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# Synthesis and Characterization of Copper Nanoparticle using *Capparis Zeylanica* leaf Extract

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**Abstract :** The development of nanotechnology is making the interest of researchers towards synthesis of nanoparticles for the bio application. Here copper nanoparticles were synthesized using *Capparis zeylanica* plant leaf extract. The leaf extract acts as both reducing and capping agent. The synthesized copper nanoparticles were confirmed by the change of colour after addition of leaf extract into the Copper Sulfate solution. The biosynthesized CuNPs were characterized by using UV-Vis analysis, Fourier Transform Infrared analysis (FTIR), X-ray diffraction analysis (XRD), Scanning Electron Microscopy (SEM), Energy Dispersive X-Ray analysis (EDX) and Transmission Electron Microscopy (TEM) analysis. The synthesized CuNPs were in cubical structure with the particle size in the range between 50-100 nm. The Antimicrobial study of the CuNPs wasestablished using both gram positive and gram negative pathogens.
**Keywords:** Bio reduction, Nanoparticles,*Capparis zeylanica,* Characterization.

## Introduction

Nanotechnology plays a very important role in modern research[1,2], it is the most capable technology that can be applied almost all fields such as pharmaceutical, electronics, health care, food and feed, biomedical science, drug and gene delivery, chemical industry, energy science, cosmetics, environmental health, mechanics and space industries. It has also been utilized for the treatments of infection[3], cancer[4], allergy[5], diabetes[6] and inflammation[7].Green chemistry is an implementation, development, design of chemical

Products and processes to minimize the use of hazardous to environment[8]. To synthesize nanoparticles there are many ways such as sol gel method, chemical reaction, solid state reaction and co-precipitation. Compared to those methods green synthesis method is one of the best method for the production of nanoparticles in recent years. This green synthesis method have several advantages over other methods namely cost effectiveness, simplicity, use of less temperature, the usage of less toxic materials, moreover it is compatible for medical and food applications[9,10]. Many researchers used green synthesis methods for different metal nanoparticles due to their growing need of eco-friendly properties[11].Green synthesis method was found to be the best method when compared to the other method such as chemical reduction, photochemical reduction, electrochemical reduction, heat evaporation etc.,[12]. In this method, the plant extract has been used as capping and reducing agent for the synthesis of copper nanoparticlesdue to their reducing properties present in the leaf extract[13,14].

Over the past few years, the metal nanoparticles are focused mainly for the research work due to their potential applications in different fields such as magnetic recording media or micro electronics, catalysis[15],

nanosensors, nanoelectronics, optoelectronics and information storage devices[16]. Some properties such as morphology, size and distribution of the particles are clearly obtained from the nanoparticles[17].

Copper is most widely used material in the world due to their electrical, optical, catalytic, biomedical and antifungal/antibacterial applications among various metal particles such as gold, silver, iron, palladium, zinc and quantum dots[18]. It can give more yields and reaction rate in mild reaction conditions when compared to other traditional catalysts[19]. Copper nanoparticles act as antimicrobial agent in various fields. The copper is highly toxic to microorganism such as bacteria (*E-Coli, Staphylococcus Aureus, Pseudomonas aeruginosa*)and non-toxic to animal cells, due to these phenomena it is considered to be an effective bactericidal metal. Itis consider to bea safe for human beings such as food package application and in water treatment applications[20-22].

Various plants were used for the synthesis of nanoparticles using green synthesis method. Nanoparticles were synthesized from all the parts of the plant separately like seed, stem, flower, leaf and skin of the fruits. The nanoparticles synthesized from plant extract were found to be covered by the medicinal properties of plant extract which could be used in drug, targeted drug delivery and cosmetic applications[23]. In this present investigation, *Capparis zeylanica* Linn own to the family of capparidaceae commonly known as Indian caper, is a climbing scandant shrub found throughout India and been used as a 'Rasayana' drug in the traditional medicine. This plant extract was used as reducing agent for the synthesis of CuNPs[24]. The plant extract mainly consists of fatty acids, alkaloids and flavonoids. *Capparis zeylanica* Linn was reported to hold antioxidant, antimicrobial, anti inflammatory and immunostimulant activity[25].

The current investigation focused on the aqueous leaves extract of *Capparis zeylanica* used to synthesize CuNPs at various experimental conditions and thereby improving the importance of plant source and involving green chemistry for the synthesis of other nanoparticles as future research.

## Materials and Methods

### Materials

All the reagents used in this experiment were obtained from Sigma Aldrich chemicals India. Double distilled water was utilized for this process. Filtration was established by using Whatman no.1 filter papers. Glasswares used for the complete reactions were washed well, rinsed with double distilled water and dried in hot air oven.

### Preparation of *Capparis zeylanica* leaf extract

The fresh *Capparis zeylanica* leaves were collected from Mannargudi, India. The leaves were thoroughly washed several times using normal water and then followed by distilled water to remove impurities. The cleaned leaves were subsequently dried under sunshade to remove moisture completely, powdered by using mechanical grinder and then stored. The 5g of powdered plant leaves were taken into a beaker along with 100 ml of distilled waterand allowed to boil at 60°C for 30 min under reflux condition then it was cooled down to room temperature. The prepared solution was initially filtered through normal filter paper thereby powdered leafy materials will be filtered out.The filtrate was again filtered through Whatman No.1 filter paper to get clear solution. The filtrate was stored at 4°C for future works.

### Synthesis of CuNPs

25 ml solution of leaf extract was introduced drop wise into 100 ml of 1mM (0.001mM) solution of copper sulphate under continuous stirring[26]. After the complete addition of leaf extract, the mixture was kept for incubation for 24h.Within a particular time,the green colour solution was changed into straw yellow, which indicates the formation of copper nanoparticles. Then the solution was centrifuged for 15 min at 10,000 rpmand dispersed in double distilled water to remove any unwanted biological materials [27].

### Characterization

The formation of copper nanoparticles was confirmed by UV- Visible spectroscopy using Jasco V-550 spectrophotometer instrument. Size of the CuNPs was analysed with UV-Spectrometerin the range between 300-700nm. To determine the biomolecules present in the leaf extract, FTIR analysis was carried out which is responsible for the reduction of Copper ions with the spectral range of 400-4000 $cm^{-1}$. Here the sample was centrifuged at 9500 rpm for 20 min, dried using hot air oven and ground with KBr to form a pellet. Then the

pellet was analyzed using Jusco 5300 model FTIR instrument. The crystalline structure of the copper nanoparticles were determined by X-Ray diffraction analysis using Rigaku X-Ray diffractometer (Miniflex, UK) instrument operating at 40 kV with 2sec time interval at room temperature 27ºC. Morphology and mean particle size of the Cu were determined by SEM and TEM analysis. The samples were prepared for SEM and TEM analysis. The SEM analysis was established by using Supra Zeiss with 1nm resolution at 30 kV with 20 mm Oxford EDS detector. The elemental composition in the reaction mixture was determined by EDX analysis. The TEM analysis was carried out using HITACHI H-7650 at an operating voltage of 80 kV.

### Antimicrobial activity

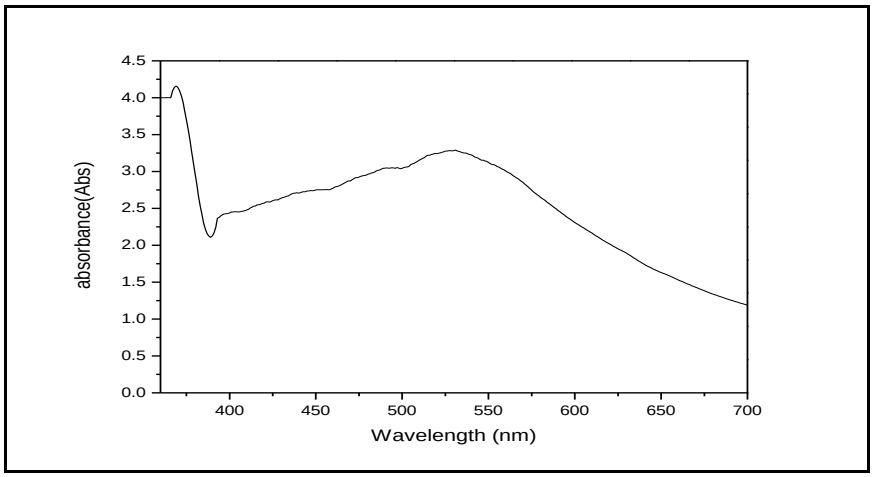
The antimicrobial activity of pathogens was established using disk diffusion method[28]. The bactericidal effect of copper nanoparticles has been attributed to their high surface to volume ratio and small size which allows them to interact very closely with microbial membranes.

The antimicrobial study of CuNPs was carried out using different pathogenic bacteria such as *E-coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. To cultivate the bacteria, nutrient agar was used.After solidification of medium, the disks were placed on the solidified medium. The prepared copper nanoparticles were added into the disks with different concentrations varying from 25 µl to 100 µl. Petri dishes were incubated for 24 h at 37°C.Antibacterial capacity of the copper nanoparticles was measured by standard Zone of Inhibition assay[29].

## Results and Discussion

### UV-Vis spectroscopy analysis

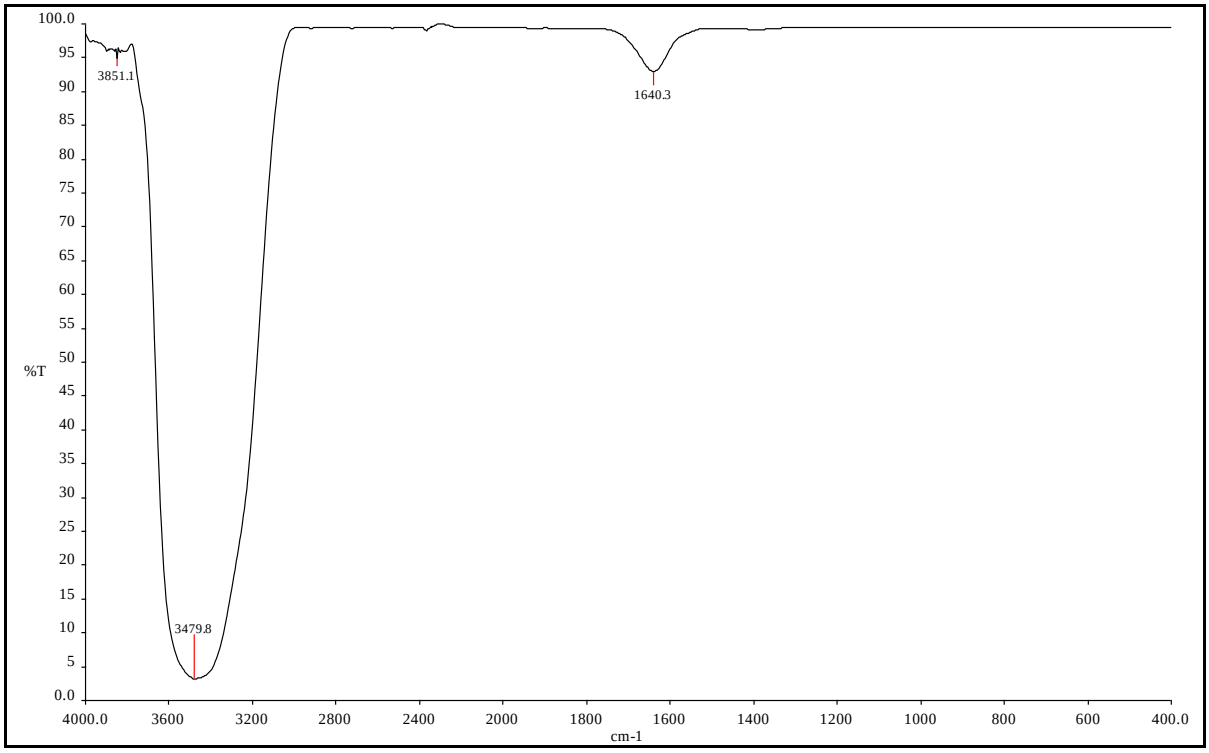
The result obtained from UV-Visible spectroscopy analysis of the sample is presented in Fig 1. It is the most important method of analysis to detect the Surface Plasmon Resonance property of CuNPs[30]. The CuNPs formation was confirmed from the peak at 531 nm, this result similar with Curtis et al results with the UV range 560- 640 nm and the UV range agreement with current result. The peak value was found to be gradually decreased with increase in particle size. Copper SPR effects decrease with the time because of the oxidation of the synthesized copper nanoparticles[39].




### FTIR analysis

The FTIR measurement of plant extract and copper nanoparticles are shown in Fig 2(a) and2(b), respectively. The FTIR analysis was used to identify the capping, reducing and stabilizing capacity of the leaf extract. In a Fig.2(a), aqueous leaf extract showed the peaks at 3851, 3479 and 1640 $cm^{-1}$. The peak at 1640 $cm^{-1}$ was due to presence of C=O stretching[31]. The peak at 3479 $cm^{-1}$ showed O-H stretching of phenolic compound[32]. The O-H stretching of hydroxyl groups were obtained from the peak at 3851 $cm^{-1}$,[33]. In Fig. 2(b) for copper nanoparticles, peak values at 3901, 3840, 3852, 3460 and 1636 $cm^{-1}$was observed. Peak at 1636, 3460 $cm^{-1}$ corresponds to C=O stretching of amides and O-H stretching of phenolic compound, respectively. The other peaks obtained in copper nanoparticle sample are 3852, 3840, 3901 $cm^{-1}$due to O-H Stretching of hydrogen bonded alcohols and phenols [1]. The FTIR analysis of CuNPs suggested that they might surround by the any of these organic molecules such as polyphenols, alkaloids and terpenoids, Kalainila et al., already reported that the same type of results[38]. The chemical constituents present in plant leaves extract such as Flavonoids, alkaloids and fatty acids

are responsible for the reduction of copper ions to copper nanoparticles due to their capping and reducing capacity.




**(b)**

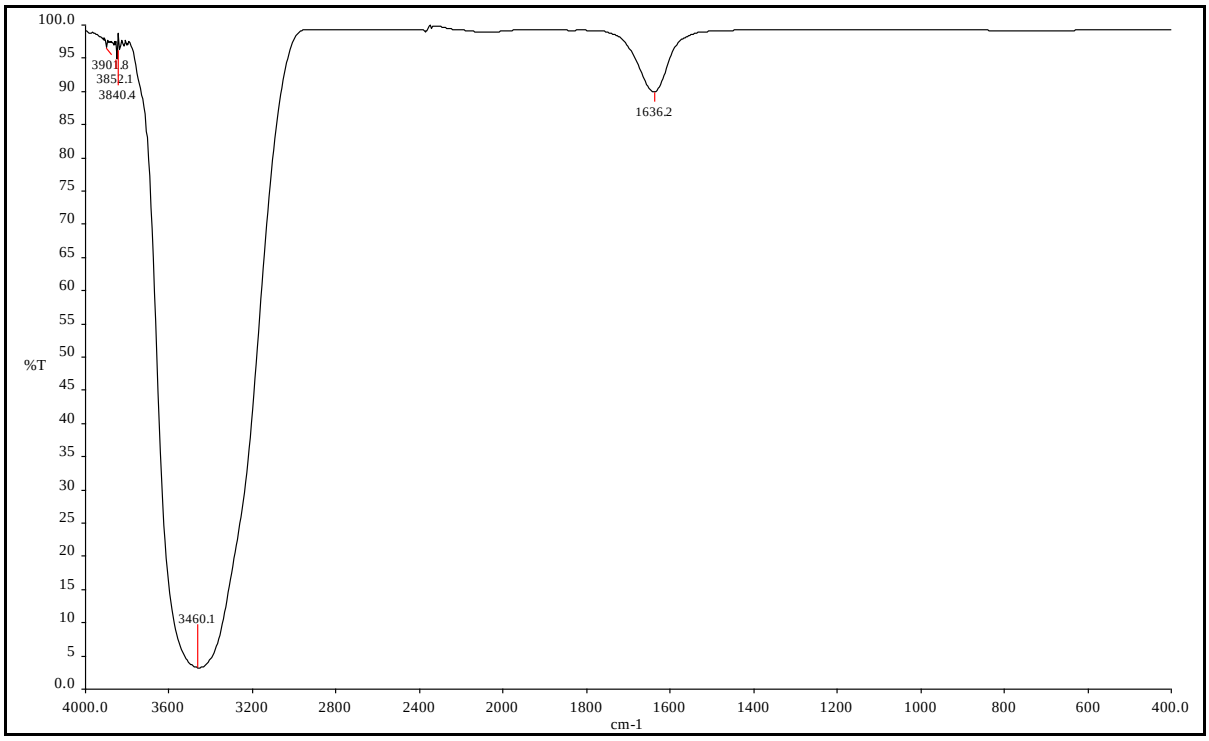



**Figure 2: FTIR analysis of plant extract (a), Copper nanoparticles (b)**

## XRD analysis

Using XRD spectrum analysis, the two different diffraction peaks at 39.1°and 68.3° are shown in Fig. 3. These diffraction peaks referred to the characteristics of cubic centred CuNPS. Diffraction peaks obtained at 2θ angle corresponds to (111) and (113) miller index[26]. To determine the average particle size of the CuNPs, the Debye-Scherrer equation is used.

$D = K \lambda / \beta \cos\theta$

Where, D is the crystalline size of NPs, (FWHM) K is the Scherer constant with a value from 0.9 to 1.λ is the wavelength of the X-ray source (0.1541 nm) used in XRD, β is the full width at half maximum of the diffraction peak and θ is the Bragg's angle. According to Debye Scherrer equation the average particle size was found to be 5 nm[23].

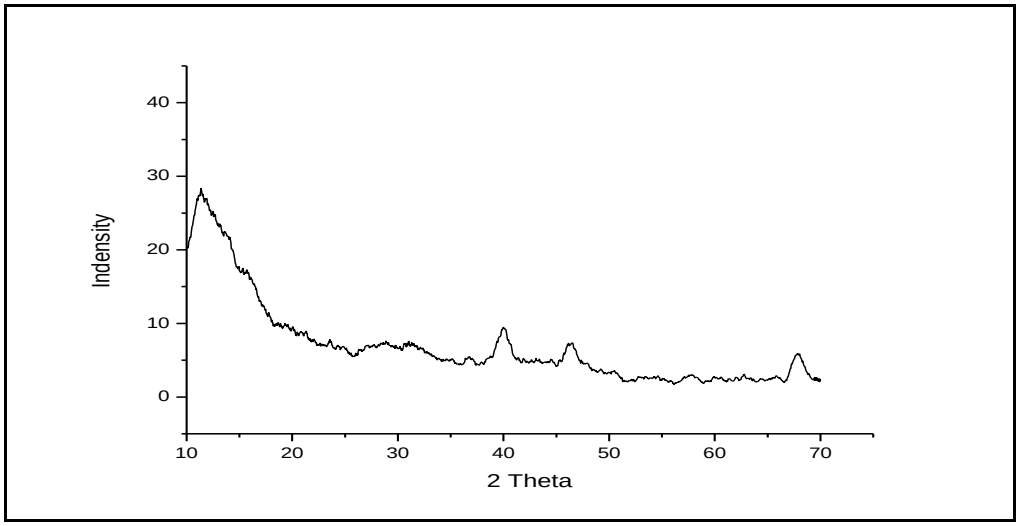
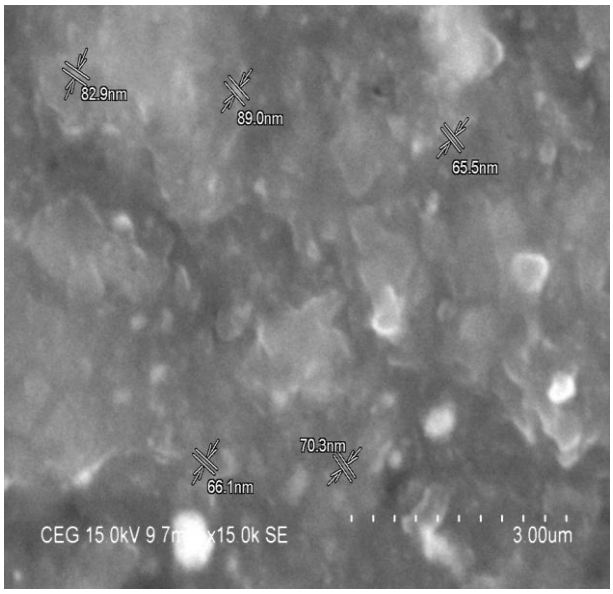



**Figure 3: XRD analysis of copper nanoparticles**

**SEM analysis**

The surface morphology and size of the nanoparticles were obtained by Scanning Electron Microscopy (SEM) analysis. The Fig. 4(a)shows the CuNPs synthesized by the plant extract of *Capparis zeylanica*. The electrostatic interactions and hydrogen bond between the bio-organic capping molecules bond are responsible for the synthesis of copper nanoparticles using plant extract. It was shown that spherical and relatively uniform shape of the copper nanoparticles was confirmed in the range of 60-100nm. The quantitative and qualitative analysis of elements may be concerned in the formation of copper nanoparticles. They were identified by EDAX analysis (Fig.4(b).Due to the Surface Plasmon Resonance, the copper nanoparticle shows the absorption peaks of higher counts.[34].

(a)



(b)

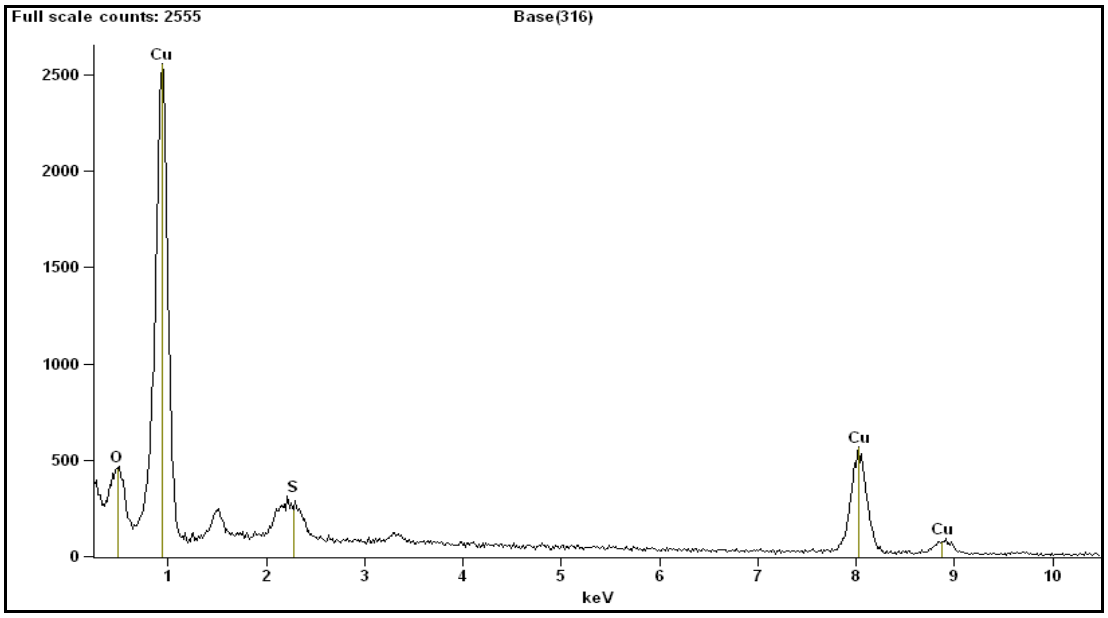



**Figure 4: SEM micrograph of CuNPs (a), EDX analysis of CuNPs (b)**

**TEM analysis**

The shape and size of the synthesized CuNPs were analysed by TEM analysis [35].Fig. 5 shows the TEM image of biosynthesized CuNPs. The synthesized CuNPs were cubical in shape with particle size in nano range. The green synthesized copper nanoparticles size is highly depending on the concentration of *Capparis zeylanica* plant leaves extract. It was confirmed that, the concentration of leaf extract was found to be increased with decrease particles size[36].

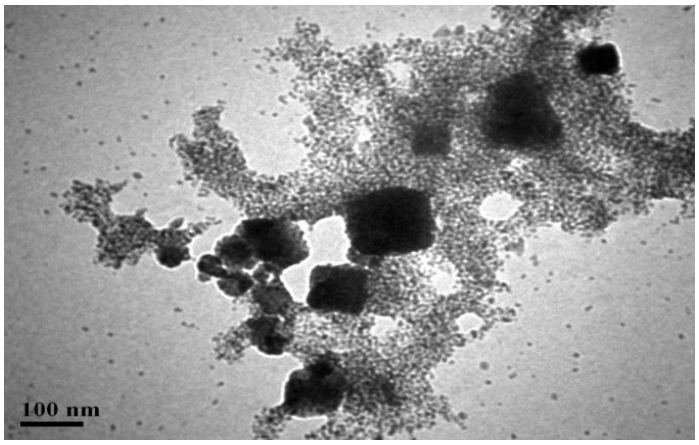



**Figure 5: TEM analysis of CuNPs**

**Antimicrobial study on CuNPs**

The antimicrobial study of green synthesized CuNPs were established against both gram negative and gram positive pathogenic bacteria such as *Staphylococcus aureus* (gram positive),*E-coli* and *Pseudomonas aeruginosa* (gram negative) using disk diffusion method as shown in Table. 1. Fig. 6 shows the zone of inhibition (ZOI) for different pathogen of copper nanoparticles. This result was effective when the concentration of copper nanoparticles was observed to be increased with increase in the zone of inhibition. However the zone of inhibition was observed to be more in gram negative bacteria when compared to gram positive bacteria. This is mainly due to the differences in bacterial pathogen's membrane structures [31]. The maximum ZOI values were observed as 11mm in *E-coli* bacteria for 100µl concentration of CuNPsas shown in Table 1. The ZOI values observed by *Pseudomonas aeruginosa* and *Staphylococcus aureus* was found to be 10mm asshown in Table 1[37].

**Table 1: Zone of inhibition of CuNPs (mm)**

| **Species** | **10 µl** | **25 µl** | **50 µl** | **100 µl** |
|---|---|---|---|---|
| *E-coli* | 6±1mm | 7±1mm | 9±1mm | 11±1mm |
| *Pseudomonas aeruginosa* | 4±1mm | 6±1mm | 7±1mm | 10±1mm |
| *Staphylococcus aureus* | 2±1mm | 4±1mm | 5±1mm | 10±1mm |

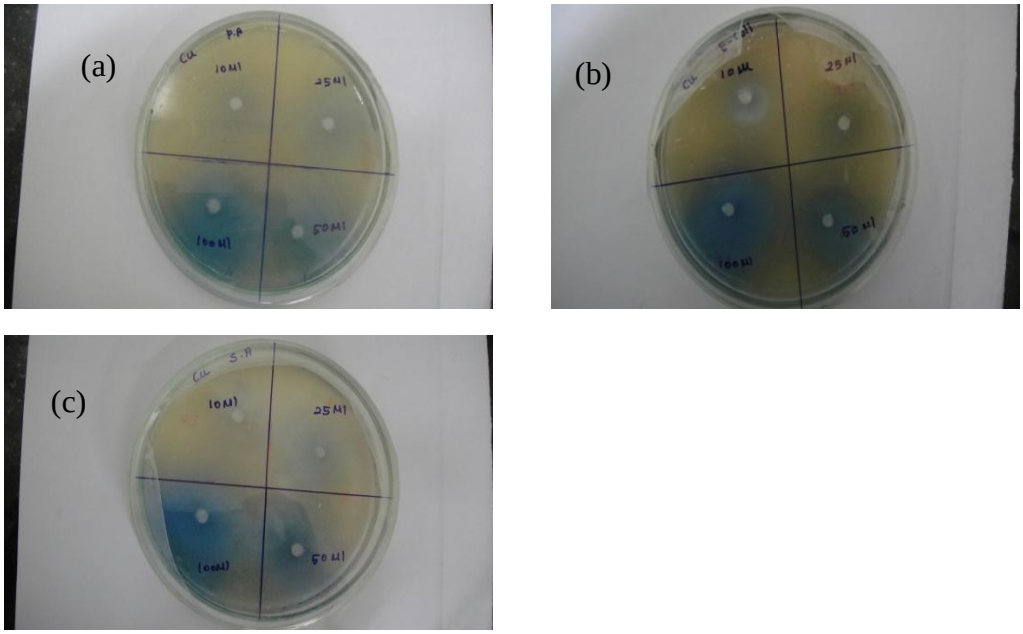



**Fig. 6 Antimicrobial activity of CuNPs with*E-coli* (a), *Pseudomonas aeruginosa* (b)and *Staphylococcus aureus*** (c)

## Conclusion

The copper nanoparticles were successfully synthesized by using novel *Capparis zeylanica* plant leaves as first time for the anti bacterial study, which provides cost effective, easy and proficient way for synthesis of CuNPs.The functional group present in the leaf extract was confirmed by FTIR analysis. These functional groups were mainly responsible for the reduction of copper metal ions into CuNPs. The synthesized copper nanoparticles were analyzed using UV-spectrophotometer, FITR, SEM with EDAX, TEM and XRD. Copper nanoparticles were effectively utilized for the antibacterial activity study. The maximum ZOI was found to be more in gram negative bacteria when compared to gram positive bacteria. The *Capparis zeylanica* plant may be effectively utilized for the production of CuNps with economically for many pharmaceutical applications.

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