Chemical Physics Letters 761 (2020) 138062

Contents lists available at ScienceDirect

Chemical Physics Letters

journal homepage: www.elsevier.com/locate/cplett

Research paper

Green synthesis of CuO nanostructures with bactericidal activities using *Simarouba glauca* leaf extract

G. Kalaiyan [a,*], K.M. Prabu [a], S. Suresh [a], N. Suresh [a]

[a] *PG & Research Department of Physics, Sri Vidya Mandir Arts & Science College (Autonomous), Uthangarai – 636 902, Krishnagiri, Tamil Nadu, India*

ARTICLE INFO

Keywords:
Simarouba glauca leaf
CuO nanoparticles
Antibacterial activity
ROS generation

ABSTRACT

For the first time, copper oxide (CuO) nanostructures were synthesized through a new green route using the leaf extract of *S. glauca* and characterized. The XRD pattern of CuO disclosed diffraction peaks correspond to monoclinic crystal structured CuO. The SEM and HR-TEM images demonstrated the presence of CuO nanostructures. The detected Cu and O elements through the EDAX analysis reveal the pristine nature of the hierarchical CuO nanostructures. Hence, this work complete that the potential bactericidal activity exhibited by CuO nanostructures against Gram negative *E. coli (MTCC 1698)* bacterial strain with zone of inhibition of 28 mm.

1. Introduction

Recently, scientists have targeted green routes for the synthesis and fabrication of nanoparticles [1]. The mechanism of the formation of nanoparticles from plant extracts is assumed that natural merchandise containing bioactive alkaloids, tannins, flavonoids, glycosides, proteins, and terpenoids, possessing useful teams that are meant to play a vital role in reduction and stabilization [2,3].

Simarouba is a distinctive tree that each one of its elements is helpful in industrial manufacture, sensible manure, and therapeutic tools. WHO, currently advised and encourages standard herbal remedies in National Health Care [NHC] programs as a result of the herbal drug out there at a low value and comparatively safe [4,5]. It has eleven medicinally essential quassinoids, the active principles within the tree [6]. Metal and metal oxide nanoparticles like silver, zinc oxide, gold, copper oxide, and mineral have familiar antimicrobial activity [7]. Although biogenesis of gold nanoparticles by plants, like neem, *Emblica officinalis*, & lemon grass and ZnO nanoparticles by plants, such as *Aloe barbadensis miller*, *Cassia fistula* are reported, the potential of plants as biological materials for the synthesis of nanoparticles is however to be explored [8].

In present days, green synthesis of copper oxide (CuO) nanoparticles has received considerable attention due to nontoxic, low-cost and eco-friendly. CuO could be used in a semiconductor device; it is a low-priced, non-toxic conversion metal compound exuberant within nature, with a bandgap of 1.2 eV. The copper oxide has a bunch of applications in numerous areas, like gas sensing, catalysis, photocatalysis, batteries, capacitors, magnetic storage medium media, lithium-ion batteries, and solar power cells [9–13].

The differences in CuO nanostructures with different dimensions, such as nanotubes, nanoplates, nanoshuttles, nanosheets, spindles, nanoflakes, nanowires, and flower-shaped nanostructures, have also been synthesized in a range of ways [14,15]. CuO nanomaterials are thought-about to possess higher antibacterial activity as a result of they possess high-quality chemical and physical stability, that allows it to simply act with biomolecules, leading to wonderful antimicrobial activity against Gram-positive and Gram-negative strains. A little farther, current results indicate that CuO nanomaterials promote the generation of reactive oxygen species (ROS) and stimulate high oxidative stress resulting in any toxicity and damage to deoxyribonucleic acid [16,17].

In the current work, we report on green synthesis of CuO nanostructures using leaf extract of *S. glauca*, which was not yet carried out by any other researchers, and this study was devoted to fulfilling the research gap. Leaf extract of *S. glauca* was used as reducing and stabilizing agents for synthesizing CuO nanostructures. The antibacterial activity of the synthesized CuO nanostructures was investigated against selected antimicrobial pathogenic Gram-positive microorganism species and Gram-negative microorganism species [18,19].

2. Materials and methods

2.1. Chemical and reagents

High purity (≥99.5%) copper nitrate trihydrate ($Cu(NO_3)_2{\cdot}3H_2O$) was purchased from the Merck. Mueller Hinton Agar medium was

* Corresponding author.
E-mail address: kalaigphy@gmail.com (G. Kalaiyan).

https://doi.org/10.1016/j.cplett.2020.138062
Received 15 July 2020; Received in revised form 26 September 2020; Accepted 4 October 2020
Available online 11 October 2020

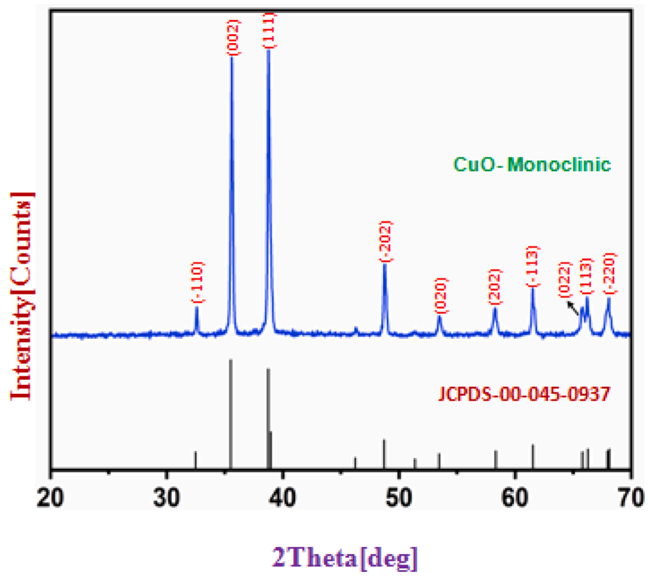


Fig. 1. XRD pattern of CuO nanostructures synthesized by *S. glauca* leaf extract.

received from the Himedia. The commercial and standard antibiotic agent, tetracycline ($C_{22}H_{24}N_2O_8$) with a purity of $\geq$ 98.0% was procured from the Sigma-Aldrich. Gram-positive bacterial strians, *B. cereus, S. aureus* and Gram-negative bacterial strains, such as *E. coli* and *K. pneumoniae* were obtained from the Institute of Microbial Technology, Chandigarh, India. Deionized distilled water was employed towards the preparation of aqueous solutions and washings.

2.2. Preparation of leaf extract

Fresh *S. glauca* leaves were cleaned by double distilled water several times. The leaves were shadow dried at room temperature to remove surface adsorbed water molecules. Cleaned leaves (5 g) were cut into small pieces, ground in mortar and pestle, and transferred into a beaker containing 150 mL distilled water. The beaker content was stirred at 650 rpm for 1 h at 80 °C and the resultant *S. glauca* leaf extract was filtered using Whatman No. 1 filter paper.

2.3. Synthesis of copper oxide nanostructures

Leaf extract (30 mL) was taken in a separate beaker, in which $Cu(NO_3)_2{\cdot}3H_2O$ (1 g) was added under stirring at 80 °C. The stirring was continued until the solution was turned from dark green to green color paste indicating formation of copper hydroxide ($Cu(OH)_2$), which was washed two or three times by distilled water. The $Cu(OH)_2$ precipitate was transferred into a silica crucible and calcinated at 250 °C for 1 h in a muffle furnace, the resultant dark color powder indicates the formation of CuO nanostructures.

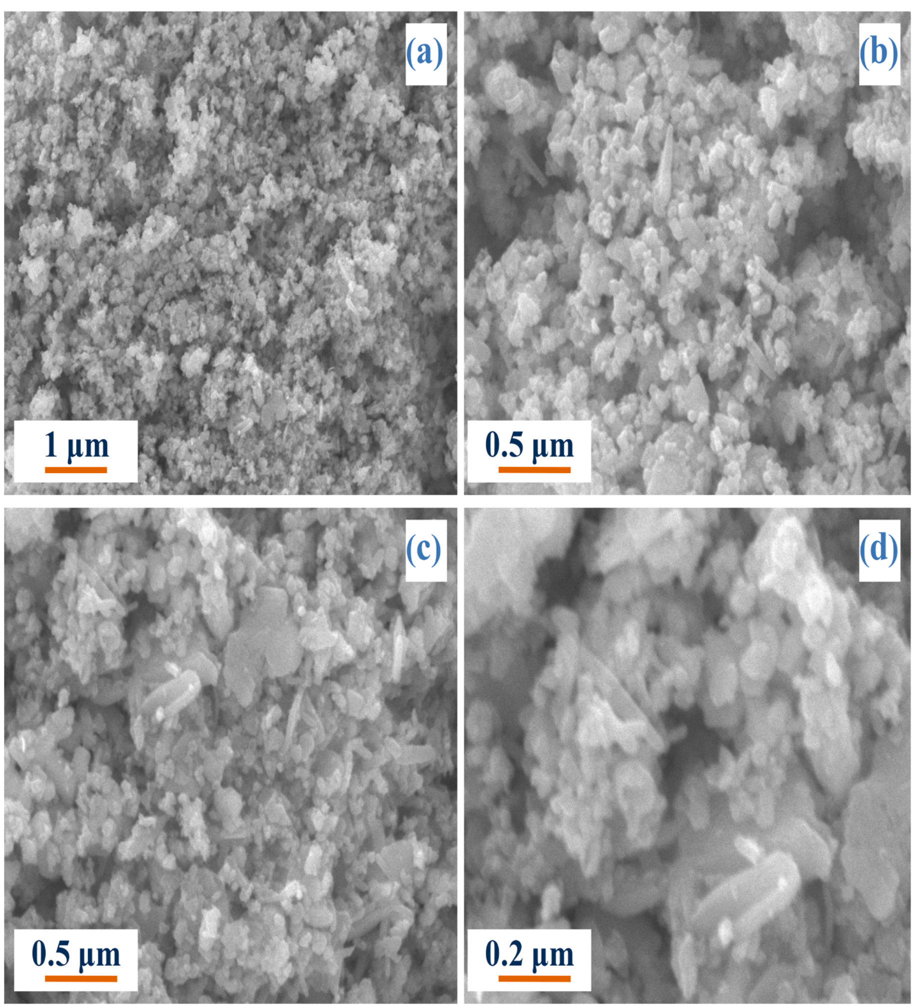
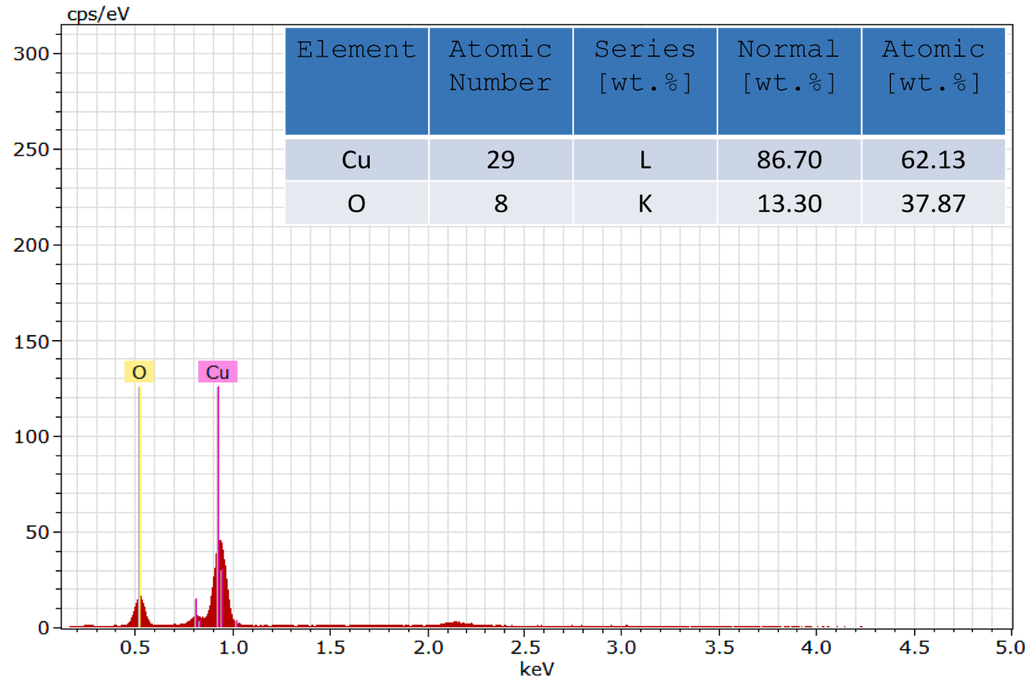


Fig. 2. SEM images of (a) 1 µm, (b, c) 0.5 µm, and (d) 0.2 µm CuO nanostructures synthesized by *S. glauca* leaf extract.



Element	Atomic Number	Series [wt.%]	Normal [wt.%]	Atomic [wt.%]
Cu	29	L	86.70	62.13
O	8	K	13.30	37.87

Fig. 3. EDAX spectrum of CuO nanostructures synthesized by *S. glauca* leaf extract. *Inset:* Table shows weight percentage of Cu and O atoms.

2.4. Characterization studies

The XRD pattern of the CuO sample was recorded in a PANalytical X'pert^3 powder X-ray Diffractometer with Cu Kα radiation ($\lambda = 1.5418$ Å). The SEM images were recorded in VEGA 3 TESCAN scanning electron microscopy. The EDAX spectrum was recorded in a Bruker Energy-dispersive X-ray spectrometer. HR-TEM images were obtained using FEI Tecnai G^2 20 TWIN transmission electron microscopy operated at 200 kV.

2.5. Antimicrobial activity evaluation of CuO nanostructures

Antibacterial activity of CuO against Gram-positive (*Bacillus cereus (MTCC 430)* & *Staphylococcus aureus (MTCC 3160)*) and Gram-negative (*Escherichia coli* (*MTCC 1698*) & *Klebsiella pneumoniae* (*MTCC 10309*)) bacterial strains was evaluated by well diffusion method based on the reported procedure. The liquid Mueller Hinton Agar media and Petri dishes were sterilized by the autoclave at a temperature of 121 °C and a pressure of 103421 Pa for 30 min. By maintaining aseptic conditions in laminar air flow chamber, agar medium (20 mL) was distributed into different petri dishes to get a uniform depth of 4 mm. After media solidification, 18 h culture of Gram-positive and Gram-negative bacterial strains were swabbed on the agar plates surface. The wells were prepared with an aid of cork borer followed by impregnating suspension having 50 and 100 μL dosage of the hierarchical CuO nanostructures, sterile distilled water as the negative control, and tetracycline (30 μg/disc) as a positive control. The samples loaded plates were incubated at a temperature of 37 °C for 24 h to find out the zone of inhibition [20–22].

3. Results and discussion

3.1. X-ray diffraction investigation

The crystal structure of CuO sample green synthesized using *S. glauca* leaf extract was investigated by X-ray diffractometry (XRD) investigation, and the recorded XRD pattern is shown in Fig. 1. The XRD pattern discloses presence of well-resolved characteristic diffraction peaks of CuO at 2θ values of 32.49, 35.49, 38.73, 48.72, 53.45, 58.33, 61.53, 65.78, 67.94, and 68.09° that are generated by (–1 1 0), (0 0 2), (1 1 1), (–2 0 2), (0 2 0), (2 0 2), (–1 1 3), (0 2 2), (1 1 3), and (–2 2 0) diffraction planes, respectively. All these diffraction peaks can be assigned to the monoclinic crystal structured CuO (JCPDS Card No.00–045-0937) with lattice constants of $a = 4.685$ Å, $b = 3.425$ Å and $c = 5.130$ Å. The highly intense diffraction peak displayed at 2θ value of 35.49 correspond to the (0 0 2) diffraction plane, implies high crystallinity of the CuO sample prepared through the present green synthesis method. CuO nanocrystallite size was calculated to be 44.15 nm using Williamson–Hall (W-H) formula.

$$D = k\lambda/\mathrm{Y\ Intercept}$$

where, D is mean size of crystalline material, k is shape factor, λ is wavelength of X-ray.

3.2. SEM analysis

It is amazingly clear from SEM pictures that *S. glauca* leaf extract have played an important role as reducing and capping mediator towards the formation of CuO nanostructures. The size of the nanostructures could not be detected from lower exaggerated (1 μm) SEM pictures. In distinction, higher exaggerated (0.2 μm) SEM picture show the presence of CuO leaf, spherical, and rod-like nanostructures exactly on the surface shown in Fig. 2(a-d). This CuO nanostructure morphology could be favorable for higher antibacterial activity.

3.3. EDAX analysis

Energy-dispersive X-ray (EDAX) spectrum of CuO nanostructures synthesized by *S. glauca* leaf extract was recorded through SEM analysis to look at the presence of components. EDAX spectrum of CuO nanostructures prepared using *S. glauca* leaf extract indicates the occurrence of peaks corresponding to Cu and O elements without any foreign impurities that confirms the formation of pristine CuO (Fig. 3). Moreover, the atomic weight percentage between Cu and O elements is determined as 62.13 and 37.87%, which also reveals the presence of CuO only.

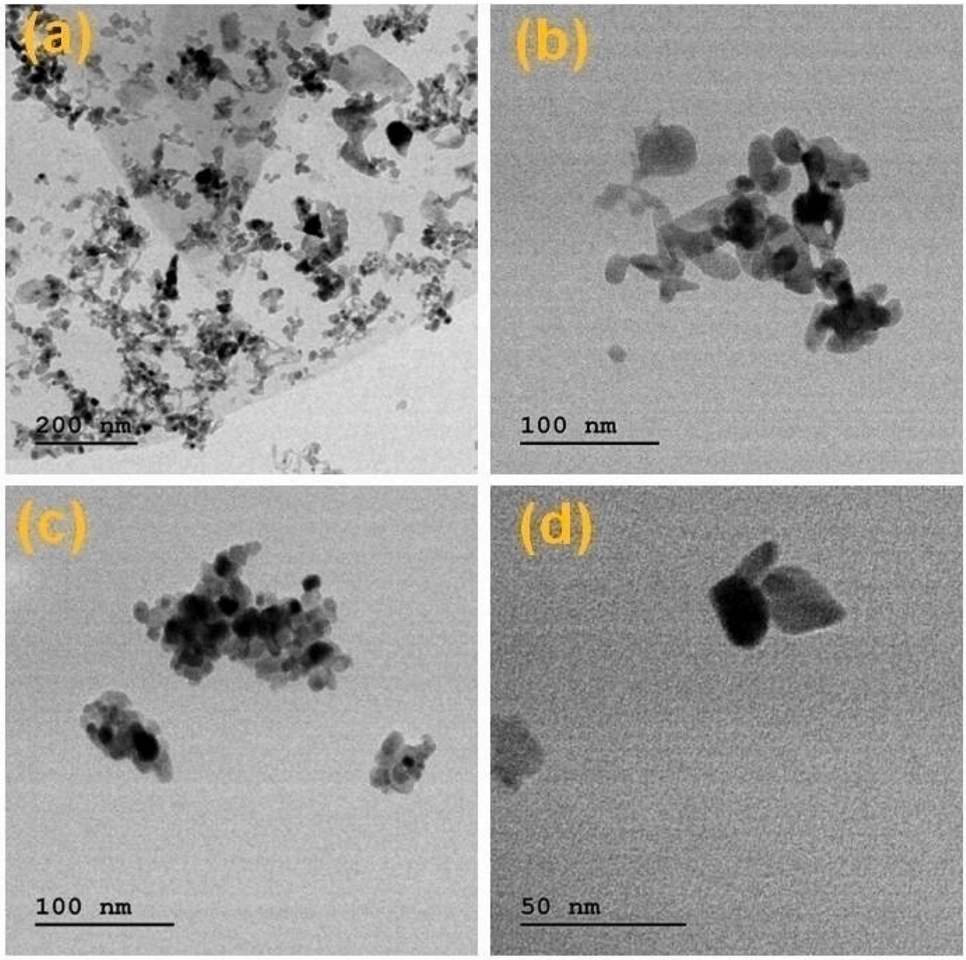


Fig. 4. TEM images of (a) 200 nm, (b,c) 100 nm, and (d) 50 nm CuO nanostructures synthesized by *S. glauca* leaf extract.

3.4. HR-TEM analysis

The size, shape, and morphology of CuO nanostructures was examined by HR-TEM images. Higher and lower exaggerated HR-TEM images of CuO nanostructures synthesized by *S. glauca* leaf extract are measured as shown in Fig. 4**(a-d)**. The HR-TEM image affirms that the CuO nanoparticles are measured in nano scale, higher exaggerated (100 nm) HR-TEM pictures show the presence of CuO leaf, spherical, and rod like nanostructures. Average length and breadth of CuO nanoleaves are 10–30 nm and 10–15 nm respectively, and a couple of particles are measured in a large scale.

3.5. Antibacterial activity evaluation

CuO nanoparticles displayed diverse microbial activity against Gram-positive and Gram-negative pathogenic bacteria. In any case, variations within the action against bacterial strains are not substantial. Most elevated antibacterial activity was noticed against *E. coli* with a circular area around the spot of the antibiotic values of 26 and 28 mm for CuO amount concentration of 50 and 100 mL individually. Next, the best activity was observed against *K. pneumonia*, followed by *S. aureus* with a circular area around the spot of the antibiotic values of 24 & 26 mm and 22 & 24 mm, respectively, for CuO nanostructures dosage of 50 and 100 mL. Least antibacterial activity was obtained against *B. cereus* with a circular area around the spot of the antibiotic values of 20 and 22 mm for CuO nanostructures dosage of 50 and 100 mL, respectively (Fig. 5(a-d)).

CuO nanostructures display better bactericidal activity for Gram-negative bacterial strain compared to Gram-positive bacterial strain. The cell membrane of the Gram-negative bacterial strain consists of a thin layer of peptidoglycan, whereas Gram-positive bacterial strain consists of a thick layer of peptidoglycan, consist of linear polysaccharide chains cross-linked by short peptides, therefore, forming a lot of rigid structures resulting in hard penetration of CuO nanoparticles, leading to low bacterial response [12]. This improved antibacterial activity can be ascribed to the favorable morphology of CuO composed of nanostructures that could effectively bind on the surface of bacterial strains through electrostatic forces and consequently damage cell wall by releasing copper ions directly in Gram-positive bacterial strains, whereas through cell membrane in Gram-negative bacterial strains, by which intercellular constituents leakage and cell death take place. Besides, the surface attached CuO nanostructures promote the generation of reactive oxygen species that considerably obstruct bacterial strains growth (Fig. 5(e)) [23]. The current antibacterial activities of CuO nanostructures are compared with recently reported works **(**Table 1**)**.

4. Conclusion

Copper oxide (CuO) nanoparticles were synthesized by *S. glauca* leaf extract. CuO sample exhibited peaks at 2θ values of 32.49, 35.49, 38.73, 48.72, 53.45, 58.33, 61.53, 65.78, 67.94, and 68.09° that are generated by (–1 1 0), (002), (111), (–2 0 2), (020), (202), (–1 1 3), (022), (113), and (–2 2 0) planes, severally made by monoclinic structured CuO. The XRD pattern revealed the formation of monoclinic crystal structured CuO with an average crystallite size of 44.15 nm. SEM and HR-TEM pictures showed the formation morphology that is composed of CuO leaf, spherical, and rod-like nanostructures. The average length and

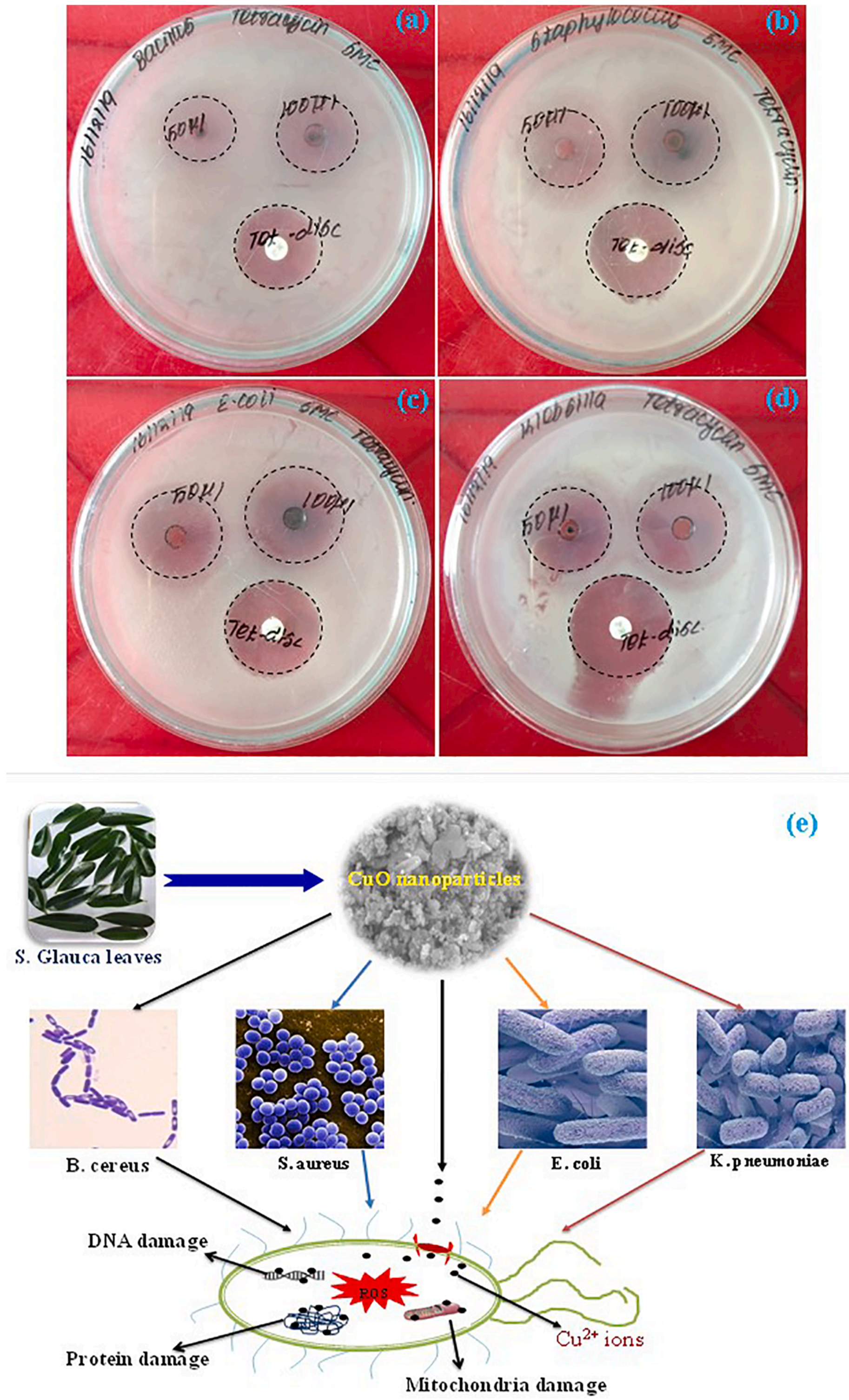


Fig. 5. Antibacterial activity of CuO nanostructures synthesized by *S. glauca* leaf extract, positive and negative controls against selected bacterial species (a) Bacillus cereus, (b) Staphylococcus aureus, (c) Escherichia coli, (d) Klebsiella pneumonia, and (e) ROS generation.

breadth of CuO nanoleaves were found to have 10–30 nm and 10–15 nm. EDAX spectrum indicated the presence of solely peaks analogous to Cu and O components that ensure the pristine nature of CuO nanostructures. This current work completes that the green synthesis of CuO nanoparticles with higher antibacterial activity against selected Gram-positive and Gram-negative microorganism species.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 1
Antibacterial activities of CuO nanostructures compared with recently reported works.

Bacterial Species	Nanosystem	Zone of Inhibition (mm)	Reference
B. cereus	CuO	24	[19]
	CuO	21	[25]
	CuO nanoplatelets	15	[16]
	CuO	22	Present Work
S. aureus	CuO	26	[19]
	CuO	16	[25]
	CuO	22	[26]
	CuO	19	[24]
	CuO	5	[27]
	CuO	28	[17]
	CuO	15	[28]
	CuO	24	Present Work
E. coli	CuO	26	[19]
	CuO	16	[25]
	CuO	20	[26]
	CuO	17	[24]
	CuO	8	[27]
	CuO	24	[17]
	CuO	17	[28]
	CuO	28	Present Work
K. pneumonia	CuO	28	[19]
	CuO	19	[25]
	CuO nanoplatelets	13	[16]
	CuO	26	Present Work

Acknowledgment

Authors are thankful to the DST-FIST, Government of India, New Delhi for generously providing Research Instruments Facility (Grant No. SR/FST/College-2017/140 (C), dt. 14.08.2018).

References

[1] F. Ijaz, S. Shahid, S.A. Khan, W. Ahmad, S. Zaman, Green synthesis of copper oxide nanoparticles using *Abutilon indicum* leaf extract: Antimicrobial, antioxidant and photocatalytic dye degradation activitie, Trop. J. Pharma. Res. 16 (2017) 743–753.
[2] G. Sreekala, A.F. Beevi, B. Beena, Adsorption of Lead (Ii) Ions by Ecofriendly Copper Oxide Nanoparticles, Orient J Chem 35 (2019) 1731–1736.
[3] N. Thangamani, N. Bhuvaneshwari, Green synthesis of gold nanoparticles using *Simarouba glauca* leaf extract and their biological activity of micro-organism, Chem. Phys. Lett. 732 (2019), 136587.
[4] K. Govindaraju, J. Darukeshwara, A.K. Srivastava, Studies on protein characteristics and toxic constituents of *Simarouba glauca* oilseed meal, Food Chem. Toxicol. 47 (2009) 1327–1332.
[5] J. Antony, A. Thomas, D. Gnanasekaran, H. Elizabeth, S, Review Study on Pharmacological Importance of Simarouba Glauca, IJNTR 1 (2) (2016) 59–62.
[6] V.K. Sharanya, K. Gayathiri, M. Sangeetha, et al., A Pharmacological Review on S. glauca DC, Int. J. Pharma Research & Review 5 (6) (2016) 32–36.
[7] M. Mirhosseini, Synergistic Antibacterial Effect of Metal Oxid Nanoparticles and Ultrasound Stimulation, J. Biol. Today's World 4 (6) (2015) 138–144.
[8] G. Sangeetha, S. Rajeshwari, R. Venckatesh, Green synthesis of zinc oxide nanoparticles by aloe barbadensis miller leaf extract: Structure and optical properties, Mat. Res. Bull. 46 (2011) 2560–2566.
[9] M. Lugo-Ruelas, P. Amézaga-Madrid, O. Esquivel-Pereyra, W. Autunez-Flores, P. Piza-Ruiz, C. Ornelas-Gutierrez, M. Miki-Yoshida, Synthesis, microstructural characterization and optical properties of CuO nanorods and nanowires obtained by aerosol assisted CVD, J. Alloys Compd. 643 (2015) S46–S50.
[10] O. Mahapatraa, M. Bhagatb, C. Gopalakrishnan, K.D. Arunachalam, Ultrafine dispersed CuO nanoparticles and their antibacterial activity, J. Exper. Nanoscience 3 (3) (2008) 185–193.
[11] N. Murugesan, A.M. Remona, S.K. Kumar, S. Suresh, Facile preparation of diverse copper oxide nanostructures and their characterization, Mater. Lett. 222 (2018) 100–104.
[12] K. Mageshwari, R. Sathyamoorthy, Flower-shaped CuO Nanostructures: Synthesis, Characterization and Antimicrobial Activity, J. Mater. Sci. Technol. 29 (2013) 909–914.
[13] Hafsa Siddiqui, M. S. Qureshi, Fozia Zia Haque, Biosynthesis of Flower–Shaped CuO Nanostructures and Their Photocatalytic and Antibacterial Activities, Nano-Micro Lett. (2020) 12:29.
[14] S. Harish, M. Navaneethan, J. Archana, S. Ponnusamy, C. Muthamizhchelvan, Y. Hayakawa, Chemical synthesis and properties of spindle-like CuO nanostructures with porous nature, Mat Lett 139 (2015) 59–62.
[15] O. Lupan, V. Postica, N. Ababii, M. Hoppe, V. Cretu, I. Tiginyanu, V. Sontea, Th. Pauporte, B. Viana, R. Adelung, Influence of CuO nanostructures morphology on hydrogen gas sensing performances, Microele, Eng., 164 (2016) 63-70.
[16] R. Uma Maheswari, B. Jansi Rani, G. Ravi, R. Yuvakkumar, Fuad Ameen, A. Al-Sabri, Structural, morphological, optical and antibacterial properties of pentagon CuO nanoplatelets, J Sol-Gel Sci Tech, 87(3) (2018) 515–527.
[17] N.P.S. Acharyulu, R.S. Dubey, V. Swaminadham, S.V.N. Pammi, Green Synthesis of CuO Nanoparticles using Phyllanthus Amarus Leaf Extract, IJERT 3 (4) (2014).
[18] S.K. Santhosh, A. Venugopal, M.C. Radhakrishnan, Study on the phytochemical, antibacterial activities of S.glauca, South Ind. J. Bio. Sciences (2016) 119–124.
[19] S. Suresh, R. Ilakiya, G. Kalaiyan, S. Thambidurai, P. Kannan, K.M. Prabu, N. Suresh, R. Jothilakshmi, S.K. Kumar, M. Kandasamy, Green synthesis of copper oxide nanostructures using *Cynodon dactylon* and *Cyperus rotundus* grass extracts for antibacterial applications, Ceram. Int. 46 (2020) 12525–12537.
[20] S. Magaldi, S. Mata-Essayag, C.H. de Capriles, C. Perez, M.T. Colella, C. Olaizola, Y. Ontiveros, Well diffusion for antifungal susceptibility testing, Int. J. Infect. Dis. 8 (1) (2004) 39–45.
[21] M. Balouiri, M. Sadiki, S.K. Ibnsouda, Methods for *in vitro* evaluating antimicrobial activity: A review, J. Pharm. Anal. 6 (2) (2016) 71–79.
[22] G. Kalaiyan, S. Suresh, S. Thambidurai, K.M. Prabu, S.K. Kumar, N. Pugazhenthiran, M. Kandasamy, Green synthesis of hierarchical copper oxide microleaf bundles using *Hibiscus cannabinus* leaf extract for antibacterial application, J. Mol. Struct. 1217 (2020), 128379.
[23] T. Cherian, K. Ali, S. Fatima, et al., Myristica fragrans bio-active ester functionalized ZnO nanoparticles exhibit antibacterial and antibiofilm activities in clinical isolates, J. Microbiol. Methods 166 (2019), 105716.
[24] M.S. Jadhav, S. Kulkarni, P. Raikar, D.A. Barretto, S.K. Vootla, U.S. Raikar, Green biosynthesis of CuO & Ag–CuO nanoparticles from *Malus domestica* leaf extract and evaluation of antibacterial, antioxidant and DNA cleavage activities by reflux condensation method, New J. Chem. 42 (2018) 204–213.
[25] Mohammad, J. Al-Jassani, Haider Qassim Raheem, Anti-bacterial activity of CuO nanoparticles against some pathogenic bacteria, Int J. of ChemTech Research, 10 (2) (2017) 818-822.
[26] A. Azam, A.S. Ahmed, M. Oves, M.S. Khan, A. Memic, Size-dependent antimicrobial properties of CuO nanoparticles against Gram-positive and Gram-negative bacterial strains, Int. J. Nanomedicine 7 (2012) 3527–3535.
[27] M. Elango, M. Deepa, R. Subramanian, A. Musthafa, Synthesis, characterization, and antibacterial activity of polyindole/Ag–Cuo nanocomposites, Polym. Plast. Technol. Eng. 57 (2017) 1440–1451.
[28] V. Vellora, T. Padil, M. Cernik, Green synthesis of copper oxide nanoparticles using gum karaya as a biotemplate and their antibacterial application, Int. J. Nanomedicine 8 (2013) 889–898.