



Biofabrication of Copper Nanoparticles: A Next-generation Antibacterial Agent Against Wound-associated Pathogens

Bakır Nanopartiküllerin Biyofabrikasyonu: Yara ile İlişkili Patojenlere Karşı Yeni Nesil Antibakteriyel Ajan

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ABSTRACT

Objectives: Impaired wound healing is a major complication. A few factors such as blood glucose level, poor circulation, immune system deficiency, and infection are the root causes of impaired wound healing. The aim of the present study was to bio-synthesize copper nanoparticles with potential antibacterial activity against wound-associated pathogens.

Materials and Methods: Copper nanoparticles were fabricated using the sol-gel method with the mixing of *Syzygium cumini* leaf extract in metal salt solution. The particles were then later characterized using UV spectroscopy, SEM, TEM, FTIR, and XRD, and evaluated for their antibacterial activity and its MIC against four wound-associated pathogens.

Results: The results obtained from TEM, SEM, and XRD characterization showed that the particle size was below 100 nm and of spherical shape. FTIR analysis showed the possibility of various biomolecules, which have a role in capping and stabilizing copper nanoparticles. The particles synthesized showed antibacterial activity against four wound-associated pathogens (*P. mirabilis*, *S. saprophyticus*, *S. pyogenes*, and *P. aeruginosa*).

Conclusion: The biosynthesized copper nanoparticles showed potent antimicrobial activity, thus the antibacterial activity of the synthesized copper nanoparticles could be used in several biomedical applications. Additionally, they can be exploited as a better therapeutic agent for treating infection seen in impaired diabetic wounds. The particles synthesized by the biological route are eco-friendly, less toxic, feasible, and cost effective.

Key words: Nanoparticles, sol-gel process, biosynthesis, characterization, wound-associated pathogens, biomedical applications

ÖZ

Amaç: Düzensiz olmayan yara iyileşmesi önemli bir komplikasyondur. Kan glukoz düzeyi, zayıf dolaşım, bağışıklık sistemi eksikliği ve enfeksiyon gibi birkaç faktör düzensiz seyretmeyen iyileşmenin temel nedenleridir. Bu çalışmanın amacı, yara ile ilişkili patojenlere karşı potansiyel antibakteriyel aktivitesi olan bakır nanopartiküllerin biyosentetik olarak üretilmesidir.

Gereç ve Yöntemler: Bakır nanopartikülleri, *Syzygium cumini* yaprak ekstresinin metal tuzu çözeltisi ile karıştırılmasıyla sol-jel yöntemi kullanılarak üretilmiştir. Parçacıklar daha sonra UV spektroskopisi, SEM, TEM, FTIR ve XRD kullanılarak karakterize edildi ve antibakteriyel aktiviteleri yara ile ilişkili olan dört patojene karşı MIC değerleri belirlenerek araştırıldı.

Bulgular: TEM, SEM ve XRD karakterizasyonlarından elde edilen sonuçlar, parçacık boyutunun 100 nm'nin altında ve küresel şekilde olduğunu göstermiştir. FTIR analizi, bakır nanopartiküllerin kapatılmasında ve stabilize edilmesinde rol oynayan çeşitli biyomoleküllerin olasılığını göstermiştir. Sentezlenen parçacıklar, yara ile ilişkili dört patojene karşı (*P. mirabilis*, *S. saprophyticus*, *S. pyogenes* ve *P. aeruginosa*) antibakteriyel aktivite göstermiştir.

Sonuç: Biyosentezlenmiş bakır nanopartiküller güçlü antimikrobiyal aktivite göstermiştir, bu nedenle sentezlenmiş bakır nanopartikülleri antibakteriyel aktiviteleri için çeşitli biyomedikal uygulamalarda kullanılabilir. Bununla birlikte, iyileşmeyen diyabetik yaralarda görülen enfeksiyonu tedavi etmek için daha iyi bir terapötik ajan olarak kullanılabilirler. Biyolojik yolla sentezlenen parçacıklar çevre dostu, daha az toksik, uygulanabilir ve uygun maliyetlidir.

Anahtar kelimeler: Nanopartiküller, sol-jel süreci, biyosentez, karakterizasyon, yara ilişkili patojenler, biyomedikal uygulamalar

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Received: 18.05.2017, Accepted: 09.11.2017

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INTRODUCTION

Impaired or delayed wound healing is a major complication seen in various patients, especially in patients with diabetes.^{1,2} There are several factors responsible for impaired wound healing, such as poor circulation,³ diabetic neuropathy,^{4,5} immune system deficiency,^{6,7} infection, and stiffness of the arteries,⁸ which lowers the supply of blood, nutrients, and oxygen to tissues and ultimately lowers the efficiency of white blood cells to fight against infection.⁹ These factors may lead to impaired wound healing,^{10,11} thus close monitoring is very essential.¹² The poor replication of immune cells is a sign of infection development, which ultimately lowers rate of wound healing.¹³

Since ancient times, metals have been known to have good antimicrobial activity, thus in daily life metals have been used for disinfecting water,¹⁴ preservation of victuals.¹⁵ During World War 2, the Japanese dropped metal coins into water and milk to treat dysentery.¹⁶

In India, nanobiotechnology is providing an incipient insight in employing Indian greeneries, which is a great source of various plant products used in Ayurveda for the synthesis of eco-friendly and non-hazardous nanoparticles.¹⁷ Particles smaller than 100 nm are considered as nanoparticles, which have unique particle size along with advanced physical, chemical, and biochemical properties.¹⁸ Both physical and chemical methods are a commercial way of synthesizing metal nanoparticles, which are hazardous to the environment, thus it is imperative to develop an economically and commercially feasible as well environmentally sustainable route for synthesizing metal nanoparticles to meet demand.¹⁹ These phyto fabrications of metal nanoparticles undergo a highly controlled single-step protocol with green principles.²⁰ Phytoconstituents present in plant extracts can be used to synthesize metal nanoparticles in a single step.²¹ Studies have shown that a few metal nanoparticles have consequential wound rejuvenating activity.²² Thus, this study may provide insight into methods for nanoparticles synthesis and a direction for future research in impaired wound treatment.

MATERIALS AND METHODS

Material requirement

Copper sulfate metal salt was purchased from Fisher-Scientific. Nutrient agar and nutrient broth was purchased from HiMedia Ltd. *P. aeruginosa* (MTCC No. 3542), *S. saprophyticus* (MTCC NO. 6155), *S. pyogenes* (MTCC No. 5969) and *P. mirabilis* (MTCC No. 3310) were the standard cultures, which were procured from the Institute of Microbial Technology, Chandigarh, India.

Materials used for bioreduction of metal nanoparticles are *Syzygium cumini* leaf extract, double-distilled water, ethanol, magnetic beads, conical flask, and test tubes etc.

The study were approved by the Institutional Animal Ethics Committee Jiwaji University (protocol number: EAC/JU/27, date: 28/01/17).

Method

Preparation of leaf extract and phytochemical profiling

Syzygium cumini plant leaves were used for the study, which

were collected, air dried, and then coarsely powdered. Extraction was performed using ethanol as a solvent in a soxhlet extractor. The extract was then concentrated. The phytochemical profiling of the plant leaf extract was performed using an alkaloids test (Mayer's test), flavonoids test, glycosides test, steroids test (Salkowski's test), cardiac glycosides test (Keller-Killiani's test), saponins test, resins test, phenols test (ferric chloride test), tannins test (FeCl₃/lead acetate test), and a terpenoid test.²³

Biosynthesis of copper nanoparticles

0.01 M of copper sulphate was prepared and then mixed properly by placing it on magnetic stirrer. *Syzygium cumini* leaf extract was used for the purpose of reduction, where plant phytochemicals may themselves act as capping agents. The solution was then sanctioned for mixing on magnetic stirrer at a temperature of 60-70°C. After 2 hours, the sample was accumulated and sanctioned to centrifuge at 14,000 rpm. Pellets were collected and then washed three times by means of ethanol and then kept for drying on a dry bath. Samples were then collected and sanctioned for further characterization.

Characterization of copper nanoparticles

The synthesized nanoparticles were characterized using ultraviolet (UV)-visible spectrophotometry, a Fourier-transform infrared (FTIR) spectrophotometer Model RZX (Perkin Elmer), scanning electron microscope (SEM) Model JSM6100 (Joel) with image analyzer, an X-ray diffractometer (XRD) (powder method), and transmission electron microscope (TEM) Hitachi (H-7500).

Antimicrobial activity of copper nanoparticles

Antimicrobial susceptibility testing of bio-synthesized copper nanoparticles was performed using the Kirby-Bauer well diffusion method,^{24,25} where Mueller-Hinton Agar was taken as a medium, the well diameter was 5 mm and the amount of material used was 30 μ L. McFarland standard (0.5)²⁶ was used. *P. mirabilis*, *S. saprophyticus*, *S. pyogenes*, and *P. aeruginosa* were the four different wound-associated pathogens against which the antimicrobial potential of copper nanoparticles was tested. A solvent blank was used as a negative control. Pre-existing drug (povidone iodine), metal salt solution, and *Syzygium cumini* plant leaf extract were used as positive controls.

Minimum inhibitory concentration (MIC) of copper nanoparticles

The MIC of the bio-synthesized copper nanoparticles was then calculated at different concentrations (0.1 mg/mL, 0.3 mg/mL, 0.5 mg/mL, 0.7 mg/mL, and 0.9 mg/mL) against *P. mirabilis*, *S. saprophyticus*, *S. pyogenes*, and *P. aeruginosa* using the broth dilution method in nutrient broth. The concentration of culture was adjusted to 0.2 at 568 nm (1 \times 10⁸ CFU/mL, 0.5 McFarland's standard). Positive and negative controls were used as standard. MICs were denoted by analyzing the turbidity of the tubes. A small aliquot of the sample (approx. 50 μ L) from the culture tubes showing the least or no turbidity was taken and poured on an agar plate for 24 h at the optimum temperature for bacterial growth and was examined for growth. The experiment was performed in triplicate.^{27,28}

RESULTS AND DISCUSSION

Availability of phyto chemicals in Syzigium cumini leaf extract

The qualitative estimation of plant extract was performed and the results showed the availability of various phyto chemicals, which are presented in Table 1.

Nanoparticles synthesis and visible observation changes

There are three main phases of metal nanoparticle synthesis using plant extracts i.e. the activation phase, which includes metal ion reduction and then their nucleation, the second is the growth phase, which involves coalescence of small nanoparticles, and the last is the termination phase, which provides the final shape to the nanoparticles. Various studies have shown that in the bio-reduction, when the metal salt (copper sulfate) is dissolved in distilled water, it soon gets dissociated into its ionic form i.e. Cu^{2+} and SO_4^{2-} . After mixing the plant extract into the metal salt solution, there is a possibility that the chemical functional groups present within the plant extract interact with metal ions (Cu^{2+}) and reduce it to its zerovalent state (Cu^0), thus leading to the formation of metallic copper nuclei followed by the growth phase, leaving the rest of the components as by-product.²⁹

Thus, the addition of plant extract converts the bulk of the copper to copper nanoparticles, leaving the by-product aside, and ultimately changes the color of the solution. After integration of the plant extract to the metal salt solution, the color of the copper sulfate salt solution turns from bluish-greenish to brownish-reddish, which can be seen in Figure 1A and 1B. Bio-reduction and bio-sorption are the two major steps required for nanoparticle synthesis, by using various phyto products such as plant phytochemicals, carboxylic and amino groups, proteins, and carbohydrates.³⁰ The

colorimetric changes given by nanoparticles are due to the property of quantum confinement, which is a size-dependent property of nanoparticles that affects the optical properties of the nanoparticles.³¹⁻³³ The resulting color change may be due to the quantum confinement property of copper nanoparticles.

UV spectroscopy

Applied electromagnetic fields cause the excitation of surface plasmons on the periphery of nanoparticles, which leads to the occurrence of the phenomena called surface plasmon resonance.³⁴ The UV absorption apex range of copper nanoparticles is 573-600 nm.^{35,36} The result obtained from UV-Vis spectra (Figure 2) showed the absorption peak approximately at 582 nm, indicating the formation of copper nanoparticles. An additional peak of 558 nm was also obtained. A broad absorption peak at 582 nm is due to the surface plasmon resonance absorption band along with free electronic vibrations of copper nanoparticles in resonance with a light wave.³⁷

FTIR spectroscopy

FTIR is a characterization technique that is used to quantify the vibration frequencies (Table 2) of bonds in the molecule,

Table 1. The qualitative estimation of phytoconstituents in *Syzigium cumini* leaf extract

S. No.	Phytoconstituents	Availability in ethanol extract
1.	Flavonoids	+
2.	Alkaloids	+
3.	Glycosides	+
4.	Steroids	+
5.	Phenols	+
6.	Terpenoid	+
7.	Saponins	-
8.	Resins	+
9.	Tannins	+
10.	Cardiac glycosides	-
11.	Phytosterols and triterpenoids	+
12.	Carbohydrates	+
13.	Fixed oils and fats	-

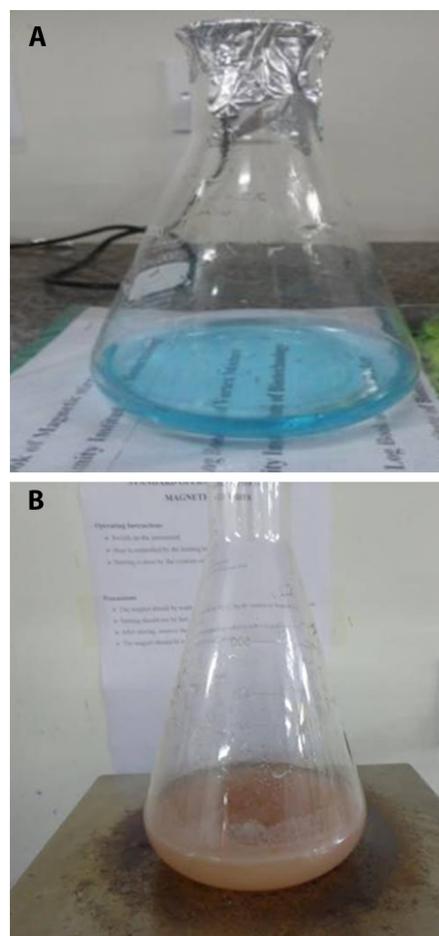


Figure 1. A) Visible observation of copper sulfate salt solution before adding plant leaf extract. B) Visible observation of copper sulfate salt solution after adding plant leaf extract

which can be seen in (Figure 3). FTIR analysis is performed to understand the vibrational kinetics of atoms or molecules, and to identify the possible phytoconstituents responsible for the reduction, as well as capping of reduced copper nanoparticles along with the nature of surface adsorbents.³⁸⁻⁴⁰ The alternate modification by such adsorbents (functional groups) may generate different properties. The FTIR spectra due to such adsorbents over the surface of the nanoparticles thus show a number of absorption peaks, each peak designating the availability of particular functional groups present in the plant extract.⁴¹ It is thus possible to understand the oxidation levels of synthesized nanoparticles prepared at different partial oxygen pressures. From FTIR data, it is possible to study the oxidation levels of nanoparticles prepared at different partial oxygen pressures.⁴²

Previous studies have shown that terpenoids are often associated with nanoparticles as analysed in FTIR

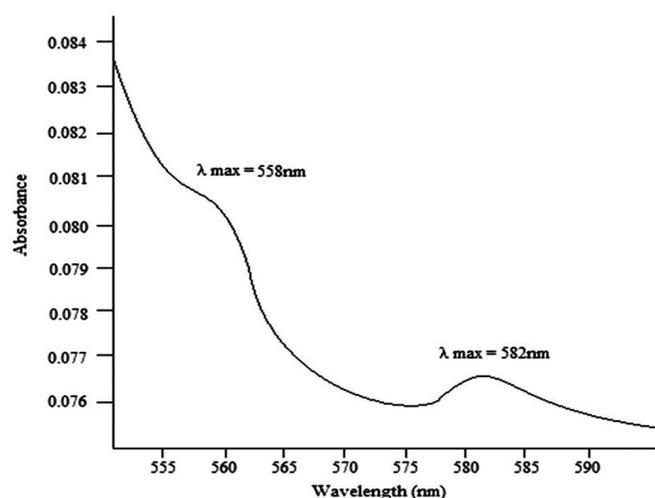


Figure 2. UV analysis of copper nanoparticles

Table 2. Vibrational frequencies of functional groups of possible phytoconstituents obtained by FTIR analysis

S. no.	Frequency (cm ⁻¹)	Possible functional groups
1.	3377.6 cm ⁻¹	O-H stretch vibration of phenols
2.	1632.12 cm ⁻¹	N-H bend of primary amines
3.	1514.13 cm ⁻¹	N-O asymmetric stretch vibration of nitro compounds
4.	1198.6 cm ⁻¹ , 1117.3 cm ⁻¹ , and 1107.3 cm ⁻¹	C-N stretch vibration of aliphatic amines
5.	864.11 cm ⁻¹	N-H bond of primary and secondary amines
6.	803.11 cm ⁻¹ , 676.10 cm ⁻¹ , and 626.8 cm ⁻¹	C-Cl stretch vibration of alkyl halides
7.	594.9 cm ⁻¹	Cu-O stretching vibration

FTIR: Fourier-transform infrared spectroscopy

spectroscopy results. Also, terpenoids have an essential role in transforming metal ions into nanoparticles⁴³ by dissociating eugenol OH-group protons, thus generating structures that can be further oxidized, leading to the reduction of metal ions, and ultimately the formation of nanoparticles.⁴⁴ The flavonoid tautomeric shift, i.e. from enol to keto, results in the release of reactive hydrogen, resulting in the reduction of metal ions and nanoparticle formation.⁴⁵ In plant sugars, by means of the nucleophilic addition of OH⁻, oxidation of the aldehyde group to a carboxyl group occurs, which leads to metal ion reduction and nanoparticle synthesis.⁴⁶ Similarly, different functional groups have different mechanisms for nanoparticle synthesis. The exact mechanism behind nanoparticle synthesis is still unknown and this area thus needs further exploration.

XRD analysis

X-ray diffraction patterns of copper nanoparticles were recorded using an XRD (powder method), which can be seen in Figure 4. Debye-Scherrer's equation i.e. $D = 0.9 \lambda / \beta \cos \theta$, was habituated to calculate the size of copper nanoparticles, where D represents crystalline size, λ represents wavelength of X-ray, β represents full width at half maximum of the diffraction peak and θ represents Bragg's angle. At 2θ values, a number of Bragg reflection peaks were observed at 26.79, 32.4, 35.5, 36.4, 44.1, 48.7, 50.6, 58.3 and 75.6, which were indexed to (111), (110), (002), (111), (200), (202), (200), (202) and (220) crystallographic planes of face-centred cubic, (JCPDS, File No. 04-0836 and JCPDS No. 45-0937). Additional peaks obtained seen during XRD analysis (35.09, 35.90 and 36.52) revealed the availability of CuO nanoparticles and 47.49 revealed the availability of Cu₂O nanoparticles, which may have occurred due to exposure of the nanoparticles to the surrounding environment during characterization. The estimated particle size was below 100 nm (calculated using Debye-Scherrer's equation). The width of the peaks obtained in XRD pattern is cognate to the crystallite size of the particle.⁴⁷ The small size of the nanoparticles synthesized thus increases their high surface area, and surface area to volume ratio.⁴⁸

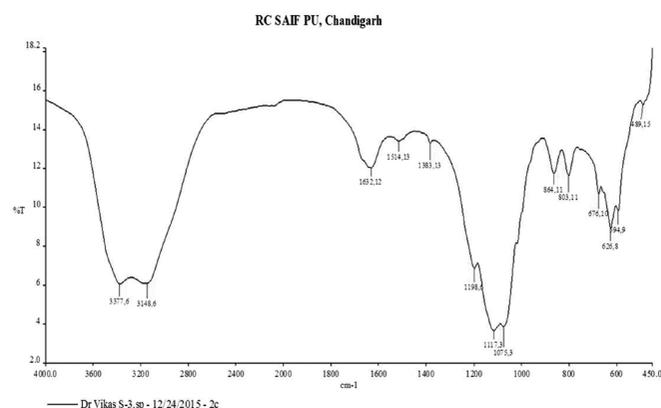


Figure 3. FTIR analysis of copper nanoparticles

TEM and SEM analysis

The synthesized nanoparticles had spherical or ellipsoidal symmetry. The copper nanoparticles were smaller than 100 nm, which can be seen in Figure 5. The obtained result supports the result obtained in TEM analysis, which can be seen in Figure 6. Both TEM and SEM confirmed the presence of copper nanoparticles (i.e. size <100 nm).

Antimicrobial activity of copper nanoparticles

Antimicrobial activity of the copper nanoparticles revealed that they had consequential antibacterial activity against wound-associated pathogens as compared with the plant extract and pre-subsisting drug (povidone iodine), which

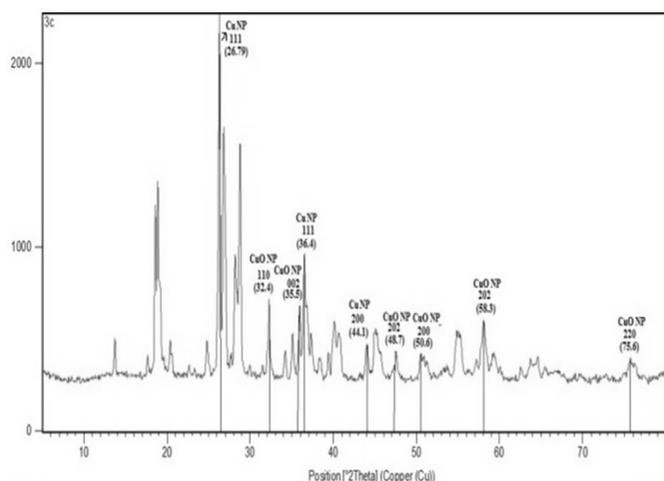


Figure 4. XRD analysis of copper nanoparticles

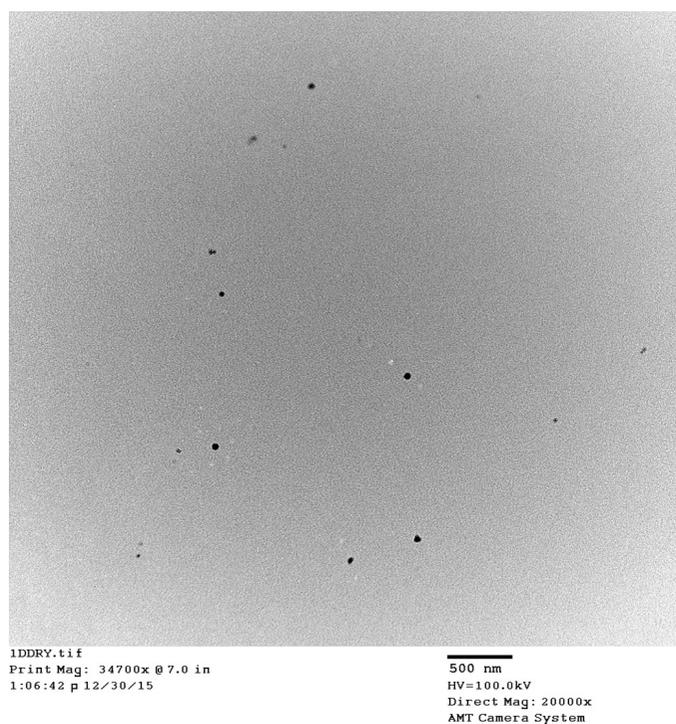


Figure 5. TEM analysis of copper nanoparticles (below 100 nm)

can be seen in Figures 7A-H. The biosynthesized copper nanoparticles exhibited good antibacterial activity against *P. mirabilis*, *S. saprophyticus*, *S. pyogenes*, and *P. aeruginosa* (i.e. 16 mm, 15 mm, 14 mm, and 12 mm, respectively).

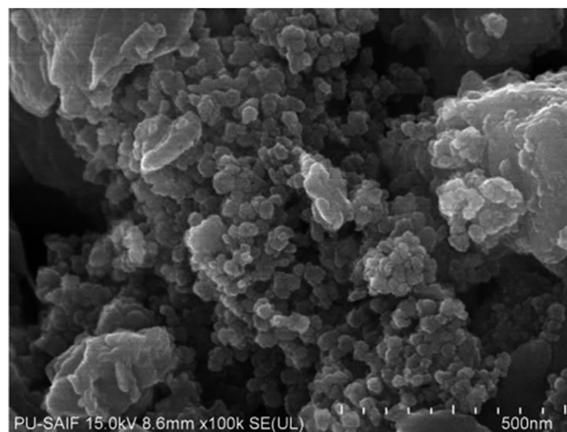


Figure 6. SEM analysis of copper nanoparticles

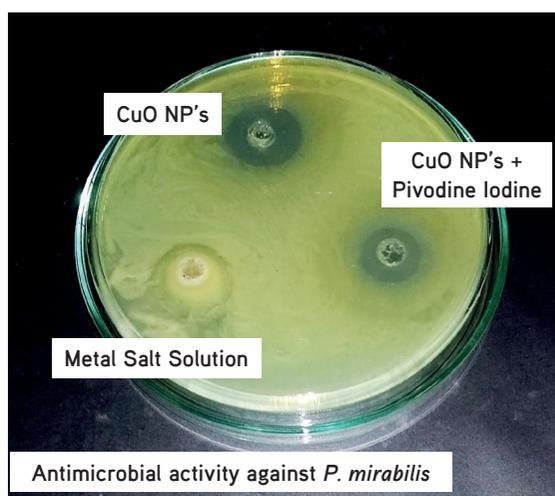


Figure 7. A) Antibacterial activity of copper nanoparticles against *P. mirabilis*

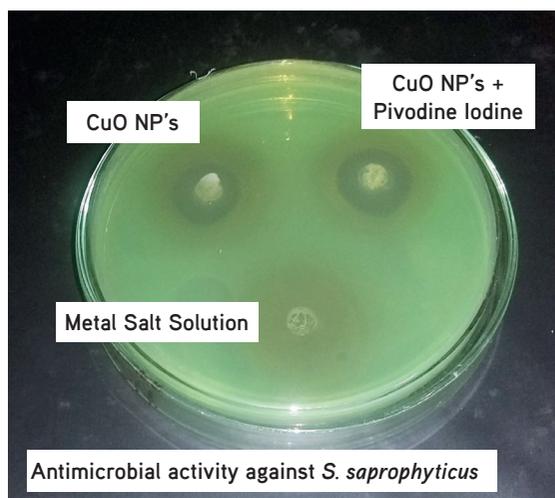


Figure 7. B) Antibacterial activity of copper nanoparticles against *S. saprophyticus*

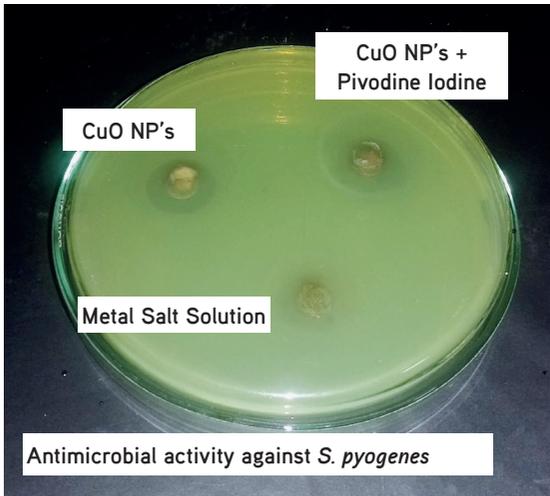


Figure 7. C) Antibacterial activity of copper nanoparticles against *S. pyogenes*

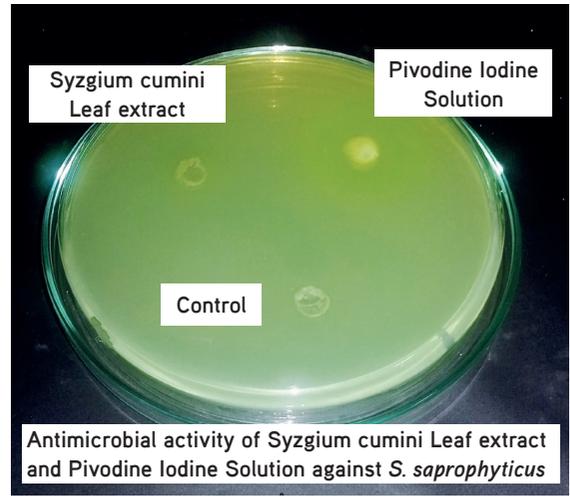


Figure 7. F) Antibacterial activity of plant leaf extract and povidone iodine against *S. saprophyticus*

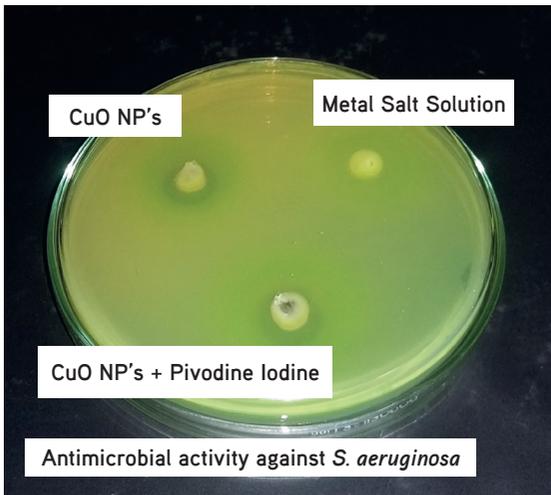


Figure 7. D) Antibacterial activity of copper nanoparticles against *P. aeruginosa*

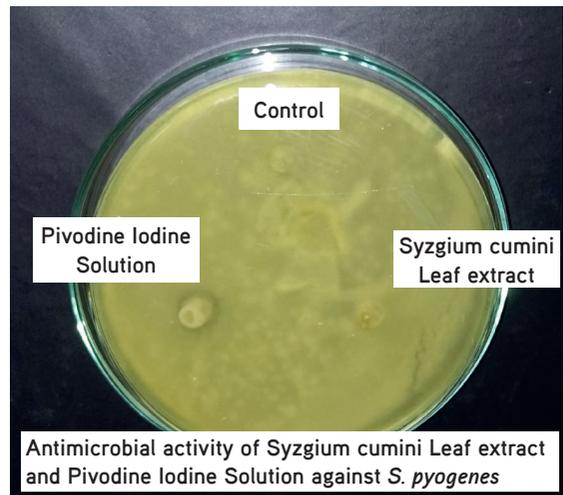


Figure 7. G) Antibacterial activity of plant leaf extract and povidone iodine against *S. pyogenes*

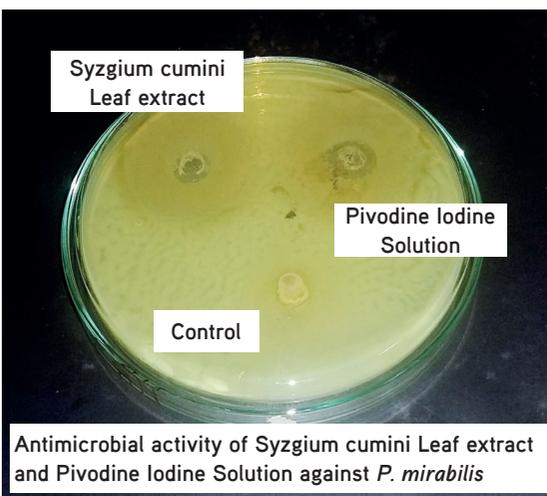


Figure 7. E) Antibacterial activity of plant leaf extract and povidone iodine against *P. mirabilis*

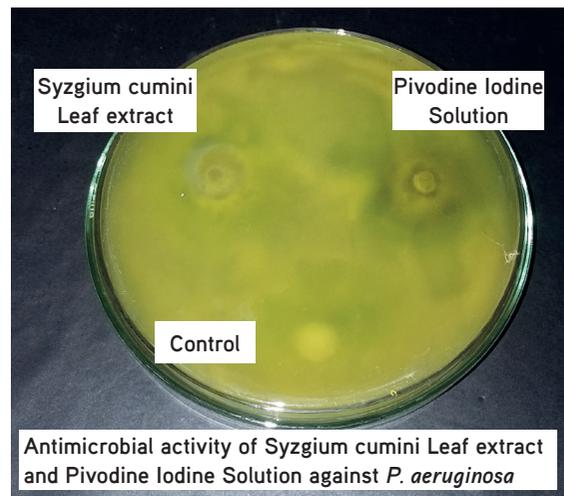


Figure 7. H) Antibacterial activity of Plant leaf extract and povidone iodine against *P. aeruginosa*

Table 3. Bacterial growth at different concentration of copper nanoparticles

Dilution for same bacterial concentration	Sets	Bacterial growth at different concentrations of copper nanoparticles				
		0.9 mg/mL	0.7 mg/mL	0.5 mg/mL	0.3 mg/mL	0.1 mg/mL
<i>P. mirabilis</i>	Set 1	-	-	-	+	+
	Set 2	-	-	-	+	+
	Set 3	-	-	-	+	+
<i>S. saprophyticus</i>	Set 1	-	-	+	+	+
	Set 2	-	-	-	+	+
	Set 3	-	-	-	+	+
<i>S. pyogenes</i>	Set 1	-	-	-	+	+
	Set 2	-	-	-	+	+
	Set 3	-	-	+	+	+
<i>P. aeruginosa</i>	Set 1	-	-	-	+	+
	Set 2	-	-	+	+	+
	Set 3	-	-	+	+	+

+: Turbidity due to microbial growth, -: No turbidity

The biosynthesized copper nanoparticles + pre-subsisting drug (povidone iodine) also exhibited good antibacterial activity against *P. mirabilis*, *S. saprophyticus*, *S. pyogenes*, and *P. aeruginosa* (20 mm, 17 mm, 18 mm, and 14 mm, respectively). Integration with povidone iodine exhibited less activity against *P. mirabilis*, *S. saprophyticus*, and *S. pyogenes* (11 mm, 8 mm, 8 mm), but no activity against *P. aeruginosa*. Moreover, the metal salt solution exhibited less activity against *P. mirabilis* and *S. saprophyticus* (10 mm, 9 mm) and no activity against *S. pyogenes* and *P. aeruginosa*.

MIC and minimum bacterial concentration of copper nanoparticles

The MIC and minimum bacterial concentration of copper nanoparticles was evaluated analysing the turbidity of the culture tubes. Culture tubes containing nanoparticles ranging from 0.1 mg/mL to 0.5 mg/mL showed bacterial growth, whereas no growth was seen in culture tubes containing nanoparticles 0.7 mg/mL and 0.9 mg/mL. A small aliquot of the sample poured on an agar plate showed no bacterial growth when allowed to grow for 24 hours in optimum temperature conditions, showing a bactericidal property of the copper nanoparticles at this particular concentration. Thus, it can be concluded that both the MIC (Table 3) and minimum bacterial concentration (Table 4) of the copper nanoparticles were effective at a concentration of 0.7 mg/mL.

The antibacterial activity results revealed that copper nanoparticles and copper nanoparticles + pre-existing drug acted as potent antibacterial agents against wound-associated pathogens when compared with pre-subsisting drug (povidone iodine), copper sulfate salt solution, and plant extract used for nanoparticle synthesis. The potential antimicrobial activity of the synthesized copper nanoparticles may be due to the grain size of the nanoparticles having a high surface to volume ratio.

Table 4. Minimum bactericidal concentrations of copper nanoparticles

Dilution of copper nanoparticles	Sets	Different concentration of copper nanoparticles	
		0.9 mg/mL	0.7 mg/mL
<i>P. mirabilis</i>	Set 1	-	-
	Set 2	-	-
	Set 3	-	-
<i>S. saprophyticus</i>	Set 1	-	-
	Set 2	-	-
	Set 3	-	-
<i>S. pyogenes</i>	Set 1	-	-
	Set 2	-	-
	Set 3	-	-
<i>P. aeruginosa</i>	Set 1	-	-
	Set 2	-	-
	Set 3	-	-

+: Bacterial growth, -: No bacterial growth

Nanoparticles are known to have bactericidal activity the ability to reduce flora without affecting surrounding tissue.⁴⁹ Antimicrobial agents have two modes of action, they are either bactericidal or bacteriostatic.⁵⁰ The antibacterial properties of such agents can be used to fight infectious diseases by reducing bacterial load. There is a significant difference between strains of bacteria, thus the use of antibacterial

agents should also be specific to the respective strains. Nanoparticles thus exert toxic effects against bacteria.⁵¹ There are several factors that affect the antimicrobial activity of nanoparticles against various microbial species, some of which are discussed below.

The cell wall protects the cell from damage and rupture because it provides stability, protection, rigidity, and shape to the cell. Tolerance as well as susceptibility is dependent on the structure of the cell wall, and there are several factors that affect the tolerability as well as susceptibility to nanoparticles such as bacterial growth rate and biofilm formation.⁵²

The bacterial growth rate is another factor that affects the tolerance of bacteria against nanoparticles. Susceptibility of fast-growing bacteria is more for nanoparticles and antibiotics as compared with slow-growing bacteria (in relation to the expression of stress-response genes).⁵³

The formation of biofilm (adhesion of microbial species to a solid surface together with matrix secretion covering them) by bacteria is a major drawback for antibacterial drugs as well as for nanoparticles to fight against bacteria. The interaction of biofilm as well as nanoparticles is dependent on their electrostatic properties.⁵⁴

CONCLUSIONS

The plant leaf extract we used showed great capability to synthesize copper nanoparticles at optimum temperature conditions. The UV absorption peak at 582.00 nm designates the synthesis of copper nanoparticles. The SEM and TEM studies were used with the aim at deciphering the morphology and size of the particle. FTIR studies showed the bio fabrication of the copper nanoparticles by the action of different phytochemicals with its different functional groups present in the extract solution. The XRD patterns showed the purity, phase composition, and nature of the synthesised nanoparticles. The following study justified the synthesis of stable nanoparticles, which could be due to the presence of capping and stabilizing materials such as flavonoids and terpenoids within the plant extract.

Additionally, the bio-synthesized copper nanoparticles showed potential antimicrobial activity against four different wound-associated pathogens as compared with the pre-subsisting drug povidone iodine. Thus the present work focuses on highlighting approaches of bio reduction approaches to synthesize copper nanoparticles using plant extract and antibacterial activity of synthesized nanoparticles. Various studies have already reported the synthesis of metal nanoparticles using physical and chemical methods, but the methods generally employed rigorous chemicals and stringent protocols, which are hazardous to the environment. Thus it is important to develop a protocol that is simple, cost-effective, eco-friendly, with the ability for scale up. The exact mechanism of metal nanoparticles synthesis using plant products is still not clear, but there are several studies which somehow focus on the possible mechanisms behind it. Bio-reduction and bio-sorption are two major steps required for

nanoparticles synthesis, governed by the use of various phyto products such as plant phytochemicals, carboxylic and amino groups, proteins and carbohydrates. Accordingly, the present study may support proper wound management with special reference to antimicrobial activity of bio-fabricated copper nanoparticles.

Conflict of Interest: No conflict of interest was declared by the authors.

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