Green Synthesis and Characterization of Copper Nanoparticles Using Madhunashini Leaf Extract and Evaluation of Its Antibacterial Property

Sweety Singh
Deenbandhu Chhotu Ram University of Science and Technology Murthal-131039

***Abstract*- Development of an environment friendly process for synthesis of NPs is significant step in the field of Nanotechnology. Among all the metallic nanoparticles, copper nanoparticles have drawn attention due to its unique physical, chemical and biological properties. In the present study, biosynthesis of copper nanoparticles was done using leaf extract of Gymnema sylvestre. The leaf extract of Gymnema sylvestre resulted in successful reduction of copper sulphate to Cu NPs as confirmed by UV-Visible Spectrophotometer by the presence of absorption peak at 651 nm. The average particle size of synthesized Cu NPs was found to be 111.9 nm by PSA. HRTEM images of Cu NPs showed the irregular ball like structures and their size was measured in the ranges from 10-24 nm. The antibacterial activity of the Cu NPs was evaluated against E. coli MTCC 40 and B. subtilis MTCC 2616 by agar well diffusion method. These results showed that Cu NPs synthesized from leaf extract of Gymnema sylvestre exhibited good antibacterial activity against gram +ve than gram –ve bacteria. These results confirmed that this method can be used for rapid and ecofriendly biosynthesis of stable Cu NPs.**

***Index-Terms*-Copper nanoparticles, Gymnema sylvestre, E. coli MTCC 40 and B. subtilis MTCC 2616, agar diffusion method**

INTRODUCTION

Nanotechnology is growing field in biomedical [1]. Nanotechnology includes design, synthesis and manipulation of particles [2]. Nanoparticles are having one or more dimensions and having size 100nm or less [3]. Due to low cost and specific properties Cu NPs have special attention than that of bulk metals [4]. Cu NPs can interact with other particles due to high surface to volume ratio. Cu NPs can be synthesized using various methods including chemical reduction and biological [5, 6]. Chemical method used for NPs synthesis is not environment friendly. Biological method includes NPs synthesis using plant extract or using microbes. This method of using plant extract is beneficial over other biological methods which involve the very complex procedure of maintaining cell cultures [7]. Green synthesis of Cu NPs using plants has received lots of attention due to its low cost, fast, environment friendly and providing natural capping agent for the stabilization of Cu NPs [8]. The biomolecules present in the plant extracts are carbohydrates, flavonoids, steroids, glycosides, saponins, triterpenoids, phenols and aromatic compounds which are responsible for reduction and stabilization of copper ions [9]. Due to its cytotoxic and bactericidal properties Cu NPs have applications as antimicrobial material in wound dressing, as heat transfer system, as sensors [10, 11]. Lots of plant has been already reported for synthesis of Cu NPs. The present study was carried out to synthesize the Cu NPs using Gymnema sylvestre (Madhunashini) leaf extract.

MATERIALS AND METHODS

Materials

Copper sulphate pentahydrate i.e. $CuSO_4.5H_2O$, Ethanol, AOT, Gymnema sylvestre leaf extract, Nutrient broth, Agar powder.

Preparation of Gymnema sylvestre leaf extract 5 g Gymnema sylvestre leaves extract powder was dissolved in 100 ml double distilled water and stirred for 30 min to make 5% solution. The solution was then filtered using Whatman filter paper no. 1 and stored in refrigerator for further experiments.

Synthesis of Copper nanoparticles

0.038 g $CuSO_4.5H_2O$ was dissolved in 100 ml of double distilled water to make 1.5 mM solution. 20 ml of the above $CuSO_4$ solution was taken in a conical flask and kept on stirrer at 350 rpm for 30 min. AOT (Dioctyl sodium sulphosuccinate) was then added drop wise with continuous stirring. 4 ml Gymnema sylvestre leaves extract was added to above solution in drop wise in 1:5 ratios. The stirring (at 500rpm) was continued for 3 h for complete reduction of copper sulphate to Cu NPs. The sample was incubated overnight at room temperature. Finally, the solution was centrifuged at 10,000 rpm and washed several times with ethanol and the nanoparticles were collected. The nanoparticles thus obtained were dried on hot plate to get powder form. The black colored product so produced was subjected to further characterization.

Characterization of $CuSO_4$ NPs

The synthesized $CuSO_4$ nanoparticles were subjected to characterization by UV- Visible Spectroscopy, PSA, Zeta potential, SEM and HRTEM.

UV-Vis Spectroscopy study

The reduction of pure copper sulphate to Cu NPs was monitored by studying the UV-Vis spectrum of the sample. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer (SPECTRO star Nano BMG labtech, Germany). Small amount of synthesized Cu NPs sample was taken in distilled water and sonicated for proper mixing of the sample. Then, this sample was used for UV-Vis analysis in 1 cm well-stoppered quartz cuvette in the range of 200-800 nm.

Particle size analysis

Average particle size of synthesized Cu NPs was determined using particle size analysis. For PSA, the samples of Cu NPs suspensions in distilled water was made in ratio 3:1 (distilled water: sample) and ultrasonicated for mixing. Small amount of suspension was taken in well stoppered disposable cuvette and characterized using Zeta-sizer (Malvern, UK).

Zeta Potential

Stability of synthesized nanoparticles can be determined using zeta sizer (ZS-90). For zeta potential analysis, suspensions in distilled water was made in 4:1 (distilled water: sample) and ultrasonicated for mixing. Small amount of suspension was taken in disposable polystyrene cuvette and then characterized by Zeta-sizer (ZS-90, Malvern, UK).

SEM (Scanning electron microscopy) Analysis

The scanning electron microscope (SEM) is a type of EM (electron microscope) that images the sample surface by scanning it at high- resolution. For SEM characterization, suspension of Cu NPs was made in distilled water and ultrasonication was done for proper mixing. Then, thin film were made on cleaned glass slides and dried under lamp. The surface morphology was studied by scanning electron microscopy.

HRTEM Analysis

High resolution transmission electron microscopy is a powerful technique to study size of nanoparticles. Particle size, shape, orientation etc. were characterized by "Tecnai G2 F30 S-Twin (FEI; Super Twin lens with CS=1.2 mm)" HRTEM instrument.

The Cu NPs suspension was made and then a drop of suspension was loaded on copper grid and dried in desiccators. Observation under different magnification was performed.

Antibacterial Properties

The antimicrobial activity of as synthesized Cu NPs was studied against E. coli MTCC 40 and B. subtilis MTCC 2616.

Revival of Culture

A loop full of bacterial cultures from slants was aseptically transferred to nutrient agar medium plates to obtained fresh culture strain. The inoculated plates were then incubated for 18 to 20 h at 30^o C and bacterial growth was observed for purity check. A loop full of these purified bacteria was then transferred to 50 ml sterilized NB (nutrient broth) medium of and incubated for another 18 to 20 h in incubator shaker.

Seeding of test organisms

100 µl of freshly grown culture was plated with 20 ml of molten and cooled nutrient agar media. For setting of agar completely the plates were kept undisturbed for 30 min.

Antimicrobial activity assay

The antimicrobial activity of sample was evaluated using Agar well diffusion method. Three wells were made on the solidified media plates seeded with test organism using sterile hole-borer. The wells were labeled and 80 µl of Cu NPs suspension (50 mM), positive control i.e. 80 µl of Ciprofloxacin (0.25 mg/ml), negative control i.e. 80 µl of distilled water were transferred to respective wells. The plates were then incubated for 12 to 16 h at 30° C. The diameter of zone of inhibition was measured using scale and divider.

RESULT AND DISCUSSION

Synthesis of Cu nanoparticles

The Cu NPs were synthesized due to reduction of $CuSO_4.5H_2O$ to Cu by plant extract. Leaf extract of Gymnema sylvestre was used to synthesize Cu NPs.

Figure: 1(a) Copper sulphate solution, (b) Cu NPs synthesized using Gymnema sylvestre

The greenish colour of extract change to greenish black colour, which indicates the formation of Cu NPs in case of Gymnema sylvestre leaf extract as shown in (Fig. 1, b)

Characterization of $CuSO_4$ NPs

The biosynthesized Cu NPs were identified and characterized by UV-Visible Spectroscopy, PSA, Zeta potential, SEM and HRTEM.

UV-Vis Spectroscopy study:

Leaf Extract of Gymnema sylvestre resulted in successful reduction of $CuSO_4.5H_2O$ to Cu NPs as confirmed by UV-Visible Spectrophotometer. The UV-Vis spectrum was recorded in the wavelength range of 200-800 nm for the synthesized Cu NPs. Synthesized Cu NPs using Gymnema sylvestre leaf extract showed a single SPR peak at 651 nm (Fig. 2), which indicates the formation of Copper nanoparticles.

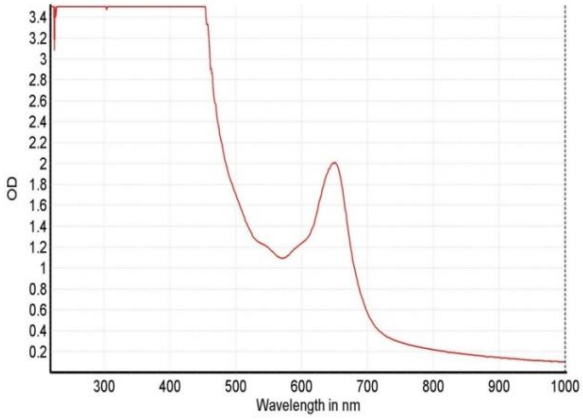

Figure 2: UV-Visible spectrum of Cu NPs synthesized using Gymnema sylvestre

Increase in the bandwidth of the SPR was observed which resulted in a decrease in size of the nanoparticles.

Particle size analysis:

Particle size analysis of Cu NPs synthesized using Gymnema sylvestre leaf extract was performed using Zeta-sizer (Malvern, UK). The particle size of Cu nanoparticles synthesized using Gymnema sylvestre leaf extract was evaluated using zeta sizer and average particle size was found to be 111.9 nm (Fig 3). It was inferred from the obtained results that

particle size of the synthesized nanoparticles was within favorable size range. Also, the sharp peaks indicated the uniform particle size distribution of synthesized Cu NPs.

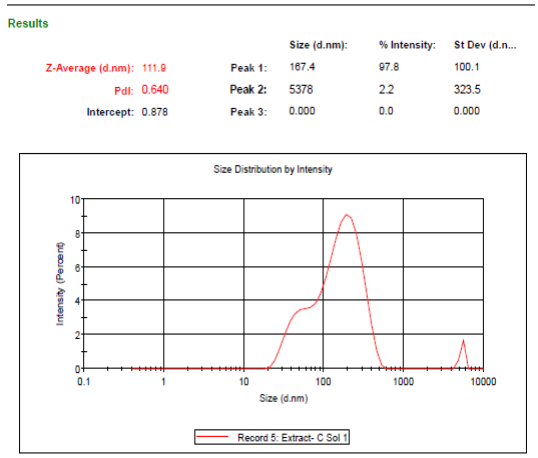

Figure 3: Particle Size of as synthesized Cu NPs (using G. sylvestre).

Zeta potential:

Zeta potential of Cu NPs synthesized using Gymnema sylvestre leaf extract was performed carefully. It determines the stability of synthesized Cu nanoparticles. The zeta potential of Cu NPs synthesized using Gymnema sylvestre leaf extract was evaluated and was found to be -57.4 mV (Fig. 4). It was inferred from the obtained results that high negative magnitude of zeta potential denotes stability of copper nanoparticles in the suspension.

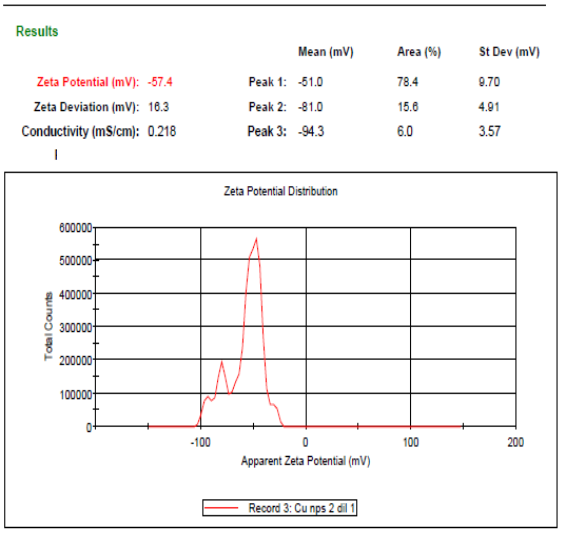

Figure 4: Zeta potential of synthesized Cu NPs using G. sylvestre

SEM Analysis

SEM analysis of Cu nanoparticles was performed using scanning electron microscope.

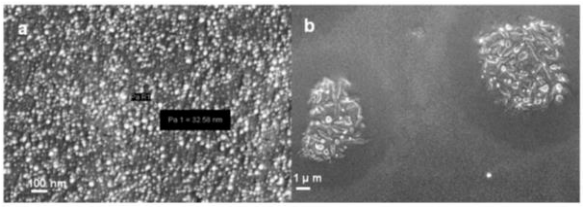

Figure 5: SEM images of Cu NPs

Above figure depicts that the grains (20-40 nm) of Cu NPs were formed, which were the result of agglomeration of the Cu nanoparticles. Flower like structures (Fig. 5 b) were also observed which may be formed due to agglomeration of Cu nanoparticles.

HRTEM Analysis

HRTEM analysis of Cu nanoparticles was performed using high resolution TEM. .

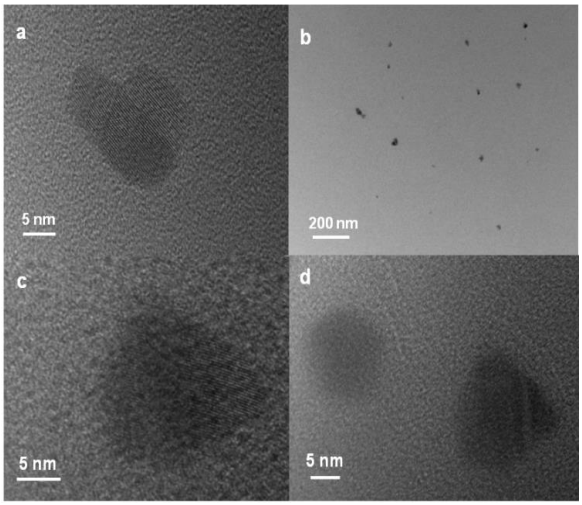

Figure 6: HRTEM images of Cu NPs

HRTEM images of Cu NPs showed the irregular ball like structures were observed and their size were measured in the ranges from 10-24 nm (Fig.6 b, c, d). In HRTEM analysis lattice spacing were also observed (Fig.6a) which showed the crystalline nature of as synthesized Cu nanoparticles

Antibacterial Screening

The antibacterial activity of the Cu nanoparticles (using Gymnema sylvestre leaf extract) was evaluated against E. coli MTCC 40 and B. subtilis MTCC 2616 by Agar well diffusion method. Fig 7 denotes A for Cu NPs , Ab for antibiotic drug and W

for distilled water. Double distilled water (sample W) was taken as –ve control and the antibiotic drug, ciprofloxacin (sample Ab) was taken as +ve control. The diameters of the zone of inhibition were measured using scale and divider.

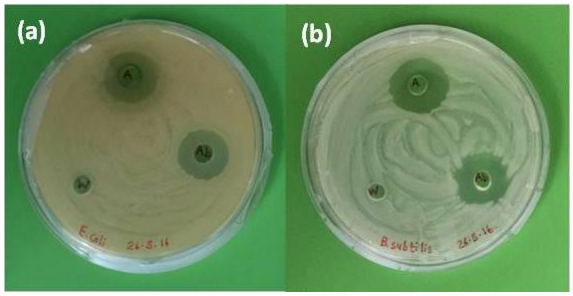

Figure 7: Antibacterial activity of Cu NPs synthesized using G. sylvestre extract against
(a) E.coli MTCC 40, (b) B. Subtilis MTCC 2616

Sr. no.	Name of bacteria	Zone of inhibition
1.	E. coli	19mm
2.	B. subtilis	21mm

Table 1: Zone of inhibition for both gram positive and gram negative bacteria

The zone of inhibition was found to be 19 mm for E. coli MTCC 40, 17 mm for Ciprofloxacin and 21 mm for B. subtilis MTCC 2616, while no zone of inhibition was observed in case of double distilled water. The plot from the above study is shown in Fig. 8 and 9 respectively.

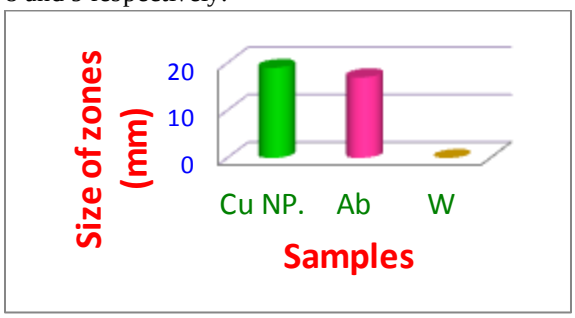

Figure 8: Graph showing relative antibacterial activity of Cu nanoparticles synthesized from Gymnema sylvestre leaves extract (against E. coli) compared with standard antibiotic (Ciprofloxacin). [Where sample Cu NP, Ab and W are Cu nanoparticles (from G. sylvestre), Antibiotic drug Ciprofloxacin (+ve control) and water (-ve control) respectively

It is evident from the graph (Fig. 8 and 9) that the synthesized Cu NPs possess good antibacterial activity against Gram +ve bacteria than Gram –ve bacteria and is a potential candidate for Antibacterial application.

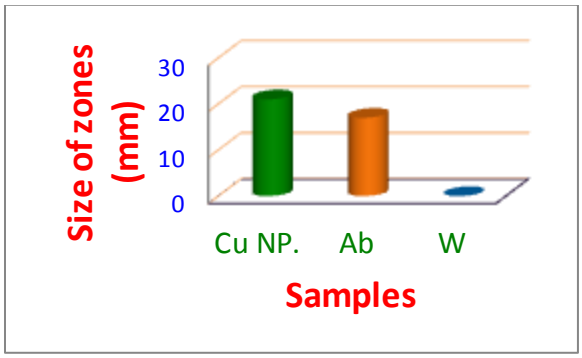

Figure 9: Graph showing relative antibacterial activity of Cu nanoparticles synthesized from Gymnema sylvestre leaves extract (against B. subtilis) compared with standard antibiotic (Ciprofloxacin). [Where Sample Cu NP, Ab and W are Cu nanoparticles (from G. sylvestre), Antibiotic drug Ciprofloxacin (+ve control) and water (-ve control) respectively.]

CONCLUSIONS

Cu nanoparticles are prepared successfully by green synthesis method using Gymnema sylvestre leaf extract with average particle size of 111.9 nm.HRTEM images of Cu NPs showed the irregular ball like structures with size ranging from 10-24 nm.The Cu nanoparticles synthesized using leaf extract of Gymnema sylvestre possess good antibacterial activity against E. coli MTCC 40 and B. subtilis MTCC 2616. It was also observed that the as synthesized Cu NPs, showed more antibacterial activity towards gram +ve bacteria than gram –ve bacteria

ACKNOWLEDGEMENT

The author is thankful to Dr. Geeta Singh (Assistant Professor), Department of Biomedical engineering for providing facilities to carry out this piece of research work.

REFERENCES

[1] Prabhu N., Divya TR, Yamuna G. “Synthesis of silver phyto nanoparticles and their antibacterial efficacy” Digest Journal of Nanomaterials and Biostructures, vol 5 No. 1, p. 185-189 (2010).

[2] Hoffman AJ, mills G, Gee H, Hoffmann M, "Q-sized cadmium sulfide: synthesis, characterization and efficiency of photo initiation of polymerization of several vinylic monomers". The journal of physical chemistry, 96(13), pp: 5546-5552, (1992).

[3] Daniel M.C. and Astruc D., "Gold Nanoparticles: assembly, supramolecular chemistry, quantum-size related properties and applications toward biology, catalysis and nanotechnology", chem. Rev., 104,293-346(2004).

[4] Brust M, Kiely CJ "some recent advances in nanostructure preparation from gold and silver particles". Colloids surf A 202:175-186. (2002)

[5] Kobayashi Y, Ishida S, Ihara K, Yasuda Y, Morita T, Yamada S, "Synthesis of copper Nanoparticles coated with polypyrole". Chemistry of materials, 287:877-880, (2009).

[6] Zhang HX, siegert U, Liv R, caiwB, "Facile fabrication of ultrafine copper Nanoparticles in organic solvent". Nanoscale research letters,Y: 705-708,(2009).

[7] Sastry M, Ahmad A, Islam NI, Kumar R. "Biosynthesis of metal Nanoparticles using fungi and actinomycctes". Current sci; 85:162-170, (2003).

[8] Saxena A, tripathi RM, singh RP. "Biological synthesis of silver Nanoparticles by using onion (allium cepa) extract and their antibacterial activity", Dig J nanometer bios. 5:427-432 (2010).

[9] Sushmita choudhary, latika sharan, manoranjan Prasad sinha "Phytochemical and antibacterial screening of psidium guajava L leaf extract against clinically important gastrointestinal pathogens". Ranchi, 2(4): 524-529 (2012).

[10] Borkow G., Molecular mechanisms of enhanced wound healing by copper oxide- impregnated dressings wound Repair Regen 18(2), 266-275, (2010).

[11] Li Y., Liang J., Tao Z., Chen J., CuO particles and plates: synthesis and gas-sensor application Mater. Res. Bull. 43(8-9), 2380-2385 (2008)