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Bioconstruction of copper nanoparticles using stem bark extract of *Picralima nitida* and their antibacterial potency

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Abstract

Copper nanoparticles are involved in several applications due to the fact that they possess certain desirable properties. In this research report, copper nanoparticles have been synthesized by a green approach using the stem bark extract of Picralima nitida. The nanoparticles were characterised using UV-visible spectroscopy, FT-IR spectroscopy, scanning electron microscopy (SEM) and X-ray diffraction (XRD) method. The change in colour observed during copper nanoparticles synthesis from orange to golden yellow within 10 minutes confirms the formation of copper nanoparticles. The surface Plasmon peak indicating the formation of copper nanoparticles appeared at 213 nm. FT-IR spectroscopy was used to investigate interactions and changes in chemical compositions of the mixtures during biosynthesis. The FT-IR spectra of the copper nanoparticles and that of the stem bark extract were similar with minor differences. The similarity of the two spectra indicates that the components of P. nitida stem bark extract got attached to the copper nanoparticles retaining their essential features. The morphology of the copper nanoparticles reveals that the particles consist of spherical, cubic and irregular shaped structures with smooth surfaces. XRD analysis reveals the crystalline nature of the bio-synthesized copper nanoparticles with average size to be in the range of 35-61 nm. The copper nanoparticles showed potent inhibition against Pseudomonas aeruginosa which is a Gram-negative bacterium but with lesser effect on Staphylococcus aureus which is a Gram-positive bacterium. The copper nanoparticles synthesized here could be employed in the treatment of diseases caused by P. aeruginosa and S. aureus.

Keywords: Copper nanoparticles, *Picralima nitida*, antibacterial potency, bark extract.

Introduction

Copper is one of the most widely used materials in the world due to its electrical, optical, catalytic, biomedical, antifungal and antibacterial applications[1]. Among the metal nanoparticles, copper nanoparticles are potentially attractive, which may be due to their good optical, electrical and thermal properties, superior strength, and use as sensors, catalysts, and its bactericidal effect as antimicrobial and antifungal agents because they are very reactive and their high surface-to-volume ratio helps to interact with other materials effectively[2,3]. Copper is highly toxic to most microorganisms and non-toxic to animal cells, therefore, it is considered an effective bactericidal metal. It is also considered safe for applications in food packaging and in water treatment[4-7]. Copper and copper oxide nanoparticles have been studied as potential antimicrobial agents against infectious organisms such as *Escherichia coli*, *Bacillus subtilis*, *Vibria cholera*, *Pseudomonas aeruginosa*, *Syphilis typhus* and *Staphylococcus aureus*[8,9].

Copper nanoparticles have been successfully synthesized by radiolysis, laser irradiation, thermal decomposition, thiol-induced reduction in supercritical water, reduction in microemulsions, reverse micelles, vapour deposition, sonoelectrochemical, flame spray and chemical reduction[10]. The use of toxic chemicals for the synthesis of nanoparticles limits their applications in clinical fields. Therefore, development of clean, biocompatible, nontoxic and eco-friendly methods for nanoparticles synthesis deserves merit. The interest in this field has shifted toward 'green' chemistry and bio-processor approach. These approaches focus on utilization of environmental-friendly, cost-effective and biocompatible reducing agents for synthesis of copper nanoparticles[11].

Natural plant materials such as magnolia leaf extract and stem latex of *Euphorbia nivulia* have been used for the synthesis of copper nanoparticles[12]. Other ones include extracts of lemon and curcumin[2], gum karaya[13], *Gloriosa superba* L.[14], *Eucalyptus* sp.[10], *Cassia alata*[15], *Phyllanthus embilica*[11], *Phyllanthus amarus*[16] and *Rubia cardifolia*[17]. The copper nanoparticles synthesized from these plant materials showed antimicrobial activities.

Amongst various natural materials used for nanoparticle construction, plants seem to be the best candidates, and nanoparticles produced by plants are more stable, possess various sizes and shapes, and the rate of production is faster than in the case of microorganisms[12,13]. In this study, we report the bioconstruction of copper nanoparticles using the stem bark extract of *Picralima nitida* and the assessment of their antibacterial potency.

Materials and methods

Collection of Plant Materials: The stem bark of *P. nitida* was collected from the tree plant located at Ubakala, Umuahia south L.G.A., Abia State, Nigeria. The plant sample was sun-dried, mortared and then milled into a fine powder. Meanwhile, leaves from the plant were taken alongside the stem bark for identification and authentication at the Taxonomy Section of Forestry Department of Michael Okpara University.

Preparation of Aqueous Plant Extract: The powdered stem bark material was dispersed in 200 ml of sterile distilled water in a 500 ml glass beaker and boiled at 80^0C for 15 min and was allowed to cool. After that, the solution was filtered through Whatman No. 1 filter paper (Springfield Mill. Maidstone. Kent, England) and the filtrate was used immediately for the synthesis of copper nanoparticles.

Synthesis of Copper Nanoparticles: For the synthesis of copper nanoparticles, 10 ml of the aqueous stem bark extract was added to 90ml of 1×10^{-3} M aqueous $CuSO_4.5H_2O$ solution in a 250ml Erlenmeyer flask. Within 10 min. change in colour was observed from orange to golden yellow indicating the formation of copper nanoparticles. The copper nanoparticles solution obtained was purified by repeated centrifugation at 10000 rpm for 15 min followed by re-dispersion of the pellet in deionized water. Then the copper nanoparticles were dried in an oven at 80^0C and then allowed to cool before storing in an airtight container.

UV-visible Spectroscopy Analysis: The bioreduction process of copper ions in aqueous solution was measured by the sampling of 1 ml aliquot compared with 1 ml of distilled water used as blank and subsequently measuring the UV-visible spectrum of the solution. UV-visible spectrum was monitored on Cary Series UV-vis spectrophotometer Agilent Technology, operated within the wavelength range of 200 to 800nm.

FT-IR Spectroscopy Measurement: This was carried out on *P. nitida* stem bark extract and on the copper nanoparticles. FT-IR measurement of the samples was performed using FTIR-Cary 630 Fourier Transform Infrared Spectrophotometer, Agilent Technology, in a transmittance method at a resolution of $8cm^{-1}$ in potassium bromide (KBr) pellets in the wave number range of $4000\text{-}650cm^{-1}$.

Scanning Electron Microscopy (SEM) Analysis: Morphology of the nanoparticles was studied using SEM analysis (Model - Phenom ProX Scanning Element Microscope manufactured by PhenomWorld Eindhoven, the Netherlands).

X-ray Diffraction (XRD) Analysis: XRD (PAN analytical, Netherlands) patterns were obtained with a diffractometer (Empyrean model, Netherlands) operated at a voltage of 45 KV and a current of 40mA using Cu-Kα radiation in a θ-2θ configuration with a wavelength (λ) of 1.541 Å. The sample was made smoother and was imparted on a slide which was then charged into the machine after adjusting the machine parameters and was operated via a monitor.

Antibacterial Assay: The agar well diffusion assay method was used to evaluate the antibacterial potency of the copper nanoparticles against the test microorganisms. Concentrations of 100, 50 and 25mg/mL prepared from the nanoparticles in a 2-fold dilution process were tested against the organisms. Sterile Mueller Hinton Agar (MHA) was poured into sterile Petri dishes and allowed to set. Standardized concentrations (0.5 McFarland Turbidity Standard) of overnight cultures of test isolates were swabbed aseptically on the agar plates and holes (6 mm) were made in the agar plates using a sterile metal cork-borer. 50µl of the various dilutions of the copper nanoparticles and control standard were put in each hole under aseptic condition, kept at room temperature for one hour to allow the agents to diffuse into the agar medium and incubated accordingly. Gentamycin (10µg) was used as a positive control in the antibacterial evaluation. The MHA plates were then incubated at 37^0C for 24h. The diameter zones of inhibition produced were measured and recorded. This procedure was conducted in duplicates for each of the test organisms.

Results and discussion

UV-visible Spectroscopy: The change in colour observed during copper nanoparticles synthesis from orange to golden yellow within 10 minutes confirms the formation of copper nanoparticles. UV-vis spectroscopy which remains one of the most convenient methods for the assessment of metal nanoparticles formation and characterization was employed. Figure-1 shows the UV-vis absorption spectrum of the synthesized copper nanoparticles. The surface Plasmon peak indicating the formation of copper nanoparticles appeared at 213 nm. Although many researchers have reported the surface Plasmon peak of copper nanoparticles to appear above 500nm[3,4], Caroling *et al*[11] reported a value of 294nm for copper nanoparticles biosynthesized using the extract of *Phyllanthus embilica*. It is noteworthy that Plasmon absorption position may depend on certain factors which include particle size, shape, solvent type as well as capping and stabilizing agent, hence a value of 213nm might be as a result of any of these factors. The type and concentration of phytochemicals present in the stem bark extract of *P. nitida* might have influenced the arrangement of molecules around the copper particles.

FT-IR Spectroscopy: FT-IR spectroscopy was used to investigate the interactions between different species and changes in chemical compositions of the mixtures during biosynthesis. FT-IR measurement was carried out on both the plant extract and copper nanoparticles to identify the possible functional groups responsible for the reduction of copper ions and the capping as well as stabilization of the copper nanoparticles by *P. nitida* stem bark extract. Figures-2 and 3 show the FT-IR spectra of the stem bark extract of *P. nitida* and

the copper nanoparticles bioconstructed from it respectively. In the spectra, it could be noticed that there were very little changes in the peak locations between the *P. nitida* stem bark extract and the copper nanoparticles. Concomitant considerations of the two spectra show that absorption peaks at 3306.1 and 3302.4cm^{-1} were due to O-H stretching vibrations while that of 2944.6 and 2948.3cm^{-1} were due to alkyl or alkane C-H stretching vibrations. Also, absorption peaks observed at 1651.2 and 1654.9cm^{-1} were as a result of C=C stretching vibration of alkenes while the ones at 1449.9cm^{-1} (for both spectra) were due to C=C stretching of aromatics. Absorption peaks at 1408.9cm^{-1} (for both spectra) correspond to O-H bending vibration due to *tert*-alcohols or phenols. Another peak observed at 1114.5cm^{-1} (for both spectra) was assigned to C-O stretching vibration. The sharp absorption peaks at 1017.6 and 1013.8cm^{-1} were also as a result of C-O stretching vibrations. The similarity of the two spectra indicates that the components of *P. nitida* stem bark extract got attached to the copper nanoparticles retaining their essential features. Observations regarding the similarity of FT-IR spectrum of precursor and that of nanoparticles synthesized from it have been reported by other researchers[4,18].

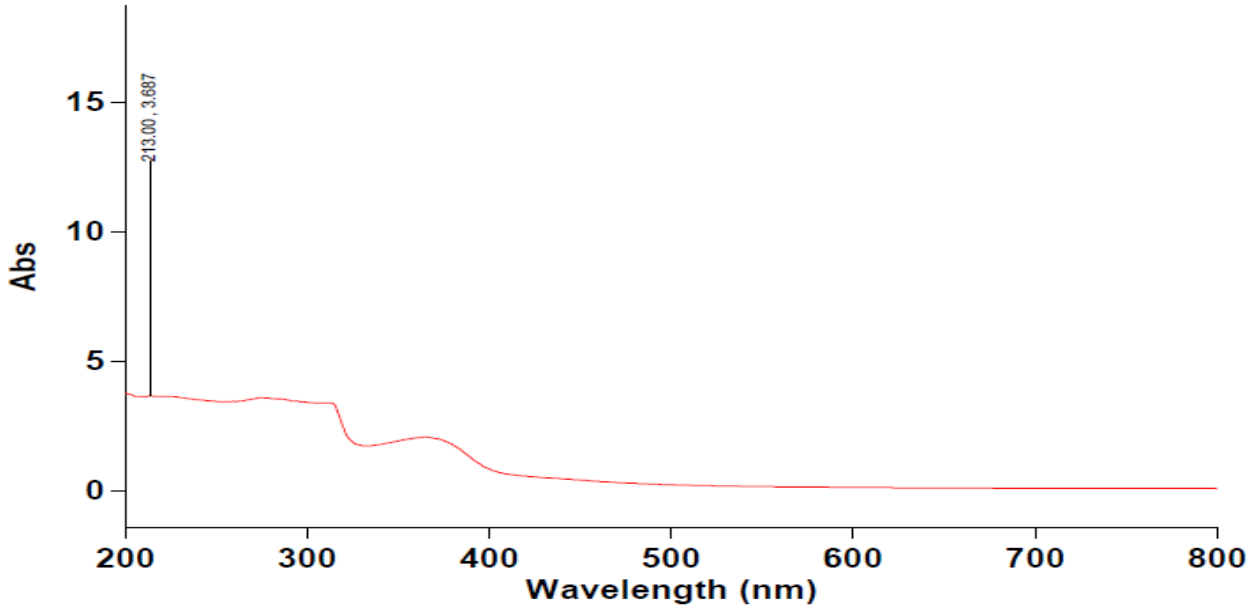

Figure-1: UV-vis absorption spectrum of copper nanoparticles bioconstructed using stem bark extract of *P. Nitida.*

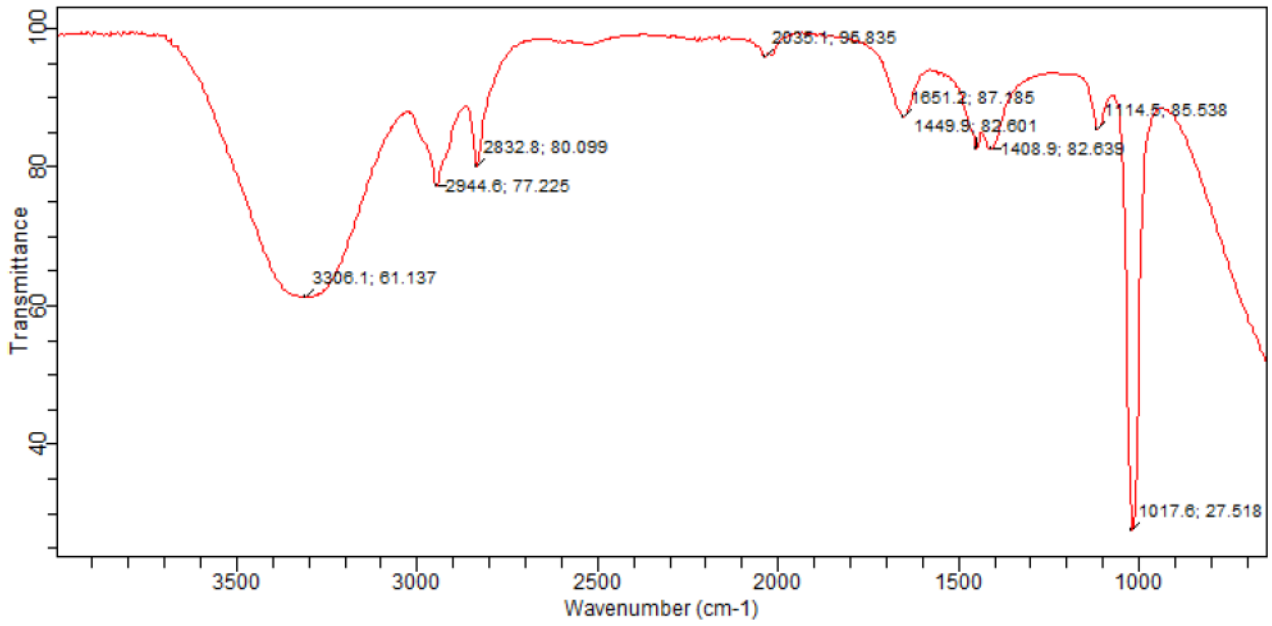

Figure-2: FT-IR spectrum of the stem bark extract of *P. Nitida*.

SEM Studies: The SEM images of copper nanoparticles bioconstructed from the stem bark extract of *P. nitida* is shown in Figure-4. The morphology of the copper nanoparticles reveals that the particles consist of spherical, cubic and irregular shaped structures with smooth surfaces. Observations made with higher magnifications reveal that there are tiny individual copper particles as well as a number of aggregates with minimal uniformity.

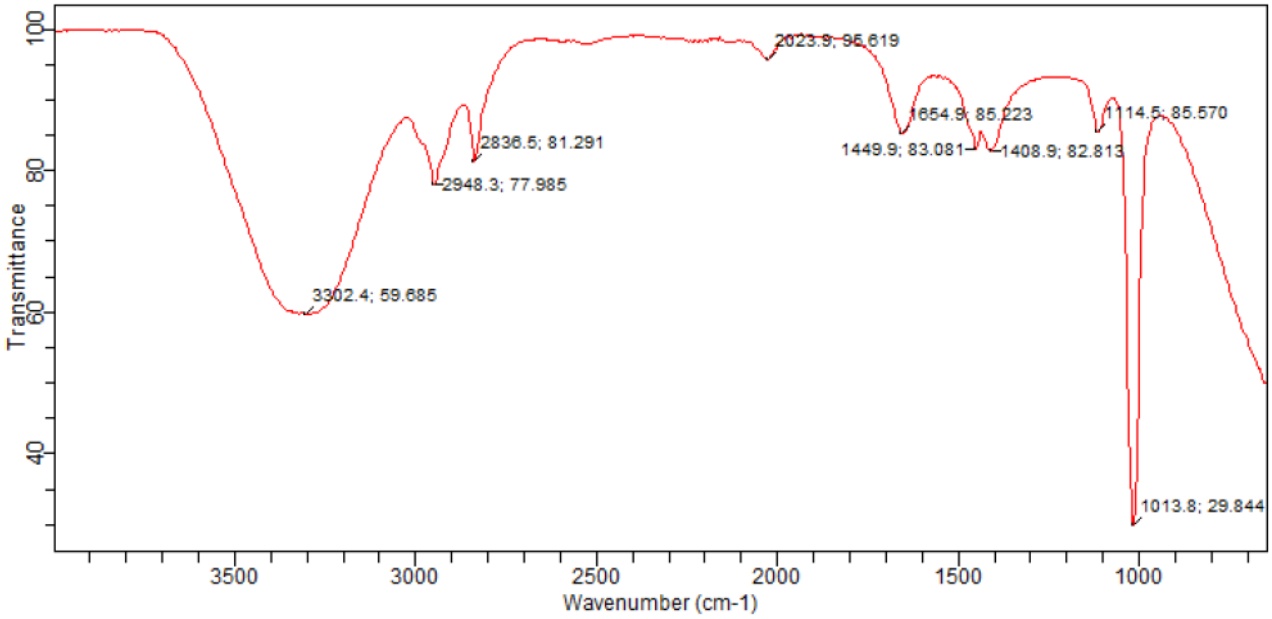

Figure-3: FT-IR spectrum of copper nanoparticles bioconstructed from the stem bark extract of *P. nitida*.

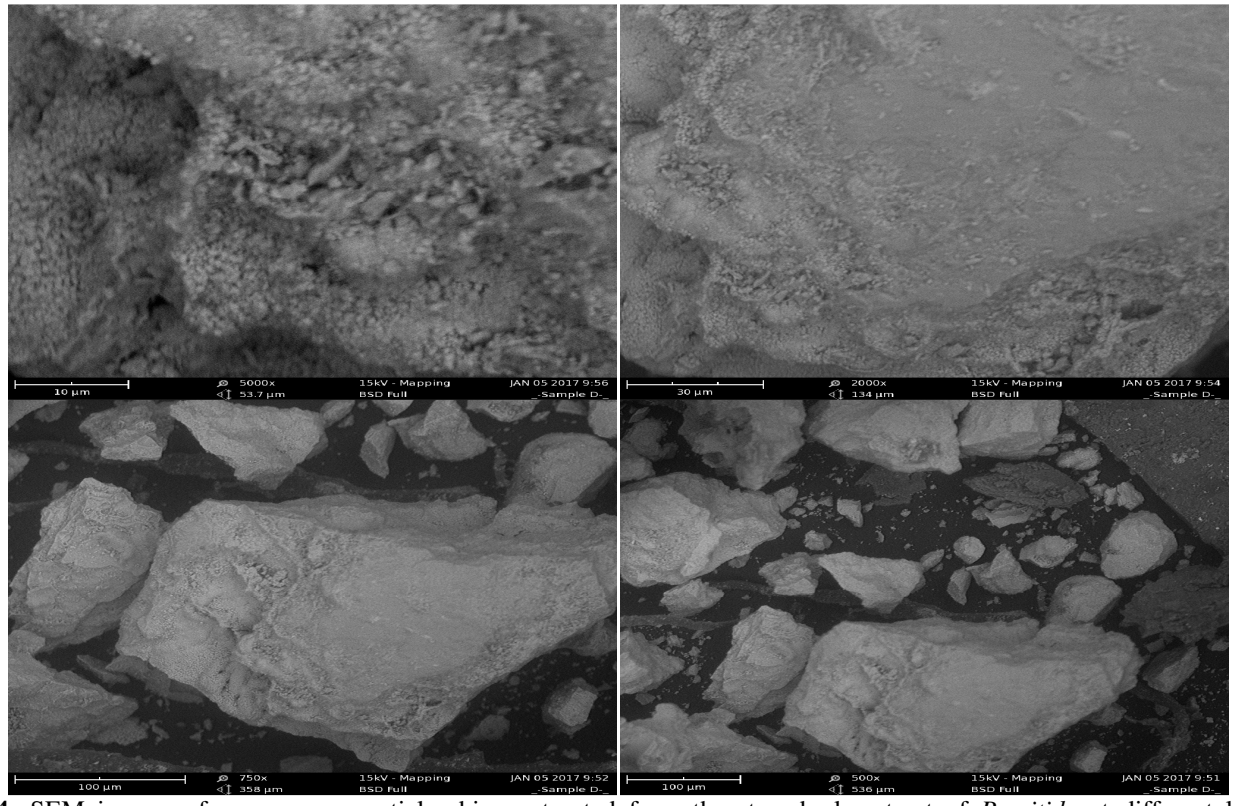

Figure-4: SEM images of copper nanoparticles bioconstructed from the stem bark extract of *P. nitida* at different levels of magnification.

XRD Studies: The XRD pattern of the copper nanoparticles bioconstructed from the stem bark extract of *P. nitida* is shown in Figure-5. The pattern confirms the crystalline nature of the copper nanoparticles. Bragg diffraction angles with 2θ values of 10.11^0, 23.87^0, 26.52^0, 30.99^0, 36.09^0, 49.79^0 and 65.62^0 were observed within the range of $5.5\text{-}74.985^0$ in a continuous scan. The average particle size of the copper nanoparticles was calculated from the XRD pattern using the Debye-Scherrer equation shown below:

$$D = \frac{k\lambda}{\beta \cos\theta}$$

where D is the average diameter size of nanoparticles (in nm), K is the Scherrer constant related to the shape and index (*hkl*) of the crystals with a value of 0.9, λ is the wavelength (1.541 Å) of the x-rays, β is the additional broadening which is the full-width at half maximum (FWHM) of the peak (in radians), and θ is the Bragg angle (in degrees). The average size of the copper nanoparticles was obtained to be in the range of 35-61 nm.

Antibacterial Assay: The antibacterial potency of the copper nanoparticles was assessed on two bacteria organisms which included *P. aeruginosa* (a Gram-negative bacterium) and *S. aureus* (a Gram-positive bacterium) as shown in Table-1. The experiment revealed that the nanoparticles successfully inhibited the growth of *P. aeruginosa* and *S. aureus* at concentrations of 100 and 50 mg/ml. This observation could be attributed to the difference in thickness of the cell walls of these organisms. The bacteria cell wall is made up of polysaccharides and peptides called 'peptidoglycon'[19]. The Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan. In contrast, gram-negative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan[19]. It is noteworthy that the cell wall thickness for Gram-positive bacteria is 20-30 nm while that of the Gram-negative is 8-12 nm [20]. So, it was easier for the nanoparticles to pierce through the cell wall of *P. aeruginosa* than through that of *S. aureus*. This explains the reason for the drastic difference in the observed antibacterial sensitivity.

Conclusion

Copper nanoparticles with a size distribution of 35-61 nm have been synthesized using an aqueous extract of *P. nitida* stem bark as a precursor. This method adopts the principles of green chemistry as no harmful chemical is discharged into the environment coupled with procedural cost effectiveness. The copper nanoparticles exhibited antibacterial potency against *P. aeruginosa* and *S. aureus*. This means that the copper nanoparticles could be used to treat diseases and infections caused by these organisms.

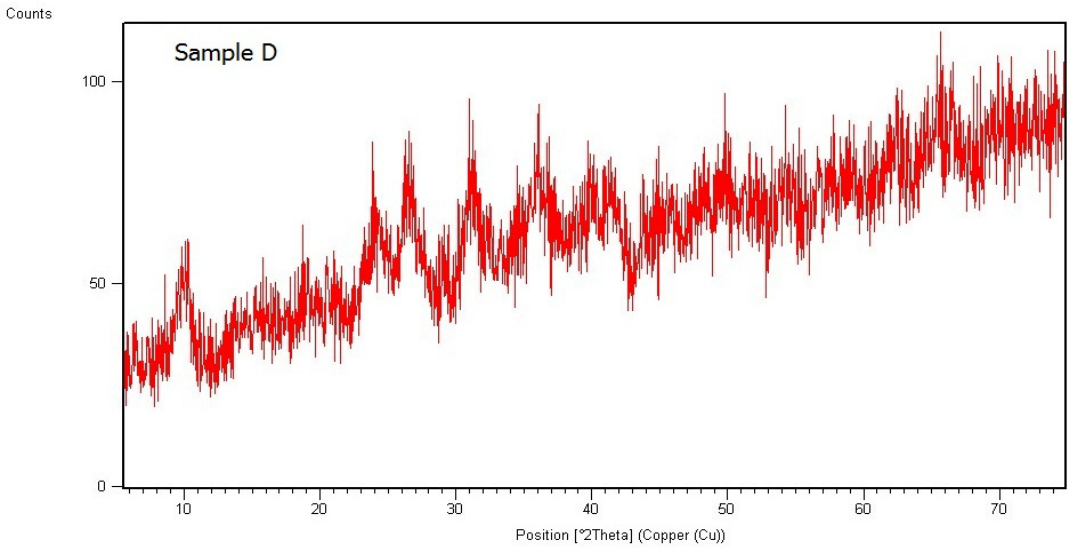

Figure-5: XRD pattern of copper nanoparticles bioconstructed from the stem bark extract of *P. Nitida.*

Table-1: Antibacterial potency of copper nanoparticles bioconstructed from the stem bark extract of *P. nitida*

Test organisms	Mean zone of inhibition (mm)			
	Concentrations			
	100 mg/ml	50 mg/ml	25 mg/ml	Gentamycin
P. aeruginosa	12.5	12.0	0.0	31.0
S. aureus	4.0	2.5	0.0	29.0

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