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# Biosynthesis of Copper Nanoparticles using *Vitis vinifera* Leaf Extract and Its Antimicrobial Activity

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## ABSTRACT

*Present paper describes a rapid and eco friendly, non toxic and cost effective approach for green synthesis of Copper nanoparticle (CuNPs) using Vitis vinifera (Black and Green Grapes) leaf extract. The synthesized Copper nanoparticles were characterized by UV Visible Absorption Spectrophotometer, X-Ray Diffraction (XRD) and Fourier Transform Infrared (FTIR). The X-Ray diffraction analysis showed that the Copper nanoparticles are crystalline in nature with average 3 – 6 nm in size. The synthesized Copper nanoparticles showed effective activity against Escherichia coli and Bacillus subtilis.*

**Key words**: *Vitis Vinifera* Leaf, Copper nanoparticles, XRD, FTIR, UV Visible Spectrophotometer and antibacterial activity.

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## INTRODUCTION

Nanotechnology is emerging as cutting edge technology interdisciplinary with physics, chemistry, biology, material science and medicine. Nanoparticles have found wide range of applications in area such as catalysis, anti microbial, optics and biomaterial production. [1, 2]

Synthesizing Copper nanoparticles has also attracted considerable attention due to their wide applications and properties. Many works have been done on synthesizing Copper nanoparticles by thermal decomposition, electrochemical reduction, radiolytic reduction etc. [3, 4]. Most of these methods require high energy, hazardous chemicals and wasteful purifications which may create environmental and biological risk. To overcome these problems plant extract have been used to synthesis nanoparticles which have many advantages over chemical, physical and microbial synthesis. [5 – 9]

In this study, we have biosynthesized Copper nanoparticles (CuNPs) using *Vitis Vinefera* (Black and Green grapes) leaf extract. By X-Ray diffraction (XRD) it was shown that biosynthesized Copper nanoparticles are found to be highly effective against *Escherichia Coli* and *Bacillus subtilus*.

## MATERIALS AND METHODS

Leaves from *Vitis vinifera* (Black Grapes as well as Green Grapes) were collected from the local area of Ahmednagar, Maharashtra, India. (Fig 1). The leaves were washed with distilled water to remove the dust particles and then were dried for six days to remove the moisture. The dried leaves were grind into fine powder. 5 gm of powdered leaves sample was boiled with 50 ml Sterile distilled water for 5 minutes at 40 ˚C. The aqueous extract was separated by filtration using Whatmann No. 1 filter paper. Both the extract, one from Black grape leaf sample and one Green grape leaf sample were stored at 4 ˚C to be used for biosynthesis of Copper nanoparticles.

**Black Grape** **Green Grape**

**Fig 1: *Vitis vinifera***

**SYNTHESIS OF CuNPs**

10 ml of *Vitis vinifera* leaf extract was added to 100 ml of 1 % Copper acetate solution with magnetically stirring for 3 hours. After 2 hours, the colour of the solution changed from green to blue indicating formation of Copper nanoparticles. (Fig 2)

**Before** **After two hrs**

**Fig 2: Solution showing formation of Copper nanoparticles**

The solution was aged for 12 hours. The nanoparticles formed were washed with double distilled water. Then, it was dried in an oven at 60 ˚C for further analysis.

**CHARACTERISATION TECHNIQUES**

The biosynthesized Copper nanoparticles were analyzed for absorption in UV-Visible region using UV-Visible Spetrophotometer in the range of 200 – 800 nm. The crystalline metallic CuNPs were examined using X-Ray diffractometer. Fourier transform infrared (FT IR) Spectra was obtained in the range 800 – 400 $cm^{-1}$.

**ANTIBACTERIAL ACTIVITY**

Antimicrobial activity of biosynthesized CuNPs was tested against two pathogenic bacteria, viz. *Escherichia Coli* and *Bacillus subtilus*.

## RESULTS AND DISCUSSION

**UV – Visible absorbance studies**

The absorbance peak for biosynthesized CuNPs from both the extracts was at 337 nm indicating the formation of CuNPs from Copper acetate. Fig 3 & 4

**Fig 3: UV Absorption Spectra for CuNPs biosynthesized from Black Grape leaves extract**

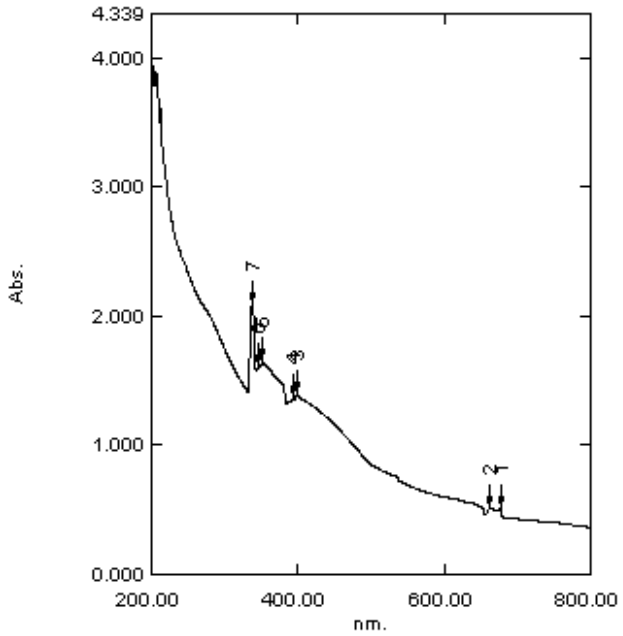
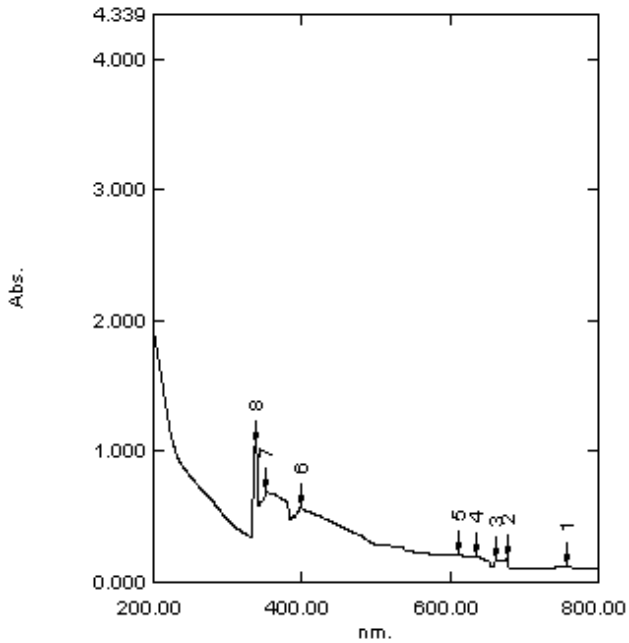



**Fig 4: UV Absorption Spectra for CuNPs biosynthesized from Green Grape leaves extract**




**FTIR Spectrum**

Results of the FTIR study of biosynthesized CuNPs from both the extract showed absorption peaks as shown in Fig 5 and Fig 6.

**Fig 5: FTIR Spectra for CuNPs biosynthesized from Black Grape leaves extract**

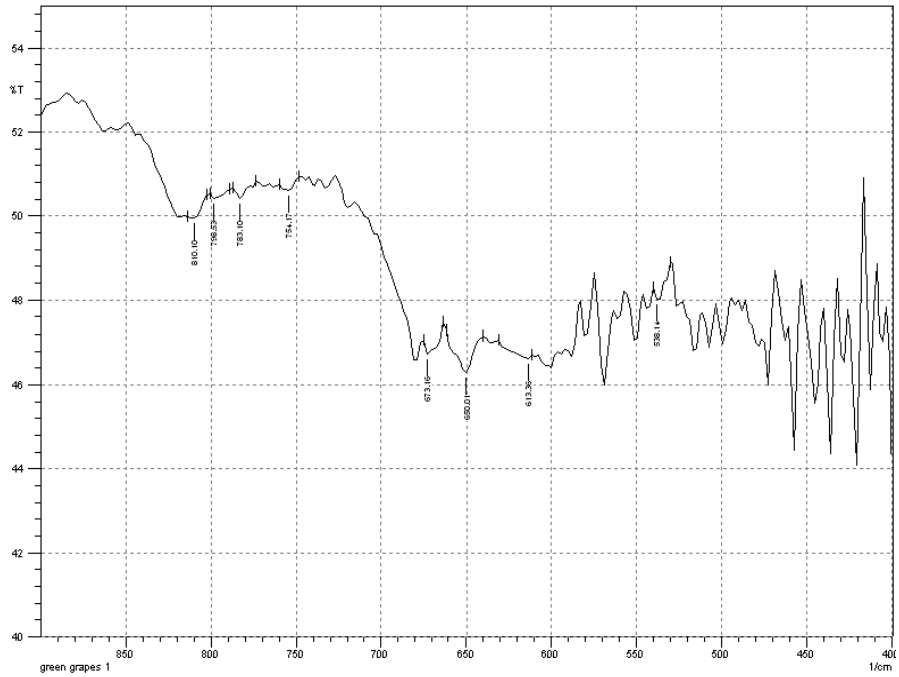
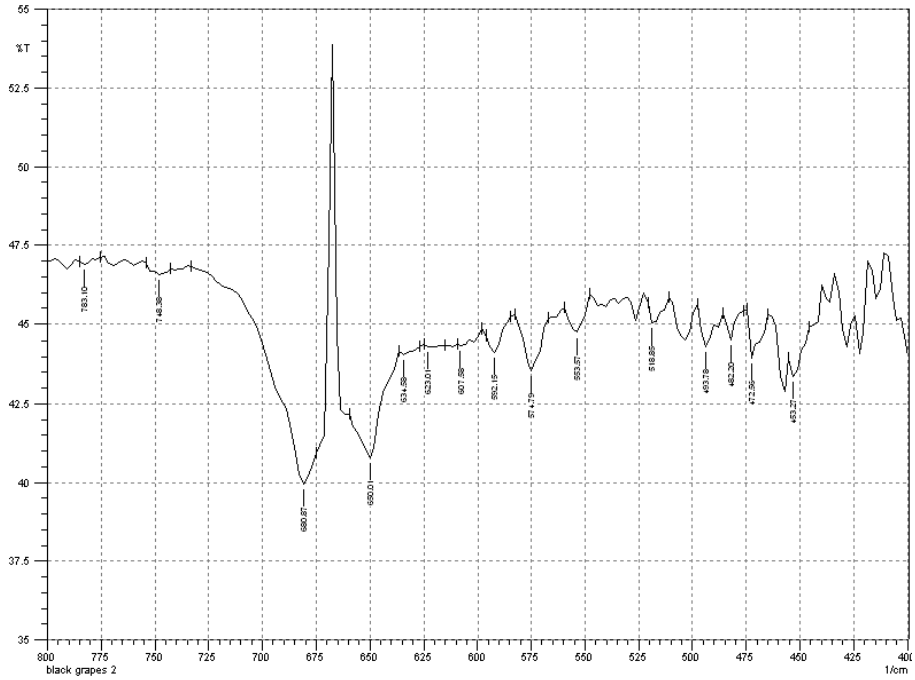



**Fig 6: FTIR Spectra for CuNPs biosynthesized from Green Grape leaves extract**




The possibility of functional group according to absorption peak is discussed in Table below.

| Observation No. | Frequencies ($cm^{-1}$) | Functional Group |
|---|---|---|
| 1 | 3228.84, 3327.21, 3458.37, 3520.09 | -O-H bonded, intramolecular hydrogen bond |
| 2 | 2870.08,2891.3,2910.58, 2993.52 | -C-H saturated alkane (stretch) |
| 3 | 2731.20,2843.07, | Chelated hydrogen bond |
| 4 | 2353.16 | Due to absorption atmospheric $CO_2$ |
| 5 | 1360.1, 1427.32, 1465.04 | $-CH_3$ bend, $-CH_2-$ bend |
| 6 | 1103.28, 1219.01, 1354.03 | -C-N (amines) |
| 7 | Below 600 (430.13,476.42,547.78, 594.08) | Metal ligand bond |
| 8 | 501.49,530.42,555.5,588.29 | -C-X bond (X=bromide) |

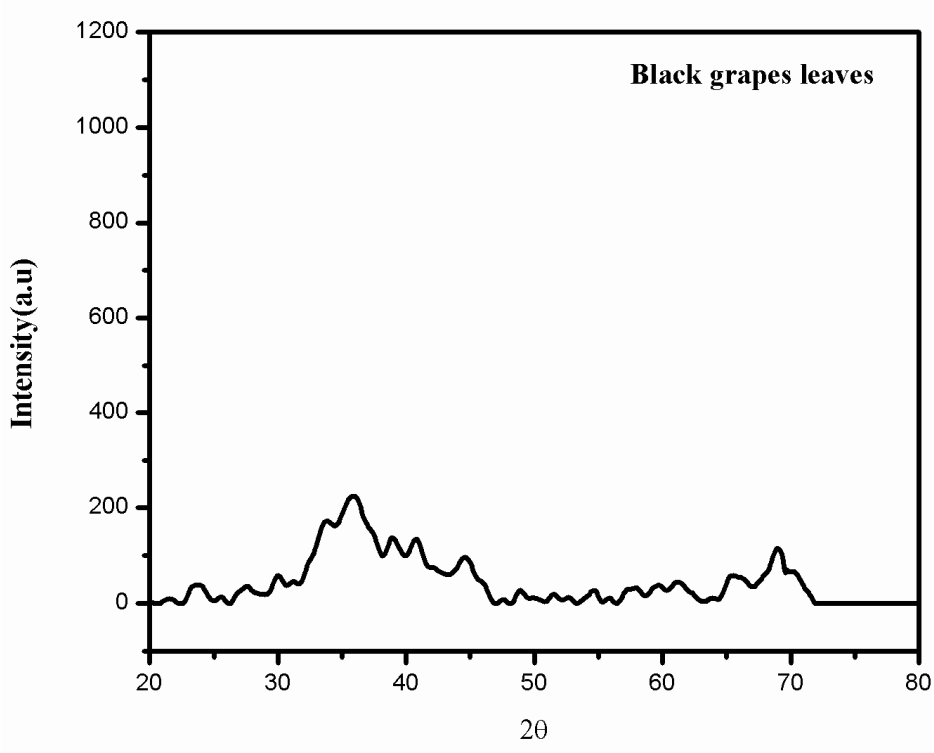



**Fig 7: XRD pattern of CuNPs biosynthesized from Black Grape leaves extract**

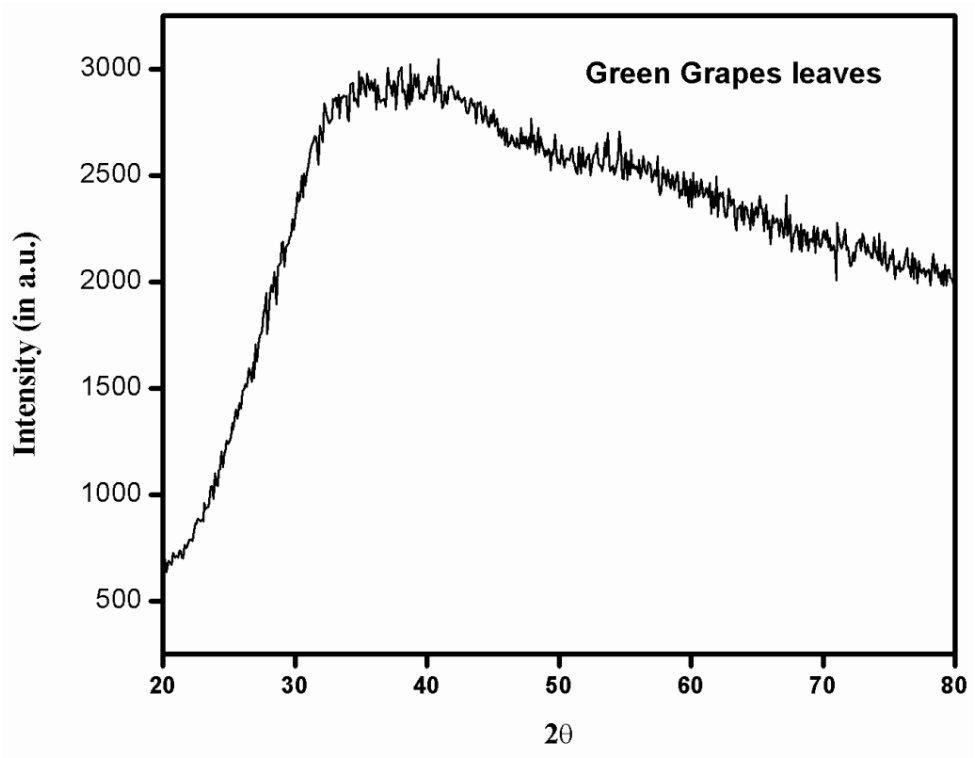



**Fig 8: XRD pattern of CuNPs synthesized from Green Grape leaves extract**

**XRD Studies**

X-Ray diffraction results clearly show that Copper nanoparticles formed by both type of leaf extracts are crystalline in nature (Fig 7 & 8). Crystalline size of CuNPs formed was calculated using the Debye-Scherrer equation

$$D = \frac{K\lambda}{\beta cos\vartheta}$$

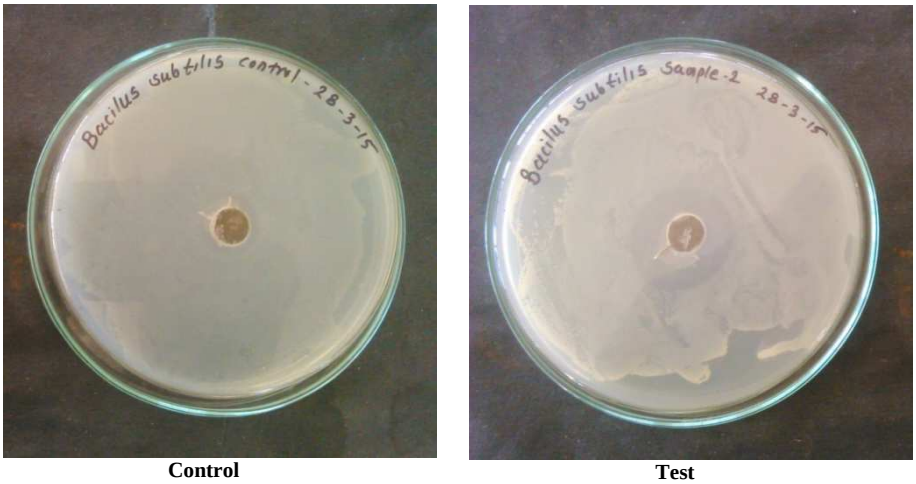
Where D is the crystallite size of CuNPs, λ is the wave-length of the X-Ray source used in XRD, β is the full width at half maximum of the diffraction peak, K is the Scherrer constant with a value from 0.9 to 1, ϑ is the Bragg angle.

**ANTIBACTERIAL ACTIVITY**

Biosynthesized CuNPs were analyzed against two pathogen bacteria, viz. *Escherichia Coli* and *Bacillus subtilus* by standard agar well diffusion method. The antibacterial activity of CuNPs was very effective against these pathogen (Fig 9 to 12).

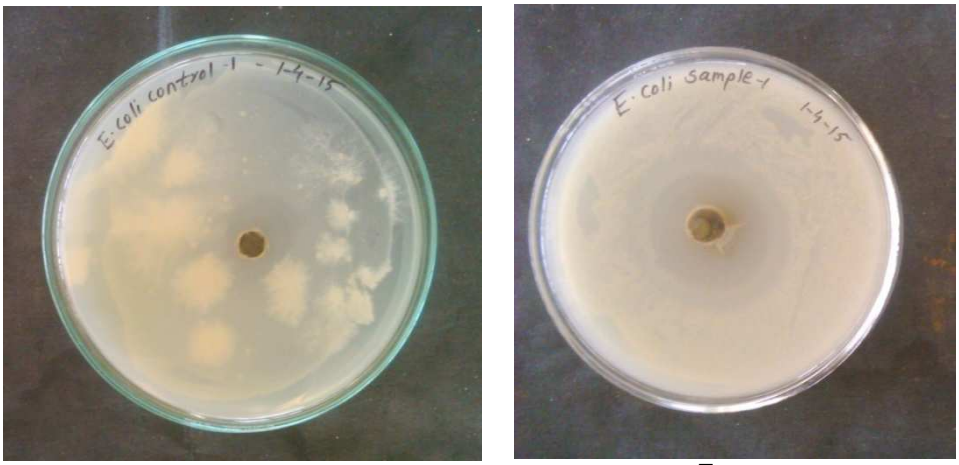

**Control** **Test**

**Fig 9: CuNPs synthesized from Black Grape Leaves extract against *E. coli***



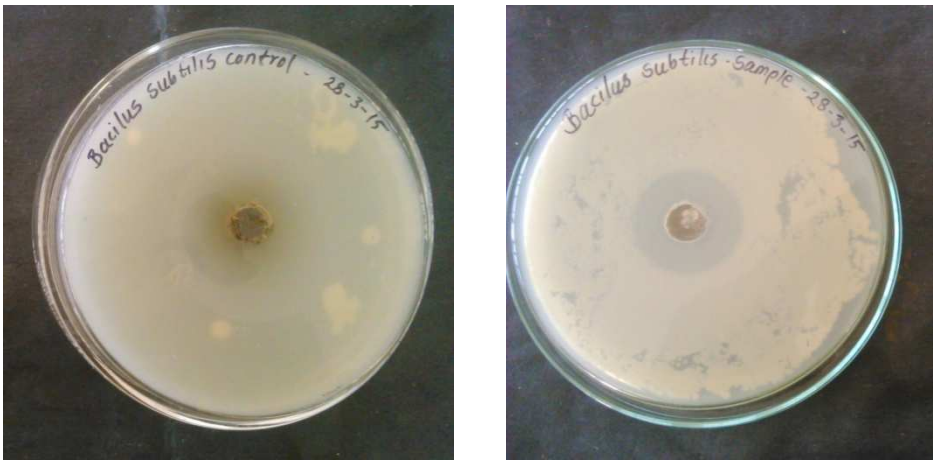

**Control** **Test**

**Fig 10: CuNPs synthesized from Black Grape Leaves extract against *B. subtilis***




**Control** **Test**

**Fig 11: CuNPs synthesized from Green Grape Leaves extract against *E. coli***




**Control** **Test**

**Fig 12: CuNPs synthesized from Green Grape Leaves extract against *B. subtilis***

**The zone of inhibition for synthesized CuNPs ranges from 14 – 20nm as given in the table below.**

| CuNPs Sample loaded 50µl | Zone of inhibition | | | |
|---|---|---|---|---|
| | *E. coli* | | *B. subtilis* | |
| | CuNPs from Black grape leaves extract | CuNPs from Green grape leaves extract | CuNPs from Black grape leaves extract | CuNPs from Green grape leaves extract |
| | 14mm | 17mm | 18mm | 19mm |

## CONCLUSION

The development of reliable and eco friendly process for synthesis of nanoparticles is a critical need in the field of nanotechnology. A simple, eco friendly and low cost approach for preparation of Copper nanoparticles by biological method using *Vitis vinifera* leaves extracts has been reported here. The characteristics of the obtained Copper nanoparticles were studied using UV-Visible spectrophotometer which conformed synthesis of Copper nanoparticles.

In IR spectra, the peaks corresponding to $–CH_2$ – (bending), C-H (stretching), -OH group, environmental $CO_2$ and metal ligand bonds are clearly seen.

From the XRD pattern, it was confirmed that the crystalline size of Copper nanoparticles from both the extract formed was between 3 – 6 nm.

Copper nanoparticles were synthesized successfully by biological method. Antibacterial activity of biologically synthesized nanoparticles was tested against two different pathogen viz. *Escherichia Coli* and *Bacillus subtilus* by agar well diffusion method. From this study, Copper nanoparticles were observed to have strong antimicrobial potential.

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