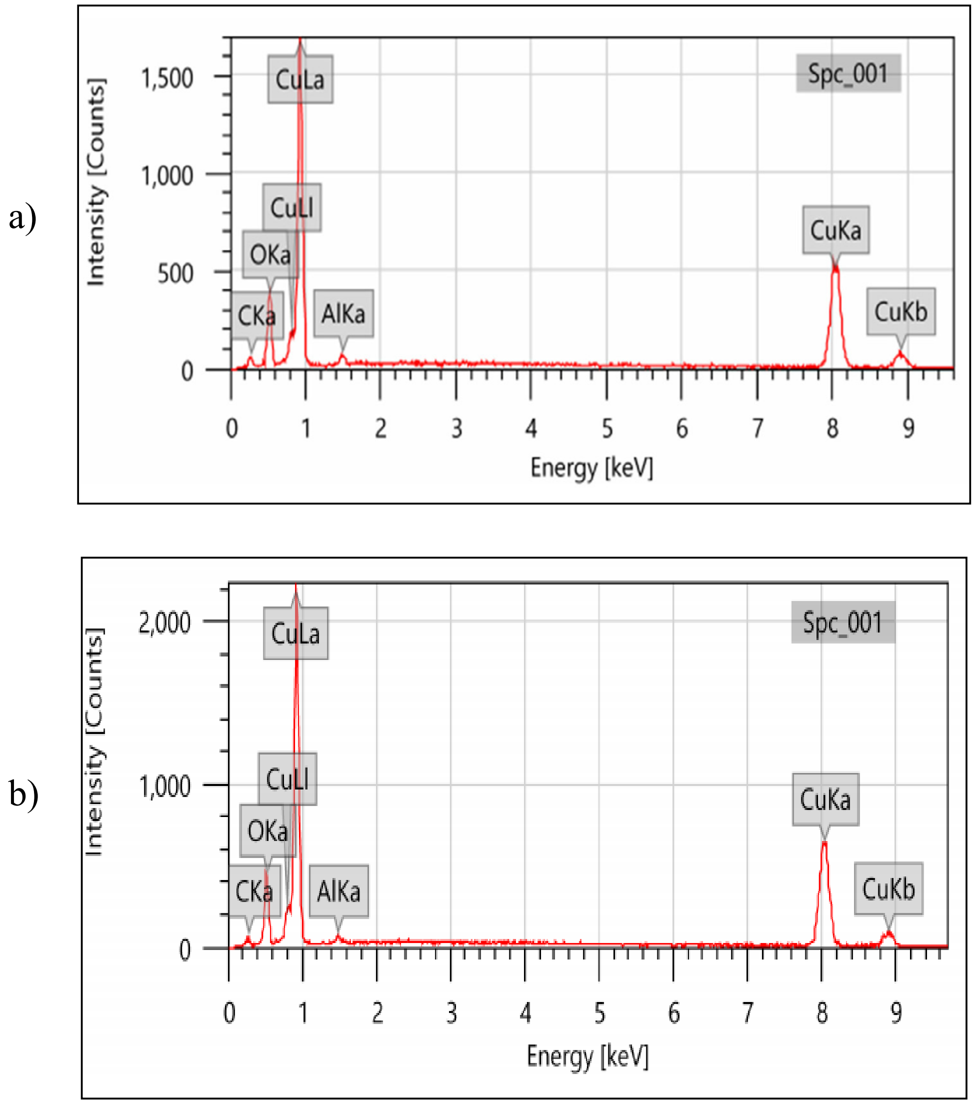
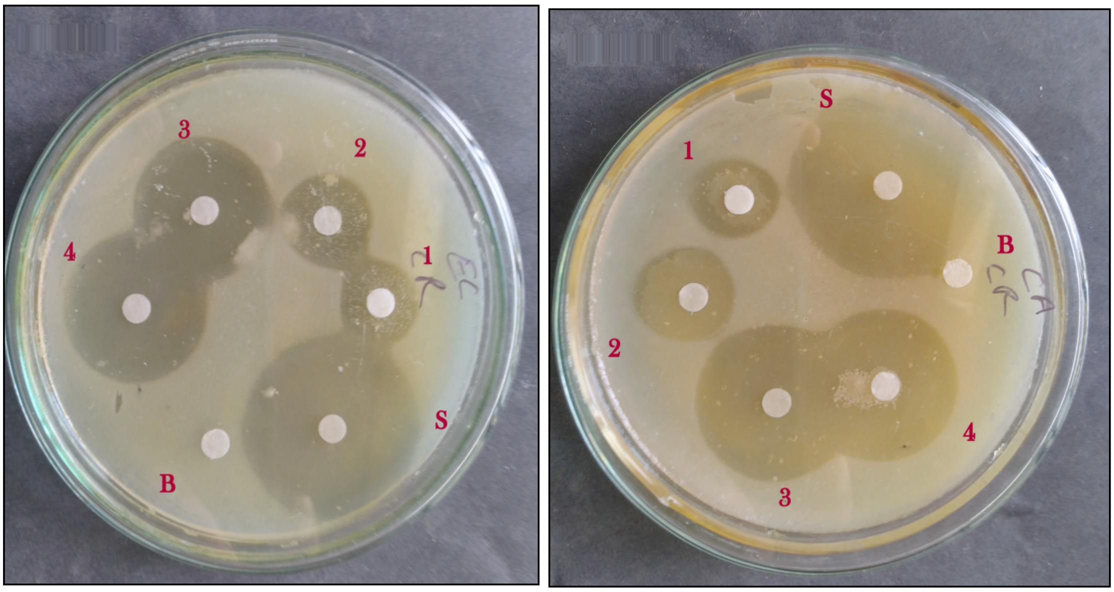


Fig. 6. A) The EDX image of AC-Cu_2ONPs, b) The EDX image of RS-Cu_2ONPs.

Table 3. Elemental composition of AC-Cu_2ONPs and RS- Cu_2ONPs as revealed by EDX.

Element line	AC-Cu_2O NPs		RS-Cu_2O NPs	
	Element Wt %	Atom %	Element Wt %	Atom %
C K	5.46 ± 0.14	16.82 ± 0.42	3.86 ± 0.11	12.55 ± 0.35
O K	15.59 ± 0.26	36.05 ± 0.60	15.10 ± 0.23	36.83 ± 0.57
Al K	1.45 ± 0.10	1.99 ± 0.14	1.02 ± 0.09	1.48 ± 0.13
Cu K	77.50 ± 0.89	45.13 ± 0.52	80.01 ± v 0.84	49.14 ± 0.52



(7a) (7b)

Fig. 7a. Photograph of inhibition zones in petri dishes for *E. coli* by RS-AgNPs and 7b. for *C. albicans* by RS-AgNPs [In the photographs, where S stands for standard (Ciprofloxacin); B for Blank; Nanoparticle concentration 1 - 100 mcg/mL; 2 - 200 mcg/mL; 3- 300 mcg/mL and 4- 500 mcg/mL].

Table 4. Zone inhibition data showing antimicrobial activity of Cu_2ONP against pathogens.

Samples	Treatment (mcg/mL)	*S aureus*	*E coli*	*C albicans*
Standard Ciprofloxacin	100	36	34	-
Standard Flucanazole	100	-	-	32
AC- Cu_2ONPs	100	8	7	9
	200	10	11	13
	300	14	14	15
	500	16	17	17
RS- Cu_2ONPs	100	9	9	10
	200	11	12	14
	300	15	16	17
	500	18	18	20

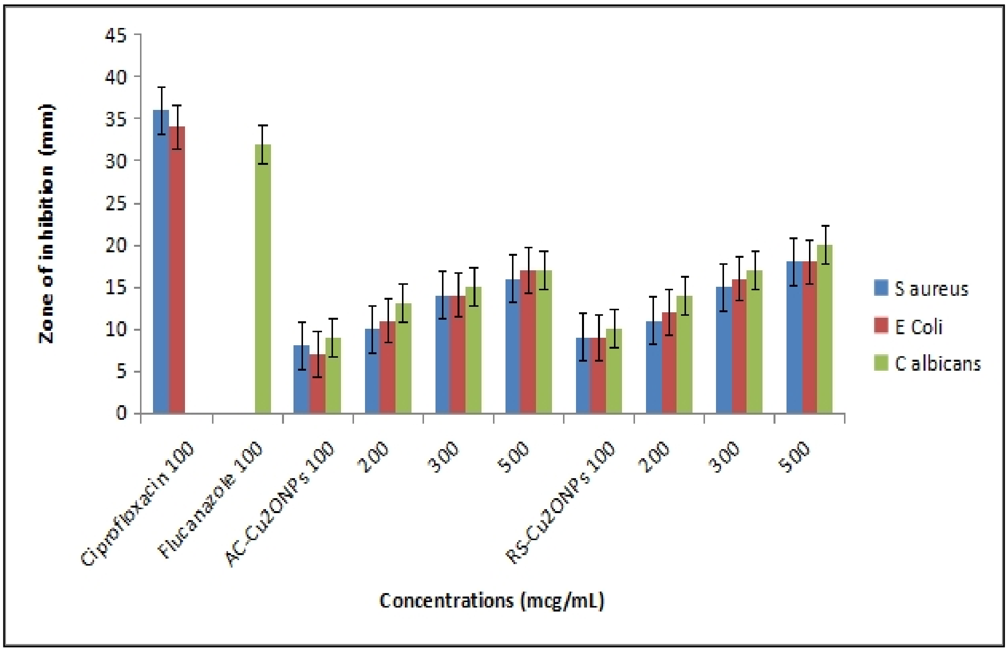


Fig. 8. Histogram showing Antimicrobial activity of Copper oxide nanoparticles at different concentrations against pathogens based on zone of inhibitions.

mechanism suggested that the metal ion (Cu^{2+}) was released in situ from the nanoparticles and then binds to bacterial cell wall which causes denaturation of protein coat and finally death of the cells [57]. Electro- static attraction between Cu^{2+} and plasma membrane and also membrane-based reductases lead to reduction some part of them to Cu^{+} [62-69]. The Cu^{+} ions are energetically easier to move across a lipid bilayer and taken up by the cell, generating reactive oxygen species, leading to lipid peroxidation and protein oxidation Haung *et al*. [58] suggested that the copper nanoparticles will bound to cell wall of *E. coli* and penetrate in to bacteria leading to the destruction of cell membrane. Some similar types of mechanisms were proposed by Line *et al*. [59], Azam *et al*. [60], Nawaz *et al*. [61] for copper nanoparticles.

CONCLUSION

The research paper reports the green synthesis of copper oxide nanoparticles using *Allium cepa* (AC) and *Raphanus sativus* (RS) extracts. The UV-Visible absorption spectra revealed the isotropic and anisotropic nature of AC-Cu_2ONP and RS-Cu_2ONP nanoparticles respectively. The powder XRD studies revealed that both the nanoparticles have face centered cubic (FCC) lattice. The SEM images revealed spherical morphology and low grain size (15-30 nm range for AS-Cu_2ONPs and 12-25 nm for RS-Cu_2O NPs). Certain limitations are noticed in the preparation of copper nanoparticles (CuNPs) using plant based extracts. Firstly, some plant leaves have to be dried for a long time. The leaves of *Catha edulus* were dried for seven days . *Catha edulus* is native to East Hararghe Zone and West Hararghe Zone of Ethiopia. These plants are localized in some parts and restricted in other countries. Secondly, many methods require heating at high temperature in the synthesis of CuNPs. The methods using extracts from (i) leaves of *Calotropis procera* , (ii) flowers of *Lantana camara* and (iii) seeds of *Caesalpinia bonducella* require heating of reaction mixture (copper and extract) at 700 °C, 400 °C and 450 °C respectively. Thirdly, large grain size and low copper content of CuNPs are serious limitations of previous green methods. The present methods are rapid and have the advantage of using readily available vegetables (onion and radish) in the markets of any country. In the present methods CuNPs were prepared at low temperature (80 °C) and in a small period of time (30 minutes). Higher copper content and low particle size of present copper nanoparticles are attractive features of our CuNPs. Low particle size of present Cu_2O NPs ranks among the recently reported plant based nanoparticles. The present Cu_2O NPs significantly inhibits fungi (*C. albicans)* and bacteria (*S. aureus and E. coli)*. Antifungal activity of RS-Cu_2ONP is more than that of AC-Cu_2ONP. Higher antibacterial activity and antifungal activity of RS-Cu_2ONP may be due to its lesser particle size and due to its rich copper content. The present paper demonstrates a successful use of *Allium cepa* and *Raphanus sativus* extract as an effective reducing and capping agent for the green synthesis of Cu_2ONPs and their substantial antimicrobial activity.

ACKNOWLEDGMENT

Prof. K. Hussain Reddy is thankful to UGC, New Delhi for the award of BSR Faculty Fellowship. The authors thank UGC for proving funds through SAP program for procuring analytical instruments.. The authors also thank SAIF centre, Karanataka University, Dharwad for providing P -XRD, SEM and EDX data. We also thank Prof. P. Bayapa Reddy, Professor of English (Retired) for checking the grammatical aspects of the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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