Original Research Article

Synthesis and Biological activities of Cu-Nanoparticles from *Aspergillus niger*

ABSTRACT

Nanotechnology has received tremendous attention because its applications have expanded in a variety of fields. The biological route for the synthesis of nanoparticles become more demanding as it is eco-friendly, low cost, and not time taking procedure. In this research,*Aspergillus niger*filtrateis used as a reducing agent to biosynthesize copper nanoparticles (CuNPs) under controlled parameters i.e., pH, temperature, and time. Synthesized CuNPswere confirmed by UV-Visible spectroscopy and further characterized by scanning transmission electron microscopy (STEM), and Fourier transforms infrared (FTIR) spectroscopy. The UV- Visible spectroscopy exhibit a maximum peak of 540nm which confirmed the formation of CuNPs. FTIR showed two maximum peaks 3339cm^{-1} and 1638 cm^{-1}.These peaks representthe presence of O-H stretching and –C=C- stretching respectively. The size ranges of CuNPs between 15nm-85nm with spherical shapes. The anti-microbial activity was tested against gram-positive andgram-negative bacteria and showed the significant antibacterial potential of CuNPs. Radical scavenging activity was confirmed by DPPH assay. The results of antioxidant activity indicated the IC50 value ofCuNPs was59.10ug/ml. Thus, CuNPs synthesized through a biological route could act as good antibacterial as well as antioxidant agents.

Keywords:*Aspergillus niger*,nanoparticles, copper, antibacterial, antioxidant

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1. INTRODUCTION

Nanotechnology is the field of science where we study small particles having a size of approximately 0.1 to 100 nanometres, measured at the nanoscale.Nanotechnology involves the synthesis of particles at the nanoscale level. Present-day nanotechnology continuously gaining attention because of its potential to produce nanoparticles and their use in drug delivery systems as nanomedicine. This technology preciselydeals with human diseases before time[1]. Advanced developments in science lead toward fast progress inthe synthesisof

different types of nanoparticles. Nanoparticles have been extensively used as carriers for target-specific drug delivery and to maintain stability against enzyme degradation[2].Production of by-products using nanotechnologies in science disciplines is known as nanobiotechnology. Biotechnology advancements have created limitless possibilities for genetic diagnostics and clinical therapy. Although there are many types of nanoparticles due to their exclusive properties like antimicrobial, anticancer, catalytic activity, and optical properties, metal nanoparticles (NPs) have been explored extensively[3]. Drug designing at the nano-scale level needs detailed researchbecause of its potential benefits, including solubility, drug release profiles, diffusion rate, bioavailability, and immunogenicity, that is why it becomes the most advanced technology in the field of nanoparticle applications.In all phases of clinical treatment,nanoparticlesare effective to treat and diagnose several diseases.

The use of metal-based NPs in diagnosis and treatment is an area of study that led to more widespread use of nanomedicines in the future. As copper is the most stabilizing in nature, biosynthesis of CuNPs offers higher preferences in comparison with other metallic NPs. Their high conductivity exists naturally.They may have emerged as attractive candidates for numerous medicinal applications due to their characteristics. There are some major applications of copper-based nano-drugs including targeted drug delivery, genetherapy, molecular tracking,and detection of clinical diseases like cancer, diabetes, and atherosclerosis[4].The advancement of less hazardous technologies for producing metallic NPs is critical[5]. There isa variety of systems present for the synthesis of NPs. The two most broadly used methods are the bottom-up andthetop-down procedure.These approaches are further characterized into subgroups based on response conditions.Some common methods used forthe synthesis of NPs are chemical, mechanical, and biological methods.The chemical methods include plasma, microemulsions, sonochemical reduction, microwave irradiation, ultrasound irradiation, andpulsed laser method. These all are chemically based methods that are widely used for synthesizing NPs. It is because large numbers of nanoparticles may be produced in a short time with precise shape and size. However, it is very not an eco-friendly method[6]. Mechanical procedures are the same as the physical methods for the synthesis of NPs. Physical processes include mechanical milling, induced heating, and spraying. Although these methods are not harmful, they are difficult, time-consuming, and require a high amount of energy. Thus, chemical and physical methods are either hazardous to the environment or not cost-effective. However, biological methods are more suitable for the production ofNPs.

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As the biological routedoes not require expensive and harmful substances,it is very extensively used just due to itscost-effectiveness, greater efficiency, environment friendly, and easeof handling. Plants and microbes (bacteria, fungi, and algae) are the most common sources of green synthesis of NPs. All plants and other organisms do not need a stabilizing agent as enzymes are naturallypresent in their bodies. Biological methods are suitable for producing therapeutic NPs[7].Fungi is an important candidatefor the production of metallic NPs as it secretes enzymes and proteins in high concentrations. Moreover, fungi can be grown on a larger scale very easily. No extra stabilizing agents are required, enzymes secreted by fungi act as stabilizing and capping agents for NPs. Reaction conditions could be modified to get NPs of precise shape and size. *Aspergillus niger*is a commonly found fungi. Previous studies have explored the potential of *Aspergillus niger* for the synthesis of different metallic NPs.Gade et al.[8]exploited *Aspergillus niger*for the production of silver NPs. Results of this study showed the successful synthesis of silver NPs of 20nm diameter having significant antibacterial activity. Recently, another research study[9] reported the extracellular biosynthesis of silver NPs from the cell-free filtrate of *Aspergillus niger*. Biosynthesized silver NPs showed a significant antiamoebic potential against *Allovahlkampfiaspelaea* trophozoite and cyst. These studies showed that *Aspergillus niger* is a valuable source of eco-friendly and cost-effective production of metallic NPs having medicinal applications.

Thus present study focused on the fabrication of *Aspergillus niger* for CuNPs and exploring different biomedical applications of CuNPs.

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2. MATERIALS AND METHODS

2.1 Collection of Fungus

Spoiled mangoes were collected from the mango farm. Mango is a common source of *Aspergillusniger*[10].

2.2 Chemical Collection

20g of sabouraud dextrose agar (SDB) and potatoes dextrose agar (PDB) was obtained from the chemical store of the Institute of Molecular Biology and Biotechnology. The molecular

mass of the copper sulfate salt is 159.6g/mol. All chemicals e.g., DMSO, DPPH, and ethanol of analytical grade were purchased from Sigma Aldrich.

2.3 Isolation and Purification of Filamentous Fungus

Black spots of fungus from mango fruits were separated with the help of a sterilized wireloop. All mango fungalspores were then cultured on solidified SDAPetriplates. The fungal-growth (SDA) media (25.12g) was prepared in a 500ml flask having 400ml of distilled water, then autoclaved. Sterilized media was poured into sterilized glass Petri plates. After inoculation of fungal spores, plates were placed in the incubator for 2-3 days at 37°C temperature for fungal growth [11].The individual fungusspores were picked for further purification. Sub-cultured 2 to 3 times to obtain pure *Aspergillus niger*. After purification stored all plates at 4°C. The purified fungusspores were maintained at 28°C with pH 6.5 for longer use. The purified culture was used for the screening of strains[12].

2.4 Identification of Purified Fungus

The purified fungus was identified by the following methods:

2.4.1 Morphological Identification

Morphological identification of purified fungus was done after 4 days by observing the shape, growth, color, appearance, and diameter of the *Aspergillus niger* colonyalong blackish velvety spores. These all are macroscopic characteristics of fungus[1].

2.4.2 Microscopic Identification

Microscopic identification of fungus was done after 5 days of incubation. Spores of purified fungus were separated with the help ofa needle. Placed on a slide and teased until large structures were shattered completely. A drop of water was placed on a slide and then placed covered bya coverslip. Tissue paperwas used to remove extra water. On the slide, mentioned a name to be used later. Then, the slide was set on the microscope perfectly. The slide was observedunder a microscope at different magnifications i.e., 10X and 40X to observe the characteristic structures of*Aspergillus niger*[13,14].

2.4.3 Molecular Identification

The first step of molecular identification is DNA extraction. Fungal DNA has been extracted through Cetyl Tri-methyl Ammonium Bromide (CTAB)technique along with some

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modifications[15,16]. Fungal hyphae (0.5g) was shown fast growth in SDB, which was extracted by filtration through Whatman filter paper no.1, dried then freeze it. Freeze-dried fungal spores are used for the filtration process. For 25ml CTSB buffer prepared in 200ml distilled water, after mixing of 10ml EDTA, 25ml Tris-base, and 20g NaCl for 25 minutes. CTAB extraction buffer was used for proper grinding of fungal conidia's structures and noted that cellwalls of fungal structures were broken completely, this grinding was done through the help of a pestle and mortar. After powering homogenized mixture was shifted to 1.5ml and all tubes were kept in a water bath for 25 minutes at 65℃. Then tubes were left for 30 minutes at room temperature. Added chloroform and isoamyl alcohol (24:1) solution in Eppendorf tube as equal volume to the reaction mixture. Ependroffs tubes were inverted after every 10 minutes. Then centrifuged for 10 minutes at 9000 rpm. After that, this solution was removed and the supernatant contained DNA was transferred into another tube, and chilled isopropanol was added with the same volume. Tubes were again centrifuged at 9,000rpm for 10 minutes. Pellets of DNA were washed with ethanol after centrifuge. Centrifuged it for 5 minutes at 9000rpm. Through inverting method, tubes were inverted every 3-5 minutes. Upper impurities were removed 3 times. The tubes had pure DNA pellets. All pure pellets of fungal- DNA were stored at -20℃ for further use[16]. The UV-spectrophotometer was used to check fungal DNA quality. After that, the samples were diluted. Diluted DNA sample added into 99 well plate and samples quantified by measured absorbance of fungal-DNA at A_{260}/A_{280} nm. The blank was distilled water.For the amplification of isolates,polymerase chain reaction (PCR)amplification of 18S ribosomal DNA was done. The main components needed in PCR amplification. The reaction mixture was prepared using 4µl of pure DNA sample, nuclease, 12.5µl of 2X mastermix containingTaq-DNA polymerase, buffer, dye, dNTPs (0.4µM), and reverse and forward primers. Bio-Rad thermal cycler used for PCR amplification with reaction conditions of denaturation at 95℃, annealing at 60℃, and extension at 72℃. After this, one % Agarose-gel was used for PCR product analysis.

2.4.4 Sequence Analysis

Similarly, the PCR reaction was conducted by using 30µl of master mixture activation of *Taq* polymerase for 3 minutes at 94℃, 35 cycles of denaturation at 95℃, annealing at 55℃ and extension of primer at 72℃. Terminator-cycle Sequencing Kit used to perform sequencing of*Aspergillus niger*. Samples having amplified DNA products suspended into Hi-Di for-Amide, incubated for 6 minutes at 95℃ for heat-shock treatment, and kept in Ice for 5-6 minutes. The analysis of sequences DNA analyzer was used carefully[17].

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2.4.5 Phylogenetic Tree

The phylogenetictree can detect evolutionary relationships among organisms of the same species because of sequence similarities in genetic history. Eventually, the Clustal Omega tool was used to identify pair-wise genetic distances, and besides sequences joining used to build a phylogenetic tree. Clustal Omega is also important to achieve unweighted rDNA results easily. It is presented in dendrogram forms. The alignment of rDNA of *Aspergillus niger* using the Basic Local Alignment Search Tool (BLAST) at NCBI was used to detect the correlation of sequences of fungus *Aspergillus niger*[18,19]. The Omega tool was used for the construction of a phylogenetic tree.

2.5 Biological Synthesis of Cu-NPs

2.5.1Preparation of Biomass Mat

The two types of culture media i.e., SDB and PDB prepared in a 250ml flask. 12g of PDB and 12g of SDB powder were addedto150ml of distilled and autoclaved water and then mixed through a spatula/stirrer. This prepared media of SDB and PDB autoclaved. This autoclaved media then cool down properly. After the cool-down step, the purified fungus *Aspergillus niger*spores inoculate in both SDB and PDB media ina laminar airflow (LAF) cabin. These medial flaskswere kept in a rotatory shaker for 7-10 days, at 120rpm speed and 28°C. After incubation, flasks containing fungal biomasswere removed from the shaker[20].

2.5.2 Preparation of Fungal-Filtrate

After incubation/inoculum, the biomass mat was harvested by using Whatman filter paper No 1. All impurities present in biomass were removed by washing three times with water. The mat after 3 washing weighed and 9.8g of biomass was again poured into the washed flask containing 200ml deionized water and kept in an arbitrary shaker at 120rpm for 1-day incubation. After that, biomass was filtered 1st by using Whatman filter paper No.1 and then witha syringe filter of 0.2µm pore size. The PBD filtrate and SDB filtrate obtained were used for CuNPs synthesis[21].

2.5.3 Synthesis of CuNPs

Different dilutions of copper salt ($CuSO_4$) i.e., 10, 15, 20mM mixed with the fungalfiltrate with a ratio of 1:1 at room temperature. On addition tothe $CuSO_4$ solution, a color change was detected by the naked eye. The reaction mixture was placed in an arbitrary shaker for one-day incubation at 37°C.

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2.5.4 Drying ofCuNPs

Followed the protocol for drying CuNPs. After one day of incubation,CuNPs were centrifuged at 1200rpm for 5minutes. This process was repeated 3 times. The upper watery extract discard and washed CuNPs with distilled water. These stepswere performed thrice and after 3 washing the only NPs were left in the form of pellets. These pellets were poured into the Aluminium foil. The pellets were heated at 100 °C for one day. The next day pellets were removed from the hot air oven.Through this, the pure dried form of CuNPswas obtained[22].

2.6. Characterization of Dried CuNPs

CuNPswere characterized by different techniques including UV-spectrophotometric, scanning transmission electron microscopy and Fourier transform infrared analysis,etc.

2.6.1 UV-Spectrophotometer

The CuNPswere characterized by a UV-Visible spectrophotometer. CuNPs absorbance was measured at 1 nm resolution by UV-visible spectro-photometer after 1-day incubation, one ml of (Cu-NPs) taken at different concentrations with wavelength range was adjusted at 200-800nm[23]. For UV-spectrum Micro-plate spectrophotometry (96 well-plate) was used for the detection of CuNPs. The distilled water was used as blank. Sample data was loaded sequence-wise after adding blank. Recorded all sample data and data graph plotted on excel sheet.

2.6.2 Scanning Transmission Electron Microscopy (STEM)

Scanningtransmissionelectron microscopy (STEM)was used to analyze the sizes and shapes of CuNPs. The scanning instrument (resolution 5.25 A^o) used an electron light beam to the produced image on a screen. This providesthe exact size of CuNPs in high resolution. It can detect even 1nm small-sized particles. A scanning electron micrograph was taken from the instrument of the JSM-6380 SEM model. Before the experiment, samples were filtered and dried[24].

2.6.3 Fourier-Transform Infrared (FTIR)

FTIR spectrometer was used to determine functional groups that bind with CuNPs. FTIR analysis was performed with the help ofan interferometer. CuNPswere diluted in potassium bromide at 1:100. One drop of solution was placed on the sample holder. Spectrum noted in the series of 1000-3500cm^{-1} wavelength[25].

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2.7 Antibacterial Activity of CuNPs

For the antibacterial procedure, the microbial cultures were obtained from the laboratory of the Institute of Molecular biology and Biotechnology. Antibacterial activity was performed against gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae, Salmonella enterica,* and gram-positive bacteria *Bacillus subtilis, Micrococcus luteus,*and *Staphylococcus aureus.* For anti-bacterial activity, the agar well method was performed. Media plates containing nutrient agar were prepared by adding 4.2gof nutrient agar to 150ml of distilled water and kept in an autoclave machine for 30 minutes. Cooled down media completely then poured into glass Petri-plates in LAF hood. Prepared media was not touched until solidified after that wells were prepared by steel borer (6mm). Sterilized swabs were used for microbial culturing on the plate. Swabs rolled around the wells perfectly. CuNPsdilutions10mM, 15mM, and 20mM were added into wells using tips and a pipette 10-100ml.NPs (100ul) were added into wells. The standard was a drug (Ciprofloxacin) added to all plates and these plates were left for 5 mins to allow them to diffuse properly. All plates were kept in the incubator for one-day incubation at 37°C. After one day, plates were removed from the incubator and measured the zone of inhibitions (ZOIs) using a scale and maker. The experiment was repeated 3 times[26].

2.8 Antioxidant Activity of CuNPs

DPPH scavenging activity was performed to evaluate the antioxidant potential of CuNPs. Prepared NPs sample dilutions at different concentrations (10-100µg/ml) then prepared dilution of ascorbic acid similar to samples. 50µl of methanolic DPPH solution added in 50µl to all Eppendorf containing NPs and standard (Ascorbic acid). Incubated all samples at 37°C for 30 mins in dark at room temperature with blank and control. DPPH solution in methanol was used as a control. The color change observed showed the breakdown of oxygen. The 96-well plate was used for spectrophotometer analysis. Absorbance was taken at 517nm and % age inhibition of DPPH was calculated using the formula[25]:

$$\text{Scavenging Percentage} = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100$$

Inhibition graph curve prepared after IC50 value calculated on excel sheet.

3. RESULTS

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3.1 Identification of Isolated Fungus

After screening, the purified fungus was obtained after 5-7 days. The dark-colored purified fungus was obtained. The purified fungus contained a single colony and black velvety spores spread all over the colonies clearly shown below in Figure 1. The fungal-isolate change its colorbehavior from whitish to deep brownish-blackcolor andstraw pale pattern of colonies from the backside. The growth of fungus with a globular shape is shown in Figure 1.

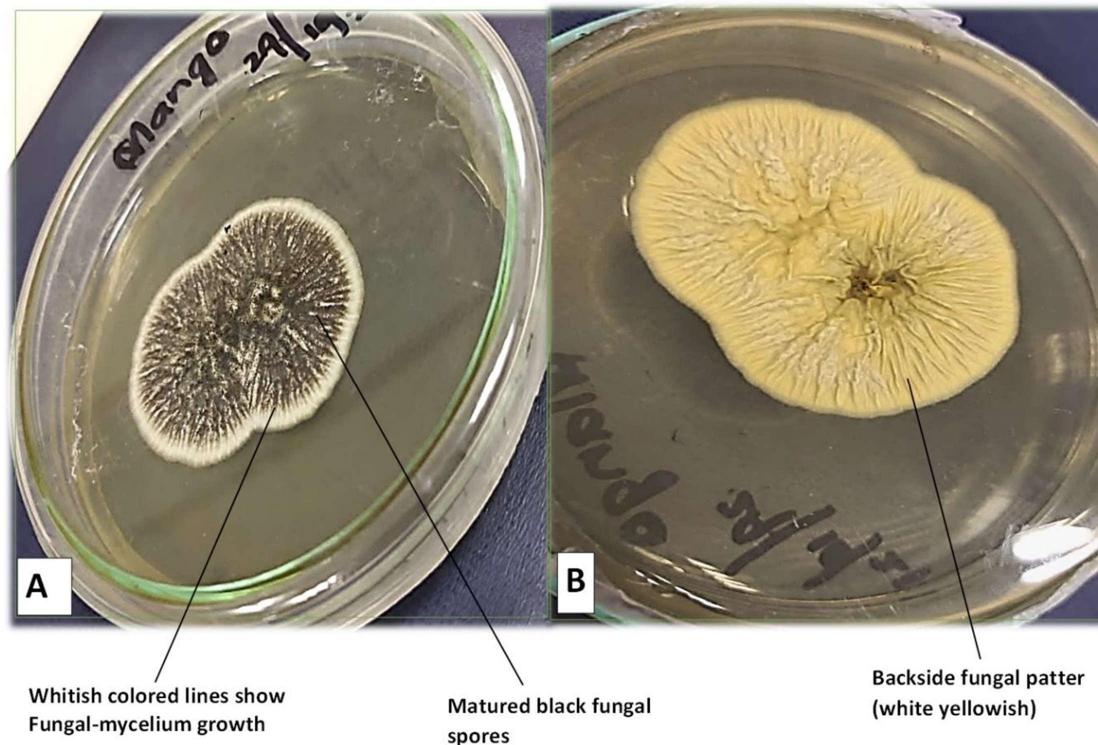

Figure 1 Greyish black velvety textured *Aspergillus niger*

The microscopic characterization showed hyphae without septa, globular shaped matured conidia had dispersed conidiophores. Microscopy identification also showed that produced spores were between 200-250nm and the spores' shape was spinose. The stipes were white around the apex. The flask-shaped phialides completely covered the vesicles. Long thin tubular hyphae were unbranched (Figure 2).

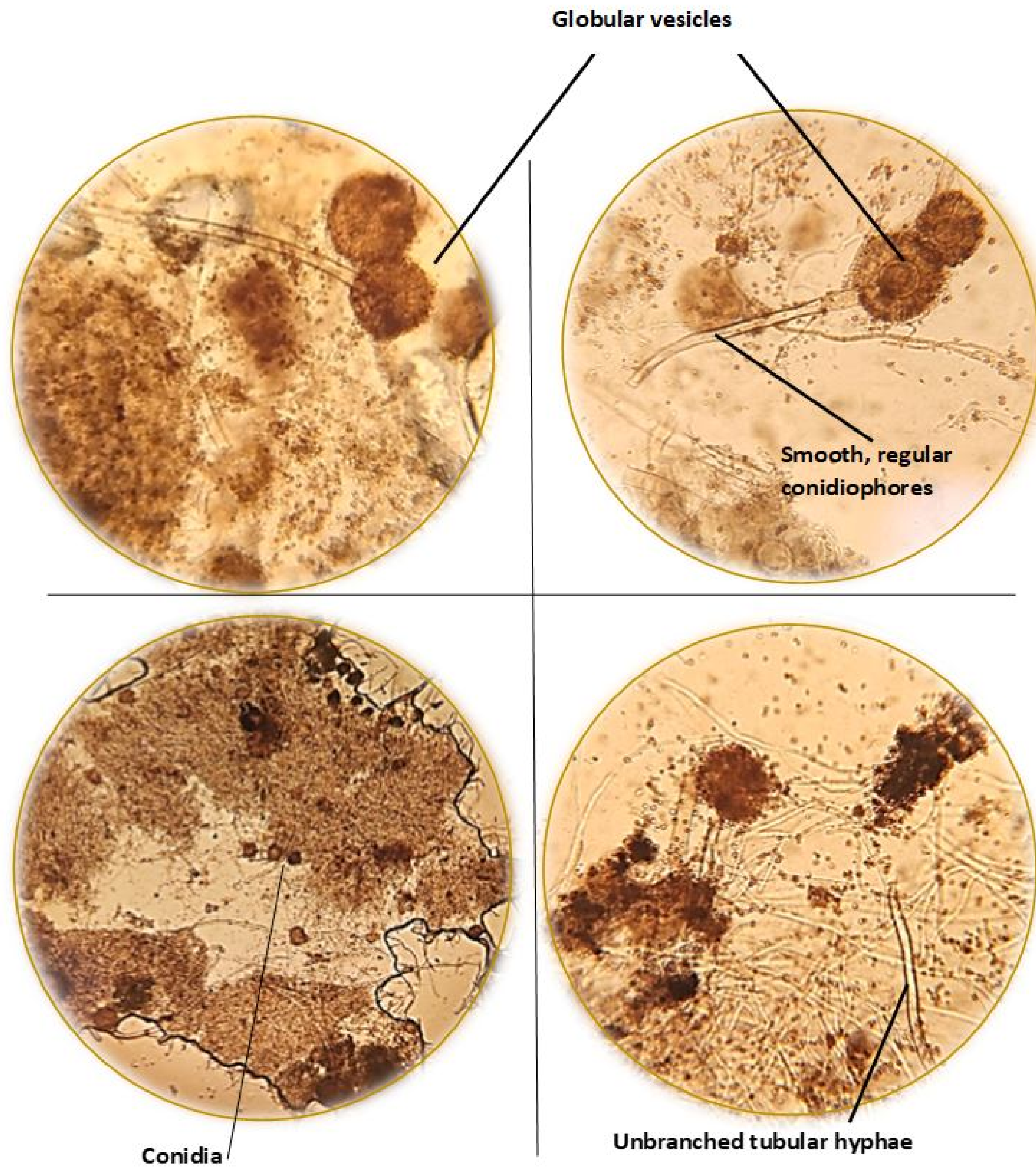

Figure 2 Microscopic evaluation showing a detailed structure of a fungus, containing long unbranched hyphae, globular conidiophores

3.2 Molecular Characterization

The fungus-isolate was characterized by sequence analysis with the 18S-rDNA region. The molecular analysis clearly showed that the purified fungus strain was *Aspergillus niger*. It was proved by the NCBI website. NCBI-server has given BLAST results. The confirmation

of fungal-strain sequences resembled*Aspergillus niger* sequences present in the NCBI database.

The phylogenetic tree was constructed through an online tool, Clustal Omega by using the option MUSCLE. The sequence was submitted to NCBI with its allotted number. The phylogenetic overview is shown in Figure 3.

Phylogenetic tree

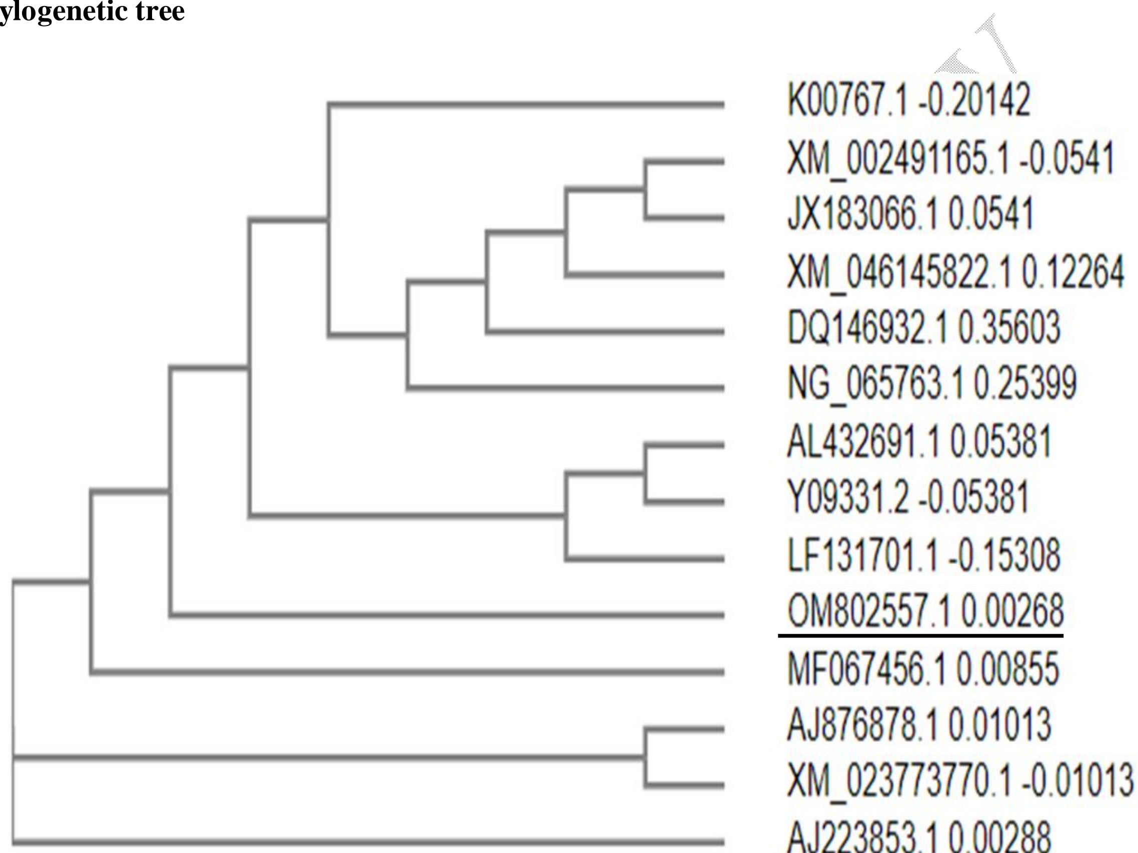

Figure 3____ *Aspergillus niger* strain with 0M80255.1 allotted number, the partial sequences of the small subunit of gene rRNA

The phylogenetic tree representsthe evolutionary linkage of the *Aspergillus niger* strain.ClustalOmega was used for designing the tree. Maximum likelihood process used to compute distance and units by number-based substitution per site.

3.3 Biologically Synthesized CuNPs

The biosynthesis of CuNPs by mixing *Aspergillus niger*filtrate with $CuSO_4$ salt. The filtrate was cell-free so used as a reducing system in CuNPs production. Filtrate of *Aspergillus*

*niger*reduced the copper-salt and it was proved by a change in color from pale white to light blue. The formation is clearly shown in Figure 4. Controlled parameters e.g., pH 7 and reaction temperature 37 ^{o}C.1:1 ratio (filtrate: salt) was used.

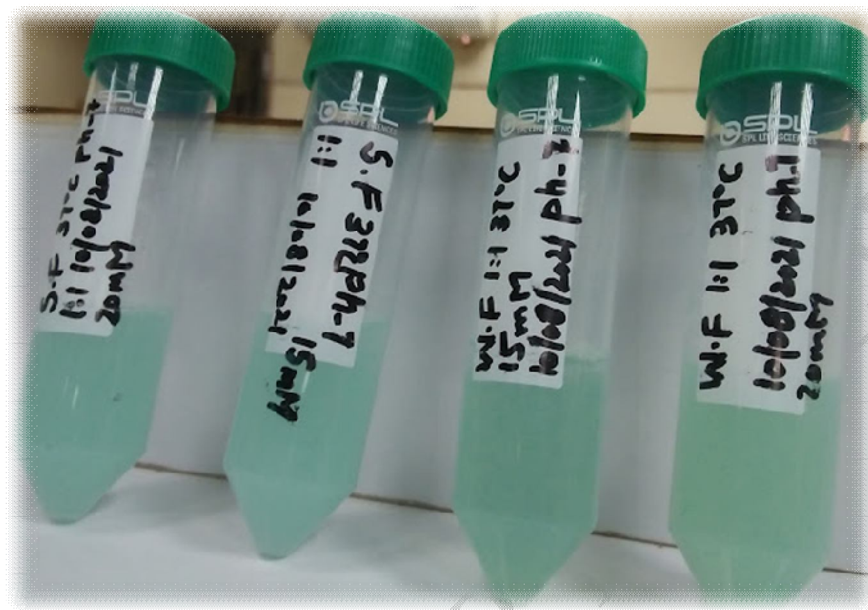

Figure 4 Change in color supported the CuNPs formation

3.4 Characterization of Biosynthesized CuNPs

3.4.1 UV-Visible SpectrumAnalysis

The UVspectrum was carried out from the 200-800 range of wavelength which helped to confirm the synthesis of copper-nanoparticles from *Aspergillus niger.* Similarly, the broad-spectrum range was 350-550nm, this range supported the maximum absorbance spectrum. It was observed that CuNPs showed a maximum absorption peak at 540nm. It was clearly shown in the graph that filtrate of fungus has zero absorptionpeak between 220-600nm wavelength. The copper nanoparticles showed maximum excitation at different points by absorbance.The CuNPs indicate maximum peaks at different points because of the surface-plasmon resonance spectrum by this reduction of $CuSO_4$ into NPs. The peaks are shown below in Figure 5.

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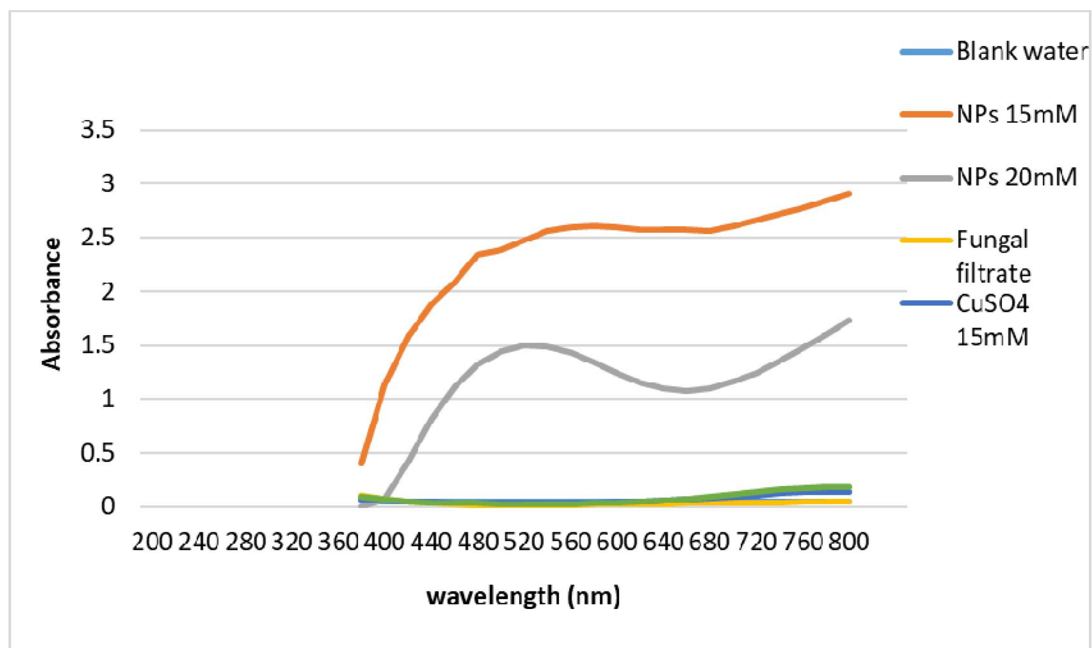

Figure 5 Graph curve showed the absorbance of NPs at a different wavelength

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3.4.2 Fourier Transform Infrared Spectroscopy (FTIR)

Biosynthesized CuNPs absorbance peaks were observed by the FTIR apparatus. The peaks were obtained which analyze the stabilized CuNPs and a high peak was observed at 3330.07cm-^{1}by this, phenolic ions bind with the O-H functional group expressing stretching of the O-H bond. The wave region of FTIR spectroscopy was at 1000-3500cm^{-1}.The -C=C- bond stretching supported the presence of (alkene) functionalgroup in CuNPs at a high absorption peak of 1636.91cm^{-1}.The results are shown in Figure 6. These peaks identify the functional bonds involved in the capping and binding of CuNPs.

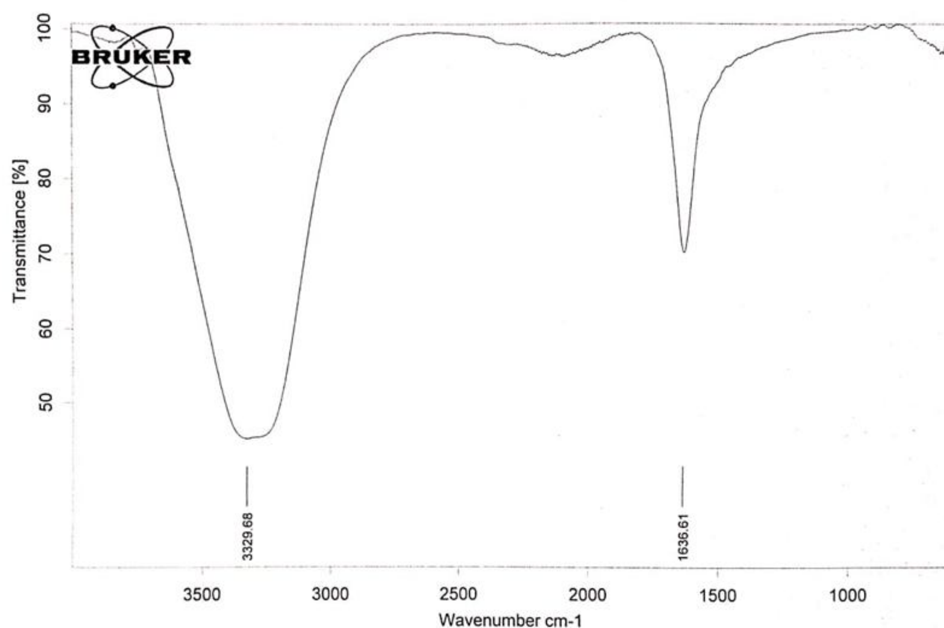

Figure 6 CuNPs analysis by FTIR spectrum

3.4.3 Scanning Transmission Electron Microscopy (STEM)

The STEM images provided information about the elemental composition of a single atom. Its principleis the same as SEM and TEM.STEM is a fast mode of analysis. This microscopy supported theimaging of secondary electrons. Its importance is that multiple operations of characterization are done simultaneously. It gives actual atomic bonds present inside nanoparticles.STEM characterization supported 0.2nm resolution at 200kV accelerating voltage.In this analysis not only size and shape were observed but the size distribution of NPs also counted. Different magnification compute results 25000X-200,000X nm range. The STEM micrograph and histogram showed the size of CuNPs range between 15nm-85nm while exhibitinga spherical shape (Figure 7a&b).

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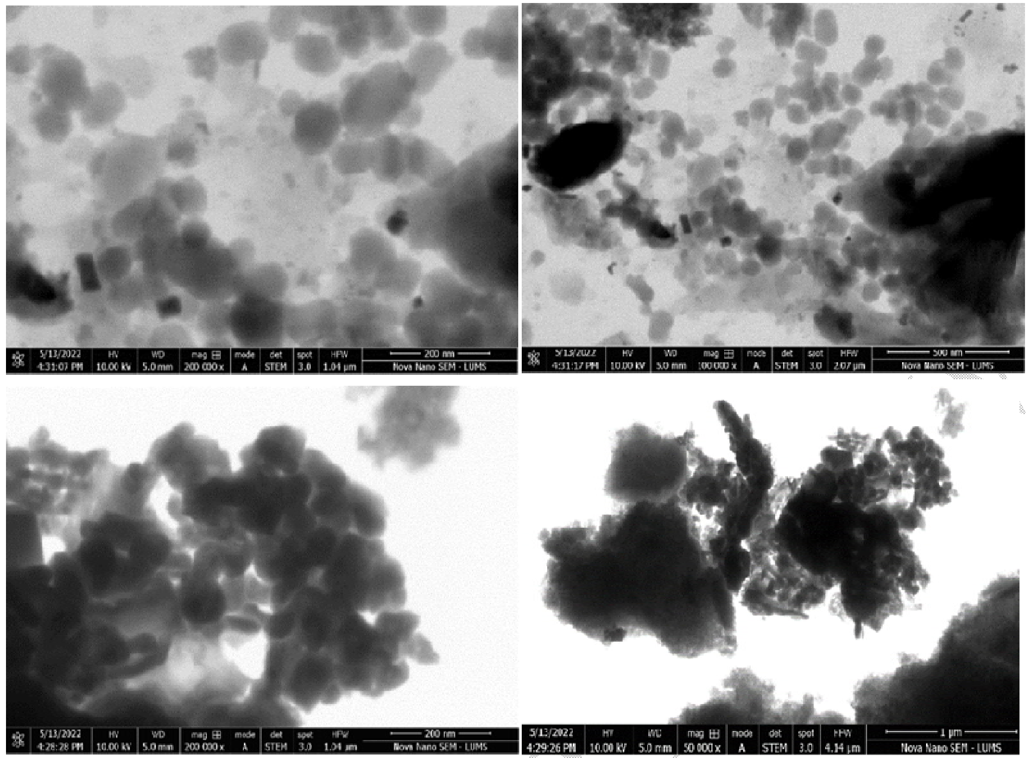
a)



b)

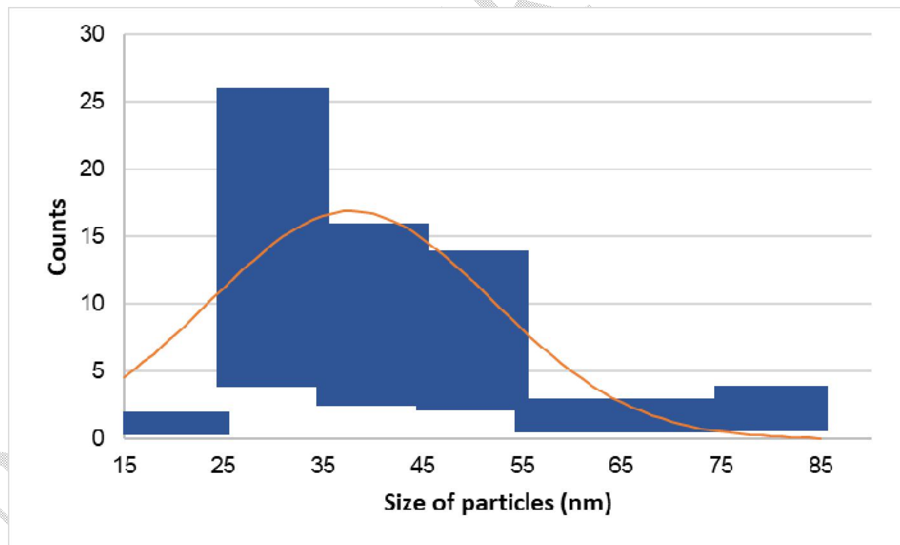


Figure 7a) STEM analysis and**b)** particle size histogram of CuNPs

3.5Biological Activities

3.5.1 Antimicrobial Activity of CuNPs

Anti-bacterial activity of fungal-based CuNPswas analyzed against 6bacteria including *Escherichia coli, Pseudomonas aeruginosa,Staphylococcus aureus, Micrococcusluteus, Bacillus subtilis,*and *Salmonella enterica*. The antibacterial activity of CuNPssynthesized from 15mM salt concentration was observed to be 27mm, 15mm23mm,14mm, 13mm,and 11mm respectively. Copper nanoparticles showed significant antibacterial efficacy results against bacteria(Figure 8, Table 1).

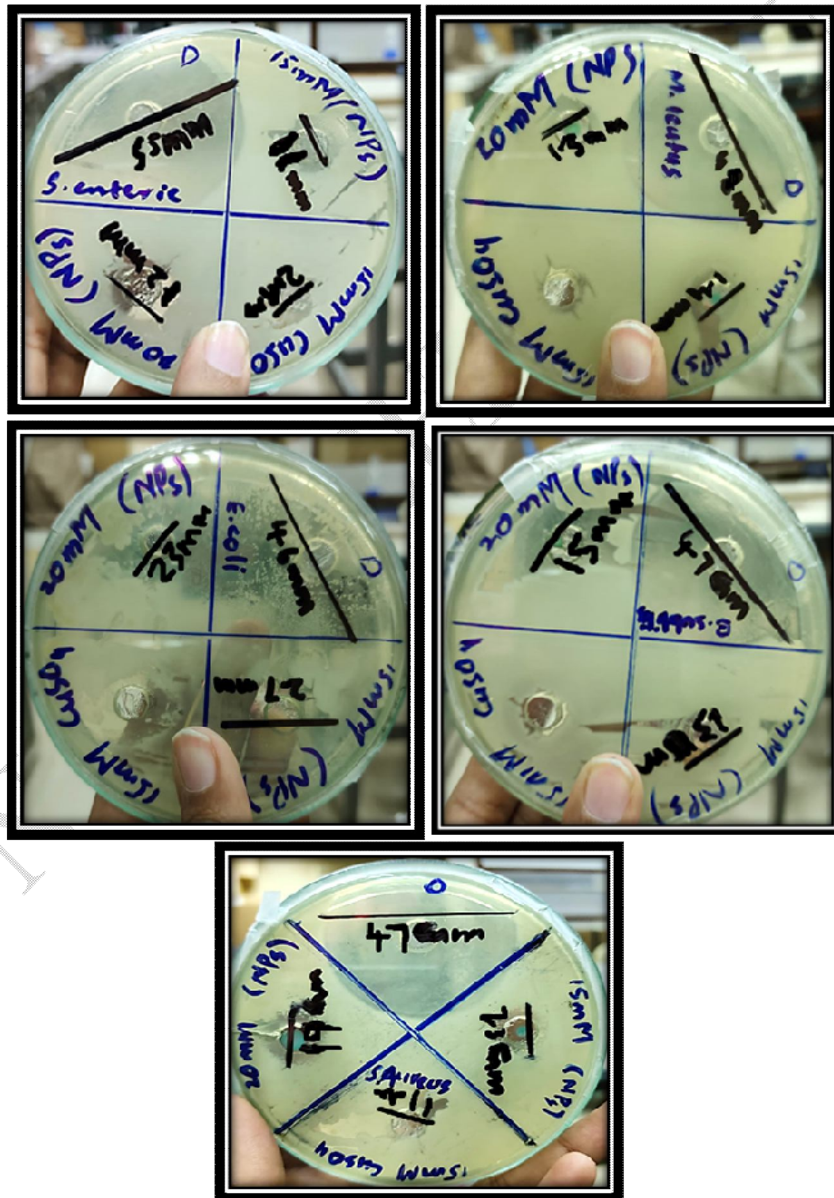


Figure8 Antibacterialactivity of biosynthesizedCuNPs

Table 1 CuNPs activity against different bacterial strains

MICROBIAL STRAINS	ZOI of DRUG (Ciprofloxacin)	ZOI of CuNPs 15mM NPs	ZOI of CuNPs 20mM NPs
Escherichia coli	46mm	27mm	23mm
Pseudomonas aeruginosa	55mm	15mm	16mm
Staphylococcus aureus	47mm	23mm	19mm
Micrococcusluteus	48mm	14mm	13mm
Bacillus subtilis	47mm	13mm	15mm
Salmonella enterica	55mm	11mm	12mm

3.5.2 Antioxidant Activity of CuNPs

Antioxidant activity of NPs and standard (ascorbic acid) was evaluated at varying concentrations (10-100µg/ml). The IC50 value was calculated, and the results showed that the IC50 value of CuNPs was 59.10µg/ml as shown inTable 2.

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Table 2 Antioxidant activity of Standard and CuNPs

Concentration	% Inhibition of Standard	IC50(μg/ml)	% Inhibition of CuNPs	IC50(μg/ml)
10	44.05	**38.134**	37.06	**59.10**
20	46.85		39.86	
40	51.04		44.40	
60	52.44		50.34	
80	57.69		54.89	
100	65.73		61.53	

4. DISCUSSION

This study aims to expand a microbial-based method to produce nanoparticles.CuNPswere produced using the reduction of Cu^{+} with supernatants of *Aspergillus niger* at room temperature. It is reported that CuNPs produced cloudy blue color in an aqueous medium due to the reduction of Cu metal.CuNPs solution containing cell filtrate of *Aspergillus niger* changed from light white to blue in a few minutes, while no color change was observed in the culture supernatant without a salt solution. Thus, the solution's color changed, clearly indicating the synthesis of CuNPs. CuNPs were scattered in the solution and there was no evident aggregation, as evidenced by the fact that the color intensity of the cell filtrate with CuNPs was maintained even after a 24-hour incubation [27].

CuNPsare a good candidate for their high metal resistance, ease of treatment, and biogenic production. Numerous physical and chemical methods are being employed for the synthesis of CuNPs, however owing to constraints, the development of more acceptable and appropriate procedures and techniques that are eco-friendly, cost-efficient, and readily scale-able is a crucial demand. *Aspergillus niger* was used for the synthesis of CuNPs. Several studies report the use of *Aspergillus* and many other fungal species for nanoparticle synthesis. CuNPs micro-synthesis by *Aspergillus niger* is a highly effective approach. Because copper ions must be reduced and CuNPs must be stabilized, an external reducing and capping agent

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is required [28]. Furthermore, there are no hazardous chemicals involved, making this procedure ideal. Because of its simplicity, applicability as a one-step process, and production at scalable parameters, biological synthesis has proven to be more important than physical and chemical methods. CuNPs were characterized by UV-spectrophotometer, FTIR, and STEM. The results of the spectrophotometer revealed that CuNPs were synthesized using the biological method, by observing a peak at 540nm. The reported peak for CuNPs is 480nm in literature so confirmation of synthesis of the nanoparticle by metallic salt [29]. The FTIR results showed the presence of functional groups on the surface of NPs. There were two peaks observed one peak showed the presence of the OH group and the other one showed the presence of the phenolic group which tightly binds to the OH group [30]. Antibiotics such as cephalosporin, tetracycline, and streptomycin are currently available. These antibiotics have limitations because bacteria have developed resistance to them. The impact of drug resistance on human health is significant. Antibiotic resistance has risen dramatically in recent years as a result of usage. CuNPs have been identified as a possible antibacterial agent for example against*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis,* and*Salmonella enterica* by observing and measuring zone of inhibition. This research shows the significant antibacterial potential of CuNPs.The inhibition zones of CuNPs against tested strains were27mm, 15mm 23mm, 14mm, 13mm, and 11mmrespectively. Previous studies [31,32] have shown similar results which support our study. It indicates the mechanisms of bacterial action of nanoparticles to damage the membrane extent of inhibition depends on the concentration of nanoparticles as well as initial bacterial concentration. The reason for this could be that smaller particles absorb more tightly on the surface of bacterial cells, disrupting the membrane and allowing intracellular components to leak out, killing the bacteria.

CONCLUSION

The CuNPs were synthesized from the biological route by using cell-free filtrate of *Aspergillus niger*as a reducing agent. Myco-synthesis of nanoparticles seems an eco-friendly and cost-effective method. CuNPs characterized through FTIR, and STEM showed functional groups attached to nanoparticles along with size and shape. Furthermore, myco-based CuNPs showed significant antibacterial activity against different pathogenic bacterial strains and antioxidant potential with an IC50 value of 59.10µg/ml. Further studies should be done to explore the other biological applications of CuNPs.

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