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Green synthesis of copper nanoparticles using natural reducer and stabilizer and an evaluation of antimicrobial activity

M. Jayandran[1], M. Muhamed Haneefa[2]* and V. Balasubramanian[2]

[1]Faculty of Chemistry, Mahendra Engineering College, Namakkal, Tamilnadu, India
[2]Faculty of Chemistry, AMET University, Chennai, Tamilnadu, India

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ABSTRACT

Synthesis of nanomaterial with the desired quality and properties is one of the key issues in current nanotechnology. Today, the green synthesis of metallic nanoparticles has received increasing attention due to the development of eco-friendly technologies in materials science. Use of natural plant extracts in the preparation of nanoparticles by greener route provides advancement over chemical and physical method as it is cost effective, environment friendly. In the present work, copper nanoparticles have been synthesized with simple and green synthesis method by using lemon extract as a reducer and curcumin as a stabilizer under certain conditions. The obtained copper nanoparticles were characterized by UV-Vis, IR, XRD, SEM and TEM techniques. The experimental results showed that the particle size of copper nanoparticles was appreciable and the antimicrobial activities of synthesized nanoparticles were significantly higher.

Keywords: Copper nanoparticles, Curcumin, Extract, Green method, Antimicrobial activity

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INTRODUCTION

The application of nanosized materials and structures which by definition should fall in the range between 1 to 100 nanometers is an emerging area of nanoscience and nanotechnology. Nanotechnology is mainly concerned with synthesis of nanoparticles of variable sizes, shapes, chemical compositions and controlled dispersity and their potential use for human benefits [1-2].Synthesis of metal nanoparticles has gained much importance because their physical-chemical properties differ from those of the bulk particles. The behavior of a material changes between these two distinct domains and the nanometer range is considered as the threshold for the transition of a material's behavior [3-4]. Due to their special properties and also small dimensions, find important applications in the fields of catalysis, optoelectronics, magnetic, thermal, sensors, fine chemical synthesis, solar energy conversion and medicine, etc. Nanomedicine is the latest advancement which the world has been the nanoparticles being the prime part of therapeutic and diagnostic agents [5-6].

The most common method employed for the synthesis of metal nanoparticles is the reduction of metal ions in solution. Although chemical and physical methods may successfully produce pure, well-defined nanoparticles, these are quite expensive and possibly dangerous to the environment [7-8]. Green chemistry has enormous potential in steering the responsible development of nanotechnology through the design of greener nanoscale materials and the discovery of green-nanomanufacturing methods. Green synthesis mainly concerns the elimination of hazardous wastes and the utilization of sustainable processes, implementation of environmental friendly chemicals, solvents and renewable materials [9-10]. In the green-nanotechnology, various metal nanoparticle synthesis have been reported using microorganisms, plant extracts and other biological natural materials, especially transition metals produced well-dispersed and ultrafine metal nanoparticles have great interest due to their distinctive physicochemical thermodynamic properties, which have made them proper for use in several applications [11-13].

Copper nanoparticles have attracted considerable attention due to its optical, catalytic, mechanical and electrical properties. Moreover its cheap, high yields and short reaction times under normal reaction conditions are the advantages in green-nano preparation [14-15]. Copper nanoparticles have wide applications as super strong materials, sensors especially antimicrobial materials because they are very reactive and their high surface-to-volume ratio helps to interact with other materials effectively [16]. The copper nanoparticles proved its superior antimicrobial activity against various bacterial and fungal strains from many researches [17-18].

Curcumin is of considerable interest and is well known compound because of its antioxidant, anti-inflammatory, antimicrobial and anti-carcinogenic activities [19-23]. In addition it is unique among active compounds because it is extremely safe even at high doses. On the other hand and due to the presence of olefinic groups in its structure this β-diketone of poor aqueous solubility rendering it of relatively low bioavailability [24]. One of the possible approaches to increase the bioavailability of curcumin is its conjugation on the surface of metal nanoparticles [25-26].

Based upon the above considerations here in the present investigation we have reported the synthesis of curcumin (Scheme 1) as well as the synthesis of copper nanoparticles (Scheme 2) using the naturally available materials, i.e., lemon extract as a reducing agent and curcumin isolated from turmeric used as a stabilizing agent. Thus, the surface functionalization of CNPs with curcumin may give a new way of using the curcuminoids towards possible drug delivery and therapeutics. The rate of nanoparticle synthesis was very high and the produced nanoparticles were pure and stable. Moreover this study clearly reported that CNPs have a significant antibacterial and antifungal activity.

EXPERIMENTAL SECTION

All the chemicals and solvents used were of analytical reagent grade and procured from Merck (Indi) Ltd and all samples were prepared by using fresh double-distilled water throughout the experiment. Curcumin was isolated from turmeric (BSR-01) which was purchased from Agricultural College and Research Institute, Madurai.

Collection of extracts

Lemon fruits were collected from the local markets. They were washed in double distilled water, cut into pieces and squeezed well to make 5 to 10 ml pure extract. The extract was then filtered using Whatman's No. 1 filter paper. The filtrate was collected in a clean and dried container and it was stored for further uses.

Isolation of curcumin (CR) from turmeric

Curcumin (CR) was quantitatively extracted from turmeric in soxhlet apparatus by using 95% ethanol (Scheme 1) as per our previous work [27]and the curcumin content was estimated by Manjunath et al. 1991 [28]. BSR-01 turmeric variety was used in this method for better curcumin yield. The process is described as follows briefly. 5.0 g of turmeric dried powder weighed and taken in soxhlet apparatus with 250 ml of ethanol. The extraction process was carried out for 2-3 hour and the final curcumin extract absorbance was measured at 425 nm against alcohol blank and the curcumin percentage was calculated. The above ethanol residual extract was evaporated and dried then recrystallized by 95% ethanol for further uses.

Characterization

A pH meter (Metrohm, model 913, Switzerland) with combined glass electrode was used for pH measurements. A single pan analytical balance (Mettler Toledo, model ML 104, Switzerland) was employed for weighing the samples. The UV-Visible absorption spectra of the samples were measured on a Shimadzu UV-Vis V-530Aspectrophotometer in the range of 425nm.The nanoparticles were examined for FT-IR spectra analysis and recorded on a Jasco FT-IR/4100 spectrophotometer with $4cm^{-1}$ resolution in the range of 4000 to 400 cm^{-1}.X-ray measurement of the prepared solids was carried out using a P analytical X'Pert Powder X'Celerator Diffractometer (Netherland) in the range of 10^o to 80^o 2θ of 2^o min^{-1}. Scanning electron microscopy (SEM) images were recorded by using a JEOL Model JSM - 6390LV scanning electron microscope. High resolution transmission electron microscopy (HRTEM) was carried out using a 300 kV JEOL-3011 instrument with an ultrahigh resolution pole piece to determine the morphological changes.

Synthesis of copper nanoparticles (CNPs)
Double distilled water has been used throughout the synthesis process. 1mM aqueous solution of copper chloride was prepared and used for the synthesis of CNPs. The filtered stored pure lemon extract (10 ml) was taken in a beaker and freshly prepared copper solution (10 ml) was mixed with the extract with constant stirring for the reduction of copper ions. The reaction mixture was kept in the magnetic hot stirrer at 50-60^{0}C for a particular time to colour change from pale bluish yellow to pale yellow which denoted the metal ion reduction. Then freshly prepared curcumin extract (1mM) was added with above solution mixture for stabilizing the nanoparticle and the stirring was continued for about an hour. The solution colour was changed from yellow to yellowish brown slowly and finally a permanent dark brown colour which indicated the complete stabilized CNPs. P^H was maintained between 3-4 throughout the experiment for better result. The solution was centrifuged with washing several times to obtain the pure CNPs. The supernatant was decanted and kept in oven to dryness (Scheme 2).

Biological assay
The antibacterial and antifungal activities of the synthesized CNPs were tested against two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), two gram negative bacteria (*Escherichia coli* and *Staphylococcus bacillus*) and four funguses (*Candida albicans*, *Curvularia lunata, Aspergillus niger* and *Trichophyton simii*).

Antibacterial activity test
The disc diffusion method was used to screen the antimicrobial activity [29]. Stock cultures were maintained at 4^{o}C on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loopful of cells from the stock cultures to test tube of Muller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 hour at 37^{o}C and 25^{o}C respectively. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to 2.0×10^6 colony forming units (CFU/ml) for bacteria. The Muller Hinton Agar (MHA) plates were prepared by pouring 15 ml of molten media into sterile petri plates. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly and allowed to dry for 5 minutes. The concentration of sample at 40 mg/disc was loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the extract was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37^{o}C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

Antifungal activity test
The fungal strains were inoculated separately in sabouraud's dextrose broth for 6 hour and the suspensions were checked to provide approximately 10^5 CFU/ml. The agar well diffusion method was modified [30]. Sabouraud's dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in sabouraud's dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with the sample and solvent blanks (hydro alcohol, and hexane). Standard antibiotic (Fluconazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37^{o}C for 72 h. The diameters of zone of inhibition observed were measured.

RESULTS AND DISCUSSION

Synthesized CNPs are known in the solution by the colour changing from pale bluish green to pale yellow due to metal ion reduction and from yellow to dark brown colour due to capping of stabilizing agent. The colour change can be easily identified by the naked eye. It was clearly indicates that the formation of well reduced and stabilized CNPs.

UV-Vis spectra studies
One of the most convenient techniques for characterization of copper nanoparticles is UV-Vis spectroscopy. The synthesized turmeric curcumin (CR) was confirmed by the strong broad absorption peak at around 425 nm. This can be due either to an $n\rightarrow\pi^*$ transition or to a combination of $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ transitions which is shown in Figure 1. The absorption spectra of CNPs exhibit a broad band at around 585 nm corresponding to the absorption of copper nanoparticles. The important another one peak observed at 415 nm could be assigned to curcumin moiety which is shown in Figure 2.

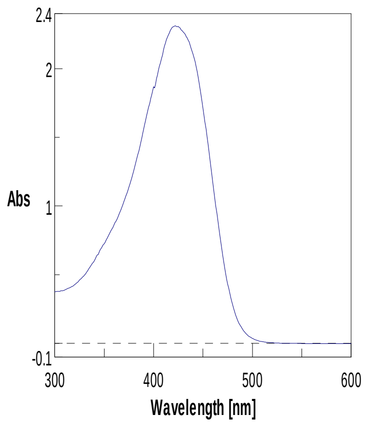


Fig.1 UV-Vis spectrum of CR

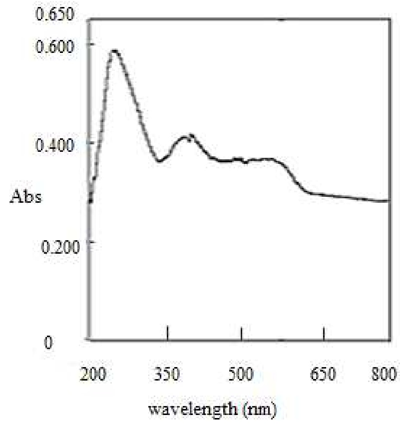


Fig.2 UV-Vis spectrum of CNPs

FT-IR spectra studies

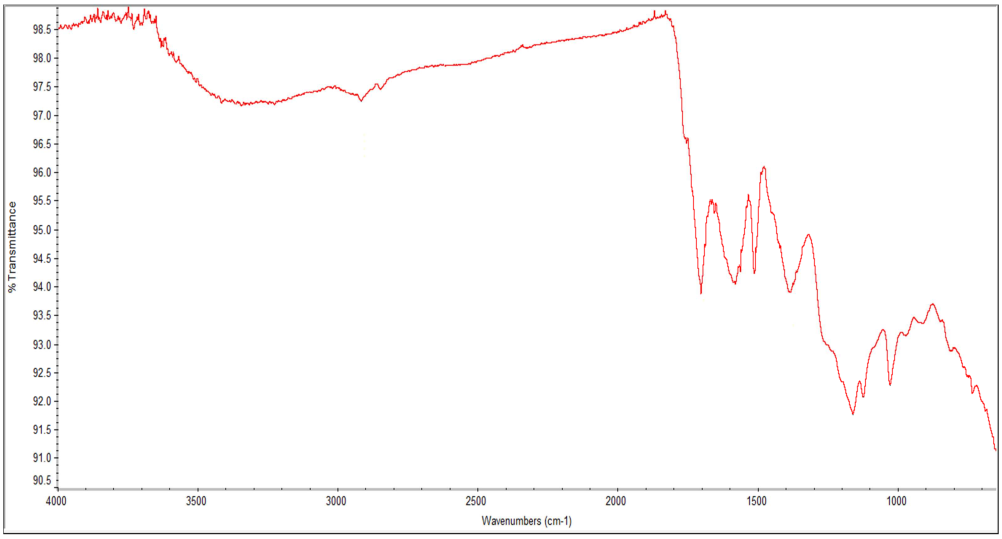


Fig. 3 FT-IR spectra of copper nanoparticles

FT-IR spectroscopy was used to investigate the interactions between different species and changes in chemical compositions of the mixtures. Figure 3 shows the FT-IR spectrum of curcumin stabilized copper nanoparticles. From the data obtained, phenolic OH showed its weak broad band in the range of 3500-3200 cm^{-1} which is assigned to phenolic (OH) group of curcumin moiety. The peak observed at 2913 cm^{-1}which can be assigned to the -OH stretching of water or ethanol present in the system. The C=O stretching of curcumin at 1625 cm^{-1} was shifted to a higher wave number at 1700 cm^{-1} due to interaction with copper nanoparticles. Three characteristic peaks in the range of 1520 – 1350 cm^{-1} conforms the aromatic unsaturation (C=C) of stabilized curcumin system. The (C-O) band presence was assigned by the peaks found at 1000-1250 cm^{-1}.

XRD studies

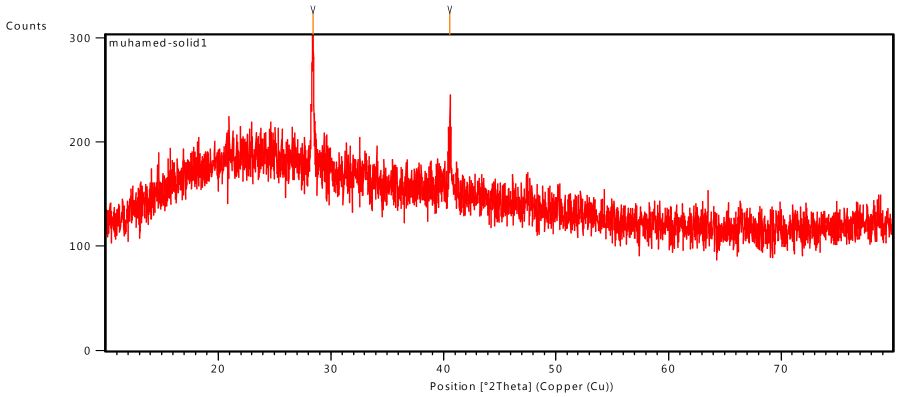


Fig. 4 XRD pattern of copper nanoparticles

The crystallite size of material, packing and morphology tested using XRD spectrometer with Cu source on the basis of powder diffraction method and the size was calculated by using Scherrer equation.

$$d= \frac{B\lambda}{\beta \cos \theta} \qquad (1)$$

where d is the average crystallite size of the phase under investigation, B is the Scherrer constant (0.89), λ is the wavelength of X-ray beam used, β is the full-width half maximum (FWHM) of diffraction and θ is the Bragg's angle. X ray diffraction studies of CNPs were investigated from the angle of 10^0 to 80^0. The intensity plotted against angle (2θ in degrees) showed the average crystallite size. The average particle size is calculated to be 45 nm by using Scherrer formula. The crystal structures of copper nanoparticles were shown in the Figure 4.

SEM studies

Morphology of synthesized copper nanoparticles was characterized by SEM analysis. The samples were placed in an evacuated chamber and scanned in a controlled pattern by an electron beam. Interaction of the electron beam with the specimen produces a variety of physical phenomenon that detected, were used to form images and provide information about the specimens. The SEM images of copper nanoparticles are shown in Figure 5. It can be view that the CNPs formed are well dispersed and evenly distributed in all direction. SEM images of those compounds had shown very clear that most of the particles are cubic and rod shaped morphology of material.

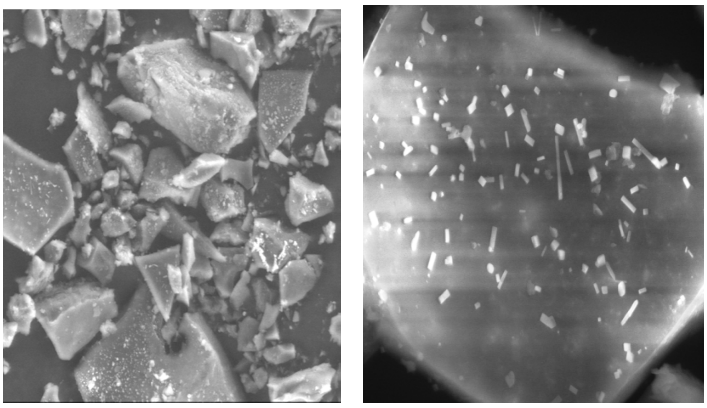


Fig. 5 SEM image of copper nanoparticles

TEM studies

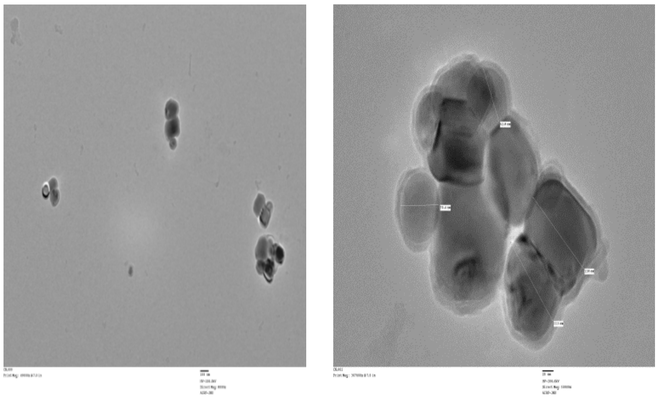


Fig. 6 TEM image of copper nanoparticles

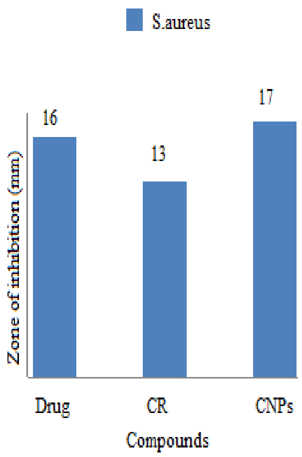
Figure 6 shows the TEM image of the copper nanoparticles. These images shows that the particles formed are of nearly spherical morphology. The nanoparticles are moderately dispersed and the average crystallite size of particles in the range of 60 to 100 nm and calculated in good agreement with the crystallite size value from XRD.

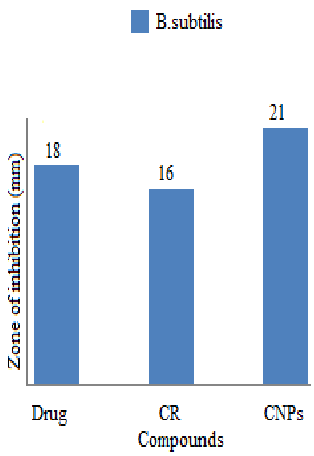
Antibacterial activity

The antibacterial activities of curcumin and curcumin stabilized copper nanoparticles against two gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram-negative bacteria (*Escherichia coli* and *Staphylococcus bacillus*) were evaluated and their activity was compared to a well-known commercial antibiotic Chloramphenicol. The results are reported in Table 1.

Table 1: Effect of curcumin and copper nanoparticles on antibacterial activity

Bacterial Species	Zone of inhibition diameter (mm sample^{-1})		
	Standard drug (C)	Curcumin(CR)	Copper nanoparticles (CNPs)
S. aureus	16	13	17
B.subtilis	18	16	21
E. coli	20	17	19
S.bacillus	21	15	18





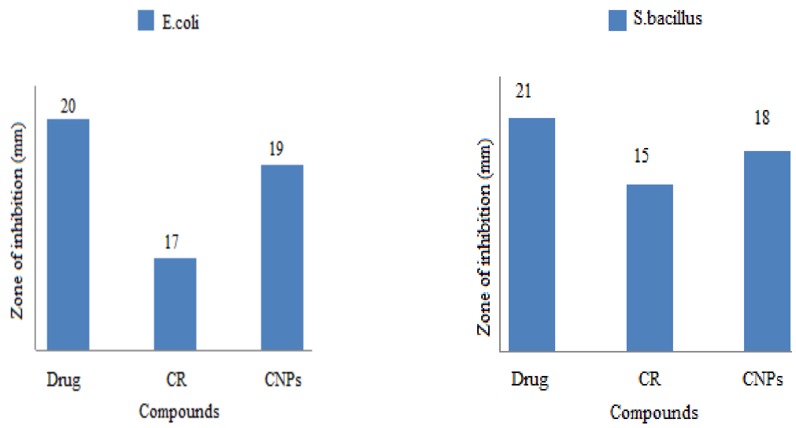


Fig. 7Antibacterial activity data of complexes

From these results, copper nanoparticles found to be more active against all the bacteria tested and curcumin was moderate active. Curcumin stabilized CNPs have a greater effect against *S.aureus*, *B.subtilis* and*E.coli* than the pure curcumin. Interestingly, the zone of inhibition observed for CNPs against *S.aureus* and *B.subtilis* showed the higher antibacterial action than the standard drug, Chloramphenicol. Moreover, it showed appreciable activity against *E.coli* and *S.bacillus* nearly similar to standard drug. Therefore the zone of inhibition exhibited by the CNPs was significantly very active. The results of antibacterial evaluation are summarized in Figure 7.

Antifungal activity

Curcumin and copper nanoparticles were determined for their antifungal activity against four fungal strains *Candida albicans*, *Curvularia lunata*, *Aspergillus niger* and *Trichophyton simii* and their activity was compared with standard antifungal drug fluconazole. The results were shown in the Table 2.

Table 2: Effect of Curcumin and copper nanoparticles on antifungal activity

Fungal Species	Zone of inhibition diameter (mm sample^{-1})		
	Standard drug (C)	Curcumin(CR)	Copper nanoparticles (CNPs)
C.albicans	19	16	21
C.lunata	17	14	16
A.niger	20	15	19
T.simii	17	16	15

From the results, it can be concluded that the activity of the CNPs were showed better inhibition compared to curcumin when tested against *C.albicans*, *C.lunata* and *A.niger* fungal species. However the activity of CNPs against *C.lunata* and *A.niger* was almost similar to the standard antibiotic, Fluconazole. Particularly, it showed higher inhibition activity against *C.albicans* than standard drug. Therefore it was indicated that the activity of curcumin stabilized CNPs was more appreciable than the activity of raw curcumin. The results of antifungal evaluation are summarized in Figure 8.

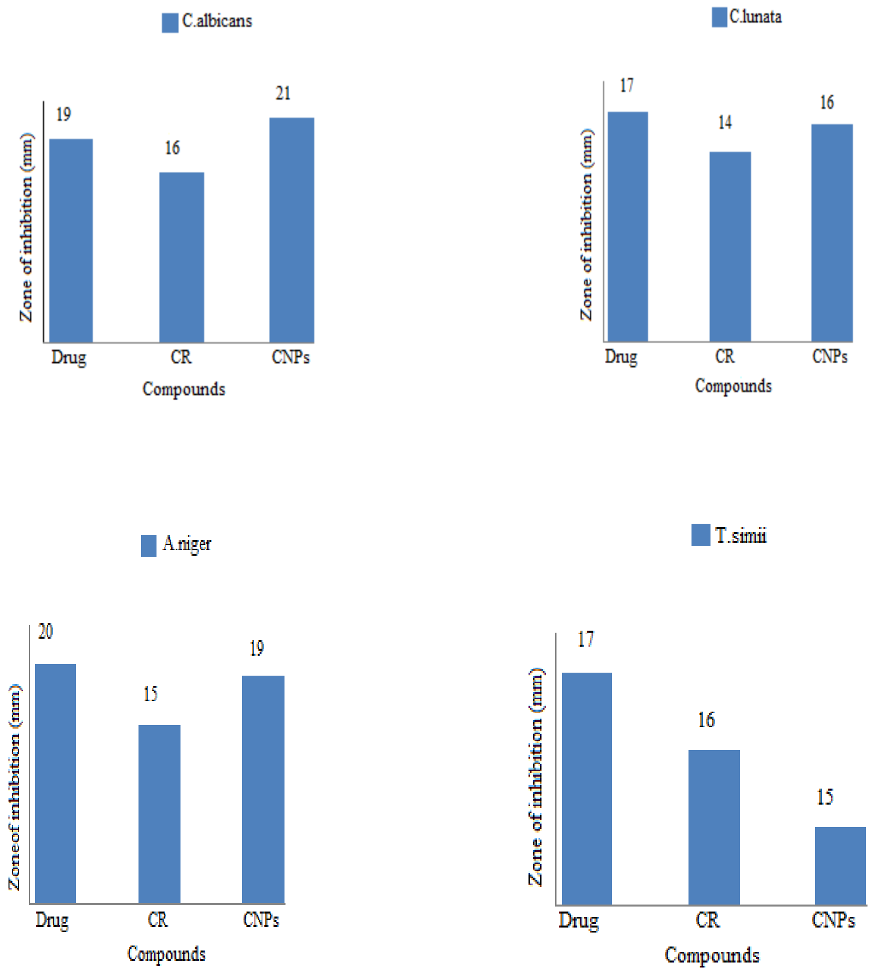


Fig. 8Antifungal activity data of complexes

CONCLUSION

We have reported a preparation method of copper nanoparticles through a simple and commercially low cost effective green chemistry route by using easily available natural lemon extract and turmeric curcumin as a reducing agent and stabilizing agent respectively. The synthesized nanoparticles are stable and smaller in particle size. Moreover, CNPs showed excellent antimicrobial activity than the standard drug against two bacterial species *S.aureus*, *B.subtilis* and a fungi *C.albicans* and also nearly similar inhibition activity was observed against *E.coli*, *S.bacillus* bacterial species and *C.lunata*, *A.niger* fungus. From this investigation it can be concluded that the synthesized copper nanoparticles are capable of significant antibacterial and antifungal activities which may possibly find in various medicinal applications.

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REFERENCES

[1] Abhishek Kaler;Navin Patel; Uttam Chand Banerjee.*Current Research & Information on Pharmaceuticals Sciences*, **2010**, 11(4), 68-71.
[2] MA Sastry;A Ahmad;MI Khan et al. Microbial nanoparticle production in Nanobiotechnology. Wiley-VCH publishers, Weinheim, **2004**; 126-135.
[3] A Alarifi; NM Deraz; S Shaban.*J of Alloy Compds.*, **2009**,486, 501-506.
[4] DP Chattopadhyay;BH Patel.*Indian Journal of Fiber & Textile Research*, **2009**, 34:368-373.
[5] RJ White;R Luque;VL Budarinet al.*Chemical Society Reviews*, **2009**, 38, 481-494. Doi:10.1039/b802654h.
[6] VJ Mohanraj; Y Chen.*Tropical journal of pharmaceutical research*, **2006**, 5, 561-573.
[7] NR Jana; ZL Wang; TK Sau et al.*Current Science*, **2000**, 79, 1367-1370.
[8] D Bhattacharya;G Rajinder.*Crit Rev Biotechnol.*, **2005**, 25:199-204.
[9] PT Anastas; JC Warner. Green chemistry: Theory and practice, Oxford University Press Publishers, New York, **1998**; 30.
[10] AS Matlack.*Introduction to green chemistry*, 3rd Edition, Marcel Decker Publishers, New York, **2001**; 3-7.
[11] P Mohanpuria; NK Rana; SK Yadav.*J. Nano Part Res.*,**2008**, 10, 507-517.
[12] A Eychmuller. *J. Phys Chem B.*, **2000**, 104, 6514-6528.
[13] OV Salata. *J. Nanobiotechnol.*,**2004**, 2,3-9.
[14] MH Kim;B Lim;EP Lee;Y Xia.*Journal of materials chemistry*, **2008**, 18, 4069-4073.
[15] M Satlavati Niasari; F Davar; N Mir.*Polyhedron*, **2008**, 27, 3514-3518.
[16] R Narayanan; El-Sayed.*M A J Am Chem Soc.*, **2003**, 125, 8340.
[17] N Cioffi;L Torsi;N Ditaranto;G Tantillo;L Ghibelli; L Sabbatini et al. *Chem Mater.*, **2005**, 17,5255-62.
[18] Z Li;D Lee;X Sheng; RE Cohen; MF Rubner.*Langmuir*,**2006**, 22, 9820-3.
[19] K Priyadarsini; D Maity; GH Naik; M Kumar; M Unnikrishnan; J Satav; H Mohan. *Free Radical Biology and Medicine*, **2003**, *35*, 475-484.
[20] A Kastori; M Chatzopoulou; K Dimas; C Kontogiorgis; A Patsilinakos; T Trangas; D Hadjipavlou-Litina. *European Journal of Medicinal Chemistry*,**2011**, *46*, 2722-2735.
[21] P Singh; V Kotia; D Ghosh; M Mohite; A Kumar; S Maji. *ACS Chem Neurosci..*, **2013**, *4*, 393–407.
[22] S Tharakan; T Inamoto; B Sung; B Aggarwal; A Kamat. *Biochemical Pharmacology*, **2010**, *79*, 218–228.
[23] M Notarbartolo; P Poma; D Perri; L Dusonchet; M Cervello; A D'Alessandro. *Cancer Letters*, **2005**, *224*, 53-65.
[24] P Anand; AP Kunnumakkara; RA Newman; BB Aggarwal. *Mol Pharmaceutics.*, **2007**, *4*, 807–818.
[25] D Jain. *J. Agric Food Chem.*, **2011**, *59*, 2056–2061.
[26] D Ghosh; TS Choudhury; S Ghosh; AK Mandal; S Sakar; A Ghosh; KD Saha; N Das. *Chemico-Biological Interactions*, **2012**, *195*, 206-214.
[27] M Muhamed haneefa; M Jayandran; B Anand; V Balasubramanian; S Muthumariappan.*International Journal of natural products research*, **2014**, 4(3),82-87.
[28] MN Manjunath;VD Sattigeri;KV Nagaraj. *Spice India*,**1991**, 12,7-9.
[29] RW Bauer; MDK Kirby; JC Sherris et al.*American Journal of Clinical Pathology*, **1966**, 45, 493-496.
[30] BPF Almeida Gomes; CCR Ferraz; ME Vianna.*Braz Dent J.*,**2002**, 13(3), 155-161.