PARIPEX - INDIAN JOURNAL OF RESEARCH | Volume - 10 | Issue - 03 | March - 2021 | PRINT ISSN No. 2250 - 1991 | DOI : 10.36106/paripex

ORIGINAL RESEARCH PAPER

Biology

# BIOSYNTHESIS AND CHARACTERIZATION OF COPPER NANOPARTICLES FROM *SPIRULINA PLATENSIS* AND ITS ANTIBACTERIAL ACTIVITY AGAINST PATHOGENIC BACTERIA *SALMONELLA TYPHI*

**KEY WORDS:** *Spirulina platensis*, Copper Nanoparticles, *Salmonella typhi*, Antibacterial activity

| | |
|---|---|
| **B. Sivasankari*** | Department of Biology, The Gandhigram Rural Institute (Deemed to be University), Gandhigram-624 302. *Corresponding Author |
| **B. Sooryakanth** | Department of Biology, The Gandhigram Rural Institute (Deemed to be University), Gandhigram-624 302. |
| **M. R. Rajan** | Department of Biology, The Gandhigram Rural Institute (Deemed to be University), Gandhigram-624 302. |
| **S. Ganesh** | Department of Physics, The Gandhigram Rural Institute (Deemed to be University), Gandhigram-624 302. |

**ABSTRACT**

Nanotechnology offers unique revolutionary impact on biology and medicine because of size dependent physical and chemical properties. The physicochemical properties of the metal NPs are totally different to that of the pure substances due to their high surface to volume ratio which alters the mechanical, thermal and catalytic properties of the material. *Spirulina* is gaining more attention in the field of medical science because of its nutritional and pharmaceutical importance. It has been demonstrated that small amounts of *Spirulina* reduced HIV-1 replication while higher concentration totally stopped its reproduction. Thus, the *Spirulina* mediated synthesis of CuO and bimetallic nanoparticles communicated in this work gains its importance in its medical application. Synthesis of copper nanoparticles by the strain of *S. platensis* was carried out by the modified method of Mahdieh *et al. S. platensis* strain were isolated and purified repeatedly through liquid subculture method. In the characterization of copper nanoparticles using FTIR analysis copper nanoparticles shown the peaks characters O-H stretch of carboxylic acid (3291.89cm-1), C- H stretch alkyls (2920.66cm-1), C-H stretch of alkanes (2187.84cm-1) and C=C stretch of alkenes (1656.55cm-1). In the UV spectrum analysis, to study characters of nanoparticle based on intensity of light: CuO nanoparticles formed in the reaction media has absorbance peak at 440 nm and broadening of peak indicated that the particles are polydispersed, the indicative of relatively small, monodisperse. XRD pattern showed a small peak indicating the crystalline nature of the reduced copper nanoparticles. In *vitro* antibacterial activity of CuO nanoparticles was tested against pathogenic *Salmonella typhi,* using agar well diffusion technique. The inhibition of bacterial growth at various applied concentrations of the test solutions are significant indicating that CuO nanoparticles exhibits good biocidal activity.

## INTRODUCTION

Nanoscience is currently a fast growing niche and nano-technology is at the cutting edge of this rapidly evolving area (Mandal *et al.*, 2006). Nano-technology collectively describes technology and science involving nano-scale particles (nanoparticles) that increases the scope of investigating and regulating the interplay at cell level between synthetic materials and biological systems (Du *et al.*, 2007). It can be employed as an efficient tool to explore the finest process in biological processes (Sondi & Salopak, Sondi, 2004) and biomedical sciences (Hutten *et al.*, 2004). One of the major developments in nanotechnology is the production and application of nano particles in biology. New methods to produce nanoparticles are constantly being studied and developed. The enormous interest in the bio-synthesis of NPs is due to their unusual optical (Krotiknowska *et al.,* 2003), chemical (Kumar *et al.,* 2003), Photochemical (Chandrasekharan *et al.*, 2000), electronic (Peto *et al.*, 2002) and magnetic (Watson *et al.,* 1999), properties, NPS are either newly created via nanotechnology or are present naturally over the earths crust or in the environment caused by weathering of Au deposits. The metal formed by evaporation is coupled with minerals and been deposited rapidly from saline ground water. Nanotechnology has been defined as a technology that mainly consist of the process of separation, consolidation and deformation of materials by one atom or molecule (Taniguchi, 1974). Silver is a non toxic safe inorganic antibacterial agent used for centuries and is capable of killing about 650 types of diseases causing micro-organisms (Jeorg *et al.*, 2005). Copper has been described or being "oligodynamic" because of its ability to exert a bactericidal effect at minute concentrations (Percivala *et al.,* 2005). Among the biological materials, microorganisms (Reddy *et al.* 2010; Kumar and Mamidyala 2011 ; Otari *et al.* 2012; Gaidhani *et al.* 2013), plants (Aravinthan *et al.* 2015 ; Sengottaiyan *et al.* 2016b ; Senthilkumar *et al.* 2016) and green algae (Mahdieh *et al.* 2012; Sinha *et al.* 2015) have been reported the synthesis of Cu NPs. Several studies revealed *S.platensis* or its extract could show physiological benefits as antioxidants, anti-microbial, antiinflammatory, antiviral or anti-fungal (Carreri *et al.*, 2001, Mendes *et al.,* 2003, Pinero, 2001, Subhashim *et al.*, 2004). The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. The majority of clinically used anti-microbial drugs have various draw back in terms of toxicity, efficacy, cost and their frequent use has led to the emergence of resistant strains. The use of nanoparticles derived from noble metals has spread too many areas including jewelry, medical fields, electronics, water treatment and sport utilities thus improving the longevity and comfort in human life. The application of nanoparticles as delivery vehicles for bactericidal agents represents a new paradigm in the design of antibacterial therapeutics. (Vijayaraghavan and Nalini, 2010). Major consumer goods manufactures like LG and Samsung already produce household items that silver nanoparticles. These products include nano-silver lined refrigerators air conditioners and washing machines (www.azom.com, 2011). The recent years infections caused by bacteria resistant to multiple antibiotics have been a significant problem. *Salmonella* is a genus of rod- shaped (bacillus) Gram negative bacteria of the family Enterobacteriaceae. *Salmonella typhi (S. typhi)* are bacteria which infect the intestinal tract and the blood. The disease is referred to as typhoid fever. *S. paratyphi* A, B and C bacteria cause a similar illness which is included under the typhoid heading. However, paratyphoid fever is generally milder and shorter in duration than typhoid fever. In the present study, attempts were made to utilize the potential of cell-free extracts of *S. platensis* as a bio-factory for the Cu NP synthesis. The objective of this study was the bactericidal effect of CuO NPs using pathogenis bacterial strain, *Salmonella typhi.* The antimicrobial activity was enumerated based on the zone of inhibition measured in agar well diffusion tests conducted in plates.

## METHODOLOGY

### Algal strain and growth condition

The experimental organism *S. platensis* was cultivated in Zarrouk's medium illuminated with white fluorescent lamps at a light intensity of 2,000 lux. Conical flasks of 250 ml capacity were prepared containing 100 ml *S. platensis* culture with initial optical density 0.1. Cultures were shaken gently thrice a day to avoid clumping and enhance the growth. Typically, 5 g (dry weight) *S. platensis* biomass was suspended in 100 ml of double distilled sterile water for 15 min at 100 °C in an Erlenmeyer flask. After boiling, the mixture was cooled and centrifuged at 10,000 rpm for 15 min. Supernatant was collected and was stored at 4 °C for further analysis.

### Biosynthesis of Copper Oxide Nanoparticles

Synthesis of copper nanoparticles by the strain of *S. platensis* was carried out by the modified method of Mahdieh *et al*. A total of 5 mL of exponential growth phase culture of *S. platensis* was transferred to the 250 mL Erlenmeyer flask with 100 mL of 1 mmol/L aqueous copper (II) acetate [Cu(CH3COO)2·H2O] solution (pH 7) for 24 h under shaking condition at 28 °C.

### Characterization

UV-vis spectral analysis was performed on a Shimadzu-1800 spectrophotometer. The biosynthesized copper nanoparticles solution was centrifuged at 10000 r/min for 15 min and the suspension was redispersed in sterile distilled water. Finally, dried samples were palletized with KBr for Fourier transform infrared spectroscopy (FTIR) measurements. The spectrum was recorded in the range of 4000-500 cm-1 using Bruker OpticGmbH Tensor 27. X-ray diffraction (XRD) measurement of the copper nanoparticles was carried out using Rigaku smart lab instrument operated at a voltage of 40 kV and a current of 30 mA with Cu Ka1 radiations Field emission scanning electron microscopy-energy dispersive X-ray analysis (FESEM-EDAX) was performed by Supra 55-Carl Zeiss, Germany.

### Antibacterial activity

The antibacterial activity of coated dressing was tested against *S. typhi* adopting agar diffusion assay. Copper nanoparticles prepared by the reduction of copper ions using spirulina as reductants as well as stabilizing agent was placed on the Nutrient agar plates swabbed with respective bacterial culture incubated at 37 °C for 24 h and the plates were observed for zone of inhibition. After the incubation period the diameter of the zone was recorded.

## RESULTS AND DISCUSSION

### Synthesis and characterization of silver nanoparticles

In the present study, copper nanoparticles were synthesized from *S. platensis* and evaluation of antibacterial and has been made. Reduction of copper ions into copper nanoparticles by the algal culture was visually identified by colour change from deep blue solution gradually became colourless and then turned slowly to brick red which changed to dark brown after vigorous stirring for 24 h. The colour change was observed. It is well known that copper nanoparticles exhibit dark brown color in aqueous solution due to excitation of surface plasmon vibrations in copper Nanoparticles. UV-vis absorption spectroscopy analysis reveals a broad surface plasmon absorption maxima at 440 nm (Figure 1). Absorption spectra of copper nanoparticles formed in the reaction media has absorbance peak at 440 nm and broadening of peak indicated that the particles are polydispersed, the indicative of relatively small, monodisperse in the aqueous solution. Characterization of synthesized copper nanoparticles by scanning electron microscopy reveals spherical particles with few rod shaped crystals with the size ranging 20±5 nm (Figure 2). Some nanoparticles aggregated into large clusters with no well-defined morphology were also observed. This aggregation may be due to the presence of secondary metabolites in the *S. platensis* extracts. Further, characterization was carried out by FTIR(Figure 3). FTIR spectrum of the synthesized Nanoparticles shows strong peaks characters O-H stretch of carboxylic acid (3291.89cm-1), C- H stretch alkyls (2920.66cm-1), C-H stretch of alkanes (2187.84cm-1) and C=C stretch of alkenes (1656.55cm-1) (Table 1). The formation of synthesized copper nanoparticles was further supported by X-Ray Diffraction (XRD) measurements. This method is used to elucidate crystallinity and the lattice properties of the copper Nanoparticles. Presence of distinct high diffraction peaks at 35.5 °, 35.6°, 38.7° and 38.9° respectively, indexing the Bragg's reflection planes (6048), (6176), (6707) and (7264) confirmed the face centered cubic structure of crystalline copper nanoparticles (Figure 4). Energy Dispersive X-ray spectroscopy (EDX) was performed to confirm the formation of AgNPs and CuNPs by recording the strong signals of elemental silver. The results indicated that reaction product was composed of best purity AMPs from *s. platensis* extract 15% of metallic Cu along with occurrence of carbon and on peaks reveal the presence of covering organic moieties on the metallic nanoparticle existence of carbon, oxygen and phosphorus may have originated from me biomolecules bound to the surface of the nanoparticle. The EDX pattern clearly shows that the Cu nanoparticles were formed by the reduction of copper ions using *S. platensis* extracts. There was a strong signal of elemental Cu NPs using EDX spectroscopy.

### Antibacterial activity

strain was susceptible to the copper nanoparticles as dose dependent manner (Table2). An increase in inhibitory zone was recorded in high concentration (Figure). In the case of *Salmonella typhi*, maximum zone of inhibition was recorded at 75 µg/mL with 24.2 mm followed by 50 µg/mL with 23.0 mm, 30 µg/mL with 21.2 mm, 10 µg/mL with 16.0 mm of zone of inhibition. It can be seen that nanoparticles showed high antibacterial efficacy.

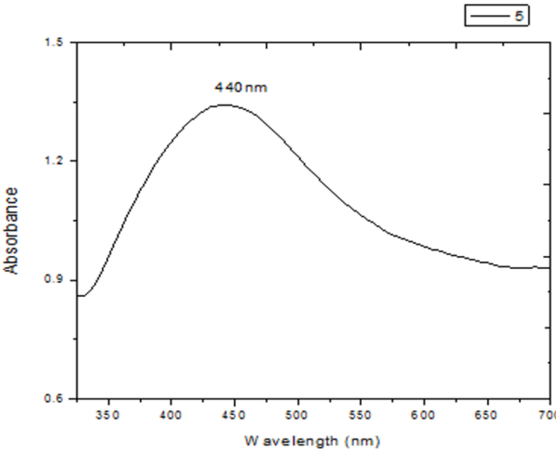


**Figure1: UV visible spectrometry of CuNPs**

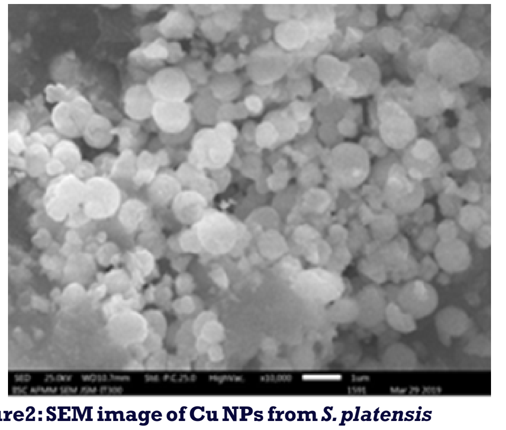

**Figure2: SEM image of Cu NPs from *S. platensis***

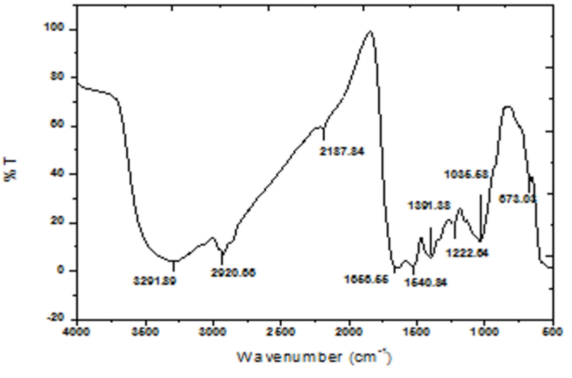


**Figure3: Infrared spectrum analysis of Cu Nps**

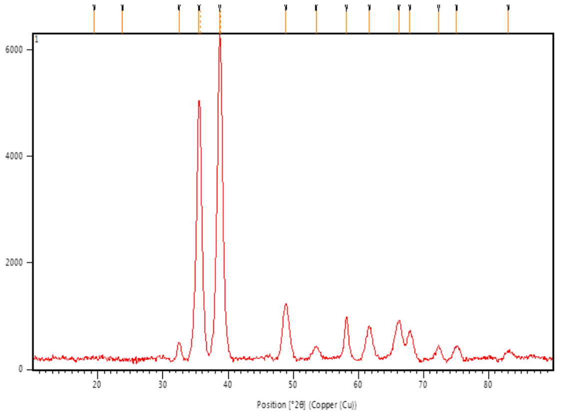


**Figure4: XRD Analysis of Cu Nps**

**Table1: Infrared spectrum analysis CuNPs**

| S. NO | Wave number ($cm^{-1}$) | Comments |
|---|---|---|
| 1 | 3291.89 | O- H stretch (carboxylic acid) |
| 2 | 2920.66 | C-H stretch (alkyls) |
| 3 | 2187.84 | C-H stretch (alkanes) |
| 4 | 1656.55 | C=C stretch (alkenes) |
| 5 | 1540.84 | Assigned to C-C skeletal vibration |
| 6 | 1222.64 | Stretching vibration for C-O from hydroxyflavones and catechins |
| 7 | 1391.38 | -NO stretch in functional group |
| 8 | 1035.58 | C-F stretch (alkyl halides) |
| 9 | 673.03 | C-Br stretch in functional groups |

**Table2: Zone of inhibition of copper nanoparticles against *S. typhi***

| Concentration (µg/mL) | Zone of inhibition (mm) | |
|---|---|---|
| | Plate 1 | Plate 2 |
| 10 | 15.2 | 16.0 |
| 30 | 21.2 | 19.5 |
| 50 | 21.8 | 23.0 |
| 75 | 24.2 | 22.3 |

## CONCLUSION

Nowadays, biological method of nanoparticles synthesis is a vast growing technique in the field of nanotechnology. Algal mediated synthesis of nanoparticles is now being extensively carried out by researchers because of the high rate of synthesis, efficacy and best compatibility. In the present work, we report an eco-benign and convenient method for the synthesis of CuO NPs using cell free aqueous extract of *S. platensis*. The bioreduction of aqueous $Cu^{2+}$ ions to copper nanoparticles using *S.platensis* as bioreductants and stabilizing agents have been carried out. The progress of $Cu^{2+}$ ions bioreduction was monitored, spectrophotometrically. The FTIR spectra revealed that the reduction of copper ions and stabilization of the resultant copper nanoparticles occur through the participation of *spirulina* proteins. As-synthesized copper nanoparticles exhibited antimicrobial activity against pathogenic bacteria: gram-negative *Salmonella typhi.* Therefore, Copper nanoparticles may be a prominent product with potential application in medicine and hygiene. An important potential benefit has numerous benefits *i.e.* non-toxic, cost effective, rapid reduction, economic viability. This biosynthesis method can be a promising method for the preparation of other metals and metaloxide nanoparticles and can be valuable in environmental, biotechnological, pharmaceutical and medical applications. Future prospects of this research would be to large scale production of CuO NPs using *S. platensis* and to ascertain its efficacy against wide spectrum of microbial population. Further investigations would involved is covering the potency of *S. platensis* to synthesize copper nanoparticles.

## REFERENCES


1. Ahmed S, Ahmad M, Swami BL, Ikram S. 2016. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. *J. Adv. Res.* 7:17e28.
2. Ali DM, Sasikala M, Gunasekaran M, Thajuddin N. 2011. Biosynthesis and characterization of silver nanoparticles using marine cyanobacterium, *Oscillatoria willei. Digest Journal of Nanomaterials and Biostructures.* 6: 385-390.
3. Azomaterials. 2006. Silver Nanoparticles – How they are bringing antibacterial properties to household appliances and products. http://www.azom.com/Details.asp/ Article ID = 3533.
4. Becker RO. 1999. Silver ions in the treatment of local infections. *Met. Based Drugs.* 6: 297–300.
5. Belay A. 2002. The potential application of *Spirulina* (*Arthospira*) as a nutritional and therapeutic supplement in health management. *J. Am. Nutracent. Assoc.* 5: 27-48.
6. Caroling G, Priyadharshin MN, Vinodhini E, Ranjitham A, Shanthi P. 2015. Biosynthesis of Copper Nanoparticles Using Aqueous Guava Extract Characterization And Study Of Antibacterial Effect. *Int. J. Pharm. Bio. Sci. 5*: 25-43. ISSN: 2230-7605.
7. Chandrasekharan N, Kamat PV. 2000. Improving the photo electrochemical performance of nanostructured TiO2 films by adsorption of gold nanoparticles. *Journal of Physical Chemistry B.* 104: 10851-10857.
8. Du L, Jiang H, Liu X, Wang E. 2007. Biosynthesis of gold nanoparticles assisted by *Escherichia coli* DH5a and its application on direct electrochemistry of haemoglobin. Electrochemistry Communications. 9: 1165-1170.
9. Chatterjee AK, Sarkar RK, Chattopadhyay AP, Aich P, Chakraborty R, Basu T. 2012. A simple robust method for synthesis of metallic copper nanoparticles of high antibacterial potency against *E. coli. Nanotechnology.* 23: 085103.
10. Dhas NA, Raj CP, Gedanken A. 1998. Synthesis, characterization, and properties of metallic copper nanoparticles. *Chem. Mater.* 10: 1446e1452.
11. Gunalan S, Sivaraj R, Venckatesh R. 2012. Aloe barbadensis Miller mediated green synthesis of mono-disperse copper oxide nanoparticles: optical properties. *Spectro chimica Acta Part A: Molecular and Bio-molecular Spectroscopy.* 97: 1140–1144.
12. Hutten A, Sudfeld D, Ennen I, Reiss G, Hachmann W, Heinzmann U, Wojczykowski K, Jutzi P, Saikaly W, Thomas G.2004. New magnetic nanoparticles for biotechnology. *Journal of Biotechnology.* 112: 47-63.
13. Joerger R, Klaus T, Granqvist CG. 2000. Biologically produced silvercarbon composite materials for optically functional thin film coatings. *Adv. Mater.* 12: 407–409.
14. Karthik AD and Kannappan G.2015. Synthesis,Spectroscopic Investigation and Biological Activities Of Synthesized Copper And Its Oxide Nanoparticles. *Int. J. of Adv. Chemical Sci. and Applications. 3*: 1-4.
15. Krolikowska A, Kudelski A, Michota A, Bukowska J. 2003. SERS studies on the structure of thioglycolic acid monolayers on silver and gold. *Surface Science.* 532: 227-232.
16. Krithiga N, Jayachitra A, Rajalakshmi A. 2013. Synthesis, characterization and analysis of the effect of copper oxide nanoparticles in biological systems. *Indian Journal of Nephrology.* 1: 6–15.
17. Kumar A, Mandal S, Selvakannan PR, Pasricha R, Mandal AB, Sastry M. 2003. Investigation into the interaction between surface bound alkyl amines and gold nanoparticles. *Langmuir.* 19: 6277-6282.
18. Mahdieha M, Zolanvari A, Azimeea AS. 2012. Green biosynthesis of silver nanoparticles by *Spirulina platensis. Scientia Iranica* 19(3): 926-929.
19. Mandal D, Bolander ME, Mukhopadhyay D, Sarkar G, Mukherjee P. 2006. The use of microorganisms for the formation of metal nanoparticles and their application. *Applied Microbiology and Biotechnology.* 69: 485-492.
20. Percivala SL, Bowler PG, Russell D. 2005. *J. Hosp. Infect.* 60(1): 1.
21. Monson CF, Woolley AT. 2003. DNA-templated construction of copper nanowires, *Nano Lett.* 3: 359e363.
22. Peto G, Molnar GL, Paszti Z, Geszti O, Beck A, Guczi L. 2002. Electronic structure of gold nanoparticles deposited on SiOx/Si. *Materials Science and Engineering.* C 19: 95–99.
23. Saran S, Sharma G, Manoj Kumar, Ali MI. 2017. Biosynthesis of copper oxide nanoparticles using Cyanobacteria *Spirulina platensis* and its antibacterial activity. *IJPSR, Vol. 8(9): 3887-3892.*
24. Sharma G, Jasuja ND, Kumar M, Ali MI. 2015. Biological Synthesis of Silver Nanoparticles by Cell-Free Extract of *Spirulina platensis. Journal of nanotechnology.* 2015: 6.
25. Sondi I, Salopek-Sondi B. 2004. Silver nanoparticles as antimicrobial agent: A case study of *E. coli* as a model for Gram-negative bacteria. *J. Colloid Inferf. Sci.* 275 – 177.
26. Taniguchi N. 1974. On the basic concept of 'Nano-Technology'. Proceedings of International Conference on Production Engineering Tokyo, Part II. Japan Society of Precision Engineering, Japan.

27. Umer A, Naveed S, Ramzan N, Rafique MS, Imra MA. 2014. Green Method For The synthesis Of Copper Nanoparticles Using L-ascorbic Acid. *Matéria*. *19*(3): 197- 203.
28. Vijayaraghavan K, Kamala Nalini SP. 2010. Biotemplates in the green synthesis of silver nanoparticles. *Bioechnology J*. 5 (10): 1098 – 1110.
29. Watson JHP, Ellwood DC, Soper AK, Charnock J. 1999. Nanosized strongly magnetic bacterially-produced iron sulfide materials. *Journal of Magnetism and Magnetic Materials.* 203: 69-72.
30. Yin M, Wu CK, Lou Y, Burda C, Koberstein JT, Zhu Y, O'Brien S. 2005. Copper oxide nanocrystals. *Journal of the American Chemical Society*. 127: 9506–9511.