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## Bio-synthesis of copper nanoparticles (CuNPs) using garlic extract to investigate antibacterial activity

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### ABSTRACT

Bio-synthesis of metal nanoparticles is regarded as one of the recently developed environmentally benign method. In the present investigation, Copper nanoparticles were synthesized reacting garlic (*Allium sativum*) extract with Copper Sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) solution over magnetic stirrer at 80 °C for 1 hour. So-prepared CuNPs were studied by observing the color change at various time intervals. Further, the nanoparticles were characterized using UV-Visible spectroscopy, Energy Dispersive X-ray spectroscopy (EDX) and Fourier Transform Infrared spectroscopy (FTIR). The results of UV-Vis spectroscopy clearly showed presence of absorption peak at 595 nm which confirmed the formation of copper nanoparticles. Likewise, the EDX spectrum depicts the presence of optical band at 8 eV which is the characteristic peak of Copper consisting of 38.747 % by weight and FTIR spectra revealed presence of various phytochemicals possessing characteristic functional groups such as carbonyl and phenolic at the surface of CuNPs. Thus, natural products available in the garlic extract help in reduction and stabilization of Copper nanoparticles. The antibacterial activity of Copper nanoparticles was investigated against Gram +ve (*Staphylococcus aureus*) and Gram -ve bacteria (*Escherichia coli*) using Agarwell diffusion method. The results of antibacterial test showed that CuNPs were found to be much sensitive towards Gram -ve bacteria compared to gram +ve bacteria.

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## 1. Introduction

In the current era, most of the research activities belong to nanotechnology as it can be applied in innumerable fields such as textile, pharmaceuticals, electronics, food industries, biomedical science, drug and gene delivery, engineering, environmental science, automobiles, mechanics

and space industries. Nanotechnology is mainly concerned with synthesis of nanoparticles of various sizes, shapes, chemical compositions and controlled disparity and their potential use for human benefits [1-4]. Nanomaterials depict the class of materials with at least one of their dimensions in the range of 1-100 nm i.e

nanoscopic range. These nanomaterials are significant for possessing improved properties different from bulk materials simply by virtue of their small size, larger surface to volume ratio, distribution and morphology. Nanomaterials are of varied types such as metallic, insulating, semiconducting, and hybrid type [5-7]. Among the various kinds of nanomaterials, bio-synthesized metallic nanoparticles is increasing commercial demand for their unique properties such as physical, catalytic, chemical, electrical, electronic and antibacterial properties. Because of these various properties, metallic nanoparticles have been used in many applications such as catalysis, photonics, electronics, medicine, electric and many more [8-10].

Copper nanoparticles have been found to be extensively studied by many researchers for their excellent electrical, optical, catalytic, biomedical, antifungal, and antibacterial properties. As a consequence of possessing excellent electrical property, CuNPs play major role for making future low cost nanodevices [11]. At the same time, inclusion of CuNPs at different matrixes could be potential materials for synthesizing thin film optical devices. Their efficient toxic property is considered to be an effective anti-bactericidal agent. Likewise, they have been found employed in drug delivery, textiles, electronics, biosensing, food industry, cosmetics, medical devices, catalytic process, high temperature superconductors and solar cells etc. [9-12]. Consequently, CuNPs act as low cost effective and good alternative material for noble metals such as Au, Ag, Pt, Pd [12].

In nanotechnology, various methods are known to prepare nanoparticles with controlled size and shape. These include metal vapor deposition, radiolytic reduction, thermal decomposition, thermal reduction [4, 5], electrochemical reduction [7], mechano-chemical process [8], chemical reduction etc. [7- 10]. Chemical reduction method is one of the most convenient methods for the synthesis of metallic nanoparticles as one can simply synthesize shape

and size-controlled nanoparticles by this process [12-16]. While talking about synthesis of CuNPs, the process is quite challenging due to its high tendency for oxidation. It is extremely sensitive to air, and the oxide phases are thermodynamically more stable. The high oxidation state of copper nanoparticle may limit their applications. To avoid oxidation, these methods were usually performed in non-aqueous media, at low precursor concentration, and under an inert atmosphere (argon, nitrogen) [6-9]. The nanoparticles are also found encapsulated using different capping agent to prevent it from oxidation during synthesis.

Garlic (*Allium sativum*), perennial plant of the family Alliaceae, belongs to the order of plant liliales, grown for its flavourful bulbs. The plant is native to central Asia and is a classic ingredient in many national cuisines. Chiefly, garlic possesses antibacterial, antifungal, antiparasitic, antiviral, antioxidant, anti-cancerous and vasodilators characteristics which motivate to carry research over this vegetable. The medicinal values of garlic has been attributed due to presence of many significant phytochemicals such as allicin, allinin, ajoene, diallylsulfide, enzymes, B-vitamins, proteins, minerals, saponins, and flavonoids etc. [17-19].

Many of the research have been focused on exploring the bactericidal effect of CuNPs as many of bacteria have been found resistant towards popular antibiotics. Hence, aim of the current investigation is to synthesize Copper nanoparticles using easily available garlic extract and its characterization. In addition to this, it will also explore the anti- bacterial property of synthesized nanoparticles.

## 2. Experimental

### Chemicals

Analytical grade Copper (II) Sulphate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ), hydrazine hydrate, nutrient agar media, Dimethyl Sulphoxide (DMSO) were used. Bulbs of garlic were purchased from local market.

### **Test organisms**

Antibacterial activity of the as-synthesized copper nanoparticles was investigated against Gram +ve bacteria (*Staphylococcus aureus*), and Gram -ve bacteria (*Escherichia coli*).

### **Biosynthesis of copper nanoparticles**

10 mL of freshly prepared garlic extract was treated with 80 mL of 0.01M Copper sulphate solution and 10mL of hydrazine hydrate [2]. Then after, the resulting solution was incubated for 24 hours at room temperature and centrifuged for 20 minutes at 3000 rpm. Centrifugation-decantation-washing processes were repeatedly done three times to remove impurities. The obtained precipitate was dried in an oven at 50 °C for 5 hours. So prepared, CuNPs were subjected to various characterizations techniques.

### **Characterization techniques**

#### **Visual observation**

Bio-reduction of the Copper Sulphate using aqueous garlic extract was monitored by observing the gradual color change at different time intervals.

#### **UV-visible spectroscopic studies**

UV-Visible spectrum of the solution containing CuNPs was carried by using double beam UV-Visible spectrophotometer (LABROTONIC, Model LT-2802) in wavelength range of 300-700 nm, at resolution of 1 nm. The measurements were recorded at every 15 minutes of time interval.

#### **Energy dispersive X-ray (EDX) spectroscopic studies**

Energy Dispersive X-ray (EDX) spectroscopy (EDX) is used for quantitative elemental analysis which was carried out by using EDX-8000 instrument. For EDX analysis, about 3 mL of the solution containing CuNPs was used to observe the energy absorption pattern of different elements.

#### **Fourier transforms infrared (FTIR)**

#### **spectroscopic studies**

Fourier transform infrared (FTIR) spectroscopy is well known method for determining different types of bonding associated with characteristic functional groups present in the sample. FTIR measurements were done in Attenuated Total Reflectance (ATR) mode in the wavenumber range 4000 to 750  $\text{cm}^{-1}$  at the resolution of 8  $\text{cm}^{-1}$ .

#### **Antibacterial activity**

Antibacterial assay was performed by agar well diffusion method using Muller Hilton Agar (MHA) in the petri dish [20]. Three MHA agar plates were loaded with corresponding sterile bacteria, garlic extracts, positive control (Ofloxacin 50  $\mu\text{g}/\text{mL}$ ), and negative control (DMSO) at their respective well. It was allowed to diffuse for about 30 minutes at room temperature and incubated for 24 hours. After incubation the clear zone formed around the well were measured.

### **3. Results and discussion**

#### **Visual observation**

Preliminarily, the formation of CuNPs was studied by visual observation of the reacting solutions at different intervals of time. Initially, the color of the precursor solution and garlic extract was dark green (Fig. 1a). The color of the reaction solution gradually started to change to light green (Fig. 1b), reddish brown (Fig. 1c and 1d) with increase in stirring time. Finally, the deposition of the shiny reddish-brown precipitate was observed on the inner wall of the vessel after 1 hour stirring indicating the formation of Copper nanoparticles. The change in coloration attributed to the bio reduction of  $\text{Cu}^{2+}$  ions to nanoparticles (i.e  $\text{Cu}^0$ , CuNPs) [5, 10]. Hence, visual observation provides the preliminary information for the formation of nanoparticles. Variations of color observed at different time intervals are shown in Fig. 1.

#### **UV-Visible absorption spectroscopic studies**

UV-Visible absorption spectroscopy is important tool for characterizing metal nanoparticle as the maximum absorbance corresponds to the particle

size [17]. The result of UV-Visible spectroscopy of the as synthesized nanoparticle is presented in the Fig. 2. It shows a broad absorption peak of at around 595 nm which is due to the Surface Plasmon Resonance (SPR) of electrons present at the surface of metal nanoparticles. Hence, this result signifies the formation of CuNPs which is supported by the result presented by Eman et al and Suresh et al. [20, 21].

Similarly, the wavelength of maximum absorption peaks of samples recorded at every 15 minutes of time intervals are presented in the table 1. It depicts that the wavelength of maximum absorbance ( $\lambda_{max}$ ) of reacting solutions shift towards higher wavelength with increase of time. The absorption peaks of the reacting solutions at 30, 45 and 60 minutes were observed at 495 nm, 545 nm and 595 nm, respectively. The maximum absorption peak of Copper nanoparticles appeared at 595 nm (Fig. 2) after 60 minutes indicates the formation of separated Copper nanoparticles which is quite similar to the maximum absorption peak at 560-570 nm as reported by Suresh et al. and Suramwar et al. [21, 22]. It clearly revealed that formation of nanoparticles take place after stirring the solution for 60 minutes while the absorption peaks at 495 and 545 nm attributes for the agglomerated particles.

#### **Energy dispersive X-ray (EDX) spectroscopic study**

Elemental analysis of the sample was determined from EDX spectrum which is presented in Fig. 3. The EDX pattern shows the existence of Copper along with some other elements like Silicon, Sulphur, Phosphorous, Oxygen etc. as impurities. The EDX peak position is consistent with Copper hence it confirmed the formation of Copper nanoparticles as reported in the literature [7]. Further, the EDX signal at 8 eV indicates presence of Copper of about 24.6% by weight which may be due to presence of other impurities.

#### **Fourier transform infrared spectroscopic studies**

The FTIR spectrum of biosynthesized CuNPs is presented in Fig. 4. which shows the existence of various biomolecules at around the CuNPs. Broad bands at around 3200-3600  $cm^{-1}$ , 1600-1800  $cm^{-1}$  and 2400  $cm^{-1}$  attribute for the presence of (-OH) stretching of phenolic group, ( $>C=O$ ) and ( $>N-H$ ) stretching.

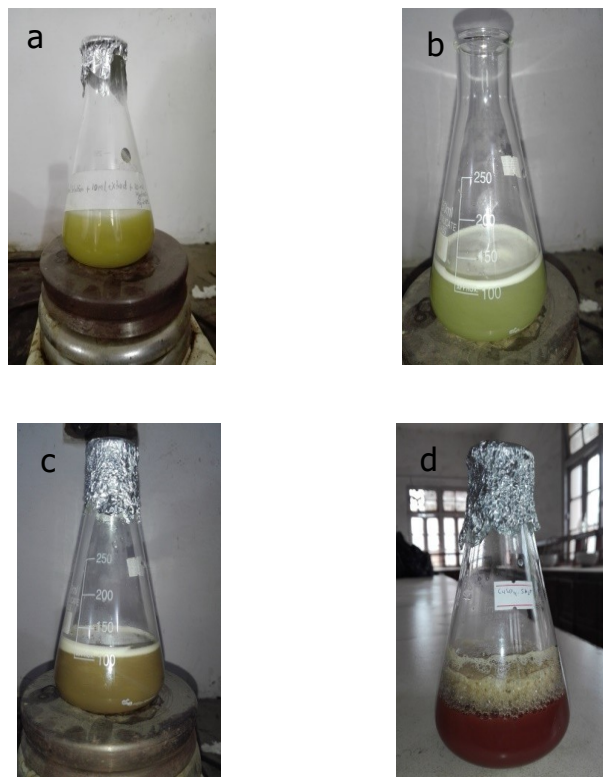
Hence, FTIR spectra of copper nanoparticles confirmed the presence of various biomolecules such as saponins, tannins, flavonoids, alkaloids and polyphenols etc. surrounding Copper nanoparticles which are responsible for reduction as well as in the stabilization of nanoparticles [8, 23].

#### **Antibacterial activity of copper nanoparticles**

Result of antibacterial test of as synthesized Copper nanoparticles against Gram negative bacteria (*Escherichia coli*), and Gram positive bacteria (*Staphylococcus aureus*) are shown in Table 2. The result showed that the bactericidal effect of Copper nanoparticles was found to be satisfactory. The zone of inhibition (ZOI) for Gram negative bacteria was observed to be more than that of Gram positive bacteria. The ZOI of Gram negative bacteria (*Escherichia coli*) and that of Gram positive bacteria are found to be 20 mm, and 13 mm, respectively. The difference in activity against these two types of bacteria is attributed to the difference of structure of cell membrane. Gram positive bacteria have thicker peptidoglycan cell membranes compared to the Gram negative bacteria, hence, it is harder for CuNPs to penetrate it resulting lower ZOI [18]. These recorded (ZOI) values of corresponding bacteria seemed to be lower than that of standard antibiotic Ofloxacin, (positive control) may be due to weak solution of Copper nanoparticle.

Further, the photographs of antibacterial test are presented in Fig. 5. which clearly depict differences in the zone of inhibitions for two different types of bacteria as reported by Padil et al. and Kartik et al. [24, 25].

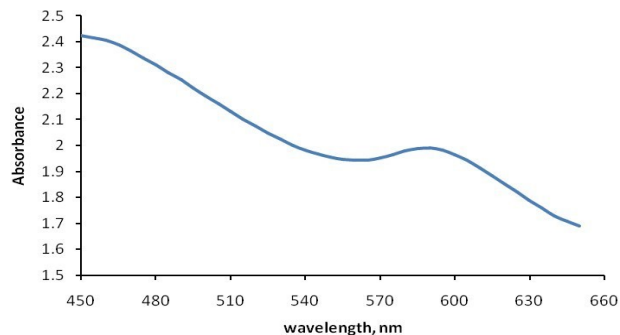
Figure 5: Photographs showing zone of inhibition formed by bio-synthesized Copper nanoparticles using garlic extract against: a) Gram +ve, bacteria, *Staphylococcus aureus* and b) Gram -ve, bacteria, *Escherichia coli*.



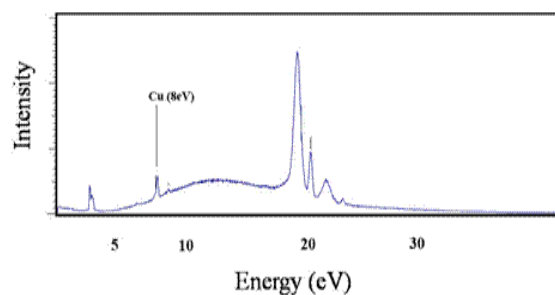
**Fig. 1:** Variation of colour of the garlic extract and CuSO<sub>4</sub> solution at different time intervals a) after 15 minutes b) 30 minutes c) 45 minutes and d) 60 minutes

**Table 1: Color observed and maximum absorption peak of bio-synthesized CuNPs at different time intervals.**

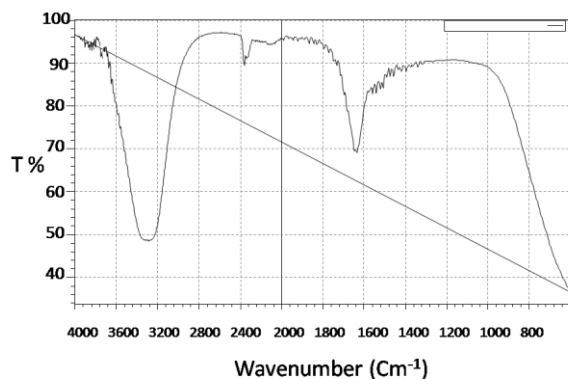
Color	Time	$\lambda_{max}$ (nm)
Dark green	Immediately after mixing	
Light green	30 min	495
Greenish brown	45 min	545
Reddish brown	60 min	595



**Fig. 2:** UV-Visible spectrum of bio-synthesized copper nanoparticles using Copper sulphate and garlic extract in presence of hydrazine hydrate (HH).



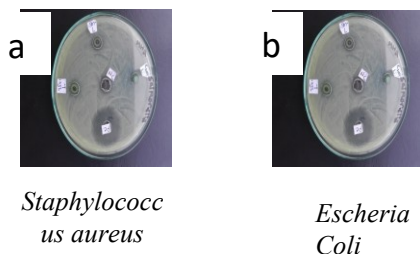
**Fig. 3:** Energy Dispersive X-ray (EDX) Spectrum of as synthesized Cu NPs using garlic extract (*Allium sativum*) and Copper sulphate solution.



**Fig. 4:** Fourier Transform Infrared Spectrum of as synthesized Cu NPs using garlic extract.

**Table 2: Measured inhibition zone of bio-synthesized copper nanoparticles using *Allium sativum* and positive control, of loxacin.**

Test Organism	Zone of inhibition	
	CuNPs	Ofloxacin
Staphylococcus aureus	13 mm	36 mm
Escherichia coli	20 mm	38 mm

**Fig. 5:** Photographs showing zone of inhibition formed by bio-synthesized Copper nanoparticles using garlic extract against: a) Gram +ve, bacteria, *Staphylococcus aureus* and b) Gram –ve, bacteria, *Escheria coli*

#### 4. Conclusion

Bio-synthesis of copper nanoparticles using *Allium sativum* (garlic) extract was successfully carried out at room temperature. During the synthesis, biomolecules present in the garlic extract are mainly responsible for the reduction and stabilization of the nanoparticles. Optical properties, compositional analysis and functional group content of as-synthesized copper nanoparticles were studied using UV-Vis spectrophotometer, Energy Dispersive X-ray spectroscopy (EDX) and Fourier Transform Infrared Spectroscopy (FTIR) respectively. The absorption spectrum of CuNPs prepared with 15 mins of stirring showed maximum peak at 495 nm. This was found to be shifted towards higher wavelength for increase in stirring time indicates the formation of CuNPs. FTIR analysis illustrated the presence of chief functional groups; phenol, carbonyl and amide groups at the surface of CuNPs which are responsible for reducing and stabilizing the nanoparticules. Similarly, EDX result depicts the presence of

Copper of 24 wt % at 8 eV along with traces of Si, S, P etc. as impurities. As-synthesized CuNPs are quite stable and have antibacterial action towards both types of bacteria though its power of killing the different bacteria is slightly different. Hence, we conclude that bio-synthesized CuNPs could be used as alternative antibiotics.

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