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Green synthesis of copper nanoparticle from *Cordia dichotoma* leaf extract and its antimicrobial activity

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Abstract
The development of nanotechnology is making the interest of researchers towards synthesis of nanoparticles for the bio application. Here copper nanoparticles were synthesized using *Cordia dichotoma* plant leaf extract. The leaf extract acts as both reducing and capping agent. The synthesized copper nanoparticles were confirmed by the change of colour after addition of leaf extract into the Copper Sulfate solution. The biosynthesized CuNPs were characterized by using UV-Vis analysis, Fourier Transform Infrared analysis (FTIR), X-ray diffraction analysis (XRD), Scanning Electron Microscopy (SEM), Energy Dispersive X-Ray analysis (EDX) and Transmission Electron Microscopy (TEM) analysis. The synthesized CuNPs were in cubical structure with the particle size in the range between 50-100 nm. The antibacterial test conducted on E. coli, Pseudomonas aeruginosa and Staphylococcus aureus, showed good zone of inhibitions 9.60, 9.45 and 9.50 mm, respectively, proving potentiality of g-Cu NPs as a remedy for infectious diseases caused by tested pathogens. Antibacterial and antifungal activities were performed by disk diffusion method. Cu NPs showed remarkable activity against bacterial strains.

Keywords: copper oxide nanoparticles, *Cordia dichotoma*, antibacterial activity

Introduction

Nanotechnology plays a very important role in modern research [1] it is the most capable technology that can be applied almost all fields such as pharmaceutical, electronics, health care, food and feed, biomedical science, drug and gene delivery, chemical industry, energy science, cosmetics, environmental health, mechanics and space industries. It has also been utilized for the treatments of infection [2], cancer allergy, diabetes6 and inflammation. Green chemistry is an implementation, development, design of chemical. In recent years nanotechnology has provided extensive research with emergence in Physics, Chemistry, Engineering, Biotechnology, Food-technology, Medical sciences and forming impact on all forms of life with an important aspect due to its innumerable applications (Baker and Satish 2012) [3]. The prefix nano means a billionth (1×10-9) and nanotechnology is a field of science which deals with production and manipulation of metal nanoparticles which are of interest because of their unique optical, electronic and magnetic properties. The nanoparticles of transition metals are an important class of semiconductors, which have expressed significant advances owing to wide range of applications in the field of electronics, sensors, automobiles, nano-fabrics, bio-medical, agriculture, bio-engineering, medicines, and other areas (Ramgir *et al*., 2013) [4]. A wide range of copper nanoparticles can be produced using chemical methods and physical methods.

These chemical or physical methods for the synthesis of copper nanoparticles are suffering with various limitations such as expensive reagents, generation of hazardous toxic chemicals, and tedious process to isolate nanoparticles etc. Hence, upsurge the researchers to develop new methods for the synthesis of nanoparticles which should be required inexpensive reagent, develop safe as well as less drastic reaction condition and eco-friendly alternative approaches. Synthesis using bioorganisms is compatible with the green chemistry principles. "Green synthesis" of nanoparticles makes use of environmental friendly, non-toxic and safe reagents (Salam *et al*., 2012) [5]. If the exact mechanism of biological synthesis is explained, it could offer an extra advantage over the chemical methods by means of higher productivity and lower cost. In biosynthesis of nanoparticles the oxidation/reduction is the main reaction that occurs during the production of nanoparticles. Metal compounds usually reduce into their respective nanoparticles because of proteins, enzymes and the phytochemicals with antioxidant or reducing properties present in plant extracts.

Synthesis of Cu2O nanoparticles using *Cordia dichotoma* leaf extract was reported and water-soluble carbohydrates present in the plant materials were mainly responsible for the reduction of copper ions to nano-sized Cu2O particles. By taking Escherichia coli as a model for Gram negative bacteria, which always causes a variety of suppurative infections and toxinoses in humans, as a model bio-particle, the negative bio-effect of nano-Cu2O on E. coli, Staphylococcus aureus and Pseudomonas aeruginos was evaluated by disc diffusion method (Gopala krishnan *et al*., 2012) [6]. A novel biological approach for the formation of copper nanoparticles using Clove extract has been done. X-Ray diffraction pattern reveals the formation of Cu nanoparticles, which shows crystalline structure. Transmission electron microscopy suggested particles size and shape in the range of 5-40 nm. Scanning electron microscopy image reveals that the particles are of spherical and granular nature (Subhankari and Nayak 2013) [7]. Analyzer and SEM. The prepared particles were elementally analyzed by EDS and FTIR. Copper nanoparticles were biologically synthesized using Magnolia leaf extract as reducing agent. UV-vis spectroscopy, TEM and SEM was used to monitor the quantitative formation of copper nanoparticles which indicated that they ranged in size from 40 to 100 nm and copper nanoparticles shows antibacterial activity against Escherichia coli bacteria (Lee *et al*., 2011) [8].

Hence the present study was carried out to synthesize and characterize the copper nanoparticles using *Cordia dichotoma* leaf extract at room temperature and its antibacterial activity against pathogenic bacteria's.

Materials and method

Materials

All the reagents used in this experiment were obtained from Sigma-Aldrich Chemicals, India. Double-distilled water was utilized for this process. Filtration was established using Whatman No. 1 filter papers. Glasswares used for the complete reactions were washed well, rinsed with double-distilled water and dried in hot air oven.

Collection of plant leaf

C. dichotoma leaf was collected from Thanjavur district. The collected leaf was tightly packed with a polyethene bag and then transfer to the laboratory. Then, it was washed with distilled water twice and kept under room temperature for 2 weeks in the dark condition. Then, it was made into powder using blender.

Preparation of leaf extracts

Plant leaf extract of *C. dichotoma* was prepared by first washing the leaves surface with running tap water followed by distilled water. The leaves were then dried in oven and crushed in powder form. To prepare the plant broth solution, 5 g powder of *C. dichotoma* leaves was taken in a 250 ml beaker with 100 ml of distilled water. Stirred vigorously and boiled the solution for 20 minutes at 80°C. The extract was filtered through Whatman No. 1 filter paper. The filtrate thus obtained was stored in refrigerator for further experiments.

Synthesis of CuNPs using of *C. dichotoma* leaf extracts

About 40 ml of of *C. dichotoma* aqueous leaf extract was added to 20 ml of 20 mM Cu acetate solution in a 250 ml flask under vigorous stirring. After 15 minutes, the green color of the solution was turned into reddish brown on keeping the reaction mixture in an oven at 80°C for 4 hrs. The appearance of reddish brown color indicated the complete reduction of CuSO4 solution and formation of CuNPs. The CuNPs thus obtained were purified by repeated centrifugation method at 9000 rpm for 30 minutes followed by redispersion of the pellet in distilled water. The formation of CuNPs was strongly inferred by visual observation leaf extracts followed by ultraviolet-visible (UV-Vis) spectrum, Fourier transform infrared (FTIR), scanning electron microscope (SEM), X-ray diffraction (XRD), and energy dispersive analysis X-ray (EDS) studies. [17]

Characterization of Copper NPs

The synthesized CuNPs were characterized through UV-Vis spectrophotometer HITACHI U2300. The reduction of CuNPs was monitored by UV-spectrophotometer range of absorbance from 250 to 480 nm. Synthesized AgNPs were characterized by FTIR to identify the biomolecules that were responsible for the reduction of CuNPs Jusco 5300 model with the wavelength range from 400 to 4000 cm−1 were used. SEM analysis was used to characterize surface morphology, using Supra Zeiss with a resolution of 1 nm at 30 kV with 20 mm Oxford EDS detector. XRD studies were carried out to study the crystal structure of the synthesized CuNPs, the dried samples were coated on XRD grid, and the spectra were recorded using PANalytical X-pert X-ray generator operated at a voltage of 40 kV and a current of 30 Ma [17].

Antimicrobial activity

Antibacterial activity of CuNPs synthesized from *C. dichotoma* aqueous leaf extract was determined by Agar well diffusion method. Nutrient agar medium (Hi-Media Pvt. Ltd., Mumbai) was used as a medium for this study [18]. The medium and other required glass wares were sterilized through autoclave process at 121°C for 15 minutes. After some time, MHA medium is poured into the petri plate. Following the solidification of medium, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. The disc was placed in the wells made on MHA plates, and 20 µl of sample (Concentration: 30µg/ml) were placed in the disc. The plates were incubated at 37°C for 24 hrs. Then, the antimicrobial activity was determined by measuring the diameter of zone of inhibition.

Results and Discussion

Synthesis of Copper oxide nanoparticle (CuONPs)

Copper nano-particles were prepared by bioreduction of copper salt as discussed earlier. The change in colour of the copper sulphate solution was observed at different stages during synthesis of copper nanoparticle. (Figure1) show how the blue-green colour of the copper sulphate solution gradually changed at different stages during synthesis. The optical properties of metal nano-particles depend strongly upon the particle size and shape as reported by Zhu *et al.*, 1996 [9]. The formation of nano sized Cu particles was noticed by the change in the optical properties of the reaction solution. The blue-green colour of CuSO4 solution gradually changes to intense yellow when the particle size of copper is reduced to nano level.

Characterization of copper oxide nanoparticle (CuONPs)

UV-analysis

UV-Vis spectroscopy measures the extinction (scatter + absorption) of light passing through a sample. NPs have unique optical properties that are sensitive to the size, shape, concentration, agglomeration state, and refractive index near the NP surface, which makes UV-Vis a valuable tool for identifying, characterizing, and studying nanomaterials. The synthesized CuNPs were characterized through UV/Vis spectrophotometer Lambda 35. The biosynthesized CuNP was monitored by UV-spectrophotometer range of absorbance from 340 to 790 nm. UV-Vis absorption spectrum shows peaks characteristics of the surface plasmon resonance of CuNPs [10]. The spectroscopic analysis of synthesized CuNPs showed the maximum absorbance at 350 nm indicating the presence of biosynthesized CuNPs in the reaction mixture. These experimental investigations were found to be in good agreement with the results already presented in the literature by Ashajyothi *et al.*, 2014 (Fig. 2) [10].

FTIR analysis

FTIR measurements were done for the aqueous fresh *C. dichotoma* leaf extract and the synthesized dried CuNPs to recognize the possible phytoconstituents responsible for the bioreduction, capping and efficient stabilization of the synthesized CuNPs. The FTIR spectra of the leaf extract and the synthesized CuNPs are shown in Table1. The spectrum was recorded in the wavelength region between 400 and 4000 cm−1. The spectrum of aqueous fresh leaf extract (Fig. 3) shows the peaks at wave numbers 3410.68cm^{-1}, 1644.87cm−1 and 1452.08, 1111.65cm−1. The peak at 1644 cm−1 was due to the presence of -C=C stretching. The peak at 3410 cm−1 showed broad O-H stretching of phenolic compound. The C-N stretching of primary amine was obtained from the peak at 1111.65 cm−1. The Alkanes stretching frequency was obtained at 1452.08 cm−1. FTIR measurement of CuNPs showed the absorption peaks at 2950.37, 2523.63, 1060.68, 668.36cm−1. Peak at 2950.37 corresponds to O-H stretch stretching of amides and 2523.63 corresponds to O-H stretching of Carboxylic acids. The other peaks obtained at 1060.68 and 668.36 cm−1 were due to C-N stretching of primary aliphatic amines. On the basis of the peaks obtained for CuNPs, it is concluded that biosynthesized CuNPs might be surrounded by any one of these bioactive molecules such as polyphenols, alkaloids, and terpenoids which are in compliance with the already established facts in the literature [11]. The FTIR spectrum of the CuNPs indicates the lowering of peak intensity for O-H stretch of phenolic compounds, confirming the reduction of CuNPs, which have been possibly proceeded via these groups. This indicates that water soluble compound such as polyphenols and terpenoids are present in extract. Therefore, it can be inferred that the biomolecules present in leaf extract, namely, flavonoids, alkaloids, and terpenoids might be responsible for the reduction of Cu ions to CuNPs due to their markable reducing capacity.

SEM analysis

The morphology of the biosynthesized dried CuNPs was examined by S-3400N SEM. The obtained SEM image shows that the product is mainly made of particle-like Cu nanoclusters with size ranges from 150 to 200 nm. However, with high magnification, further observation reveals that these Cu nanoclusters are assembled by smaller NPs, which exhibit good uniformity, and the average diameter is about 08. 56 to 23nm nm (Fig. 4) [12].

TEM analysis

The shape and size of the synthesized CuNPs were analysed by TEM analysis [13] Fig. 5 shows the TEM image of biosynthesized CuNPs. The synthesized CuNPs were cubical in shape with particle size 04–08nm in nano range. The green synthesized copper nanoparticles size is highly depending on the concentration of C. dichotoma plant leaves extract. It was confirmed that, the concentration of leaf extract was found to be increased with decrease particles size.

Energy Dispersive Spectroscopy (EDS) analysis of CuONPs

To gain further insight into the features of the biosynthesized CuO NPs, the chemical composition of the NPs was analyzed using EDS the energy-dispersive spectra of the samples obtained from the EDS revealed that the sample prepared by using *C. dichotoma plant* extract has pure CuO phases. The EDS studies of (Figure 6) present three peaks between 1 kV and 10 kV that are directly related to Cu in the tested material [14]. The weight composition obtained from EDS analysis of the normalized spectrum was Cu (55.04%) and oxygen (40.31%). EDS also revealed the formation of nonstoichiometric CuO NPs with oxygen vacancy which could lead to better antibacterial activity.

XRD analysis

XRD pattern analysis revealed the crystalline nature of CuONPs as shown in (Fig 7& Table 2, 3). The XRD spectrum showed various small distinct diffraction peaks at 40.23, 49.05, 55.00, 67. 72. This represents (110), (111), (200) and (210) of primitive structure of copper oxide nanoparticles respectively. XRD pattern of NPs was matched with a database of Joint Committee on Powder Diffraction Standards (JCPDS Card No. 05–0667, [15]. The mean grain size of CuONPs formed in the bioreduction process was measured using the Debye-Scherrer formula D= kλ/βcosθ, where D is the average crystalline size (Å), k is a constant 1, 'λ' is the wavelength of X-ray source (0.1541nm), β is the angular line full width at half maximum (FWHM) intensity in radians and 'θ' the Bragg's angle [18.16nm]. The XRD pattern showed the average particle size 19.83nm.

Antimicrobial Activity

Antibacterial activity

Antimicrobial activity of CuNPs Antimicrobial activity of biosynthesized CuNPs was studied against various pathogenic bacteria using agar well diffusion method. The test organisms used were Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginos.Biosynthesized CuNPs showed clear zone of inhibition as indicated in Table 4. Maximum zone of inhibition (9.60±0.67mm) was observed with E. coli, next was P.aeruginos with (9.50±0.68 mm) zone of Inhibition and S.aureus showed least zone of inhibition of (9.45±0.66) mm at 30 µg/ml of CuNPs (Plate 1). From the above result, it can be concluded that the zone of inhibition increases with increase in concentration of NPs. In this study, zone of inhibition of extracts was compared with that of standards like chloramphenicol for antibacterial activity. From the obtained results, it was found that the bactericidal action of the CuONPs was found to be more when compared to plant leaves extract. The occurrence of an inhibition zone clearly indicates the antimicrobial potential of CuNPs, disrupting the membrane. The reason could be that the smaller size of the CuNPs along with bound phytoconstituents, *viz*., flavonoids (chrysoeriol and apigenin), polyphenols and other biomolecules get tightly adsorbed on the surface of the bacterial cells. The presence of these bound phytoconstituents might be responsible for further enhancing the antimicrobial potential of biosynthesized CuNPs, leading to the disruption of bacterial membrane, thereby causing the leakage of intracellular components, thus killing the bacterial cells. Another proposed mechanism involves the association of Cu with oxygen and its reaction with sulfhydryl (-S-H) groups on the cell wall to form R-S-S-R bonds, thereby blocking respiration and causing cell death [16].

Conclusion

The copper nanoparticles were successfully synthesized by using novel *Cordia dichotoma* plant leaves as first time for the anti-bacterial study, which provides cost effective, easy and proficient way for synthesis of CuNPs. The functional group present in the leaf extract was confirmed by FTIR analysis. These functional groups were mainly responsible for the reduction of copper metal ions into CuNPs. The synthesized copper nanoparticles were analyzed using UV-spectrophotometer, FITR, SEM with EDS, TEM and XRD. Copper nanoparticles were effectively utilized for the antibacterial activity study. The maximum ZOI was found to be more in gram negative bacteria when compared to gram positive bacteria. The *Cordia dichotoma* plant may be effectively utilized for the production of CuNps with economically for many pharmaceutical applications.

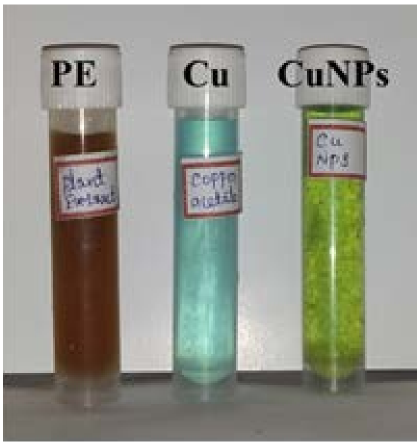


Fig 1: Synthesis of copper nanoparticles from *Cordia dichotoma* leaves

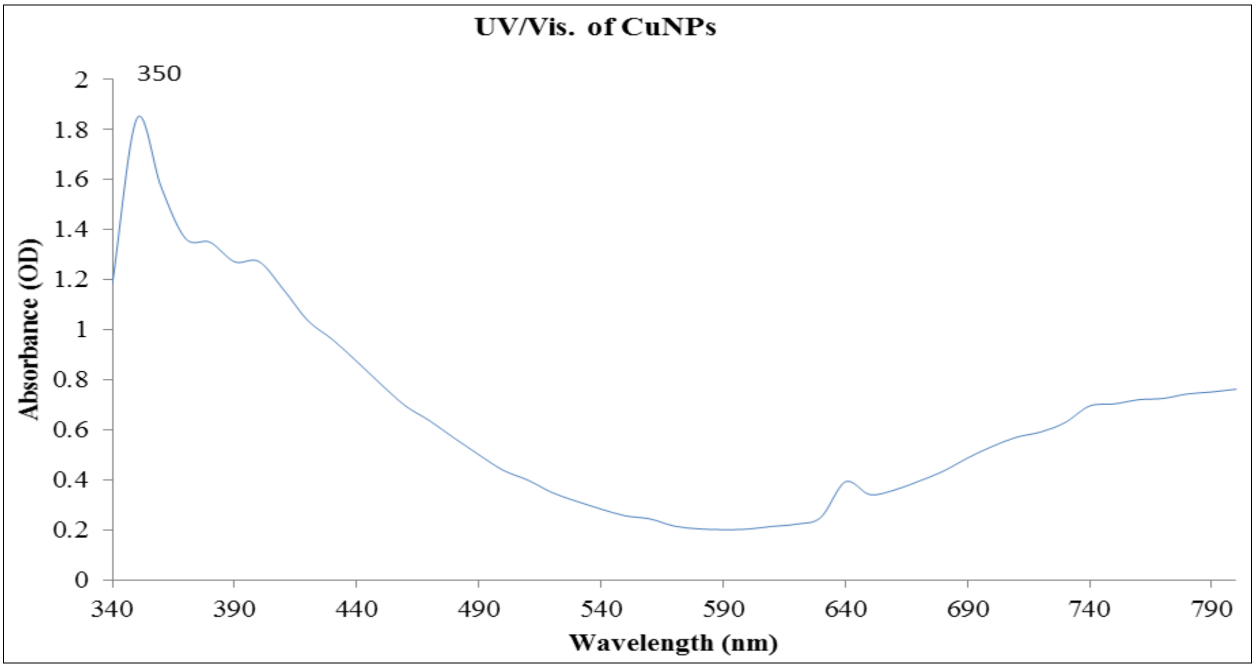


Fig 2: Ultraviolet-visible spectrum of copper nanoparticles From *C.dichotoma* leaves

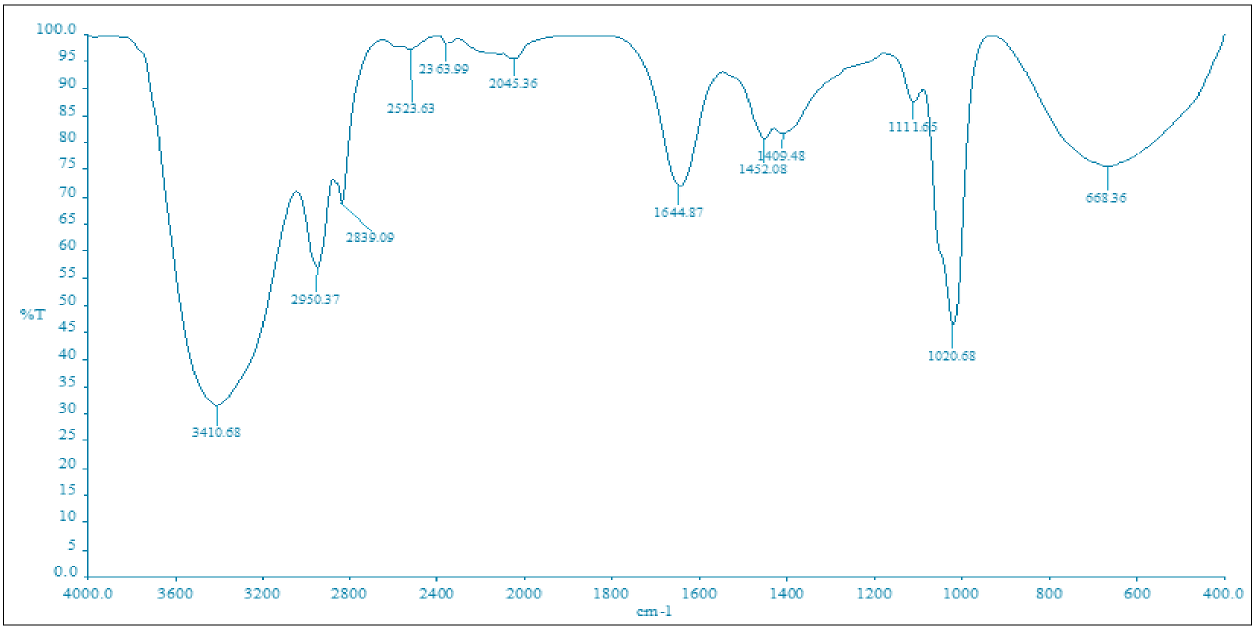


Fig 3: FT-IR spectrum of synthesized CuONPs using *C.dichotoma* leaf extract

Table 1: FT-IR spectrum of synthesized Cu ONPs using *C. dichotoma* leaf extract.

Peak	Bond	Functional group
3410.68	O-H stretch, H-bonded	Alcohols, Phenols
2950.37	O-H stretch	Carboxylic acids
2839.09	O-H stretch	Carboxylic acids
2523.63	O-H stretch	Carboxylic acids
1644.87	-C=C- stretch	Alkenes
1452.08	C-H bend	Alkanes
1409.48	C-C stretch (in-ring)	Aromatics
1111.65	C-N stretch	Aliphatic amines
1060.68	C-N stretch	Aliphatic amines
668.36	C-Br stretch	Alkyl halides

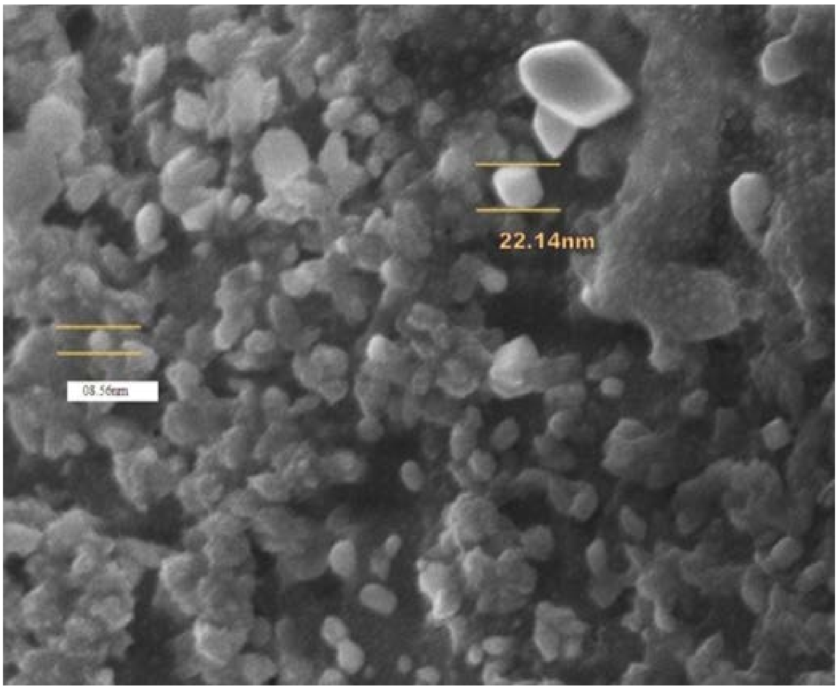


Fig 4: Polydispersed (Cluster) CuONPs ranged between 08–23nm analyzed by Scanning Electron Microscopic (SEM)

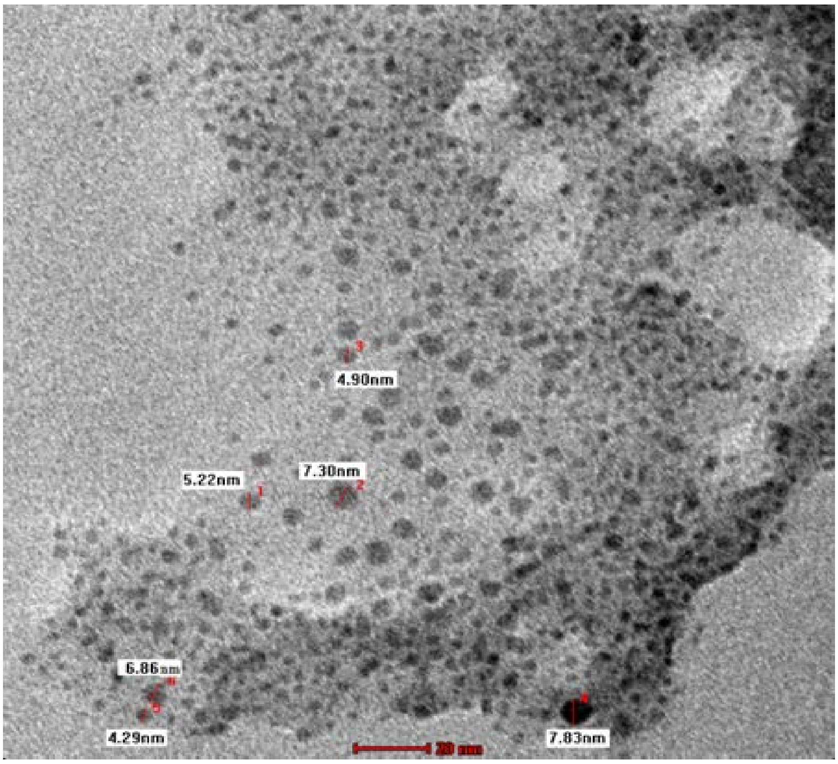
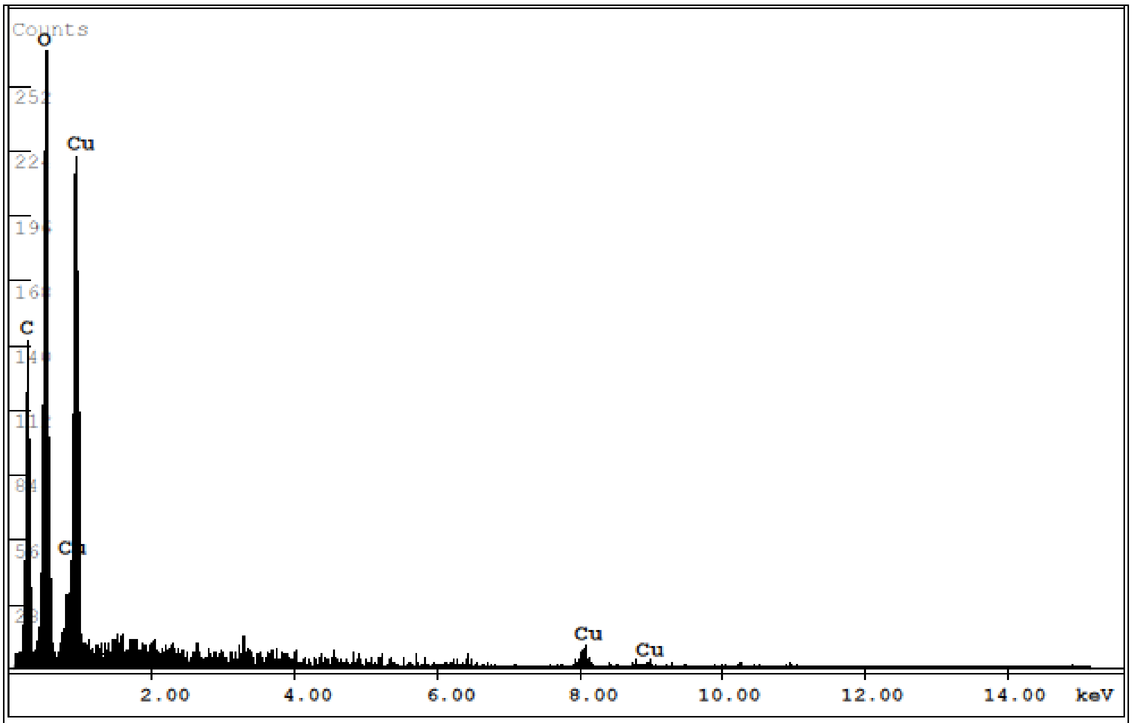


Fig 5: Transmission electron microscopic (TEM) Image of copper oxide nanoparticles with Cluster in the range between 04–08nm.



EDAX ZAF Quantification (Standardless)
Element Normalized
SEC Table : Default

Element	Wt %	At %	K-Ratio	Z	A	F
O K	40.31	48.85	0.1886	1.0166	0.4600	1.0006
Cu K	55.04	50.08	0.1543	0.9997	0.2804	1.0001
C K	4.65	1.06	0.0384	0.8123	1.0171	1.0000
Total	100.00	100.00				

Fig 6: EDS-Spectroscopy view of the *Cordia dichotoma leaves extract* showing synthesis of copper oxide nanoparticles and elemental copper signal in higher

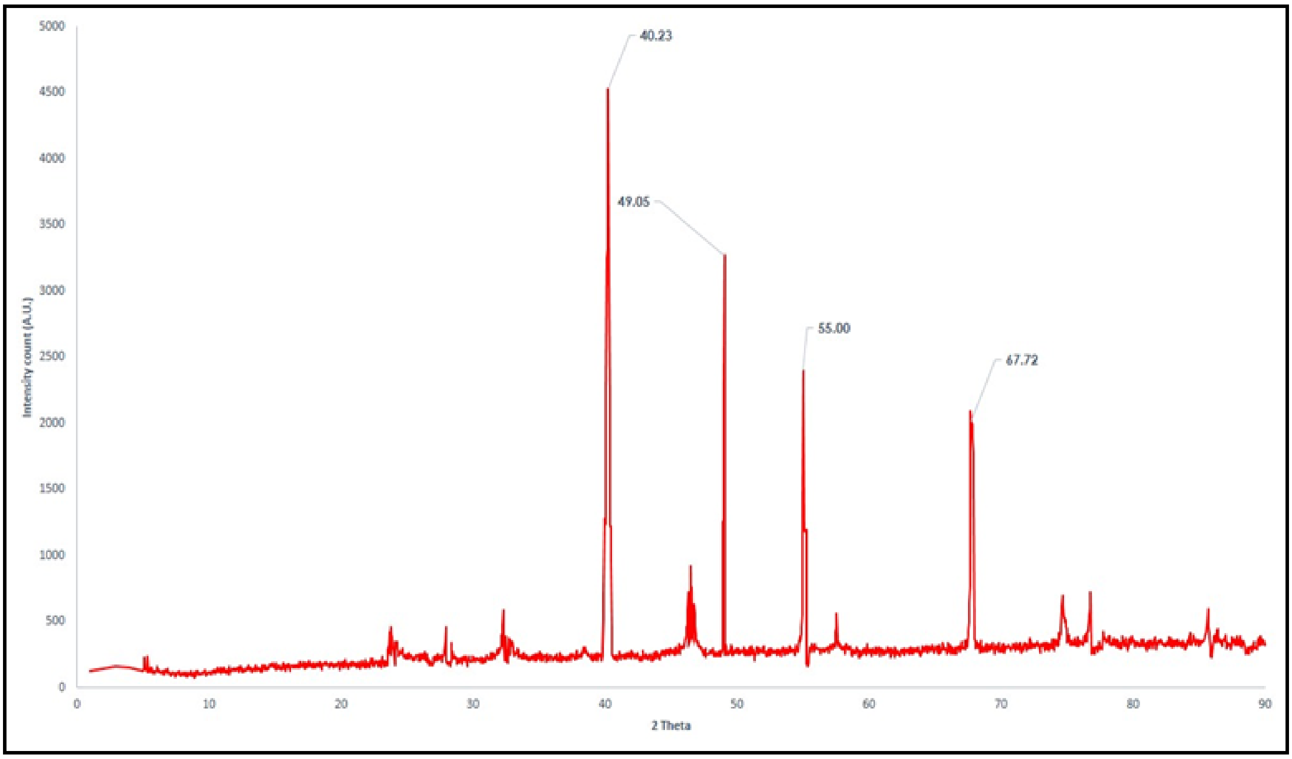


Fig 7: XRD patterns of copper nanoparticles synthesized by *Cordia dichotoma* leaves

Table 2: XRD pattern of Copper nanoparticles Synthesized by *C. dichotoma plant* Simple peak indexing

Peak position 2θ	1000 × Sin2θ	1000 × Sin2θ/102.03	Reflection	Remarks
40.23	206.61	2.02	110	$1^2 \times 1^2 \times 0$
49.05	308.64	3.02	111	$1^2 \times 1^2 \times 1^2$
55.00	390.62	3.82	200	$2^2 \times 0^2 \times 0^2$
67.72	510.20	5.00	210	$2^2 \times 1^2 \times 0^2$

Table 3: XRD The grain size of copper nanoparticle

2θ of the intense peak (deg)	Miller indices (hkl)	θ of the intense peak (deg)	FWHM of intense peak (β) radians	Size of the particle(D) nm
40.23	110	20.11	0.3511	24.82
49.05	111	24.525	0.4280	21.10
55.00	200	27.5	0.4799	18.68
67.72	210	33.86	0.5909	14.70
Average size				19.83
2θ of the intense peak (deg)	Miller indices (hkl)	θ of the intense peak (deg)	FWHM of intense peak (β) radians	Size of the particle(D) nm
40.23	110	20.11	0.3511	24.82
49.05	111	24.525	0.4280	21.10
55.00	200	27.5	0.4799	18.68
67.72	210	33.86	0.5909	14.70
Average size				19.83

Table 4: Anti-microbial activity of CuONPs in *Cordia dichotoma* leaves extract

Strains	Dose (30µl)			Std. (30µl)
	Cu	PE	CuONPs	
Bacterial strains				
Escherichia coli (mm)	2.15±0.15	3.90±0.27	9.60±0.67	11.35±0.79
Staphylococcus aureus (mm)	2.00±0.14	3.75±0.25	9.45±0.66	10.65±0.74
Pseudomonas aeruginos (mm)	2.10±0.15	3.80±0.2	9.50±0.68	11.10±0.77

Values were expressed as Mean ± SD Bacterial standard: Chloramphenicol

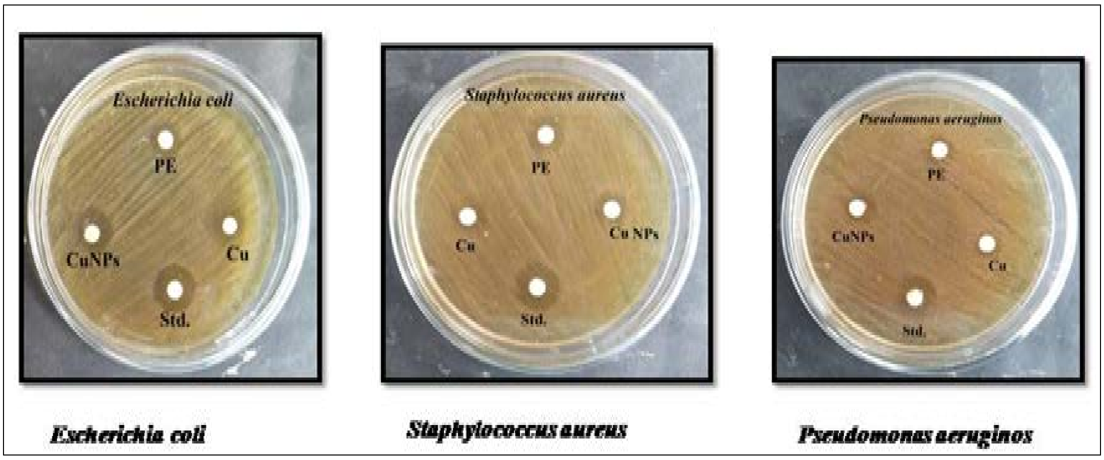


Plate 1: Anti-microbial activity of CuONPs and *Cordia dichotoma* leaves extract Bacterial plate

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Compliance with Ethical Standards

Conflict of interest

The authors declare that there is no conflict of interest.

Reference

1. Vasudev D. Kulkarni, Pramod S. Kulkarni Green Synthesis of Copper Nanoparticles Using *Ocimum Sanctum* Leaf Extract International Journal of Chemical Studies ISSN: 2321-4902 Volume 1 Issue 3.
2. Furno F, Morley KS, Wong B, Sharp BL, Arnold PL, Howdle SM *et al*. Silver nanoparticles and polymeric medical devices: a new approach to prevention of infection. J Antimicrob Chemother,2004:54:1019-1024.
3. Baker S, Satish S. Endophytes: Toward a Vision in Synthesis of Nanoparticle for Future Therapeutic Agents. Int. J. Bio-Inorg. Hybd. Nanomat,2012:1(2):67-77.
4. Ramgir N. Metal oxide nanowires for chemiresistive gas sensors: issues, challenges and prospects. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2013.
5. Salam HA. Plants: green route for nanoparticle synthesis. Int Res J Biol Sci,2012:1(5):85-90.
6. Gopalakrishnan K, Ramesh C, Ragunathan V, Thamilselvan M. Antibacterial activity of Cu2O nanoparticles on E. coli synthesized from Tridax procumbens leaf extract and surface coating with polyaniline. Digest J Nanomat Biostruct 2012:7:833-839. Grubben, G.J., 2004. Vegetables, 2. Prota.
7. Subhankari I, Nayak P. Synthesis of Copper Nanoparticles Using Syzygium aromaticum (Cloves) Aqueous Extract by Using Green Chemistry. World,2013:2(1):14-17.

8. Lee H. Biological synthesis of copper nanoparticles using plant extract. Nanotechnology,2011:1(1):371-374.
9. Zhu H, Zhang C, Yin Y. Nanotechnology,2005;16:3070.
10. Ashajyothi C, Kudsi J, Kelmani CR. Biosynthesis and characterization of copper nanoparticles from Enterococcus faecalis. Int J Pharm Bio Sci,2014;5(4):204-11.
11. Saranyaadevi K, Subha V, Ravindran RS, Renganathan S. Green synthesis and characterization of silver nanoparticle using leaf extract of Capparis zeylanica. Asian J Clin Res,2014:7(2):44-8.
12. Vinifera leaf aqueous extract and its antibacterial activity. Int J Curr Microbiol Appl Sci,2014:3(9):768-74.
13. Sivakumar P, Nethra Devi C, Renganathan S. Synthesis of Silver Nanoparticles using Lantana Camara Fruit Extract and its effect on pathogens. Asian. J. Pharma. Clini. Res,2012:5(3):31-5
14. Angeline Mary AP. A. -aminum Ansari, and R. Subramanian, “Sugarcane juice mediated synthesis of copper oxide nanoparticles, characterization and their antibacterial activity,” Journal of King Saud University - Science,2019:31(4):1103-1114.
15. Sasmal AK, Dutta S, Pal T. A ternary Cu2O–Cu–CuO nanocomposite: a catalyst with intriguing activity, Dalton Trans, 2016:45:3139-3150.
16. Gopinath M, Subbaiya R, Selvam MM, Suresh D. Synthesis of copper nanoparticles from Nerium oleander leaf aqueous extract and its antibacterial activity. Int J Curr Microbiol Appl Sci,2014:3(9):814-8.
17. Lanje AS, Sharma SJ, Pode RB, Ningthoujam RS. Synthesis and optical characterization of copper oxide nanoparticles. Adv Appl Sci Res,2010:1(2):36-40.
18. 18.Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol,1966:45(4):493-6.