Pharmaceutical Sciences
September 2017, 23, 198-206
doi: 10.15171/PS.2017.30
http://journals.tbzmed.ac.ir/PHARM

TUOMS Publishing Group

Research Article

Antibacterial Activity of Copper Oxide (CuO) Nanoparticles Biosynthesized by *Bacillus* sp. FU4: Optimization of Experiment Design

Mojtaba Taran[1], Maryam Rad[1], Mehran Alavi[1]*
[1]*Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran.*

Article Info

Article History:
Received: 14 April 2017
Accepted: 5 July 2017
ePublished: 30 September 2017

Keywords:
-Green chemistry
-*Bacillus* sp. FU4
-Taguchi method
-CuO nanoparticles
-*Escherichia coli* ATCC 25922

ABSTRACT

Background: There are several methods for synthesis of metallic nanoparticles (NPs) including chemical, physical and biological process. In this study, Bacillus sp. FU4 was used as biological source for biosynthesis of CuO NPs.
Methods: CuO NPs have been prepared by copper sulfate ($CuSO_4$). CuO NPs were formed after oxidation of Cu NPs. Design and analysis of Taguchi experiments (an orthogonal assay and analysis of variance (ANOVA)) carried out by the Qualitek-4 software. Average effect of $CuSO_4$ concentration (0.1, 0.01 and 0.001 M), incubation and culturing time (48, 72, 96 hours) as three controllable factors with three levels were evaluated in CuO NPs biosynthesis. Characterization of CuO NPs was determined by UV-Vis spectroscopy, X-ray diffraction (XRD), Fourier transform infra-red (FT-IR) spectroscopy and scanning electron microscopy (SEM). Also, the antimicrobial properties of CuO NPs were investigated using *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 43300 as multidrug resistant (MDR) bacteria.
Results: Results: It was evaluated that, NPs size distributions were in the range of 2-41 nm with spherical shapes. The anti-bacterial activities of CuO NPs were measured based on diameter of inhibition zone in disk diffusion tests of NPs dispersed in batch cultures. Two levels of $CuSO_4$ concentrations (0.1 and 0.01M) had antibacterial effect on E.coli (33±0.57 and 6 ±2mm). In the case of S. aureus, there was surprisingly no sign of growth.
Conclusion: CuO NPs have antibacterial activity that can be benefit in medicinal aspect for fighting against prominent pathogen bacteria such as E.coli ATCC 25922 and S.aureus ATCC 43300.

Introduction

There are several chemical and organic compounds with antibacterial activity such as, penicillins (β-lactams group) and natural products which kill bacterial or slow down their growth.[1] Among them, nanoparticles (metallic and semiconductor) have recently obtained more attention.[2] Reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (HO●) and organic hydro peroxides (OHP), NPs deposition on the surface of microorganisms and NPs accumulation in the cytoplasm/periplasmic region of bacteria, can be lead to microorganisms death.[3] In the case of bacteria, ROS can resulted in damage of cellular constituents including lipids, peptidoglycan, proteins and DNA through generating of ROS by NPs and subsequently physical disruption.[4] Metallic and semiconductor NPs are considerable materials for investigation in nano-medicine field. This interest is related to size and shape based on physicochemical properties. Surface area to volume ratio of NPs is important factor for these properties.[5-7] In this case, one of the important applied materials in the industry is copper oxide (CuO) and its alloy in nanometers scale.[8-11] Also, These metallic NPs can be utilized as an alternative for silver and gold NPs.[12,13]

There are several methods for production of CuO NPs; specifically characterized as a chemical, physical and biological process.[14,15] For instance, proton irradiation as physical methods and vacuum vapor deposition (VVD) are able for synthesis a wide range of metallic NPs.[16,17] These methods have several disadvantages. As, the costs of these methods are higher and there is not approach of environment protection. Therefore, eco-friendly view of point is essential for production of metal NPs using biological systems.[18,19]

***Corresponding Author:** Mehran Alavi, E-mail: mehranbio83@gmail.com

Plants, algae, yeasts, fungi and bacteria can be applied as green approach for biosynthesis of metal NPs.[20-22] It is well known that bacteria have mechanisms to survive in hard conditions such as high amount of toxic metal by transforming toxic metal ions into their corresponding non-toxic forms (metal sulfide/oxides).[22-30] It is noteworthy that these mechanisms have prominent role in NPs biosynthesis. Also, NPs biosynthesis by bacteria than to other organisms, have several advantages such as simple culturing, extracellular NPs fabrication in mild conditions of experiment (temperature, pH) and be affordable and not time consuming.[31,32]

In this study, *Bacillus* sp. FU4 was used as biological source for biosynthesis of CuO NPs; in addition, XRD, FT-IR spectroscopy and SEM were applied as benefitted technique for the characterization of CuO NPs. In the end, the antibacterial activity of these NPs on two pathogenic species with multidrug resistance characteristic, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 43300, was evaluated.

Materials and Methods

Taguchi methodology experimental design

In order to optimization of experimental conditions, all the combination experiments using the assigned parameter values were conducted. The Qualitek-4 software was used to design and analysis of Taguchi experiments as statistical method.[33-35] Table 1 shows three controllable factors ($CuSO_4$ concentration, incubation time and culturing time) and their levels in design of experiment.

Materials

Copper(II) sulfate pentahydrate, 98% (CuSO4.5H2O) and nutrient agar were purchased from Sigma-Aldrich and applied respectively for CuO NPs synthesis and antibacterial activity measurement without any further purification.

CuO NPs biosynthesis and preparation of supernatant

Bacteria *Bacillus* sp. FU4 was obtained from bacterial archive, Razi University, Kermanshah. Growth conditions were simple, growth in 0.5 nutrient broth (NB) medium at 37 ˚C for three levels of time culturing (48, 72, 96 hours). After growth of bacteria in these times, culture mediums with bacteria were centrifuged at 5000 for 5 minutes. Then 5cc of supernatant was added to $CuSO_4$ by three levels of concentrations (0.1, 0.01, 0.001M) at Erlenmeyer flask at three replicates for each concentration level. Afterwards, these solutions were incubated at under agitation (100 rpm) for three time levels of incubation (48, 72, 96 hours).[36]

Characterization

Structure, morphology and elemental composition of the prepared annealed samples were characterized for by XRD and SEM analysis tools. Crystallographic study was carried out using EQUNIOX 3000, diffractometer in the scanning range of 20˚ - 70˚ (2θ) using Cu K_a radiations of wavelength 1.5406 Å. A scanning electron microscope (model XL30, Philips, Eindhoven) was used to study the morphology of morphology and size of NPs. The intensity of absorption peaks of NPs was examined by UV-Vis spectrophotometer (Tomas, UV 331) from 400 to 800nm. Also, Fourier transform infrared spectroscopy measurements were done by (Germany, Bruker, Model:ALPHA) spectrophotometer.[37]

Antibacterial Effects

Escherichia coli ATCC 25922 and *Staphylococcus aureus* ATCC 45500 as multi drug resistant bacteria were utilized for measurement the effect of antibacterial properties of CuO NPs by modified agar disc diffusion.

Table 1. Biosynthesis parameters and their levels.

Symbol	Parameters	Unit	Level 1	Level 2	Level 3
A	$CuSO_4$ concentration	M	0.1	0.01	0.001
B	Incubation time	Hour	48	72	96
C	Culturing time	Hour	48	72	96

Table 2. Orthogonal array of Taguchi experimental design for biological synthesis of copper oxide NPs.

Trail No.	A (M)	B (hour)	C (hour)	Optical Density (OD)
1	0.1	48	48	0.565
2	0.1	72	72	0.308
3	0.1	96	96	0.987
4	0.01	48	72	0.988
5	0.01	72	96	1.185
6	0.01	96	48	0.790
7	0.001	48	96	0.011
8	0.001	72	48	0.064
9	0.001	96	72	0.199

MDR bacteria were cultivated on Muller Hinton agar (MHA) plates; 5 mm diameter paper discs were prepared with the help of a sterilized steel cork borer.

Afterwards, different concentrations of CuO NPs (three levels with 0.1, 0.01 and 0.001M concentrations) were loaded in paper discs followed by placing the discs on agar. Then, the plates were incubated at 37℃ for 48 hours.[38]

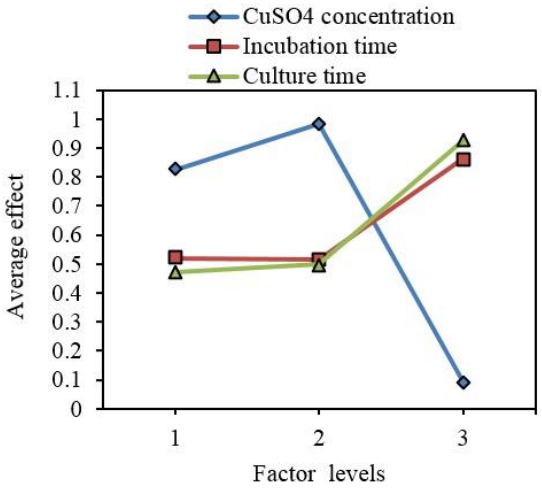

Figure 1. Taguchi results of average effect of $CuSO_4$, incubation time and culture time.

Table 3 shows effects of three different factors on the copper oxide NPs biosynthesis by *Bacillus* sp. FU4. As illustrated in this Table, $CuSO_4$ concentration in level 2 (0.984), incubation time in level 3 (0.864) and culturing time in level 3 (0.93) had higher effect on the CuO NPs biosynthesis. Similarly, Taguchi results of average effect of $CuSO_4$, incubation time and culture time are presented in Figure 1.

Effective factors in copper oxide NPs biosynthesis by *Bacillus* sp. FU4 are demonstrated by variance analysis (ANOVA) (Table 4).

Table 3. Effects of three different factors on the CuO NPs biosynthesis.

Factors	Level 1	Level 2	Level 3
A	0.826	0.984	0.091
B	0.521	0.515	0.864
C	0.473	0.498	0.93

Final column determines effect percentage of each factors which major factor is $CuSO_4$ concentration with value of 40.116%. Therefore, this result illustrates higher importance of $CuSO_4$ concentration parameter than other parameter in copper oxide NPs biosynthesis by *Bacillus* sp. FU4.[11,19,39]

In addition, optimum conditions for biosynthesis of affected CuO NPs by three factors are shown in Table 5. Expected result at optimum condition was value of 1.509% that is relatively suitable result of biosynthesis by this bacterium.

UV-Vis analysis of CuO NPs biosynthesis

When cell free supernatant of *Bacillus* sp. FU4 was added to $CuSO_4$ solution and incubated for three times of incubation (48, 72, 96), the mixture's colour reaction altered from blue to light green (Figure 2). Spectrum absorption of UV-Vis spectroscopy for this solution illustrated a distinct absorption peak in the region of 700-800nm (Figure 3).

Figure 2. Changes of reaction mixture's colour from light green (left) to light green (right) after supernatant addition.

Table 4. Analysis of variance (ANOVA) for CuO NPs biosynthesis.

Factors	DOF(f)	Sum of Sqrs.	Variance	F-Ratio (F)	Pure Sum (S′)	Percent (%)
A	2	1.362	0.681	3.404	0.962	40.116
B	2	0.239	0.119	0.599	0	0
C	2	0.396	0.198	0.99	0	0

Table 5. Optimum conditions of CuO NPs biosynthesis by bacterium.

Factors	Levels	Contribution
A	2	0.35
B	3	0.23
C	3	0.296
Total contribution from all factors	-	0.876
Current grand average of performance	-	0.633
Expected result at optimum condition	-	1.509

X-ray diffraction analysis

A high crystalline level of the CuO NPs sample can be seen with diffraction angles of 21.05, 36.2 and 43.74, 50.69, 74.2 which corresponds to the characteristic face centered cubic (fcc) of copper lines indexed at (111), (200) and (220), respectively (Figure 4). Impurities such as Cu_2O may be effective on absence of any noticeable peaks in pattern.[40]

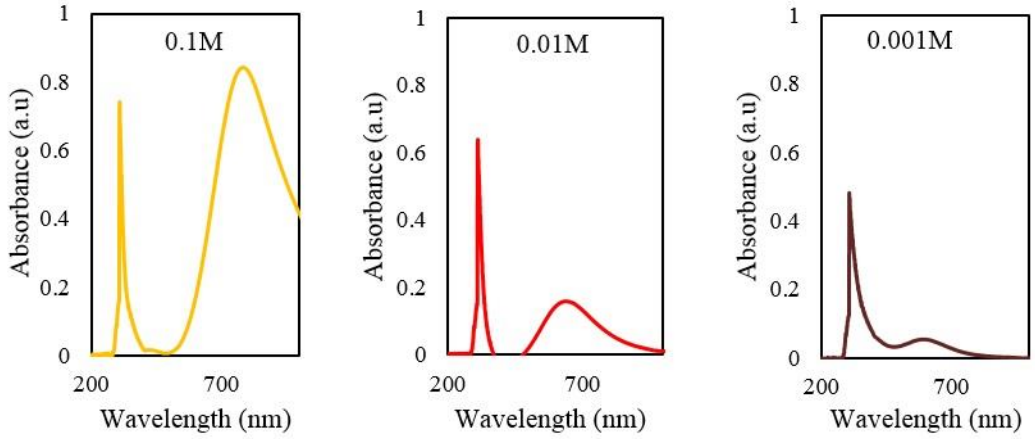

Figure 3. UV-Vis spectrum of CuO NPs produced by *Bacillus* sp. FU4 in three level of concentration 0.1, 0.01 and 0.001.

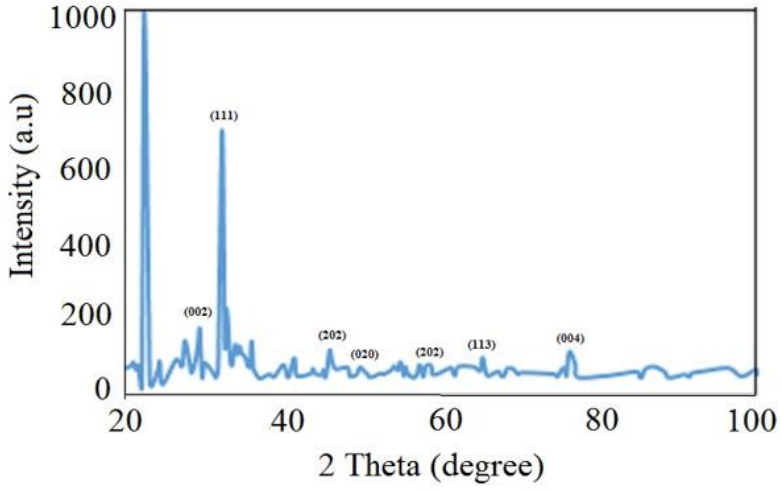

Figure 4. X-ray diffraction spectrum of CuO NPs synthesized from 0.1 M of $CuSO_4$ treated *Bacillus* sp. FU4 cell free supernatant at 28˚C.

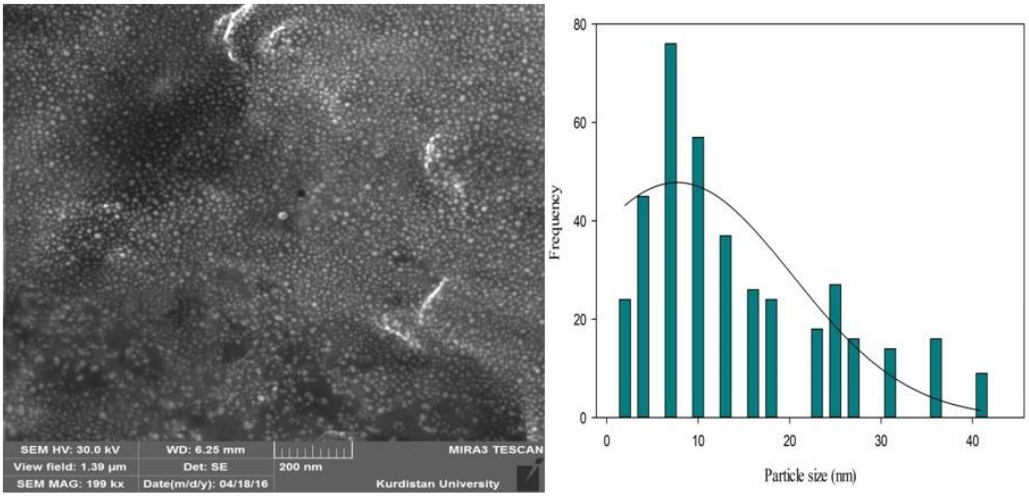

Figure 5. Scanning electron microscopy (SEM) image and particle size distributions of CuO NPs produced by culture supernatant of *Bacillus* sp. FU4.

In addition, the average grain size of NPs was 64.97 nm using Debye-Scherer Eq.(1):

$$\tau = \frac{k\lambda}{\beta cos\theta} \quad \text{Eq.(1)}$$

where K, known as Scherer's constant, ranges from 0.9 to 1.0, λ is 1.5418 Å, which is the wavelength of the X-Ray radiation source, β1/2 is the width of the XRD peak at half height and θ is the Bragg angle (Figure 4). However, XRD measurement revealed that the NPs initially formed as colloids tend to grow and react with environment oxygen.[41] Also, as shown in Figure 5, SEM images, demonstrated that CuO NPs were formed as aggregates particles with spherical shape. Particle size distributions were in the size range of 2-41nm.

FTIR analysis

FT-IR was performed in order to evaluate the molecular interactions between the CuO NPs and the media. Figure 6 demonstrates a C=O vibration band at 1098.17cm^{-1}. In addition, the spectrum illustrates at 3381.31cm^{-1} to N-H stretching, 2038.58cm^{-1} to N=C=S stretching, 1631.89 cm^{-1} to C=C stretching, 1417.22 cm^{-1} to O-H bending, 1211.90 cm^{-1} to C-O stretching, 1098.17 cm^{-1} to C-O stretching, 776.87 cm^{-1} to C-H bending.

Antibacterial activity

Antibacterial activity is defined as killing bacteria or reducing their growth without general toxic to surrounding tissue of body.[42] There are several reports about antibacterial properties of NPs.[43-45] In this study, antibacterial activity of CuO NPs was indicated by disc diffusion assay (Figure 7). Due to evaluating of antibacterial effects, maximum zone of inhibition, two important multidrug resistant pathogenesis bacteria, *E. coli* ATCC 25922 and *S. aureus* ATCC 43300 were used. Results show that two levels of CuSO4 concentrations (0.1 and 0.01M) had respectively antibacterial activity on E. coli ATCC 25922 as inhibition zone diameter of 33±0.57 and 6 ±2mm. In the case of *S. aureus* ATCC 43300, there was surprisingly no sign of obvious growth. Green synthesis of CuO NPs (5-10 nm, spherical shape) by *Gloriosa superba* illustrated antibacterial activity against *S. aureus* and *Klebsiella aeroegenes*.[46]

Discussion

Taguchi method was used to optimize the setting of the process factors values for enhancement quality properties and to identify the product factor values under the optimal process factor values.[47] In this case, Taguchi method utilizes orthogonal arrays as a specific design to investigate the entire factors space with a minimum cost of experiments.[48] In order to analyze the quality characteristics, Taguchi method applies three types of the signal/noise (S/N) ratio. In this study, we used the higher-the-better type of S/N. Three parameters ($CuSO_4$ concentration, incubation time and culturing time) and their three levels were used for design experiment. Results show higher effect of $CuSO_4$ concentrations than other parameters which can be comparative with previous reports.[49,50] This colour change illustrates the formation and oxidation of CuO NPs. In this case, Shantkriti and Rani reported similar appearance of green colour solution with addition of $CuSO_4$ to a flask containing *Pseudomonas fluorescens*.[51] Based on special particles properties such as size, shape and capping agents, the precise position of SPR band may shift.[52] As, copper oxide NPs synthesized from *Aspergillus clavatus* species confirmed the presence of copper oxide NPs at 300 nm.[53]

Also, compared to previous studies, green synthesis of CuO NPs by *Malva sylvestris* and *Phyllantus amarus* plant leaves had spherical shape.[54,55]

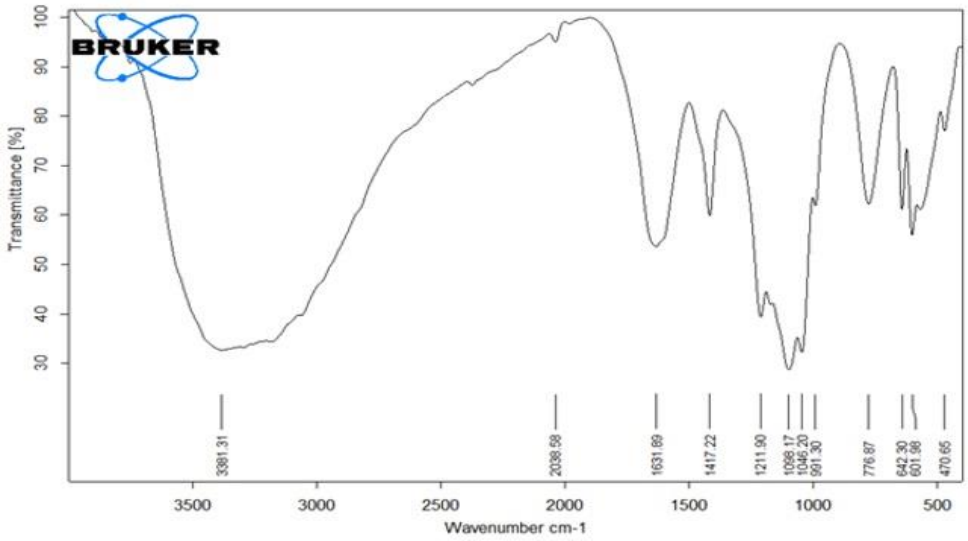

Figure 6. FTIR peaks of CuO NPs green synthesized by *Bacillus* sp. FU4.

Figure 7. Bactericidal activity of CuO NPs on *E.coli* ATCC 25922 and *S.aureus* ATCC 43300.

The interaction between the Cu-NPs and media is the cause of the corresponding vibration bond, which demonstrates a reaction between Cu-NP surface and carbonyl and hydroxyl functional groups.[56] In this case, green synthesis of copper oxide nanoparticles by *gum karaya* as a biotemplate had similar functional groups.[57] As demonstrated in similar investigation, these functional groups can contribute in NPs biosynthesis by their capping role.[58-60] The grain size of CuO NPs (64.97nm) was calculated by Scherrer formula. Similarly, Sonia et al (2016) reported the grain size of 50nm for Copper oxide NPs.[61] As shown in Figure 5, the quality and composition of biosynthesized CuO NPs were indicated by Fourier Transform Infrared spectroscopy (FTIR) in the range of 400-4000 cm^{-1}. Similar investigations illustrated Cu-O bond in CuO NPs at 430, 507, and 606 cm^{-1}.[61]

Four types of bacteria: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus vulgaris* were used to evaluate antibacterial activity of CuO NPs. Results of this study demonstrated that *E. coli* was more sensitive than other bacteria species at a highest concentration of CuO NPs (1000 µg mL) with a inhibition zone of 26.0 ± 1.00 mm.[62] Sonia and coworkers (2016) investigated the antibacterial activity of CuO NPs in three different concentrations (12.5 µg/ml, 25 µg/ml and 50 µg/ml) by agar diffusion method against four pathogenic bacteria species: *Serratia marcescens*, *Streptococcus pneumonia*, *Staphylococcus aureus*, and *Salmonella typhimurium*.[61] The minimum inhibitory concentration (MIC) for all nanostructures was in the range of 12.5 µg/ml to 50 µg/ml. Also, antibacterial effect against *E. coli*, *S. aureus* and *B. subtilis* was confirmed through green synthesis of Ag NPs.[30,63] This activity may be resulted from the attaching of copper ions (released by the NPs) to the negatively charged bacterial cell membrane.[42]

In the case of antibacterial effect mechanisms, physical disruption and oxidative stress are major cause of NPs toxicity.[64] Reactive oxygen species (ROS) including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals ($HO^\bullet$) and organic hydro peroxides (OHP), NPs deposition on the surface of bacteria and NPs accumulation in the cytoplasm/periplasmic region can be resulted in bacterial death[3]. ROS can resulted in damage of cellular constituents (lipids, peptidoglycan, proteins and DNA) through releasing from NPs and subsequently penetration into bacteria.[4]

Conclusion

In this study, CuO NPs with spherical shapes and average mean sizes in the range of 2-41 nm and an fcc crystal structure were synthesized in a green method by *Bacillus* sp. FU4. UV-Vis, XRD, FT-IR were used to characterization of NPs. There are many studies about biosynthesis of NPs by plant, fungi, and bacteria. Based on this study, green method is easy and eco-friendly way for CuO NPs synthesis with relative purity of NPs. Also, copper oxide NPs synthesized by *Bacillus* sp. FU4 extracellular and stabilizing of CuO NPs were possible without using any capping agents which are toxic. Also, these NPs have antibacterial effect that can be usable in medicinal aspect for fighting against prominent pathogen bacteria such as *E.coli* ATCC 25922 and *S.aureus* ATCC 43300. Generally, this study presents simple, low expensive, eco-friendly and high productivity in fabrication of CuO NPs.

Acknowledgments

The authors wish to appreciate Razi University for providing necessary facilities to carry out this work.

Conflict of interests

The authors claim that there is no conflict of interest.

References

1. Wright PM, Seiple IB, Myers AG. The evolving role of chemical synthesis in antibacterial drug discovery. Angew Chem Int Ed. 2014;53(34):8840-69. doi:10.1002/anie.201310843
2. Li W-R, Xie X-B, Shi Q-S, Zeng H-Y, OU-Yang Y-S, Chen Y-B. Antibacterial activity and mechanism of silver nanoparticles on escherichia coli. Appl Microbiol Biotechnol. 2010;85(4):1115-22. doi:10.1007/s00253-009-2159-5
3. Li Y, Zhang W, Niu J, Chen Y. Surface-coating-dependent dissolution, aggregation, and reactive oxygen species (ros) generation of silver nanoparticles under different irradiation conditions. Environ Sci Technol. 2013;47(18):10293-301. doi:10.1021/es400945v
4. Das B, Dash SK, Mandal D, Ghosh T, Chattopadhyay S, Tripathy S, et al. Green synthesized silver nanoparticles destroy multidrug resistant bacteria via reactive oxygen species mediated membrane damage. Arabian Journal of Chemistry. 2015; In Press. doi:10.1016/j.arabjc.2015.08.008
5. Kittler S, Greulich C, Diendorf J, Koller M, Epple M. Toxicity of silver nanoparticles increases during storage because of slow dissolution under release of silver ions. Chem Mater. 2010;22(16):4548-54. doi:10.1021/cm100023p
6. Wu W, He Q, Jiang C. Magnetic iron oxide nanoparticles: Synthesis and surface functionalization strategies. Nanoscale Res Lett. 2008;3(11):397-415. doi:10.1007/s11671-008-9174-9
7. Christian P, Von der Kammer F, Baalousha M, Hofmann T. Nanoparticles: Structure, properties, preparation and behaviour in environmental media. Ecotoxicology. 2008;17(5):326-43. doi:10.1007/s10646-008-0213-1
8. Huang Z, Cui F, Xue J, Zuo J, Chen J, Xia C. Synthesis and structural characterization of silica dispersed copper nanomaterials with unusual thermal stability prepared by precipitation-gel method. J Phys Chem C. 2010;114(39):16104-13. doi:10.1021/jp101136x
9. Longano D, Ditaranto N, Sabbatini L, Torsi L, Cioffi N. Synthesis and antimicrobial activity of copper nanomaterials. Nano-antimicrobials: Springer; 2012. p. 85-117.
10. Mudunkotuwa IA, Pettibone JM, Grassian VH. Environmental implications of nanoparticle aging in the processing and fate of copper-based nanomaterials. Environ Sci Technol. 2012;46(13):7001-10. doi:10.1021/es203851d
11. Taran M, Rad M, Alavi M. Biological synthesis of copper nanoparticles by using halomonas elongata ibrc-m 10214/sinteza biologica a nanoparticulelor de cupru prin utilizarea halomonas elongata ibrc-m 10214. Industria Textila. 2016;67(5):351-56.
12. Athanassiou EK, Grass RN, Stark WJ. Large-scale production of carbon-coated copper nanoparticles for sensor applications. Nanotechnology. 2006;17(6):1668-73. doi:10.1088/0957-4484/17/6/022
13. Rubilar O, Rai M, Tortella G, Diez MC, Seabra AB, Durán N. Biogenic nanoparticles: Copper, copper oxides, copper sulphides, complex copper nanostructures and their applications. Biotechnol Lett. 2013;35(9):1365-75. doi:10.1007/s10529-013-1239-x
14. Chatterjee AK, Sarkar RK, Chattopadhyay AP, Aich P, Chakraborty R, Basu T. A simple robust method for synthesis of metallic copper nanoparticles of high antibacterial potency against e. Coli. Nanotechnology. 2012;23(8):085103. doi:10.1088/0957-4484/23/8/085103
15. Nasrollahzadeh M, Sajadi SM. Green synthesis of copper nanoparticles using ginkgo biloba l. Leaf extract and their catalytic activity for the huisgen [3+ 2] cycloaddition of azides and alkynes at room temperature. J Colloid Interface Sci. 2015;457:141-7. doi:10.1016/j.jcis.2015.07.004
16. Ray SC, Saha A, Jana NR, Sarkar R. Fluorescent carbon nanoparticles: Synthesis, characterization, and bioimaging application. J Phys Chem C. 2009;113(43):18546-51. doi:10.1021/jp905912n
17. Chin HS, Cheong KY, Razak KA. Review on oxides of antimony nanoparticles: Synthesis, properties, and applications. J Mater Sci. 2010;45(22):5993-6008. doi:10.1007/s10853-010-4849-x
18. Lin X, Wu M, Wu D, Kuga S, Endo T, Huang Y. Platinum nanoparticles using wood nanomaterials: Eco-friendly synthesis, shape control and catalytic activity for p-nitrophenol reduction. Green Chem. 2011;13(2):283-7. doi:10.1039/c0gc00513d
19. Honary S, Barabadi H, Gharaei-Fathabad E, Naghibi F. Green synthesis of copper oxide nanoparticles using penicillium aurantiogriseum, penicillium citrinum and penicillium waksmanii. Dig J Nanomater Biostruct. 2012;7(3):999-1005.
20. Akhtar MS, Panwar J, Yun Y-S. Biogenic synthesis of metallic nanoparticles by plant extracts. ACS Sustain Chem Eng. 2013;1(6):591-602. doi:10.1021/sc300118u
21. Dhillon GS, Brar SK, Kaur S, Verma M. Green approach for nanoparticle biosynthesis by fungi: Current trends and applications. Crit Rev Biotechnol. 2012;32(1):49-73. doi:10.3109/07388551.2010.550568
22. Narayanan KB, Sakthivel N. Biological

synthesis of metal nanoparticles by microbes. Adv Colloid Interface Sci. 2010;156(1-2):1-13. doi:10.1016/j.cis.2010.02.001
23. Iravani S. Green synthesis of metal nanoparticles using plants. Green Chem. 2011;13(10):2638-50. doi:10.1039/c1gc15386b
24. MubarakAli D, Thajuddin N, Jeganathan K, Gunasekaran M. Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. Colloids Surf B Biointerfaces. 2011;85(2):360-5. doi:10.1016/j.colsurfb.2011.03.009
25. Singaravelu G, Arockiamary JS, Kumar VG, Govindaraju K. A novel extracellular synthesis of monodisperse gold nanoparticles using marine alga, sargassum wightii greville. Colloids Surf B Biointerfaces. 2007;57(1):97-101. doi:10.1016/j.colsurfb.2007.01.010
26. Ahmad A, Senapati S, Khan MI, Kumar R, Ramani R, Srinivas V, et al. Intracellular synthesis of gold nanoparticles by a novel alkalotolerant actinomycete, rhodococcus species. Nanotechnology. 2003;14(7):824-8. doi:10.1088/0957-4484/14/7/323
27. Gajbhiye M, Kesharwani J, Ingle A, Gade A, Rai M. Fungus-mediated synthesis of silver nanoparticles and their activity against pathogenic fungi in combination with fluconazole. Nanomed Nanotechnol Biol Med. 2009;5(4):382-6. doi:10.1016/j.nano.2009.06.005
28. Ahmad A, Mukherjee P, Mandal D, Senapati S, Khan MI, Kumar R, et al. Enzyme mediated extracellular synthesis of cds nanoparticles by the fungus, fusarium oxysporum. J Am Chem Soc. 2002;124(41):12108-9. doi:10.1021/ja027296o
29. Thakkar KN, Mhatre SS, Parikh RY. Biological synthesis of metallic nanoparticles. Nanomed Nanotechnol Biol Med. 2010;6(2):257-62. doi:10.1016/j.nano.2009.07.002
30. Taran M, Rad M, Alavi M. Characterization of ag nanoparticles biosynthesized by bacillus sp. Hai4 in different conditions and their antibacterial effects. J App Pharm Sci. 2016;6(11):094-9. doi:10.7324/japs.2016.601115
31. Faramarzi MA, Sadighi A. Insights into biogenic and chemical production of inorganic nanomaterials and nanostructures. Adv Colloid Interface Sci. 2013;189-190:1-20. doi:10.1016/j.cis.2012.12.001
32. Iravani S, Korbekandi H, Mirmohammadi SV, Zolfaghari B. Synthesis of silver nanoparticles: Chemical, physical and biological methods. Res Pharm Sci. 2014;9(6):385-406.
33. Taran M, Amirkhani H. Strategies of poly (3-hydroxybutyrate) synthesis by haloarcula sp. Iru1 utilizing glucose as carbon source: Optimization of culture conditions by taguchi methodology. Int J Biol Macromol. 2010;47(5):632-4. doi:10.1016/j.ijbiomac.2010.08.008
34. Taran M, Azizi E, Taran S, Asadi N. Archaeal poly (3-hydroxybutyrate) polymer production from glycerol: Optimization by taguchi methodology. J Polym Environ. 2011;19(3):750-4. doi:10.1007/s10924-011-0327-z
35. Taran M. Utilization of petrochemical wastewater for the production of poly (3-hydroxybutyrate) by haloarcula sp. Iru1. J Hazard Mater. 2011;188(1-3):26-8. doi:10.1016/j.jhazmat.2011.01.036
36. He S, Guo Z, Zhang Y, Zhang S, Wang J, Gu N. Biosynthesis of gold nanoparticles using the bacteria rhodopseudomonas capsulata. Mater Lett. 2007;61(18):3984-7. doi:10.1016/j.matlet.2007.01.018
37. Panáček A, Kvítek L, Prucek R, Kolář M, Večeřová R, Pizúrová N, et al. Silver colloid nanoparticles: Synthesis, characterization, and their antibacterial activity. J Phys Chem B. 2006;110(33):16248-53. doi:10.1021/jp063826h
38. Ruparelia JP, Chatterjee AK, Duttagupta SP, Mukherji S. Strain specificity in antimicrobial activity of silver and copper nanoparticles. Acta Biomater. 2008;4(3):707-16. doi:10.1016/j.actbio.2007.11.006
39. Zhou K, Wang R, Xu B, Li Y. Synthesis, characterization and catalytic properties of cuo nanocrystals with various shapes. Nanotechnology. 2006;17(15):3939-43. doi:10.1088/0957-4484/17/15/055
40. Yang H, Ouyang J, Tang A, Xiao Y, Li X, Dong X, et al. Electrochemical synthesis and photocatalytic property of cuprous oxide nanoparticles. Mater Res Bull. 2006;41(7):1310-8. doi:10.1016/j.materresbull.2006.01.004
41. Bortoleto-Bugs RK, Mazon T, Tarozzo Biasoli M, Pavani Filho A, Willibrordus Swart J, Roque Bugs M. Understanding the formation of the self-assembly of colloidal copper nanoparticles by surfactant: A molecular velcro. J Nanomater. 2013;2013:1-8. doi:10.1155/2013/802174
42. Hajipour MJ, Fromm KM, Ashkarran AA, de Aberasturi DJ, de Larramendi IR, Rojo T, et al. Antibacterial properties of nanoparticles. Trends Biotechnol. 2012;30(10):499-511. doi:10.1016/j.tibtech.2012.06.004
43. Ravishankar Rai, Jamuna Bai. Nanoparticles and Their Potential Application as Antimicrobials, Science against Microbial Pathogens: Communicating Current Research and Technological Advances. In: Méndez-Vilas A, editor. Formatex, Microbiology Series. 2011;1(3):197-209.
44. Singh M, Singh S, Prasad S, Gambhir IS.

Nanotechnology in medicine and antibacterial effect of silver nanoparticles. Dig J Nanomater Biostruct. 2008;3(3):115-22.
45. Zargar M, Hamid AA, Bakar FA, Shamsudin MN, Shameli K, Jahanshiri F, et al. Green synthesis and antibacterial effect of silver nanoparticles using vitex negundo L. Molecules. 2011;16(12):6667-76. doi:10.3390/molecules16086667
46. Naika HR, Lingaraju K, Manjunath K, Kumar D, Nagaraju G, Suresh D, et al. Green synthesis of cuo nanoparticles using gloriosa superba l. Extract and their antibacterial activity. J Taibah Univ Med Sci. 2015;9(1):7-12. doi:10.1016/j.jtusci.2014.04.006
47. Yang WH, Tarng YS. Design optimization of cutting parameters for turning operations based on the taguchi method. J Mater Process Technol. 1998;84(1-3):122-9. doi:10.1016/s0924-0136(98)00079-x
48. Nalbant M, Gökkaya H, Sur G. Application of taguchi method in the optimization of cutting parameters for surface roughness in turning. Mater Des. 2007;28(4):1379-85. doi:10.1016/j.matdes.2006.01.008
49. Zhu H-t, Lin Y-s, Yin Y-s. A novel one-step chemical method for preparation of copper nanofluids. J Colloid Interface Sci. 2004;277(1):100-3. doi:10.1016/j.jcis.2004.04.026
50. Saif Hasan S, Singh S, Parikh RY, Dharne MS, Patole MS, Prasad BLV, et al. Bacterial synthesis of copper/copper oxide nanoparticles. J Nanosci Nanotechnol. 2008;8(6):3191-6. doi:10.1166/jnn.2008.095
51. Shantkriti S, Rani P. Biological synthesis of copper nanoparticles using pseudomonas fluorescens. Int J Curr Microbiol App Sci. 2014;3(9):374-83.
52. Mott D, Galkowski J, Wang L, Luo J, Zhong C-J. Synthesis of size-controlled and shaped copper nanoparticles. Langmuir. 2007;23(10):5740-5. doi:10.1021/la0635092
53. Verma VC, Kharwar RN, Gange AC. Biosynthesis of antimicrobial silver nanoparticles by the endophytic fungus aspergillus clavatus. Nanomedicine. 2010;5(1):33-40. doi:10.2217/nnm.09.77
54. Acharyulu NPS, Dubey RS, Swaminadham V, Kollu P, Kalyani RL, Pammi SVN. Green synthesis of CuO nanoparticles using phyllanthus amarus leaf extract and their antibacterial activity against multidrug resistance bacteria. Int J Adv Res Technol. 2014;4(3):97-103.
55. Awwad A, Albiss BA, Salem NM. Antibacterial activity of synthesized copper oxide nanoparticles using malva sylvestris leaf extract. SMU Med J. 2015;2:91-100.
56. Usman MS, Ibrahim N, Shameli K, Zainuddin N, Yunus W. Copper nanoparticles mediated by chitosan: Synthesis and characterization via chemical methods. Molecules. 2012;17(12):14928-36. doi:10.3390/molecules171214928
57. Černík M, Padil VVT. Green synthesis of copper oxide nanoparticles using gum karaya as a biotemplate and their antibacterial application. Int J Nanomedicine. 2013;8(1):889-98. doi:10.2147/ijn.s40599
58. Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M. Synthesis of gold nanotriangles and silver nanoparticles using aloevera plant extract. Biotechnol Prog. 2006;22(2):577-83. doi:10.1021/bp0501423
59. Sanghi R, Verma P. Biomimetic synthesis and characterisation of protein capped silver nanoparticles. Bioresour Technol. 2009;100(1):501-4. doi:10.1016/j.biortech.2008.05.048
60. Wangoo N, Bhasin KK, Mehta SK, Suri CR. Synthesis and capping of water-dispersed gold nanoparticles by an amino acid: Bioconjugation and binding studies. J Colloid Interface Sci. 2008;323(2):247-54. doi:10.1016/j.jcis.2008.04.043
61. Sonia S, Jayasudha R, Jayram ND, Kumar PS, Mangalaraj D, Prabagaran SR. Synthesis of hierarchical cuo nanostructures: Biocompatible antibacterial agents for gram-positive and gram-negative bacteria. Curr Appl Phys. 2016;16(8):914-21. doi:10.1016/j.cap.2016.05.006
62. Khashan KS, Sulaiman GM, Abdulameer FA. Synthesis and antibacterial activity of cuo nanoparticles suspension induced by laser ablation in liquid. Arab J Sci Eng. 2016;41(1):301-10. doi:10.1007/s13369-015-1733-7
63. Kathiravan V, Ravi S, Ashokkumar S, Velmurugan S, Elumalai K, Khatiwada CP. Green synthesis of silver nanoparticles using croton sparsiflorus morong leaf extract and their antibacterial and antifungal activities. SpectroChim Acta A Mol Biomol Spectrosc. 2015;139:200-5. doi:10.1016/j.saa.2014.12.022
64. Von Moos N, Slaveykova VI. Oxidative stress induced by inorganic nanoparticles in bacteria and aquatic microalgae–state of the art and knowledge gaps. Nanotoxicology. 2014;8(6):605-30. doi:10.3109/17435390.2013.809810