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Antibacterial Effect of Green Synthesized Copper Nanoparticles

VIJI.P*

*Assistant Professor, Department of Zoology, VHNSN College (Autonomous), Virudhunagar, India

Corresponding Author E-mail: Viji_viswanth@yahoo.co.in

Abstract

Copper nanoparticles were synthesized from green tea leaves extract. This method was proved to be an efficient method for the preparation of copper nanoparticles. The extract was prepared in deionised water. The extract was added to 10mMol of copper sulphate solution as 1:1 ratio and 0.5 m L- ascorbic acid was added as capping agent. The colour change of the solution indicates the formation of copper nanoparticles. The biosynthesized copper nanoparticles were characterized with the help of visual inspection, SEM (Scanning electron microscopy), EDX (Energy dispersive x ray spectroscopy). The size of the particle was found to be in the range of 60-80 nm. The synthesized copper nanoparticle efficiently inhibited various pathogenic organism. This method can be used for eco-friendly biosynthesis of copper nanoparticles.

Keywords: copper nanoparticles, Green synthesis, Antibacterial effect

Introduction

Nanomaterials (NMs) are defined as materials with at least one external dimension in the size range from approximately 1-100 nanometers. A focused integration of bio and nano techniques for biological synthesis of NMs, known as bionanotechnology, has emerged from nanotechnology. Biological synthesis combains biological principles (i.e., reduction/oxidation) by microbial enzymes or plant phytochemicals with physical and chemical approaches to produce nano-sized particles. In recent years, NMs have received particular attention for its positive impact in improving many sectors of economy including consumer products, energy, transportation, cosmetics, pharmaceuticals, antimicrobial agents and agriculture. Presently, synthesis of inorganic Nps has been demonstrated by many physical and chemical means. The chemical methods have a low productivity, non eco-friendly, capital intensive and toxic. Therefore, biological synthesis either extracellularly or intracellularly from higher plants or microbes have gained an upper hand. The importance of biological synthesis of nanoparticles is being emphasized globally (Shoba *et al.*, 2014).

Copper is an essential element required by both plants and animals to live. Copper is also an industrial metal that possesses superior electrical and thermal conductivity, is easy to process and, through the incorporation of other metals, can deliver broad technical performance. This makes it a very important material in a wide range of consumer and industrial applications.

Man has exploited the natural antimicrobial properties of copper since the dawn of civilization. It has been demonstrated clearly in many scientific studies conducted over several decades that copper kills some of the most toxic species of bacteria, fungi and viruses. It has been demonstrated clearly in many scientific studies conducted over several decades that copper kills some of the most toxic species of bacteria, fungi and viruses (Nikolaos *et al.*, 2014). The antimicrobial copper surfaces have been proven to have less contamination than conventional touch surfaces in hospital trials around the world. Copper is already an active ingredient in many different types of antimicrobial products, in agriculture, in marine environments, in healthcare environments and in the home. Copper is an active ingredient in antiplaque mouthwashes, toothpastes and medicines. Copper sink strainers and scourers for pots and pans can help prevent cross-contamination in the kitchen.

The most common methods for preparing nanoparticles are wet-chemical techniques, which are generally low-cost and high-volume. However, the need for toxic solvents and the contamination from chemicals used in nanoparticle production limit their potential use in biomedical applications (Gliece *et al.*, 2012). Therefore a “green”, non-toxic way of synthesizing metallic nanoparticles is needed in order to allow them to be used in a wider range of industries. This could potentially be achieved by using biological methods.

Materials and Methods

Materials

Copper sulfate pentahydrate ($CuSO_4.5H_2O$), L- Ascorbic acid, Ethanol, Acetone, and standard antibiotic disc were purchased from Himedia (P) Ltd, Mumbai and used as starting materials without further purification. Milli-Q water was used for the synthesis of nanoparticles.

Methods

Preparation of green tea extract

The green tea leaves were purchased from tea farm, Nilgris. Thoroughly washed leaves (100g) were boiled with 100 ml of de-ionized water for 15 minutes in heating mantle at temperature 70^{o}C. The resulting product filtered and stored in refrigerator for further experiments.

Synthesis of Copper Nanoparticles using Green tea extract

The prepared 10mM $CuSO_4$ solution was purged with nitrogen (N_2) gas for 10 min to remove the dissolved oxygen (DO).Then the prepared extract was added to the copper sulphate solution in 1:1 ratio followed immediately by 0.5M L- ascorbic acid which act as capping agent and anti-oxidant. The color changed from blue to green followed by dark brown colour. The particles were then extracted by ultracentrifugation at 10,000 rpm for 30 minutes. The particles thus obtained were washed twice with alcohol and finally with acetone and vacuum dried at 60°C for 12 hrs to remove water. The dried particles were used for further characterization.

Characterization of nanoparticles

Visual observation

The reduction of metal ions was visually observed by the change of colour in the reaction solution.

Scanning electron microscopy

Size and morphology of the nanoparticles was examined by SEM (SU1510) operated at 5 kV, magnification x10 k. Thin film of the sample was prepared on a carbon coated copper grid by just dropping the suspension of nanoparticles in water on the grid, extra solution was removed using blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min. The sample surface images were taken at different magnifications.

Energy dispersive spectroscopy

EDS was used for the determination of elemental composition and purity of the samples by atom percentage of metal. Elemental analysis on nanoparticles was carried out using EDS instrument (JSM 35 CF JEOL) in a resolution of 60 Å, operated at 15.0 kV with a magnification of about 5 k. Samples were prepared on a carbon coated copper grids and kept under vacuum desiccation for 3 h before loading them onto a specimen holder.

Antibacterial studies

Bacterial culture

The following bacterial pathogens namely *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were procured from the Microbial Type Culture Collection (MTCC), Chandigarh, India. All the cultures were grown on nutrient agar plates and maintained in the nutrient agar slants at 4°C. overnight culture in the nutrient broth was used for the present study.

Evaluation of antibacterial activity

The copper nanoparticles synthesized using green tea leaves extract was tested for antimicrobial activity by agar well diffusion method against different pathogenic microorganism *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Bacillus subtilis* (*B. subtilis*), *Staphylococcus aureus* (*S. aureus*) (Gram positive) and *Klebsiella pneumonia* (*K. pneumoniae*). The pure cultures of bacteria were subcultured on Muller Hinton Agar. Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Wells of 8mm diameter were made on nutrient agar plates using gel puncture. Using a micropipette, 50uL of nanoparticle solution was poured onto each well on all plates. After incubation at 37^o C for 24h, the diameter of zone inhibition was measured in millimeter, and was recorded as mean ± SD of the duplicate experiments.

Results and Discussion

Characterization of nanoparticles

Visual inspection

The appearance of dark brown colloidal solution for Cu in the reaction mixture indicated the formation of copper nanoparticles. The formation of color in the reaction solution arises from excitation of surface Plasmon vibration in the metal nanoparticles.

Scanning electron microscopy

Scanning electron micrograph of the synthesized nanoparticles is presented in Figure 1.

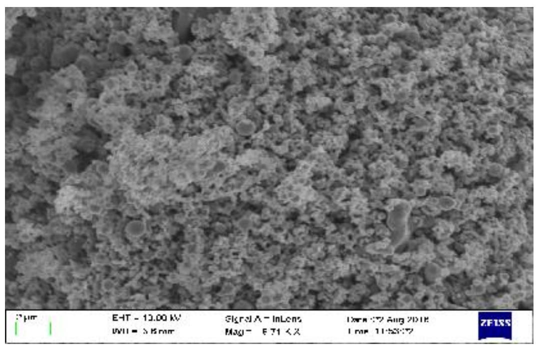

Figure 1: Scanning electron micrograph of copper nanoparticles

Micrograph shows that the appearance of the particles is spherical in shape. Synthesized particles do not appear as discrete one but form much larger particles. The observations of such larger nanoparticles are composed of van der Waals clusters of smaller entities and magnetic interactions among the particles.

Energy dispersive spectroscopy

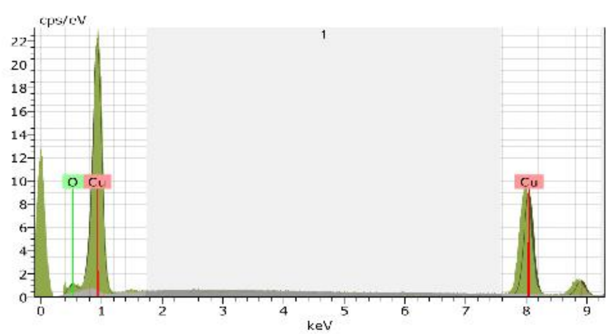

Figure 2 : Energy dispersive spectrum of copper nanoparticles

EDS micrograph explains the surface atomic distribution and chemical composition of nanoparticles. Quantitative measuring results obtained from EDS analysis reflect that 93% and 7% atom particles were of copper and oxygen respectively which confirms the purity of copper metal.

Antibacterial effect

Antibacterial activity of green synthesized Cu nanoparticles was evaluated against pathogenic bacteria using standard zone of inhibition (ZOI) assay. The greatest inhibitory effect was observed against *Pseudomonas aeruginosa* and *Bacillus subtilis* with a zone of inhibition of 21.00 mm for Cu nanoparticles.

Antibacterial activity of copper (Cu) nanoparticles against gram positive and gram negative bacterial pathogens

Bacterial Pathogen	Zone of inhibition (mm)
Escherichia coli	16±0.32
Pseudomonas aeruginosa	23±0.15
Bacillus subtilis	21±0.67
Staphylococcus aureus	14±0.27
Klebsiella pneumonia	19±0.41

An increasing amount of research is being performed on the green synthesis of metallic nanoparticles using plants or plant extracts. This area is relatively underexplored and offers promising results for the field. A very important aspect of using plants instead of bacteria or fungi for NP production is the lack of pathogenicity (Mittal *et al.*, 2013). A study suggested that Cu nanoparticles can also be used as antibacterial agents. It was also found that when Cu and Ag nanoparticles were fused together to create bi-metallic nanoparticles, their antibacterial effects increased and that nanoparticle size played a key role in the strength of the bactericidal effect

(Zain *et al.,* 2014). Much work has been done in bioreduction of metal nanoparticles by a combination of biomolecules found in plant extracts (enzymes, proteins, amino acids, vitamins, polysaccharides and organic acids such as citrates) and the respective role of phytochemicals (Asim *et al.,* 2012). The phytochemicals responsible have been identified as terpenoids, flavones, ketones, aldehydes, amides and carboxylic acids by IR spectroscopic studies. The main water soluble phytochemicals are flavones, organic acids and quinines which are responsible for immediate reduction (Abdul *et al.,* 2012).

The presence of an inhibition zone clearly indicates the bioacidal action of nanoparticles disrupting the membrane. The reason could be that the smaller size of the particles which leads to increased membrane permeability and cell destruction. Because of the large surface area of the nanoparticles, it could be tightly adsorbed on the surface of the bacterial cells so as to disrupt the membrane, which would lead to the leakage of intracellular components, thus killing the bacterial cells. Antimicrobial activity is due to its tendency to alternate between its cuprous – Cu[II], oxidation states. Differentiating Cu from other trace metals, results in the production of hydroxyl radicals that subsequently bind with DNA molecules and lead to disorder of the helical structure by cross-linking within and between the nucleic acid strands and damage essential protein by binding to the sulfhydryl amino and carboxyl groups of amino acids. This denatures the protein makes the enzymes ineffective (Yoon *et al.,* 2007). It inactivates cell surface proteins necessary for transport of materials across cell membranes, thus affecting membrane integrity and membrane lipids (Espirito *et al.,* 2008). Copper ions inside bacterial cells also disrupt biochemical process. The exact mechanism behind is not known and needs to be further studied. Based on all of these studies, the denaturing affect of Cu ion on proteins and enzymes in microbes gives Cu its antimicrobial characteristics.

Conclusion

Cu Nps are known to exhibit wide range of antibacterial activity against different strains of gram positive and gram negative bacteria. Use of plants for the fabrication of Nps have fascinated the workers of its rapid, economical, eco-friendly protocol, broad variability of metabolites that aid in reduction. Further studies are needed to fully characterize the toxicity and the mechanism involved with antimicrobial activity of this particles.

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