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Green Synthesis, Characterization of Copper Nanoparticles Derived from *Ocimum Sanctum* Leaf Extract and their Antimicrobial Activities

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ABSTRACT

The present study involves the green synthesis of copper nanoparticles using *Ocimum Sanctum* (Tulsi) leaf extract. First, we prepared leaf extract of *Ocimum Sanctum* in deionised water. This extract was added to equal amount of 0.05M copper sulphate solution and the colour change from green to brown was observed which indicates the formation of Copper nanoparticles. Here, the leaf extract may act as both reducing and capping agent. The synthesized CuNps were characterized by UV-Visible, Fourier Transform Infrared Spectroscopy (FT-IR), Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Analysis (EDX) and X-ray diffraction (XRD). The synthesized CuNps were in uniform Spherical and floret shape. The average particle size was found to be 29 nm. The antimicrobial study of the synthesized CuNps was established by using a gram positive, a gram negative and an opportunistic yeast pathogens.

Keywords: *Ocimum Sanctum*, green synthesis, CuNps, UV-Vis, FT-IR, SEM, EDX, XRD, antimicrobial.

INTRODUCTION

Nanotechnology is a fast growing area in the field of science which is an interdisciplinary field of both science and technology that increase the scope of investing and regulating at cell level between synthetic material and biological system[1]. In the recent years, the metal nanoparticles are focused mainly for the research work due to its unique properties and pharmaceutical applications. Many researchers have used green synthesis methods for

different metal nanoparticles due to their growing need of eco-friendly properties[2]. In this method, the plant extract has been used as capping and reducing agent for the synthesis of copper nanoparticles due to their reducing properties present in the leaf extract[3,4]. The biosynthesis of copper nanoparticles by employing different medical plant leaves extract is found to be simple, easy, low cost and eco-friendly green technique for the production of copper nanoparticles in bulk[5]. Hence, the present study focused on the aqueous leaves extract of *Ocimum Sanctum* used to synthesise copper nanoparticles and the characterization was carried out.

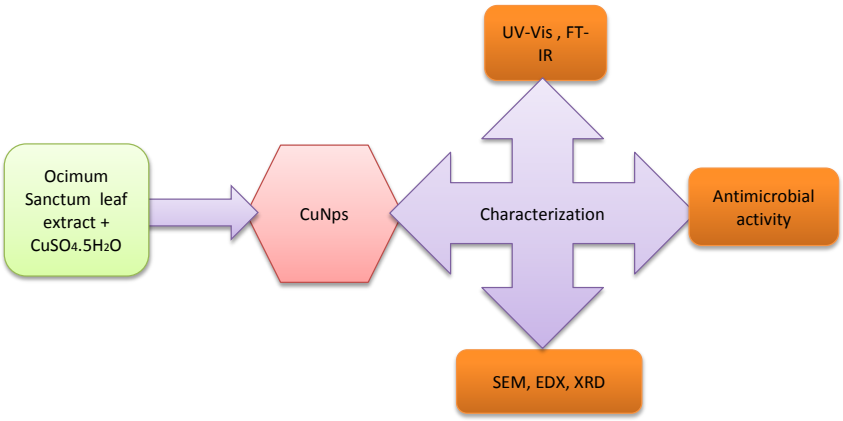

Fig 1: Outline of the Synthesis and Characterization of Copper Nanoparticles

MATERIALS AND METHODS

MATERIALS

All the Chemicals used were of Analytical grade. Double distilled water was used for this synthesis. Filteration was done by using Whatmann No.1 filter paper. All the glasswares used for the synthesis were washed well, rinsed with double distilled water and dried in hot air oven.

PREPARATION OF *OCIMUM SANCTUM* LEAF EXTRACT

The *Ocimum Sanctum* leaves were collected in Chennai, Tamilnadu, India. The fresh leaves were collected and rinsed thoroughly with tap water followed by double distilled water to remove all the dust and impurities. The washed leaves were cut into small pieces. About 100 gm of leaves were weighed separately and transferred into 250 mL beaker containing 100 mL distilled water and it was boiled for 10-15 minutes at 60^{0}C. It was then cooled down to room temperature. The prepared solution was initially filtered by using normal filter paper in order to remove leafy materials. It was again filtered by Whatmann No.1 filter paper to get a clear solution. This filterate was known as *Ocimum Sanctum* leaf extract. This extract was stored at 25^{0}C for future work. The work was done within one week of preparation of the extract.

SYNTHESIS OF COPPER NANOPARTICLES

For the synthesis of Copper Nanoparticles, 50 mL of *Ocimum Sanctum* leaf extract was added dropwise to 50 mL of 0.05M Copper sulphate solution present in 250 mL Erlenmeyer Flask. It was kept for incubation for 24 hours. After incubation, the precipitate got settled at the bottom that was confirmed by the colour change from green to black colour at the bottom. This was indicated by the formation of Copper Nanoparticles. The CuNps solution was purified by repeated centrifugation at 6000 rpm for 10 mins followed by re-dispersion of the pellet in deionised water to remove unwanted materials. The synthesized CuNps were lyophilized to get pure form and it was stored in an air tight container at room temperature.

CHARACTERISATION OF COPPER NANOPARTICLES

The synthesized CuNps were characterized by UV-Visible Spectroscopy. FT-IR analysis was carried out in order to find out the biomolecules present in the leaf extract which was responsible for the reduction of copper ions to the copper nanoparticles. Morphology and mean particle size was determined by SEM Analysis. The elemental Composition was done by EDX Analysis. The Crystallinity of CuNps were established by XRD and the average size of the CuNps was determined by using Debye-Scherrer equation.

ANTIMICROBIAL ACTIVITY

Preparation of resazurin solution

The resazurin solution was prepared by dissolving 270 mg in 40 mL of sterile distilled water. A vortex mixer was used to ensure that it was a well-dissolved and homogenous solution.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) USING RESAZURIN MICROTITRE ASSAY

Procedure for Antibacterial activity

Test was carried out in a 96 well Plates under aseptic conditions. A sterile 96 well plate was labeled. A volume of 100 µL of sample was pipetted into the first well of the plate. To all other wells, 50 µL of nutrient broth was added and serially diluted it. To each well 10 µL of resazurin indicator solution was added. 10 µL of bacterial suspension was added to each well. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. The plate was incubated at 37°C for 18–24 hours. The colour change was then assessed visually. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value.

Procedure for Antifungal activity

Test was carried out in a 96 well Plates under aseptic conditions. A sterile 96 well plate was labeled. A volume of 100 µL of sample was pipetted into the first well of the plate. To all other wells 50 µL of potato dextrose broth was added and serially diluted it. To each well 10 µL of resazurin indicator solution was added. 10 µL of fungal suspension was added to each well. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. The plate was incubated at 37°C for 18–24 hours. The colour change was then assessed visually. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value.

RESULTS AND DISCUSSION

UV – VISIBLE SPECTROSCOPY ANALYSIS

The synthesized CuNps were characterized by UV – Visible Spectroscopy. It was analysed in the range between 400 – 800 nm. UV- Visible spectra shows the peak at 659 nm which indicates the presence of CuNps. High absorbance indicates a high conversion of Cu^{2+} to Cu as nanoparticles leading to higher concentration of CuNps[6]. The result obtained from the UV – Visible Spectroscopy analysis of the CuNps was presented in Fig 2.

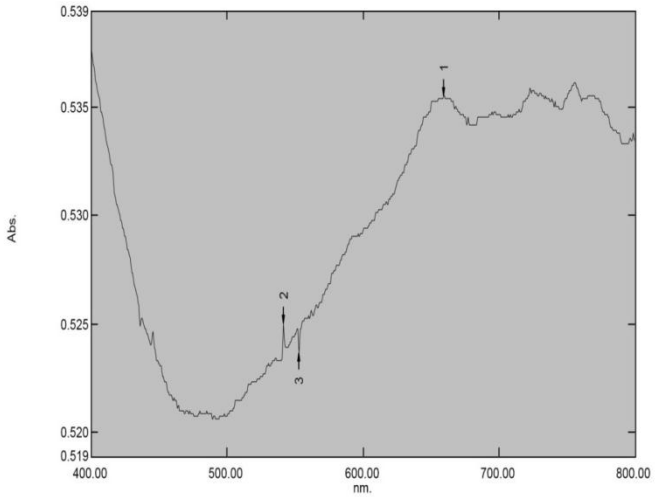

Fig 2: UV- Visible spectrum of synthesized CuNps

FT – IR ANALYSIS

The FT-IR Spectrum of CuNps was shown in Fig 3. In the spectra, the broad peak at 3317.56 cm^{-1} and 3475.73 cm^{-1} corresponds to O-H stretching H-bonded alcohols and phenols. The peak at 1633.71 cm^{-1} corresponds to carbonyl stretching of amides. The alkane C-H band

showed peak at 1406.11 cm^{-1}. The peak at 1388.75 cm^{-1} showed N-H bending primary amines. The peak at 1116.78 cm^{-1} showed C-O stretching of alcohols and ethers. The FT-IR analysis of CuNps showed that the flavonoids, alkaloids and polyphenols present in the *Ocimum Sanctum* leaf extract act as a capping and reducing agent which was surrounded by the CuNps.

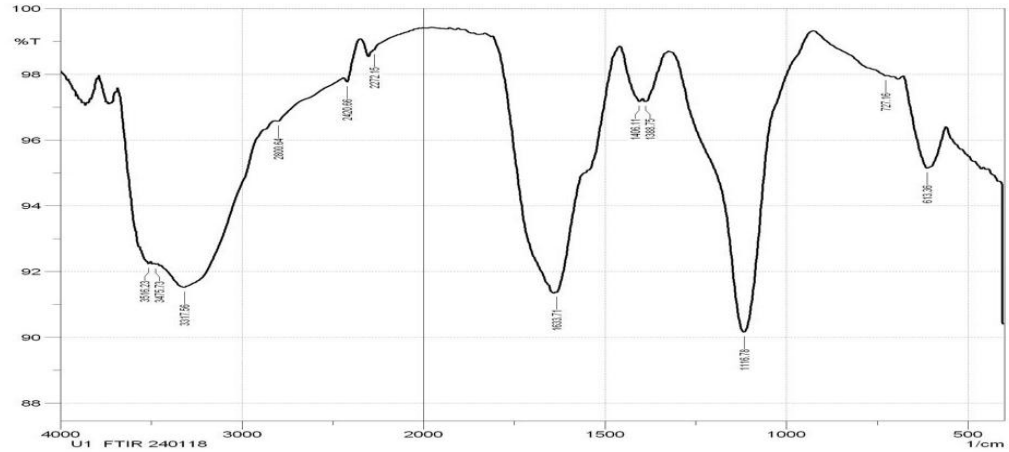

Fig 3: FT-IR Spectrum of synthesized CuNps

SEM – EDX ANALYSIS

The surface morphology of the CuNps was obtained by the Scanning Electron Microscopy (SEM) Analysis. The Fig 4 shows the morphology of CuNps synthesized by the *Ocimum Sanctum* leaf extract. It is shown that the spherical and uniform floret shape of CuNps was confirmed. The quantitative and qualitative analysis of elements was observed by the formation of CuNps. They were confirmed by EDX Analysis. EDX of the synthesized CuNps showed strong Copper signals along with O, N, C and S peaks which may originate from the biomolecules that was bound to the surface of the CuNps. CuNps show Strong peak at 1keV which is shown in the fig 5.

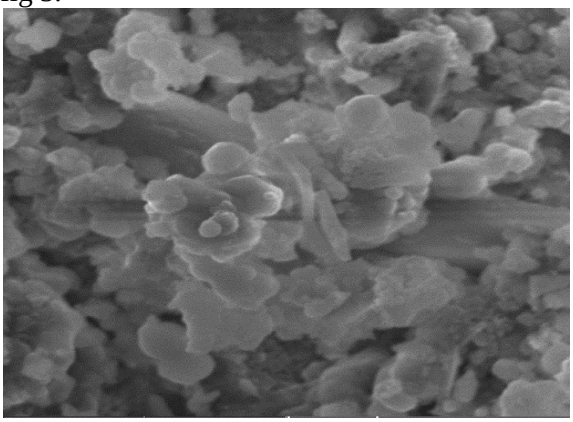

Fig 4: SEM images of synthesized CuNps

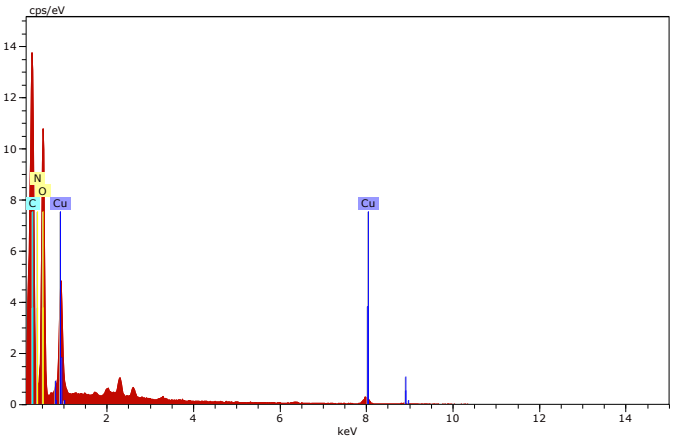

Fig 5: EDX analysis of CuNps

XRD ANALYSIS

XRD pattern of synthesized CuNps from the *Ocimum Sanctum* leaf extract was represented in the Fig 6. It shows a high crystallinity level with diffraction angles 22.16, 23.87, 24.90, 27.08, 32.48, 33.51, 37.19, 44.75, 47.96 which corresponds to Face Centered Cubic (FCC) of Copper lines indexed at (210), (111), (210) and (220)[7]. The average size of the synthesized CuNps were calculated by using Debye – Scherrer equation.

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

Where D is the Crystalline size of Nanoparticles, k is the Scherrer's constant ranges from 0.9 – 1, λ is the wavelength of the X-ray radiation source, β is the Full width at half maximum of the diffraction peak (FWHM) and θ is the Bragg's angle. According to Debye – Scherrer equation, it ranges from 24 to 35 nm. The average particle size was found to be 29 nm.

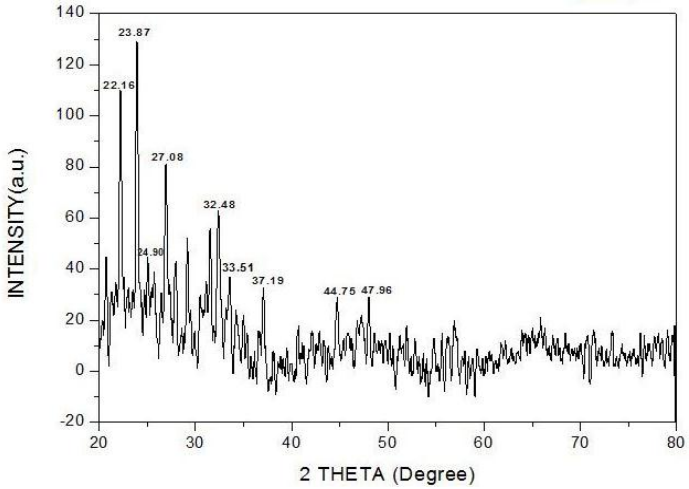

Fig 6: XRD pattern of synthesized CuNps

ANTIMICROBIAL ACTIVITY

Table 1: Antibacterial Activity of Copper Nanoparticles Synthesized from *Ocimum Sanctum* Leaf Extract

S.No.	Microorganisms	Growth of inhibition										
		1000 µg	500 µg	250 µg	125 µg	62.5 µg	31.2 µg	15.6 µg	7.8 µg	STD Streptomycin 10 µg	DMSO Negative control	Culture
1	***Escherichia coli***	-	-	-	-	-	-	+	+	-	+	+
2	***Bacillus subtilis***	-	-	-	-	-	-	-	-	-	+	+

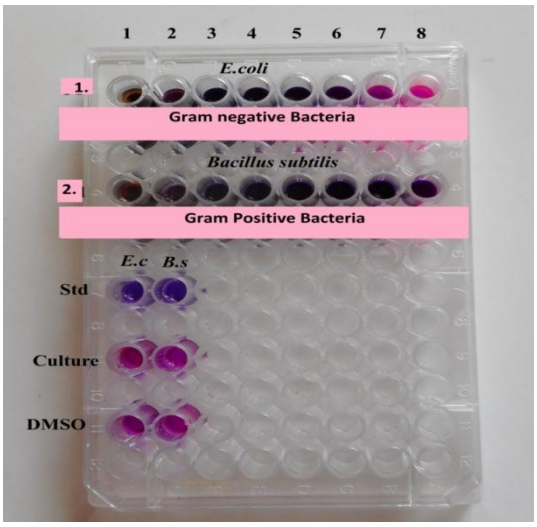

Fig 7: Antibacterial activity of CuNps using resazurin microtitre assay

The synthesized Copper nanoparticles show good antibacterial activity towards *E.coli* whose MIC value is 31.2 µg. But, it doesn't show any effect on *B.subtilis*.

Table 2: Antifungal Activity of Copper Nanoparticles Synthesized from *Ocimum Sanctum* Leaf Extract

S.No.	Microorganism	Growth of inhibition										
		1000 µg	500 µg	250 µg	125 µg	62.5 µg	31.2 µg	15.6 µg	7.8 µg	Amphotericin B 10 µg	Negative control	Culture
1	***Candida albicans***	-	-	-	-	+	+	+	+	-	+	+

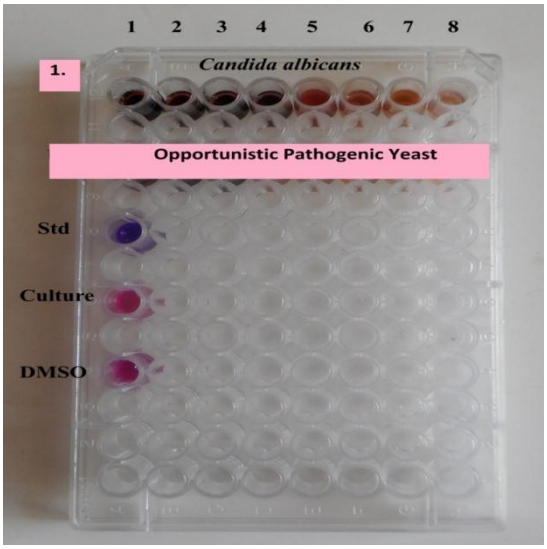

Fig 8: Antifungal activity of CuNps using resazurin microtitre assay

The synthesized copper nanoparticles show good antifungal activity towards *C.albicans* whose MIC value is 125 µg.

Table 3: Mic Value

Microorganisms	MIC Value(µg)
Escherichia coli	31.2
Bacillus subtilis	-
Candida albicans	125

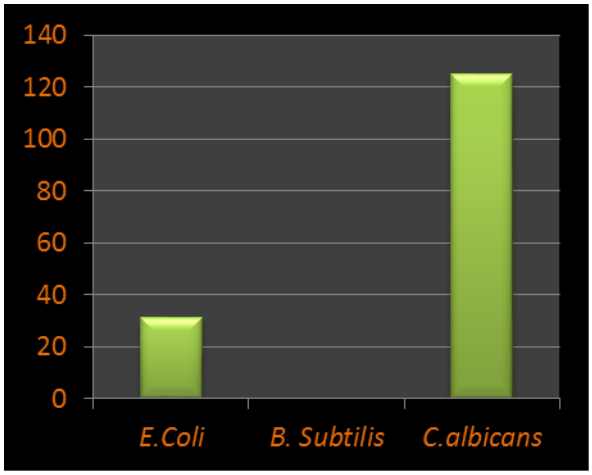

Fig 9: Antimicrobial activity of Copper Nanoparticles synthesized from *Ocimum Sanctum* leaf Extract

CONCLUSION

The Copper nanoparticles were synthesized by using *Ocimum Sanctum* leaf extract. This green synthesis of CuNps was cost- effective and eco- friendly. The synthesized CuNps were characterized by UV-Vis, FT-IR, SEM, EDX and XRD. The SEM results provide uniform spherical and floret shape of CuNps. From the XRD Analysis, the average size of the CuNps was found to be 29 nm. The synthesized CuNps were effectively used for the antimicrobial activity. It shows good antibacterial activity towards *E.Coli*. It shows good antifungal activity towards *C.albicans*. The synthesized CuNps is utilized for many Pharmaceutical applications in future use.

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