J. Appl. Biosci., 49(1&2): 22-26, January-December, 2023

ISSN (Print) 0975-685X
ISSN (Online) 0975-864X

# BIOGENIC SYNTHESIS OF GREEN-NANOPARTICLES USING ETHANOLIC EXTRACT OF *NOSTOC PUNCTIFORME* (KÜTZING EX HARIOT) HARIOT AND ASSESSMENT OF ANTIBACTERIAL ACTIVITY

**KUNTAL SARMA[1,2], RAMA KANT*[1], NARENDRA KUMAR*[2,3], MANJU SHARMA[2], AARTI MALIK[1], MANSI BALIYAN[1], SAKSHI[1], MANVI BALIYAN[1], DOLI[1], DEEPTI GUPTA[1] and GAURI[1]**

[1]Department of Botany, Chaudhary Charan Singh University, Meerut-250004, India
[2]Amity Institute of Biotechnology, Amity University, Manesar, Gurgaon, Haryana-122413, India
[3]Department of Botany, Guru Ghasidas Vishwavidyalaya (A Central University), Bilaspur-495009 (C.G.), India

## ABSTRACT

*Objective*: The aim of the study is to synthesize CuNP and AgNP from *Nostoc punctiforme* (Kützing ex Hariot) Hariot and evaluation of their antibacterial activity against *Escherichia coli* and *Klebsiella pneumonia*.

*Methodology and Results*: Copper and silver nanoparticles from ethanolic extract of 15 days old *Nostoc punctiforme* The synthesis of AgNP and CuNP was confirmed due to change in the colour of the solution from pale green to dark black and pale yellow to dark brick red respectively. The absorption spectra of the synthesized nanoparticles at the UV range between 220-260nm showing stable productions of the nanoparticles. The antimicrobial activity of synthesized nanoparticles was assessed using *E. coli* and *K. pneumoniae*. The Copper nanoparticles show more antibacterial effect against *K. pneumoniae* in comparison to the silver nanoparticles, ethanolic extract of *N. punctiforme* and Streptomycin. Similarly in case of *E. coli* copper nanoparticle is more effective than that of silver nanoparticle but less than the positive control.

*Conclusions and application of findings*: From the results obtained, it could be concluded that the antimicrobial activity of both the synthesized nanoparticles were effective against *E. coli* and *K. pneumoniae*. The Copper nanoparticles show more antibacterial effect against *K. pneumoniae* in comparison to the silver nanoparticles, ethanolic extract of *N. punctiforme* and Streptomycin. Similarly in case of *E. coli* copper nanoparticle is more effective than that of silver nanoparticle but less than the positive control.

**KEY WORDS:** Nanoparticles, *Nostoc punctiforme, Escherichia coli*, *Klebsiella pneumoniae*, antibacterial activity

## INTRODUCTION

Cyanobacteria (Blue-green Algae) are rich source of biologically active constituents and have been identified as one of the most promising groups of organisms to be able to produce bioactive compounds. These are known to produce metabolites with diverse biological activities such as antibacterial, antifungal, antiviral, anticancer, anti-plasmodial, algicide, antiplatelet aggregation, and immuno suppressive activities. Cyanobacteria from local habitats seem to be a source of potential new active substances that could contribute to reduction of the number of bacteria, fungi, viruses and other microorganisms (Ramamurthy *et al*., 2012). Cyanobacterial natural products well recognized for their bioactivity and utility in drug discovery and biotechnology applications (Safavi *et al*., 2019).

Recently nanoscience and nanotechnology became a rapidly growing field in the realm of science and technology. The green method for synthesis of nanoparticles using cyanobacteria showed highly potential antibacterial activities toward different type of pathogenic bacteria, such as *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus* (Abedin & Taha, 2008). The biologically synthesized nanoparticles are highly useful for biomedical

*Corresponding author email: narendra.microbiology@rediffmail.com

applications. The small size and the high surface of nanoparticles increase their interaction with the microbes to carry out a large range of probable antimicrobial activities and other wide range of applications (Morsy, 2014).

The nitrogen-fixing cyanobacteria of genus *Nostoc* family of Nostocaceae has ability to use atmospheric nitrogen when combined nitrogen is not available *Nostoc* is rich in water-soluble polysaccharides which may be have some positive bioactivity (Quan *et al*., 2015). The biosynthesis of green nanoparticles by microorganism is considered as non-toxic and environment-friendly technology (Vanlalveni *et al*., 2018).

In the present study, copper nanoparticles (*Np*-CuNP) and silver nanoparticles (*Np*-AgNP) were synthesized using the heterocystous, dinitrogen-fixing photosynthetic cyanobacterium *N. punctiforme* isolated from rice field of Kailashahar, Unakoti Tripura, India. This article records work on synthesis and spectral characterizations of green nanoparticles using the strain *N. punctiforme*. Antibacterial activities of ethanolic extract of *N. punctiforme*, CuNP and AgNP extracted from *N. punctiforme* against clinically isolated pathogenic multidrug resistant (MDR) gram-negative bacteria species *E. coli* and *K. pneumoniae* isolated from clinical samples.

## MATERIALS AND METHODS

**Isolation, Identification and Culturing of the cyanobacteria:** Mixotropic growth of cyanobacteria were collected from rice fields and isolated by repeated culturing and sub-culturing in nitrogenous and nitrogen deficient liquid and solid BG-11 culture medium (Stanier *et al*., 1971). The unialgal cultures were developed as per standard method described by Kant *et al*. (2005). The isolated strains were identified up to species level with the help of available literatures and monographs (Komárek, 2013).

**Preparation of *N. punctiforme* extract:** An axenic culture of *N. punctiforme* was grown as mass cultures in 1000 ml conical flask. The biomass was harvested and washed thrice with double distilled water to remove any impurities. For the ethanolic extraction, 20g of dry *N. punctiforme* biomass was dissolved in 30ml of ethanol and the mixture was boiled for 20 min, cooled at room temperature and filtered for the preparation of extract.

**Preparation of AgNP and CuNP:** Silver nanoparticles (Ag NP) were prepared by the method described by Patel *et al*. (2015). An aliquot of $AgNO_3$ (1 mM) was added to *N. punctiforme* extract and the mixture was incubated in 2000±200 lux white fluorescent light for 24-56 h. Changes in the color of the content was subsequently observed in 1 h intervals for 24 hours. After 24 h, the nanoparticles were dispersed in double distilled water and sonicated for 30 mins. The incubated mixture having AgNP was further assessed by UV-VIS spectrophotometer at OD 200-800 nm. The mixture containing *N. punctiforme*-silver nanoparticles (*Np*-AgNP) was centrifuged at 5000 rpm for 10 mins, the pellet was washed thrice in double distilled water and dehydrated at 30°C, before spectral characterizations.

Similarly Copper nanoparticles (CuNPs) were obtained by the method described by Patel *et al* (2015) by combining the prepared *N. punctiforme* extract in a 1:9 ratio with a 1 mM concentrated anhydrous copper sulfate solution. The mixture was stirred at room temperature for two hours. CuNPs were synthesized, as evidenced by the solution's brown color. The synthesized nanoparticles were dispersed in double distilled water and sonicated for 30 mins. The mixture having *N. punctiforme*-copper nanoparticles (*Np*-CuNP) was incubated and further assessed for spectral characterization using UV-VIS spectrophotometer at OD 200-800 nm.

**UV-VIS spectroscopy analysis:** The absorption spectra of ethanolic extract were obtained at room temperature using a UV-VIS spectrophotometer (Shimadzu, *UV-2600i*).

**Antibacterial activity:** Antibacterial activity of ethanolic extract, synthesized Ag and Cu nanostructures was examined by well diffusion method (Tailor *et al*., 2020). *E. coli* and *K. pneumoniae* was used to assess anti-microbial activity of nanoparticles. Pure *E. coli* and *K. pneumoniae* cultures were available at Algal Biotech Laboratory, Department of Botany, Chaudhary Charan Singh University, Meerut, India. For antimicrobial assay about 100µl nanoparticles sample of 1000ppm, was loaded in each well. Antibiotic streptomycin was used as positive control.

## RESULTS AND DISCUSSION

The isolated strain was identified as *N. punctiforme* (Kützing ex Hariot) Hariot on the basis of morphological parameters such as trichome, cell size, position and size of

Table 1: Representing the antibacterial activity of tested nanoparticles in comparison to the antibiotic Streptomycin and ethanolic extract

| Test material | *E. coli* Zone of inhibition in mm | *K. pneumoniae* Zone of inhibition in mm |
|---|---|---|
| Ethanol extract | 8 | 5 |
| Streptomycin | 24 | 10 |
| *Np*-AgNP | 7 | 7 |
| *Np*-CuNP | 13 | 16 |

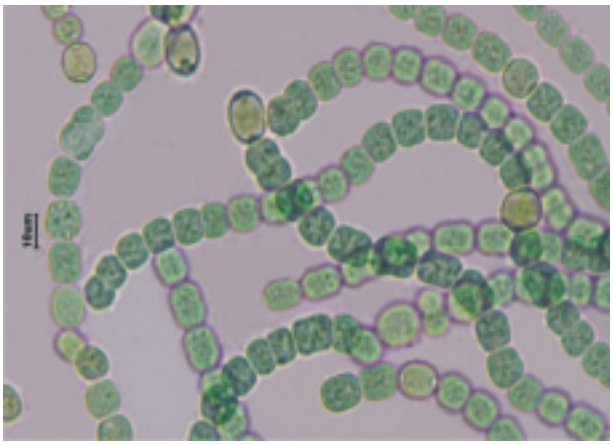

Fig. 1: Morphological details of *Nostoc punctiforme*

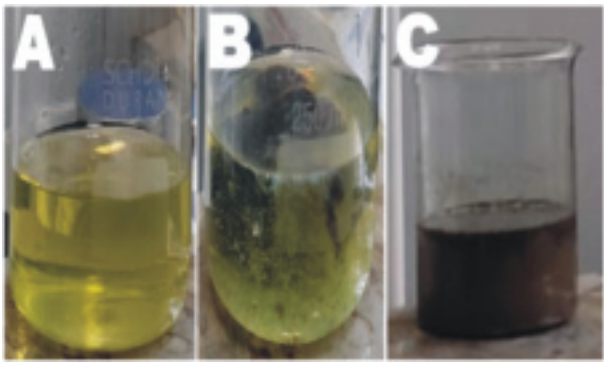


Fig. 2: Synthesis of NP from *N. punctiforme* Figures A, B, C showing stepwise synthesis of Nanoparticles

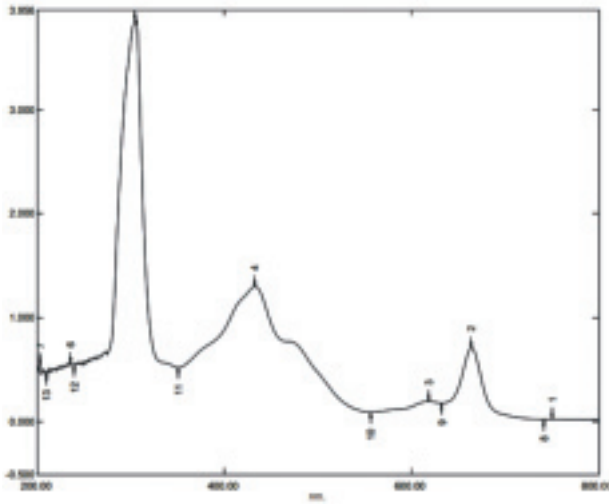

Fig. 3: UV-VIS spectrum showing absorption of ethanolic extract of *N. punctiforme*

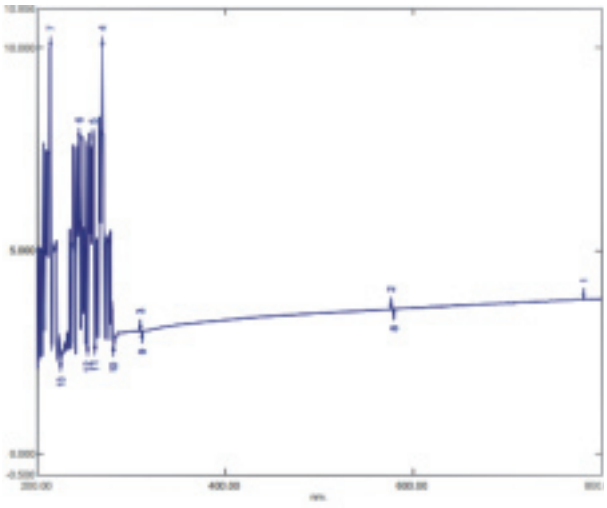

Fig. 4: UV-VIS spectrum showing absorption spectra of AgNP synthesized from *N. punctiforme*

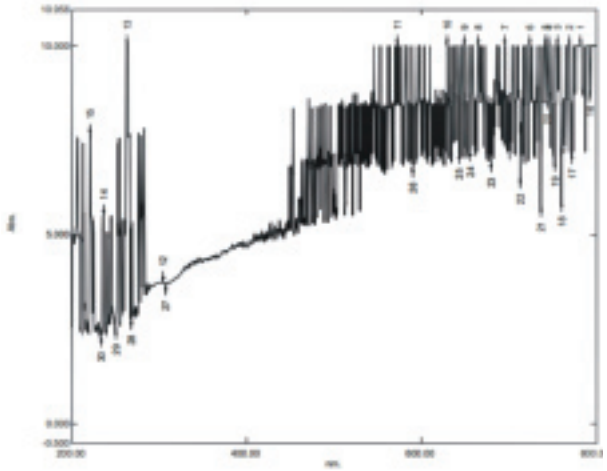

Fig. 5: UV-VIS spectrum showing absorption of CuNP synthesized from *N. punctiforme*

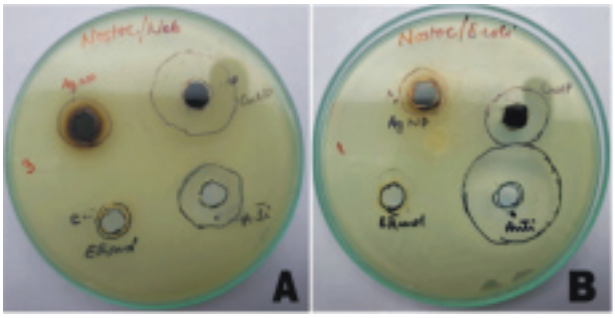


Fig. 6(A-B): Figures shows the zone of inhibition of test concentration of copper nanoparticles, silver nanoparticles, ethanolic extract of *N. punctiforme* and antibiotic Streptomycin against (A) *E. coli;* (B) *K. pneumoniae*

heterocyst (Fig. 1). The synthesis *Np*-AgNP and *Np*-CuNP from *N. punctiforme* was confirmed by the change of the color from pale green to dark black solution and pale yellow to dark brick red solution respectively. This colour change was observed immediately after the *Np*-AgNP and *Np*-CuNP synthesis (Fig. 2).

The metallic green nanoparticles produced show an interacting electromagnetic field due to the free electrons present at Peak 2, 4 and 5 obtained at room temperature using a UV-VIS spectrophotometer. Ethanolic extract of *N. punctiforme* peak 5 showing the maximum absorption spectra near to UV range while peak 2 and 4 showing absorption spectra at visible range. Detailed result on ethanolic extract of *N. punctiforme* is given in Fig. 3. The OD at the UV-VIS spectrum confirming the stable synthesis of *Np*-AgNP and *Np*-CuNP respectively likely by the reducing and capping by compounds from *N. punctiforme* extracts (Fig. 4 and Fig. 5). Peak 4 and 7 shows absorption spectra of the synthesized silver nanoparticle at the UV range between 220-260nm with stable production of the silver nanoparticles. Detailed absorption spectra of silver nanoparticles are given in Fig. 4. Similarly, peak 13 and 15 showing the absorption spectra of copper nanoparticles in the UV range while lowest absorption spectra was also observed in UV range at peak 28, 29 and 30. Detailed result on absorption spectra of copper nanoparticles is given in Fig. 5.

The antimicrobial activity of synthesized nanoparticles was tested against *E. coli* and *K. pneumoniae*. The antibiotic streptomycin was used as positive control. The ethanol extract of *N. punctiforme* was also compared with synthesized nanoparticles. The plates were incubated for 24 h at 37±2°C in incubator. Zone of inhibition was determined after overnight incubation. The plate-A shows the results of ethanol extract, *Np*-AgNP, *Np*-CuNP and positive control antibiotic streptomycin against *K. pneumoniae*. The Copper nanoparticles show more antimicrobial effect against *K. pneumoniae* in comparison to the silver nanoparticles, ethanolic extract of *Nostoc* sp. and Streptomycin. The plate B shows the results of ethanol extract, AgNP, CuNP and positive control antibiotic streptomycin against *E. coli*. The CuNP shows more antimicrobial effect in comparison to the AgNP and ethanolic extract of *Nostoc* sp. but less than the antibiotic streptomycin. A detailed result of Zone of inhibition is given in Fig. 6 (A-B). The data pertaining to the biological activity of ethanolic extract of *Nostoc* sp., AgNP, CuNP and streptomycin against *E. coli* and *K. pneumoniae* have been presented in Table 1.

Antimicrobial activity depends on both algal species and the solvents used for their extraction (Salem *et al*., 2014). The cell extracts and active constituents of various algae shown to have wide range of antifungal and antibacterial activity. Cyanobacteria have intrinsic cellular compounds, which are bioactive against microbes and are also used in the biosynthesis of silver-nanoparticles (Sahoo *et al*., 2021). Green nanoparticles synthesized from cyanobacterial bioactive compounds have great potential having anti-bacterial, anti-cancerous and anti-fungal activity. The cyanobacteria such as *Nostoc commune, Anabaena variabilis*, *Nostoc spongiaeforme*, *Anabaena flos-aquae* have been reported to produce antimicrobial substances and posses antimicrobial property. Phenolic compounds from *Nostoc muscorum*, lipopeptidases from *Anabaena sp*. fatty acids, tetramine, spermine and piperazine derivative from *Anabaena laxa* (Shaieb *et al*., 2014).

## CONCLUSION

From the results obtained it could be concluded that both the NPs synthesized from ethanolic extract of *N. punctiforme* have antibacterial activity against *K. pneumonia* than that of *E. coli*. The results also suggested that the *Np*-CuNP are more stable than that of *Np*-AgNP. The dispersion capability of *Np*-CuNP is more than *Np*-AgNP.

## ACKNOWLEDGEMENTS

Authors are thankful to the Head, Department of Botany, Chaudhary Charan Singh University, Meerut for providing necessary facilities. Authors are also thankful to Rajendra Prashad, Director AIB, AIISH, Amity Institute of Biotechnology, Gurgaon and Head, Department of Botany, Guru Ghasidas Vishwavidyalaya, Bilaspur for providing necessary facilities. Authors are thankful to Prof. G. L. Tiwari, Ex. Head, Botany Department, University of Allahabad, Prayagraj for identification of the strain. The authors (RK and KS) also thankful to CCS University (Ref. No. DEV/URGS/2022-2023/24, Dated: 22/7/2022) and UP Govt (F.No. 70/2022/ 1543/Sattar-4-2022/001-70-4099-7-2022 Dated: 07-07-2022) for providing financial support.

## REFERENCES

Abedin, R.M. & Taha, H.M. (2008). Antibacterial and antifungal activity of cyanobacteria and green microalgae. Evaluation of medium components by Plackett-Burman design for antimicrobial activity of *Spirulina platensis*. *Global J. Biotechnol. Biochem.*, 3(1): 22-31.

Kant, R., Tiwari, O.N., Tandon, R. & Tiwari, G.L. (2005). Adaptive mechanism in the developmental stages of an aerophytic Cyanoprocaryote, *Asterocapsa* Chu: a survival factor. *National Acad. Sci. Lett.*, 28(11-12): 373-378.

Komárek, J. (2013). Süßwasserflora von Mitteleuropa, Bd. 19/ 3: Cyanoprokaryota 3. Teil/3rd part: Heterocytous Genera. *Süßwasserflora von Mitteleuropa.* Spektrum Academischer Verlag, Heidelberg.

Morsy, F.M., Nafady, N.A., Abd-Alla, M.H. & Elhady, D.A. (2014). Green synthesis of silver nanoparticles by water soluble fraction of the extracellular polysaccharides/matrix of the cyanobacterium *Nostoc commune* and its application as a potent fungal surface sterilizing agent of seed crops. *Univ. J. Microbiol. Res.*, 2(2): 36-43.

Patel, V., Berthold, D., Puranik, P. & Gantar, M. (2015). Screening of cyanobacteria and microalgae for their ability to synthesize silver nanoparticles with antibacterial activity. *Biotechnol. Reports*, 5: 112-119.

Quan, Y., Yang, S., Wan, J., Su, T., Zhang, J., & Wang, Z. (2015). Optimization for the extraction of polysaccharides from *Nostoc commune* and its antioxidant and antibacterial activities. *J. Taiwan Insti. Chem. Engin.*, *52*, 14-21.

Ramamurthy, V., Raveendran, S., Thirumeni, S. & Krishnaveni, S. (2012). Antimicrobial activity of heterocytic cyanobacteria. *Inter. J. Advan. Life Sci.*, 1: 32-39.

Safavi, M., Nowruzi, B., Estalaki, S. & Shokri, M. (2019). Biological activity of methanol extract from *Nostoc sp.* N42 and *Fischerella sp*. S29 isolated from aquatic and terrestrial ecosystems. *Inter. J. Algae*, 21(4): 373-391.

Sahoo, C.R., Swain, S., Luke, A.M., Paidesetty, S.K. & Padhy, R.N. (2021). Biogenic synthesis of silver-nanoparticles with the brackish water cyanobacterium *Nostoc sphaeroides* and assessment of antibacterial activity against urinary tract infecting bacteria. *J. Taibah Univer. Sci.*, 15(1): 805-813.

Salem, O.M., Hoballah, E.M., Ghazi, S.M. & Hanna, S.N. (2014). Antimicrobial activity of microalgal extracts with special emphasize on Nostoc sp. *Life Sci. J.*, 11(12): 752-758.

Shaieb, F.A., Issa, A.A. & Meragaa, A. (2014). Antimicrobial activity of crude extracts of cyanobacteria Nostoc commune and Spirulina platensis. *Arch. Biomed. Sci.*, 2(2): 34-41.

Stanier, R.Y., Kunisawa, R., Mandel, M.C.B.G. & Cohen-Bazire, G. (1971). Purification and properties of unicellular blue-green algae (order Chroococcales). *Bacteriol. Rev.*, 35(2): 171-205.

Tailor, G., Yadav, B.L., Chaudhary, J., Joshi, M. & Suvalka, C. (2020). Green synthesis of silver nanoparticles using Ocimum canum and their anti-bacterial activity. *Biochem. Biophy. Rep.*, 24: 1-5.

Vanlalveni, C., Rajkumari, K., Biswas, A., Adhikari, P.P., Lalfakzuala, R. & Rokhum, L. (2018). Green synthesis of silver nanoparticles using Nostoc linckia and its antimicrobial activity: a novel biological approach. *Bionanosci.*, 8: 624-631.