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Green Synthesis, Characterization and Antimicrobial Activity Studies of Curcuminaniline Biofunctionalized Copper Oxide Nanoparticles

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Abstract

Background/Objectives: Biofunctionalization of nanoparticles is the recent and advanced technology in the fields of nanoscience and biotechnology which produces the environmentally benign and more efficient antimicrobial agents. Therefore, present study reported a simple, convenient and cost effective method of preparation of bioactive antimicrobial agents by the biofunctionalization process of copper oxide nanoparticles with curcuminaniline through a green process method. **Methods/Statistical analysis:** In this green process two important medicinal quality plant materials, i.e., lemon extract as a reducing agent and turmeric curcumin as stabilizing agent were used to prepare the copper oxide nanoparticles. On other hand, biomaterial curcumin was utilized to prepare the curcuminaniline for the functionalization with copper oxide nanoparticles. The synthesized curcuminaniline, copper oxide nanoparticles and biofunctionalized nanoparticles were characterized by UV- Vis, FT-IR, SEM and TEM techniques. The antibacterial activity of the samples were tested by disc diffusion method against two gram positive bacteria (S.aureus, B. subtilis), two gram negative bacteria (E.coli, S.typhi) and antifungal activity was tested by agar well diffusion method against four funguses (C.albicans, C.lunata, A.niger and T.simii). **Findings:** The size of synthesized copper oxide nanoparticles was in the ranges around 60-100 nm with dot and needle shaped morphology and biofunctionalized nanoparticles were about 100 nm with spherical shaped morphology. The antimicrobial activities of biofunctionalized copper oxide nanoparticles were observed significant inhibition activity than the non-functionalized nanoparticles and curcuminaniline against S.aureus, B.subtilis, C.lunata and A.niger species. Particularly, the results of antimicrobial activity showed higher efficiency than the standard drugs tested here. **Application/Improvements:** From this investigation, the green synthesized biofunctionalization method what we have suggested is exposed promise results in the antibacterial and antifungal activity tests. Moreover, biofunctionalized copper oxide nanoparticles are shown better performance in the resisitant of microorganisms than the non-functionalized CONPs and also modified curcumin material. This report helps further for the future scope in the preparation of effective antimicrobial agents.

Keywords: Antimicrobial Activity, Biofunctionalization, Copper Oxide, Curcuminaniline, Green Synthesis, Nanoparticles

1. Introduction

In recent years nanotechnology is being given considerable attention in a multiple way of emerging fields of science and technology over the last decades due to their interesting and potential applications in many areas of industry. Nanoparticles possess high surface to volume ratio due to its small size, which gives very distinctive features to nanoparticles and thus attracts the researchers expressively. Metal nanoparticles have been widely used

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for various applications like catalysis, optoelectronics, magnetic, thermal, sensors, fine chemical synthesis, solar energy conversion and medicine etc.[1-3]. Nanomaterials are the leading in the various fields of science, however there is an emergent need to develop environmentally benevolent nanoparticles synthesis routes, which can be proceeded by biological method instead of using toxic chemicals. Nanotechnology is a revolutionary field just at its onset, the trend in the next decades being its integration with the green chemistry approach. The green synthesis method plays a significant role on the effective consumptions of nanoparticles and also green synthesis routes are good competent over the physical and chemical techniques[4,5].

Metal nanoparticles containing magnesium oxide, silver, iron, zinc and nickel oxides are of great interest due to its well exhibited antimicrobial activities. Copper nanoparticles have concerned considerable attention because they are very reactive and their high surface-to-volume ratio helps to interact with bacterial surface effectively[6,7]. Moreover its cheap, high yields and short reaction times under normal reaction conditions are the advantages in green-nano preparation. The copper nanoparticles were prepared through various green methodologies and proved its superior antimicrobial activity against various bacterial and fungal strains from many researches[8-10].

Biofunctionalization of nanoparticles can provide them with good biocompatibility for the immobilization of biomolecules, tissues or cells and high specificity for biological recognition which led to produce a considerable effect in biological systems. Therefore, seeking suitable methods for the functionalization of nanomaterials with bioactive materials has attracted considerable attention[11,12].

Curcuminoids are dietary polyphenolic compounds that are of interest from the use of turmeric plants as herbal medicines. The pharmacological activities of curcuminoids have been widely investigated, and the antioxidative, anti-inflammatory, and antimicrobial properties of these molecules are well established[13-15]. The most attractive feature of curcuminoids is the lack of significant toxicity, as shown in several animal and human studies. Thus, the various pharmacological effects and excellent safety profile make these molecules of interest as attractive biomaterial for the biofunctionalization[16,17]. However, various researches proved modified curcumin shown highly improved biological activities in wide fields[18].

From literature various researches proved that plants are known to possess several therapeutic compounds and due to its significance plants have been explored constantly in the wide range of fields such as pharmaceutical, agricultural, industrial etc[19]. Lemons are a rich source of citric acid and ascorbic acid, easily available material and also known for its water softening properties. This extracts act as a good metal reductant in the nanoparticle preparation according to some research works[20,21].

Based upon the above discussion this study was investigated to find the highly active microbial agents in the different way of synthesis by the bio functionalization of schiff base ligand with copper oxide nanoparticles. Firstly, the raw material curcumin was isolated from its source, turmeric plant by using solvent extraction method and the isolated pure curcumin was used to prepare bioactive schiff base ligand curcuminaniline. In other hand, copper oxide nanoparticles were prepared by reducing copper chloride with the help of lemon extract and turmeric curcumin. Finally, schiff base was functionalized with synthesized copper oxide nanoparticles separately to get improved bioactive nanoparticles. The bio functionalized copper oxide nanoparticles were analyzed for antimicrobial activity against various gram positive and negative bacterial species and some fungal species and found inhibition results were more appreciable.

2. Materials and Methods

All the chemicals and solvents used were of analytical reagent grade and obtained from Merck (India) Ltd. The turmeric sample (BSR-01) was received from Agricultural College and Research Institute, Madurai, India.

2.1 Collection of Extracts

Lemon fruits were purchased from the local markets and washed well, cut into pieces and squeezed well to make 5 to 10 ml of pure lemon extract. The extract was filtered by Whatman's No. 1 filter paper and the filtrate was stored for further uses.

Literature studies have shown that the extraction of curcumin from turmeric could be done in several different ways. One of the best way to extract the compounds is to use a soxhlet apparatus and it implies that the extraction and filtration of the product is done in the same step. According to the method of Manjunath et al.[22] the weighed turmeric dried powder (5g) has been taken in the soxhlet apparatus by following with 250 ml of ethanol. The extraction process was carried out for 2-3 hour and the final curcumin extract was evaporated and dried for further uses.

2.2 Synthesis of Curcuminaniline (CA)

The synthesis process was carried out as per our previous work[18]. Curcumin (10 mM) was dissolved in ethanol and reacted with aniline (10 mM) with constant stirring. The orange color mixture was obtained and refluxed at 50°C for 6 hours in mild acidic condition. Then the solution was filtered and washed well with double distilled water and kept in vacuum oven at 100°C for 1 hr. The obtained orange powdered curcuminaniline was stored in a desiccator over silica gel.

2.3 Synthesis of Copper Oxide Nanoparticles (CONPs)

A aqueous solution of copper chloride (1mM) was used for the synthesis of CONPs. The lemon extract (10 ml) was mixed with freshly prepared copper solution (10 ml) with constant stirring and kept in the magnetic hot stirrer at 50-60°C for a particular time. The solution color change was observed from pale bluish green to pale green which indicated that metal ion reduction. Then curcumin extract (1mM) was mixed copper mixture with constant stirring for about an hour for stabilizing the nanoparticle. The solution color was changed from yellow to yellowish brown slowly and finally a permanent dark brown color was obtained which indicated the complete stabilized CONPs. For better result, the reaction pH was maintained in the range of 3 to 4 throughout the experiment. The final solution was centrifuged and washed several times to obtain pure CONPs. The upper layer was decanted and kept in oven to dryness.

2.4 Biofunctionalization of Copper Oxide Nanoparticles (BCONPs)

In this scheme bio functionalization of curcuminaniline with copper oxide nanoparticles were carried out by the normal interaction. 1 mM solution of CONPs (10 ml) added with 1mM of curcuminaniline (10 ml) with constant stirring at 60°C temperature for an hour. The reddish brown color was obtained and the reaction was continued for an hour. Finally, the brown color solution was obtained which denoted the strong functionalization of schiff base with copper nanoparticles.

2.5 Characterization

The UV-Visible absorption spectra of the samples were measured on a Shimadzu UV-Vis V-530A spectrophotometer in the range of 300 to 900 nm. The FT-IR spectra analysis was on a Jasso FT-IR/4100 spectrophotometer with 4 cm^{-1} resolution in the range of 4000 to 500 cm^{-1}. SEM analysis was carried out by using JEOL Model JSM - 6390LV scanning electron microscope. High Resolution Transmission Electron Microscopy (HRTEM) was carried out using a 300 KV JEOL-3011 instrument to determine the morphological changes.

2.6 Biological Assay

The antibacterial activity of the samples were tested by disc diffusion method against two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), two gram negative bacteria (*Escherichia coli* and *Salmonella typhi*) and antifungal activity was carried out by agar well diffusion method against four funguses (*Candida albicans*, *Curvularia lunata*, *Aspergillus niger* and *Trichophyton simii*).

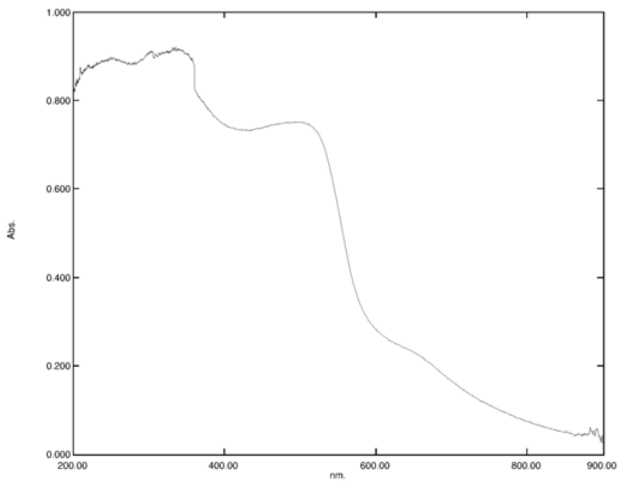
For disc diffusion method[23], stock cultures incubated in nutrient agar were transferred to Muller-Hinton Broth (MHB) test tube and kept for 24 hours at 37°C. Then the cultures were diluted with fresh Muller-Hinton Broth to get 2.0×10^6 CFU/ml for bacteria. The Muller Hinton Agar (MHA) plates were prepared by applying 15 ml of molten media into sterile petri plates. The sample was loaded placed on the surface of the cultured agar plates and incubated at 37°C for 24 hours. The inhibition zones observed around the disc were measured and the results were compared with standard antibiotic, Chloramphenicol.

For agar well diffusion method[24], the fungal strains were suspended in sabouraud's dextrose broth for 6 hours to give concentration 10^5 CFU/ml and then inoculated with the culture medium. The sample and solvent blanks (hydro alcohol and hexane) were filled in the wells which were punched into the agar with 8mm diameter. Standard antibiotic, Fluconazole (concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37°C for 72 hours. The diameters of zone of inhibition observed were measured.

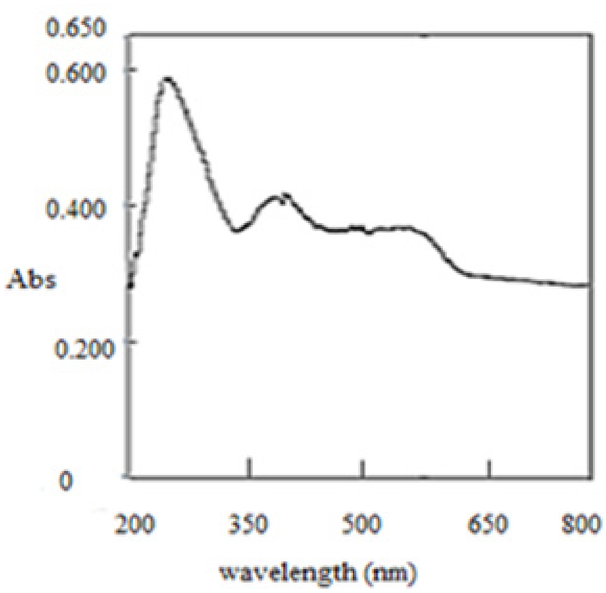
3. Results and Discussion

3.1 UV-Vis Studies

The UV-Vis absorption of Curcuminaniline (CA) displayed mainly two bands in the range of 250-500 nm. The first band showed absorption bands in the range of 340 nm which could be assigned to π - π^* and n - π^* transitions in the aromatic ring or azomethine (C = N) while the second band 481 nm was assigned to curcumin moiety (Figure 1a).



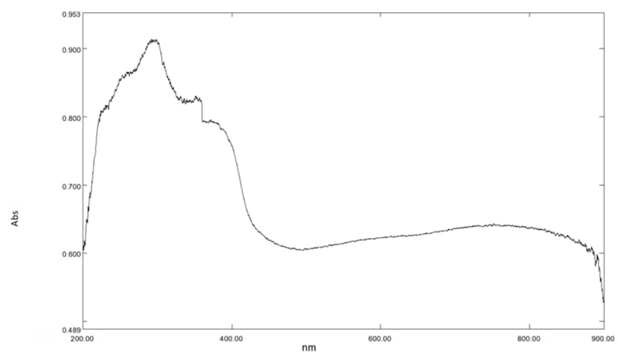
(a)



(b)

Synthesized Copper Oxide Nanoparticles (CONPs) are known in the solution by the color changing from pale bluish green to pale green color due to Cu metal ion reduction and the final dark brown color obtained due to capping of stabilizing agent, curcumin. The absorption spectra of CONPs exhibit a band at around 248 nm corresponding to the absorption of copper nanoparticles and the other small broad peak observed at 585 nm is also indicates copper oxide nanoparticles. However, these differences may be attributed to the differences in the methods used (chemical or biological) for the synthesis of the nanoparticles as was reported by Abboud et al.[25] and Phuo and Chyu[26]. The important another one peak observed at 415 nm could be assigned to curcumin moiety which is shown in Figure 1b.

Figure 1c shows a UV spectrum of Bio Functionalized Copper Oxide Nanoparticles (BCONPs).



(c)

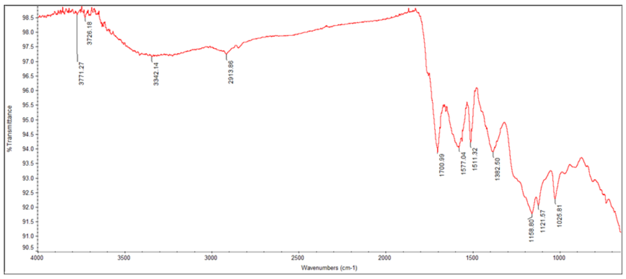
Figure 1. **(a).** UV-Vis spectrum of CA. **(b).** UV-Vis spectrum of CONPs. **(c).** UV-Vis spectrum of BCONPs.

The absorption spectra of BCONPs exhibited a sharp peak at around 298 nm which could be assigned to aromatic ring or azomethine (C = N) and the other peak observed at 351 nm corresponding to the absorption of copper oxide nanoparticles which was shifted from 248 nm due to the functionalization with azomethine compound.

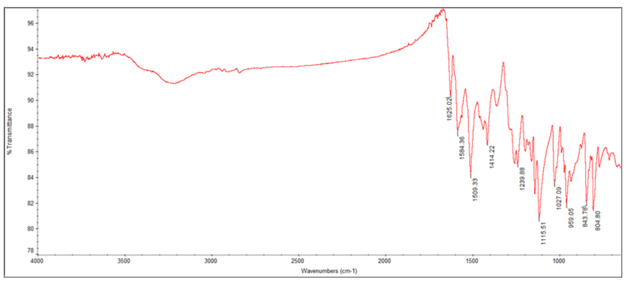
3.2 FT-IR Spectra Studies

Figure 2a shows the FT-IR spectrum of curcuminaniline. From the data obtained, the weak broad band observed at 3284 cm^{-1} which is assigned to phenolic –OH group of curcuminaniline. The weak and broadness of these peak could be observed mainly due to intra-molecular hydrogen bonding between the enolic

–OH group with azomethine nitrogen atom. The strong peak exhibited in the region of 1632 cm^{-1} was confirms the C = C unsaturation remains outside the ring and the (C-O) band presence was confirmed by the peak found at 1000–1250 cm^{-1}. The peak due to the carboxyl group (C = O) was observed at around 1575 cm^{-1}, the lower frequency shifting is suggesting that the bond breaking of oxygen with carbon and formation of azomethine C = N stretching vibration. Three characteristic peaks in the range of 1520–1350 cm^{-1} confirms the aromatic unsaturation (C = C) of curcumin.

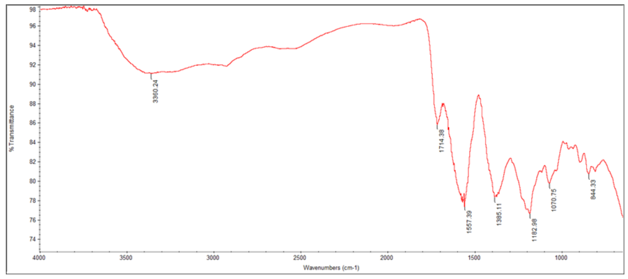


(a)



(b)

Figure 2b shows the FT-IR spectrum of curcumin stabilized copper nanoparticles. From the data obtained, the weak broad band observed in the range of 3500-3200 cm^{-1} which is assigned to ph-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} confirms 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}.

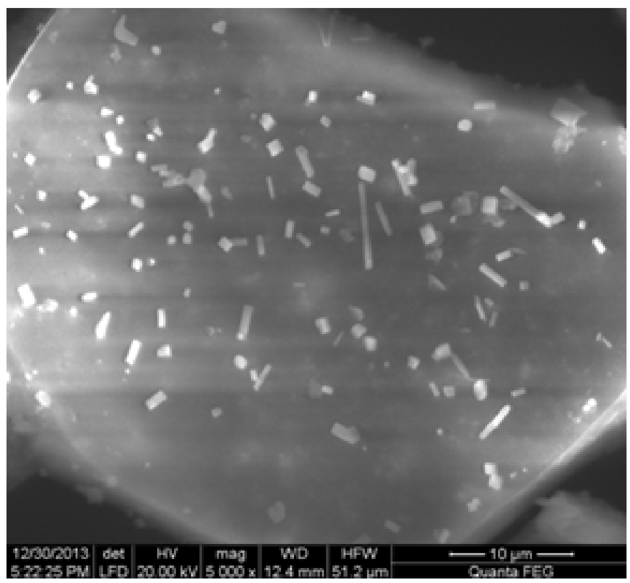


(c)

Figure 2. **(a)**. IR spectra of CA. **(b)**. IR spectra of CONPs. **(c)**. IR spectra of BCONPs.

Figure 2c shows the IR spectra of curcuminaniline functionalized copper oxide nanoparticles. The phenolic OH present in the curcumin showed its weak broad band in the range of 3360 cm^{-1}. The small sharp peak observed at 1714 cm^{-1} was assigned to C = O stretching of curcumin and the important sharp peak exhibited at 1557 cm^{-1} was assigned to curcuminaniline azomethine compound which was interacted with copper oxide nanoparticles and reached higher wave number. The characteristic peak in the range of 1385 cm^{-1} conforms the aromatic unsaturation (C = C) of curcuminaniline system. The (C-O) band presence was assigned by the peaks found at 1000-1250 cm^{-1}.

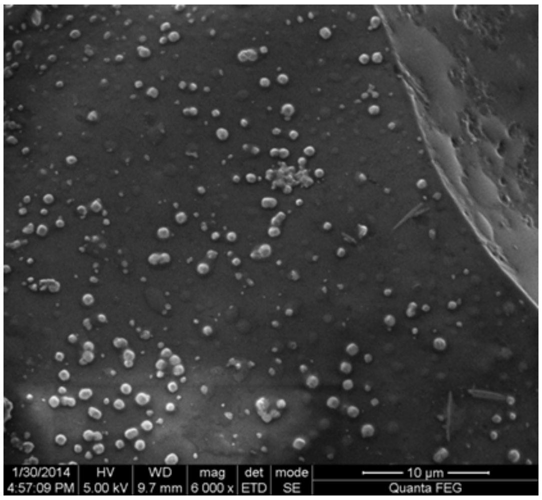
3.3 SEM Studies



(a)

Morphology of synthesized copper nanoparticles was characterized by SEM analysis. The SEM images of copper oxide nanoparticles and bio functionalized nanoparticles are shown in Figure 3a. It can be view that the CONPs formed are well dispersed and evenly distributed in all direction and SEM image had shown very clear that most of the particles are dot and needle shaped morphology of material.

In the case of biofunctionalized copper oxide nanoparticles, the image exhibits a clear dot shaped morphology with slight agglomeration due to the nanoparticles oxidation (Figure 3b).

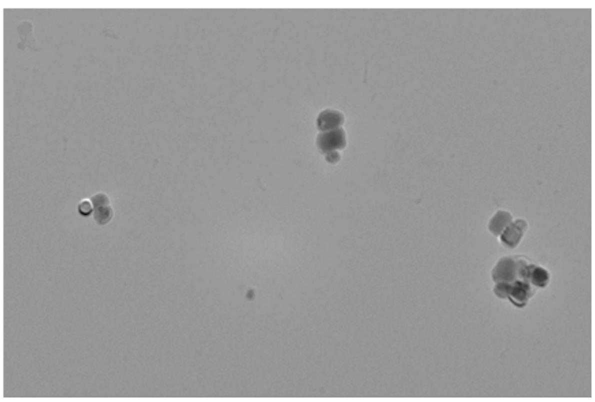


(b)

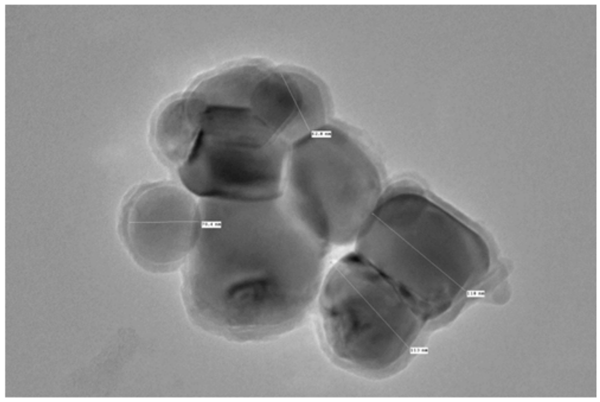
Figure 3. **(a)**. SEM images of CONPs. **(b)**. SEM images of BCONPs.

3.4 TEM Studies

Figure 4a and Figure 4b shows the TEM images of the copper oxide nanoparticles. These images shows that the particles formed are of nearly dot shaped spherical morphology.

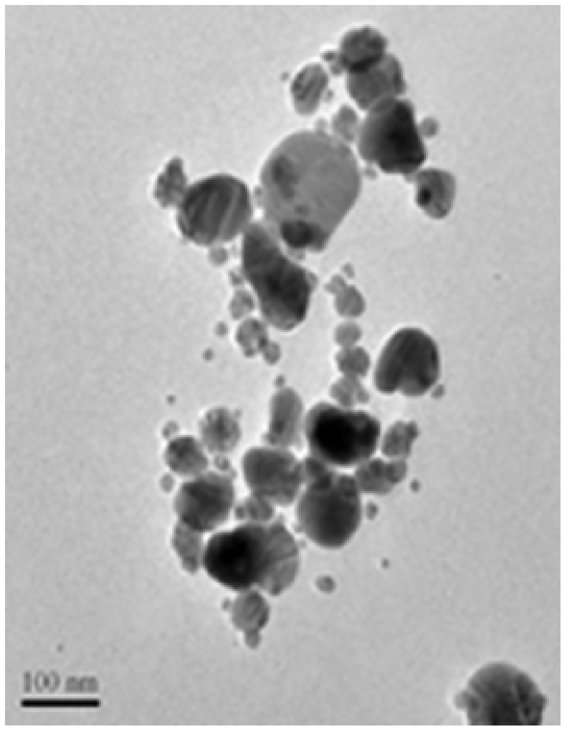


(a)



(b)

The nanoparticles are moderately dispersed and the average crystallite size of particles in the range of 60 to 100 nm. Figure 4c exhibits the TEM image of biofunctionalized copper oxide nanoparticles.



(c)

Figure 4. **(a)**. TEM image of CONPs. **(b)**. TEM image of CONPs. **(c)**. TEM image of BCONPs.

It can be view that the BCONPs formed are well dispersed spherical morphology and the average crystallite size of particles in the range of around 100 nm. From this we can conclude that the functionalization of curcuminaniline with copper oxide nanoparticles was carried out well.

3.5 Antibacterial Activity

The antibacterial activities of curcuminaniline, CONPs and BCONPs against two gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*) were evaluated and their activity was compared to a well-known commercial antibiotic Chloramphenicol. The results are reported in Table 1. From the antibacterial inhibition results, there was an acceptable improvement has been exhibited by the bio functionalized copper nanoparticles when compared with non-functionalized nanoparticles and curcuminaniline. Copper oxide nanoparticles observed moderate activity against all bacterial strains over all. The Schiff base compound showed good activity against *S.aureus* only. But while curcuminaniline functionalized copper oxide nanoparticles displayed higher inhibition activity than schiff base and copper oxide nanoparticles against *S.aureus*, *B.subtilis* and *S.typhi* bacterial strains. Especially, it showed higher activity than standard drug chloramphenicol against *S.aureus* and *B.subtilis* strains. Therefore, we can conclude that the bio functionalized copper oxide nanoparticles were shown appreciable antibacterial activity due to the functionalization when compared with schiff base and non-functionalized nanoparticles as well as standard drug.

3.6 Antifungal Activity

Curcuminaniline, CONPs and BCONPs 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. From the antifungal inhibition results, we found there was an increment in the inhibition activity observed by the biofunctionalized copper nanoparticles than non-functionalized nanoparticles and curcuminaniline. Copper oxide nanoparticles showed good activity against *C.albicans*, *A.niger* fungal strains. The schiff base compound showed good activity against *C.albicans*, *C.lunata* funguses. But in the case of curcuminaniline functionalized copper oxide nanoparticles exposed higher inhibition activity than schiff base and copper oxide nanoparticles against *C.lunata* and *A.niger* fungal strains. Interestingly, it showed significant activity similar to standard drug fluconazole against *C.lunata*, *A.niger* and *T.simii* strains. Therefore, we found that biofunctionalized copper oxide nanoparticles were shown considerable antigungal activity when compared with schiff base and non-functionalized nanoparticles as well as standard drug.

4. Conclusion

In summary, we have synthesized bioactive schiff base functionalized copper oxide nanoparticles by reacting curcuminaniline derived from curcumin with copper oxide nanoparticles. The copper nanoparticles were prepared using natural lemon extract as a reducer and

Table 1. Antibacterial activity evaluation

Bacterial Species	Zone of inhibition diameter (mm sample^{-1})			
	Standard drug (C)	Curcuminaniline (CA)	Copper oxide nanoparticles (CONPs)	Biofunctionalized Copper oxide nanoparticles (BCONPs)
S. aureus	16	18	17	18
B.subtilis	18	14	17	19
E. coli	20	15	19	17
S.typhi	21	17	18	18

Table 2. Antifungal activity evaluation

Fungal Species	Zone of inhibition diameter (mm sample^{-1})			
	Standard drug (C)	Curcuminaniline(-CA)	Copper oxide nanopar-ticles (CONPs)	Biofunctionalized Copper oxide nanoparticles (BCONPs)
C.albicans	19	19	21	14
C.lunata	17	18	16	17
A.niger	20	16	19	20
T.simii	17	13	15	16

curcumin as a stabilizer. This process was completely undertaken through easy, convenient, green synthesis method. The synthesized copper oxide nanoparticles and bio functionalized nanoparticles morphology and size were investigated by SEM and TEM analysis. The morphology study has been revealed the particle size of CONPs was around 60-100 nm and functionalized nanoparticles was 100 nm with spherical shaped morphology. The antimicrobial activity was also investigated against two gram positive bacteria and two gram negative bacteria and four funguses. From the inhibition zone results, synthesized CONPs were showed moderate inhibition activity against over all species. But the biofunctionalized nanoparticles were showed better activity than the non-functionalized nanoparticles as well as standard drug against *S.aureus*, *B.subtilis, C.lunata* and *A.niger* species. Thus our findings report the biofunctionalized copper oxide nanoparticles curcuminaniline synthesized from the above proposed green method are shown promise results in the view of pharmaceutical and therapeutic applications.

5. Acknowledgements

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6. References

1. Lakshmi YS, Banu F, Gopalakrishnan S. Green synthesis and antimicrobial activity of silver nanoparticles from the medicinal plant Vernonia Amygdalina. Indian Journal of NanoScience. 2013 Feb; 1(2):32–5.
2. Zelati A, Amirabadizadeh A, Kompany A, Salamati H, Sonier J. Manufacture and characterization of Dy_2O_3 nanoparticles via X-ray diffraction, TEM and photoluminescence. Indian Journal of Science and Technology. 2013 Dec; 6(12):5552–8.
3. Jovanova B, Raicki RS, Panovska TK. Risk assessment strategies, toxicity testing and policy aspects of nanomaterials. Indian Journal of Nanoscience. 2015 June; 3(1):19–29.
4. Braga D, Grepioni F, Maini L, Brescello R, Cotarca L. Simple and quantitative mechanochemical preparation of the first zinc and copper complexes of the neurolepticdrug gabapentin. Cryst Eng Comm. 2008; 10(5):469–71.
5. Lakshmi YS, Mala D, Gopalakrishnan S, Banu F, Brindha V, Gajendran N. Green synthesis and characterisation of Silver nanoparticles from the medicinal plant Pithecellobium dulce. Indian Journal of Nano Science. 2014 Aug; 2(8):1–9.
6. Krishna Shyla K, Natarajan N. Customizing Zinc Oxide, Silver and Titanium Dioxide nanoparticles for enhancing groundnut seed quality. Indian Journal of Science and Technology. 2014 Sep; 7(9):1376–81.
7. Theivasanthi T, Alagar M. Studies of copper nanoparticles effects on microorganisms. Annals Bio Res. 2011; 2(3):368–73.
8. Gopalakrishnan S, Philip Kaupa S, Lakshmi YS, Banu F. Antimicrobial activity of synthesized silver nanoparticles and phytochemical screening of the aqueous extract of Antiaris toxicaria. Indian Journal of Drugs and Diseases. 2015 July; 4(1):1–4.
9. Cioffi N, Torsi L, Ditaranto N, Tantillo G, Ghibelli L, Sabbatini L, Bleve-Zacheo T, D'Alessio M, Zambonin PG, Traversa E. Copper nanoparticle/polymer composites with antifungal and bacteriostatic properties. Chem Mater. 2005; 17(21):5255–62.
10. Li Z, Lee D, Sheng X, Cohen RE, Rubner MF. Two-level antibacterial coating with both release-killing and contact-killing capabilities. Langmuir. 2006; 22(24):9820–3.
11. Kumar CSSR. Biofunctionalization of Nanomaterials. Wiley-VCH; 2009; 24:398–9.
12. Yang WR, Ratinac KR, Ringer SP. Carbon nanomaterials in biosensors: should you use nanotubes or graphene?. Angewandte Chemie International Edition. 2010 Feb; 49(12):2114–38.
13. Buadonpri W, Wichitnithad W, Rojsitthisak P, Towiwat P. Synthetic curcumin inhibits carrageenan-induced paw edema in rats. Journal of Health Research. 2009; 23(1):11–6.
14. Abe Y, Hashimoto S, Horie T. Curcumin inhibition of inflammatory cytokine production by human peripheral blood monocytes and alveolar macrophages. Pharmacological Research. 1999 Jan; 39(1):41–7.
15. Lantz RC, Chen GJ, Solyom AM, Jolad SD, Timmermann BN. The effect of turmeric extracts on inflammatory mediator production. Phytomedicine. 2005 Jun; 12(6-7):445–2.
16. Eigner D, Scholz D. Ferula asa-foetida and curcuma longa in traditional medical treatment and diet in Nepal. Journal of Ethnopharmacology. 1999 Oct; 67(1):1–6.
17. Wahlstrom B, Blennow G. A study on the fate of curcumin in the rat. Acta Pharmacol Toxicol. 1978 Aug; 43(2):86–92.
18. Jayandran M, Haneefa MM, Balasubramanian V. Synthesis, characterization and biological activities of turmeric curcumin schiff base complex. International Journal of Chemical and Natural Science. 2014; 2(5):157–63.
19. Sharma VK, Yagard RA, Lin Y. Silver nanoparticles: Green synthesis and their antimicrobial activities. Advances in Colloid and Interface Science. 2009 Jan; 145(1-2):83–96.
20. Garcia OB, Castillo J, Marin JR, Ortuno A, Rio DJA. Uses and properties of Citrus flavonoids. Journal of Agricultural and Food Chemistry. 1997; 45(12):4505–15.

21. Vinson JA, Su X, Zubik L, Bose P. Phenol antioxidant quantity and quality in foods: fruits. J Agric Food Chem. 2001 Nov; 49(11): 5315–21.
22. Manjunath MN, Sattigeri VD, Nagaraj KV. Curcumin in turmeric. Spice India. 1991; 4(3):7–9.
23. Bauer RW, Kirby MD, Sherris JC, Turck M. Antibiotic susceptibility testing by a standard Bsingle disc method. American Journal of Clinical Pathology. 1966 Apr; 45(4):493–6.
24. Gomes ABP, Ferraz CC, Vianna ME. In vitro antimicrobial activity of Ca $(OH)_2$ pastes and their vehicles against selected microorganisms. Brazilian Dental Journal. 2006 Oct; 102(4):544–50.
25. Abboud Y, Saffaj T, Chagraoui A, El-Bouari A, Brouzi K, Biosynthesis TO. Characterization and antimicrobial activity of copper oxide nanoparticles (CONPs) produced using brown alga extract Bifurcaria bifurcata. Applied Nanoscience. 2014 June; 4(5):571–6.
26. Phuo TX, Chyu MK. Synthesis and characterization of copper oxide nanoparticles Synthesis and characterization of nanocomposites using the nanoscale laser soldering in liquid technique. Journal of Nanoscience and Nanotechnology. 2013; 1(1):101.