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Antimicrobial & Free Radical Scavenging Activities of Green Synthesised Copper Nanoparticles from *Musa acuminata* and *Citrus sinensis* Peel Extracts

Roopa Prasad*, Arbiya Banu, Shivpriya S. Nair & Shweta Tomar

Department of Life Sciences, Kristu Jayanti College (Autonomous), Bengaluru, Karnataka, India-560077

ABSTRACT

The biosynthesis of nanoparticles is a kind of bottom-up approach where the main reaction occurring is reduction/oxidation. Recently, green synthesis of nanoparticles using plant extracts has gained more attention, since they are simple, cost-effective, non-toxic, environment friendly and easily scaled up for large-scale synthesis. There is a great demand for synthesizing Copper nanoparticles (CuNPs) by simple and less expensive methods. The peels of a variety of fruits have gained attention as a natural source of antioxidants. Green synthesis of CuNPs was successfully obtained from bio-reduction of copper sulfate pentahydrate solutions using *Musa acuminata* and *Citrus sinensis*. CuNPs have been appropriately characterized using UV-vis spectroscopy and SEM analysis. It was observed that CuNPs synthesized from dried orange peel extract had better anti-microbial and antioxidant activity than the fresh orange peel and showed great activity than all the other three samples. Since copper is well-known for its antimicrobial properties, we assumed that fabricating CuNP from banana and orange peel extract can increase its efficiency. The antimicrobial activity of the nanoparticles was analyzed using gram-positive (*Staphylococcus aureus),* gram-negative (*Escherichia coli),* and fungal (*Aspergillus niger)* species. Due to their benign and stable nature and antimicrobial property, these CuNPs may be well utilized for industrial and medicinal purposes.

KEY WORDS: Green synthesis, Copper nanoparticles, Peel extract, Antimicrobial property, Scavenging activity

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1. INTRODUCTION

Nanomaterials like carbon nanotubes, metal nanoparticles, nanowires, etc. show distinct electrical, catalytic, and medicinal properties, due to their high surface-to-volume ratio, large surface energy, and spatial confinement. Among various nanomaterials, copper nanoparticles have caught attention due to their well-known antimicrobial property and are used widely in gas sensors, superconductors, batteries, and textile industries. Different methods are available for the synthesis of copper nanoparticles: facile method (*Nidhi et al., 2009*), thermal decomposition, microwave assistant process (*Sreeram et al., 2008*), electrochemical, sonochemical (*Esau et al., 2010*), and via green chemistry (*Begum et al., 2009*). But all these methods require the use of hazardous chemicals, high energy requirements, and waste purification. Bio-synthetic methods employing either microorganisms or plant extracts have emerged as a simple and viable alternative to chemical procedures and physical methods (*Muthusamy et al., 2014*). Thus, there is a great demand for synthesizing CuNP in a simple and less expensive way. Recently, the biological synthesis of nanoparticles using plant extracts has gained more attention, since they are simple, cost-effective, non-toxic, stable, and environment friendly (*Shindume et al., 2019*).

Banana ranks first in production and third in the area among fruit crops in India. There are few literatures where the importance of banana peels is reported, which include: medicinal uses, ethanol fermentation, application as a substrate for generating fungal biomass, and utilization as a bio-sorbent for heavy metal reduction (*Padam et al., 2014*). The most recently discovered applications of orange fruit include its antioxidant and chemical exfoliates which are widely used in cosmetics. Dried peel is used in the treatment of anorexia, cold, and coughs (*Dixit & Tiwari, 2008*).

This profusely available biowaste, which is mainly made up of lignin, pectin, and hemicellulose, can be used as the primary material for the synthesis of CuNP. Our research work emphasizes the synthesis of copper nanoparticles from *Musa acuminata* and *Citrus sinensis* peel extracts. The peels of a variety of fruits have gained attention as a natural source of antioxidants. Since copper is well-known for its antimicrobial properties, we assumed that fabricating CuNP from banana and orange peel extract can increase its efficiency. The antimicrobial activity of the nanoparticles was analyzed using gram-positive (*Staphylococcus aureus),* gram negative (*Escherichia coli),* and fungal (*Aspergillus niger)* species. We also determined the antioxidant activities of the synthesized nanoparticles and a comparison of the antioxidant activities of synthesized copper nanoparticles concerning time was also studied.

2. MATERIALS & METHODS

2.1. Preparation of dried and fresh banana and orange peel extract

Fresh red bananas and oranges were collected from the University of Agricultural Sciences, Gandhi Krishi Vignan Kendra, Bengaluru. The bananas and oranges were thoroughly washed with distilled water to remove the dirt and other impurities.

*Author for correspondence: Roopa Prasad | Email: roopa.p@kristujayanti.com

The fruit peels were removed and dried on a paper towel. 20 g of the dried banana and orange peel were taken in a 100 mL beaker with 75 mL double distilled water separately. Both the mixtures were boiled at 70 - 80 °C for 20 minutes. A light yellow color solution was obtained, which was cooled at room temperature. Filter the light yellow colored extract twice into a conical flask using Whatman filter paper. The banana peel extract (BPND) and orange peel extract (OPND) were stored at 4 °C in the refrigerator for further studies. The dried banana peel extract (DBPE) and orange peel extract (DOPE) were stored at 4 °C in the refrigerator for further studies (*Aminuzzaman et al., 2017*).

2.2. Synthesis of copper nanoparticles

For the banana and orange peel prepared extracts, 30 mL were taken and boiled at 70 - 80 °C on a magnetic stirrer. To that 170 mL of 1 mM $CuSO_4.5H_2O$ was added for the reduction of copper ions. A green-colored solution was formed immediately. The solution was further boiled until the formation of a brown color paste. It was centrifuged at 6000 rpm for 20 minutes twice. The pellet was washed thrice with deionized water. The pellet was transferred to a clean watch glass and dried using a rota evaporator. The black color powder of the respective copper nanoparticle was scraped and subjected to characterization. Nanoparticles from dried banana peel - BPD; Nanoparticles from fresh banana peel - BPND; Nanoparticles from dried orange peel - OPD and Nanoparticles from fresh orange peel - OPND.

2.3. Characterization of nanoparticles

Characterization of the synthesized nanoparticles for parameters, such as surface morphology was done by scanning electron microscopy (SEM) and Ultraviolet-visible (UV-Vis) absorption spectra were recorded by a UV-visible spectrophotometer for all four samples.

2.4. Analysis of antimicrobial activity (well-diffusion method)

Antibacterial activity was determined against gram negative (*Escherichia coli*) and gram positive (*Staphylococcus aureus*) bacteria. Pure cultures of the selected microorganisms were subcultured in a nutrient broth at 35 °C on a rotating shaker at 1600 rpm. 100 µl of 10^{-6} CFU/mL culture was pipetted and spread uniformly on a solidified nutrient agar plate. It was then left undisturbed for approximately 10 minutes to get absorbed. Then wells were punctured on the agar plate. 100 µl of the synthesized nanoparticles was added into the well. The bacterial culture plates were kept in the incubator overnight at 35 ± 2 °C. Anti-fungal activity against *Aspergillus niger* was determined using the well diffusion method. The blank solvent was kept as negative control and the tetracycline antibiotic as a positive control (*Ren et al., 2018*). Bacterial inhibition was measured through the inhibition zone (zone of clearance) around the well.

2.5. Analysis of free radical scavenging activities

Some of the reactive oxygen species, including hydrogen peroxide, singlet oxygen, hydroxyl, and superoxide radicals, have positive roles in energy production in vivo systems, phagocytosis, intercellular signal transfer, regulation of cell growth, and the synthesis of important biological compounds (*Packer et al., 2008*). Additionally, reactive oxygen species modify DNA and membranes by attacking the lipids, proteins, and carbohydrates in cell membranes and tissues (*Jung et al., 2009*). The ability of synthesized nanoparticles to scavenge hydrogen peroxide was determined (*Ruch et al., 1989*), and ascorbic acid was used as a control. The absorbance was measured at 230 nm against suitable blank and % scavenging activity was calculated using the given formula (*Lalitha et al., 2013*). All four CuNPs were analyzed for percentage scavenging activity and a comparative study of the effect of storage for one month on scavenging activity.

$$\%\ \text{Scavenging Activity} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

3. RESULTS & DISCUSSION

There are numerous methods employed for the green synthesis of metallic nanoparticles from various sources. The banana peel extract (BPND) and orange peel extract (OPND) were prepared, and these solutions were later used for the green synthesis of copper nanoparticles. The synthesized nanoparticles were confirmed by the visible change in the color to dark green and characterized for parameters, such as Ultraviolet-visible (UV-Vis) absorption spectra and surface morphology by Scanning Electron Microscopy (SEM) for all four samples. The copper nanoparticles were later used for antimicrobial and free radical scavenging activities.

3.1. Characterization by UV-Visible spectrophotometry

UV-visible spectra were analyzed for different samples (Figure 1) revealing that the CuNPs synthesized from dried orange peel extract showed the maximum absorbance λ_{max} at 591.5 nm and fresh orange peel extract-CuNPs showed the least absorbance than the other samples at 591.5 nm (Table 1). The absorption bands of CuNPs have been reported in the range of 550 – 500 nm (*Arul et al., 1998; Khanna et al., 2007*). A similar UV-Vis spectrum was observed by several researchers (*Khalid et al 2015*).

Table 1: Absorbance value of different samples

Sample	Absorbance	Wavelength (nm)
BPD	0.148	834.5
BPND	0.082	589
PD	0.155	591
OPND	0.02	591.5

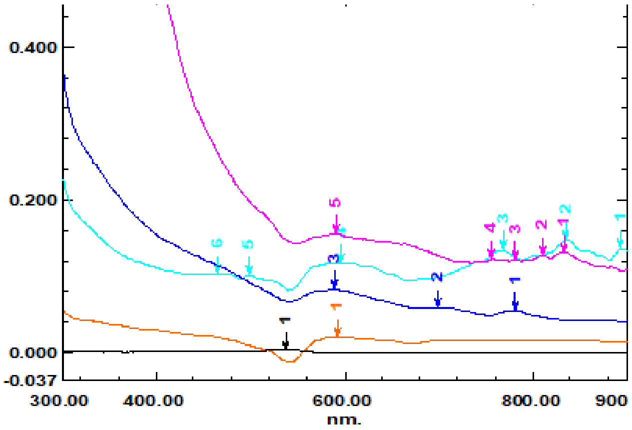

Figure 1: i) Blue- BPND; ii) Pink- OPD; iii) Red-OPND; iv) Light blue- BPD and v) Black- distilled water

3.2. Characterization of CuNPs by Scanning Electron Microscopy

The morphology of CuNPs was evaluated by SEM micrographs (Figure 3). The SEM images exhibited the nonhomogeneity of the CuNPs with respect to their shape and size. They were observed to be of irregular shapes such as hexagonal, cylindrical, triangular, and prism-like shapes, and aggregates of CuNPs with varying particle sizes were found in the micrographs (*Arya et al 2018*).

3.3. Analysis of antimicrobial activity (well-diffusion method):

Antibacterial activities of all four samples were analyzed using the well diffusion method. Antibacterial activity against gram negative (*Escherichia coli*) and gram positive (*Staphylococcus aureus*) bacteria was determined.

Table 2 shows the antibacterial activity of all four samples. Copper nanoparticles synthesized from dried orange peel extract showed the highest zone of inhibition similar to that of tetracycline, against *E.coli,* and nanoparticles from dried banana peel showed the least activity. In the case of *S. aureus,* nanoparticles from fresh banana peel extract showed the highest antibacterial activity, and nanoparticles from dried banana peel showed the least activity (Figure 3). Antifungal activity was determined against *A. niger* by well diffusion method and none of the CuNPs samples exhibited antifungal activity.

CuNPs have been proven to be an excellent antimicrobial activity (*Din et al. 2017*). The mechanism of antibacterial activity of CuNPs was due to its ability to break the bacterial cell and cause multiple toxic effects leading to the generation of reactive oxygen species and DNA degradation in the bacterial cell. (*Chatterjee et al. 2014*). CuNPs are reported to have a wide range of antibacterial activity against both gram positive and gram negative bacteria, which can be due to the presence of bioactive compounds on the surface of nanoparticles as capping and stabilizing agents (*Ananda Murthy et al. 2020*).

Table 2: Antibacterial effect of CuNPs exhibited by nanoparticle samples

Sample	Inhibition zone (mm)	
	Escherichia coli	*Staphylococcus aureus*
BPD	4	3
BPND	7	8
OPD	9	6
OPND	5	6
Tetracycline	9	

3.4. Free Radical Scavenging Activities

Percentage scavenging activity for all four samples of both samples 1 (fresh) and 2 (after one month of storage) was determined using hydrogen peroxide assay against suitable blank and ascorbic acid was taken as control. Dried orange peel copper nanoparticles showed the highest percentage of radical scavenging activity and nanoparticles from fresh orange peel extract showed the very least activity compared to that of any other sample. It was also observed that the percentage scavenging activity of the samples was reduced by 4.3 % after one month of nanoparticle synthesis (Table 3).

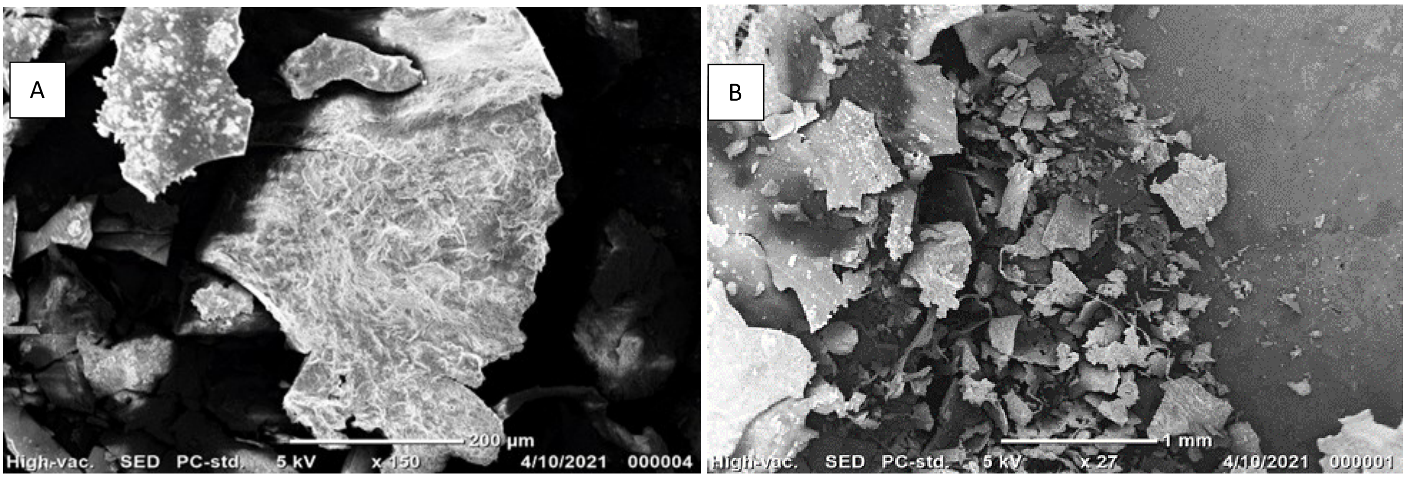

Figure 2: Scanning Electron Microscope (SEM) micrographs of the nanoparticles. (A) 200 µm resolution, (B) 1 mm resolution

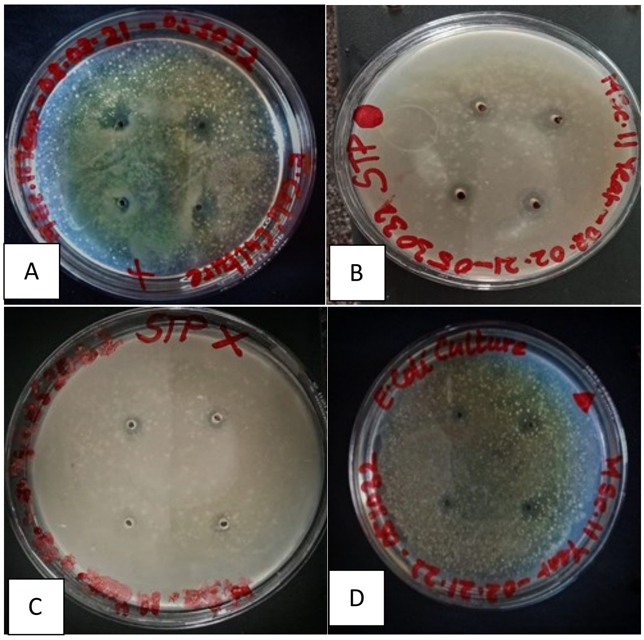

Figure 3: Petriplates showing the zone of inhibition formed by different nanoparticles against gram positive and gram negative bacteria on Mueller Hinton agar plate. Petriplates A and B: BPND sample exhibiting clear zone against *E. coli and S. aureus* respectively. Petriplates C and D: OPD sample exhibiting clear zone against *E. coli* and *S. aureus* respectively

Table 3: Percentage scavenging activity of different CuNPs

Sample	% Scavenging Activity	
	Sample 1	Sample 2
BPD	72.34	68.42
BPND	61.70	52.63
OPD	87.23	84.2
OPND	6.38	5.2

4. CONCLUSION

Green synthesis of copper nanoparticles (CuNPs) was successfully obtained from the bioreduction of copper sulfate pentahydrate solutions using *Musa acuminata* and *Citrus sinensis*. CuNPs have been appropriately characterized using UV-Vis spectroscopy and SEM analysis. It was observed that CuNPs synthesized from dried orange peel extract had better antimicrobial and antioxidant activity than the fresh orange peel and showed great activity than all the other three samples. Due to their benign and stable nature and antimicrobial property, these CuNPs may be well utilized for industrial and medicinal purposes. However, plant uptake and utilization of CuNPs require more detailed research on many issues including the uptake potential of various species, the process of uptake and translocation, and the activities of the CuNPs at the cellular and molecular levels. The biosynthesis of nanoparticles is a kind of bottom-up approach where the main reaction occurring is reduction/oxidation. The need for the biosynthesis of nanoparticles rose as the physical and chemical processes were costly. Often, the chemical synthesis method leads to the presence of some of the toxic chemicals absorbed on the surface that may have an adverse effect on medical applications in the search for cheaper pathways for nanoparticle synthesis, scientists used microbial enzymes and plant extracts (phytochemicals). Green synthesis provides advancement over chemical and physical methods as it is cost-effective, environment-friendly, and easily scaled up for large-scale synthesis and in this method, there is no need to use high pressure, energy, temperature, and toxic chemicals.

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