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BIOSYNTHESIS OF COPPER NANOPARTICLES USING *OCIMUM SANCTUM* LEAF EXTRACT AND ITS ANTIMICROBIAL PROPERTY

Anuj Bhasker, Rajalakshmi A, Krithiga N, Gurupavithra S and Jayachitra A[*]

Dept.of Plant Biotechnology, School of Biotechnology, Madurai Kamaraj University, Madurai, Tamilnadu, India.

ABSTRACT

In recent developments and implementation of new technologies, Nanorevolution unfolds the role of plants in green synthesis of nanoparticles. In this study, the copper nanoparticles were synthesized from the leaf extracts of *Ocimum sanctum* and standardization of different parameters like metal ion concentration, reaction time, pH and ratio in different concentrations were done. The synthesized nanoparticles were characterized by using UV–visible spectroscopy, Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) which shows some reducing compounds such as eugenols, terpenoids and some aromatic compounds. Finally antimicrobial activities were analysed against different pathogens.

Key Words: *Ocimum sanctum*, Nanoparticles, UV, FTIR, Antibacterial activity.

INTRODUCTION

Nanotechnology involves the production, manipulation and use of materials ranging in the size from less than a micron to that of individual atoms (Mohanpuria P *et al*., 2007). A wide variety of physical, chemical and biological processes result in the synthesis of nanoparticles, some of these are very useful and others are quite common (Satyajit *et al*., 2007). Nanomaterials are the leading in the field of Nanomedicine, Bionanotechnology and in that respect Nano toxicology research is gaining great importance. The US Environmental Protection Agency (EPA) has approved registration of copper as an antimicrobial agent which is able to reduce specific harmful bacteria linked to potentially deadly microbial infections (European Copper Institute, 2008). Copper nanoparticles, due to their excellent physical and chemical properties and low cost of synthesis, have been of great interest. Copper nanoparticles have wide applications as heat transfer systems, antimicrobial materials, super strong materials, sensors and catalysts. Copper nanoparticles are very reactive because of their high surface to volume ratio and can easily interact with other particles (Prema P, 2010) and increase their antimicrobial efficiency.

In addition, no research has discovered any bacteria that are able to develop immunity against copper as they do with antibiotics (Esumi K *et al*., 2001; Feitz A *et al*., 2006).

The emergence of nanoscience and nano technology in the last decade presents opportunities for exploring the bactericidal effect of metal nanoparticles. The bactericidal effect of metal nanoparticles have been attributed to their small size and high surface to volume ratio, which allows them to interact closely with microbial membranes and is not merely due to the release of metal ions in solution (Mohanty L *et al*., 2001)

Plant description

The *Ocimum sanctum* is a shrub that belongs to the family of *Lamiaceae*, which grows to a height ranging from 0.5 to 1.5m. The leaves are about 2-4cms in length. *Ocimum sanctum* leaves have abudancy in tannins like galllic acid, chlorogenic acid etc and also contains alkaloids, glycosides, and saponins. Traditionally *Ocimum*

Corresponding Author

Jayachitra A
Email: jchitra21@gmail.com

sanctum is used to treat malarial fever, gastric disorders and in hepatic infections. Nowdays these leaves are also used for its anticancer and antidiabetic properties, bronchitis, ringworm and other cutaneous diseases.

MATERIALS AND METHODS

Sample collection

The *Ocimum sanctum* leaves were collected from Madurai District, Tamil Nadu, India. Copper sulphate was purchased from Hi-media laboratories Mumbai, India.

Preparation of Ocimum sanctum extract

The fresh leaves of *Ocimum sanctum* were washed several times with distilled water to remove the dust. When the leaves got completely dried, they were chopped into fine pieces.5g of chopped leaves of *Ocimum sanctum* were boiled with 50 ml of distilled water at 60^0C for 1 hour. The extract obtained was filtered through Whatman No.1 filter paper and finally brown extract were collected for further experiments.

Synthesis of copper nanoparticles

For the synthesis of copper nanoparticles, 50 ml of *Ocimum sanctum* extract was mixed with 50 ml aqueous solution of $CuSO_4$ (1:1 ratio of plant and copper solution) and stirred continuously for 2 min at 30^0C. Reduction takes place rapidly which is indicated by the change in colour of the solution after the mixture was incubated at room temperature for overnight. Mixture was centrifuged at 3500 rpm for 10min to get copper nanoparticles. Wash the nanoparticles and dry it at room temperature.

Characterization of Synthesized copper nanoparticles

UV-Vis- Spectroscopy

The reduction of nanoparticles was monitored by using the UV-Visible spectrophotometer. The absorbance was recorded at 200-800nm using spectrophotometer.

Fourier Transform Infrared (FTIR) Spectroscopy Measurements

FTIR spectroscopic analysis was carried out using a Jasco Fourier Transform Infrared Spectrometer. Fourier transforms infrared spectra generated by the absorption of electromagnetic radiation in the frequency range from 400 to 4000 cm^{-1}. Different functional groups and structural features in the molecule absorb at characteristics frequencies. The Frequency and intensity of absorption are the indication of the band structures and structural geometry of the molecules.

XRD Analysis

The XRD diffraction method was used to characterize the synthesized metal nanoparticles. The size of the particle was calculated using Scherrer equation (Prema P, 2010).

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

Where CS is the crystallite size

Constant (K) = 0.94

β is the full width at half maximum (FWHM)

Full width at half maximum in radius (β) = FWHM x π/180

λ = 1.5406 x 10-10, Cos θ = Bragg angle.

OPTIMISATION OF DIFFERENT PARAMETERS

Concentration of copper sulphate solution

The above mentioned procedures were optimized for the synthesis of copper nanoparticles in the range between (0.25, 0.5, and 1.0, 1.5 and 2.0 mM) was done.

Temperature

The reaction temperatures were maintained at 30, 60, 90 and 120 degrees Celsius respectively, using water bath. The absorbance of the resulting solutions was measured.

pH

The reaction pH was maintained at 4, 6, 8 and 10 respectively. The pH was adjusted by using 0.1 N HCl and 0.1 N NaOH.

Time

The time required for the completion of reaction, where the reaction was monitored after 0, 1h, 3h, 6h, 8h, 24h of time interval.

Ratio

The optimization of copper sulphate and leaf extract concentration required for the maximum production of copper nanoparticles was done, where there action was monitored by using different ratio of copper sulphate and leaf extract solution (1:1, 1:2, 1:3, 1:4, 1:5).

ANTIBACTERIAL ACTIVITY

Disc Diffusion Method

Nutrient broth/agar containing 1g beef extract, 1g peptone, and 0.5g NaCl were dissolved in 100ml of double distilled water. The media was autoclaved, cooled and kept for inoculation. The Nutrient agar media was prepared and poured in the petri discs and kept for 30 minutes for solidification (Bauer AW *et al*., 1966). After 30 minutes the fresh overnight cultures of inoculum (100µl) of different cultures were spread on to solidified Nutrient agar plates. Sterile paper discs made of Whatman filter paper, 5 mm diameter (dipped into 100 µg/ml copper nanoparticles) along with standard antibiotic containing discs were placed in each plate. The cultured agar plate was incubated at 37^0C for 24h. After 24hof incubation the zone of inhibition was measured**.**

RESULTS AND DISCUSSION

Copper nanoparticle synthesis

The fresh leaf and aqueous extract of *Ocimum sanctum* were collected shown in Plate- 1.When the leaf extractof *Ocimum sanctum* was mixed with $CuSO_4$solution, the colour of aqueous extractwas changed immediately within 10 min, indicates the formation of copper nanoparticles.Reduction of Copper ion into copper nanoparticles during exposure to the plant extracts were observed and the result of colour change exhibits darkbrown colour shown in the Plate-2.

Fig 1. UV-Vis Spectrophotometer

The synthesized nanoparticles were characterised by using UV-Vis spectrophotometer and the maximum absorbance showed at 360-380nm in the figure-1.

Fig.2.FTIR Spectrum of Copper nanoparticles

The FTIR analysis of the copper oxide nanoparticles revealed the presence of various compounds (Krithiga N *et al*., 2013). The FTIR spectra of copper nanoparticles are shown in figure-2. The following peak were observed in spectrum and the band at 3421 cm^{-1}, due to N-H stretching of amines or O-H alcohol. The band at 2924$cm^{-1}$and 2852cm^{-1} may be due to C-H groups. The peaks at 1593and 1519 cm^{-1}show that C-C .Aromatic strong stretching. The peak at 1384,1163,1024, 528,459, and 424cm^{-1} indicated fingerprint region is complicated by the large number of different vibrations that occur here. These include single bond stretches and a wide variety of bending vibrations. This region gets its name since nearly all molecules (even very similar ones) have a unique pattern of absorptions in this region. The FTIR analysis of the copper nanoparticles revealed the presence of various compounds.

Fig 3. XRD Analysis

The X-Ray diffraction studies reveal the characterization of the synthesized nanoparticle respectively. The average size of particle was calculated using XRD formula and results shown in figure-3.The particle size are found at 21.86nm.

Optimization of different parameters

The Optimization result is as follows-Conc.- 0.5mM, Temp-60C pH7.0Incubation time - 24 hrs. Ratio of copper sulphate and plant extract is - 1:1 (Table-1)

Fig 4. Antibacterial Activity of copper nanoparticles

The antibacterial effect of copper nanoparticles were analysed on the basis of the zone of inhibition. Copper nanoparticles exhibited strong antibacterial activity against human pathogens such as *Salmonella typhi, S.aureus*, *Vibrio cholera,P. aeruginosa*. The copper nanoparticles exhibited maximum effect against *Vibrio cholera* with a zone of inhibition 12 mm compare to other pathogens.These results were also compared with standard antibiotics Tetracyclin figure-4.

Table 1.Optimization of different parameters

Parameters	Optimization values
Metal ion concentration	0.5mM
Temperature	60^{o}C
pH	7
Time	24 hours
Ratio	1:1

Plate 1. *Ocimum sanctum and* its plant extract

Plate 2. Synthesis of Copper nanoparticles

A – Fresh Leaf extracts B – coppersulphate before addition of leaf extract C – After addition of leaf extract

Fig 1. UV-Vis-Spectrophotometer

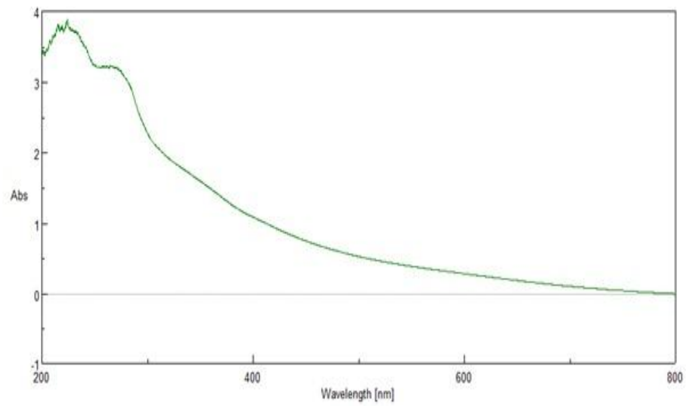


Fig 2. FTIR

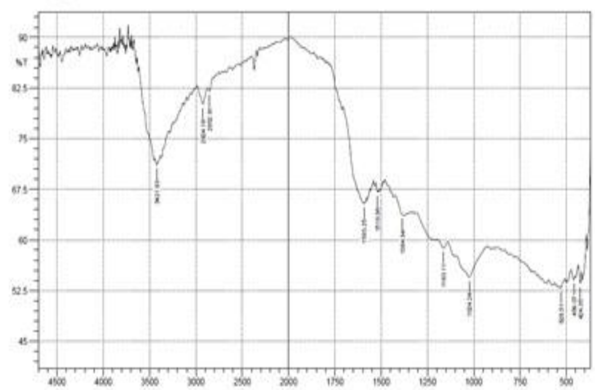


Fig 3. XRD Analysis

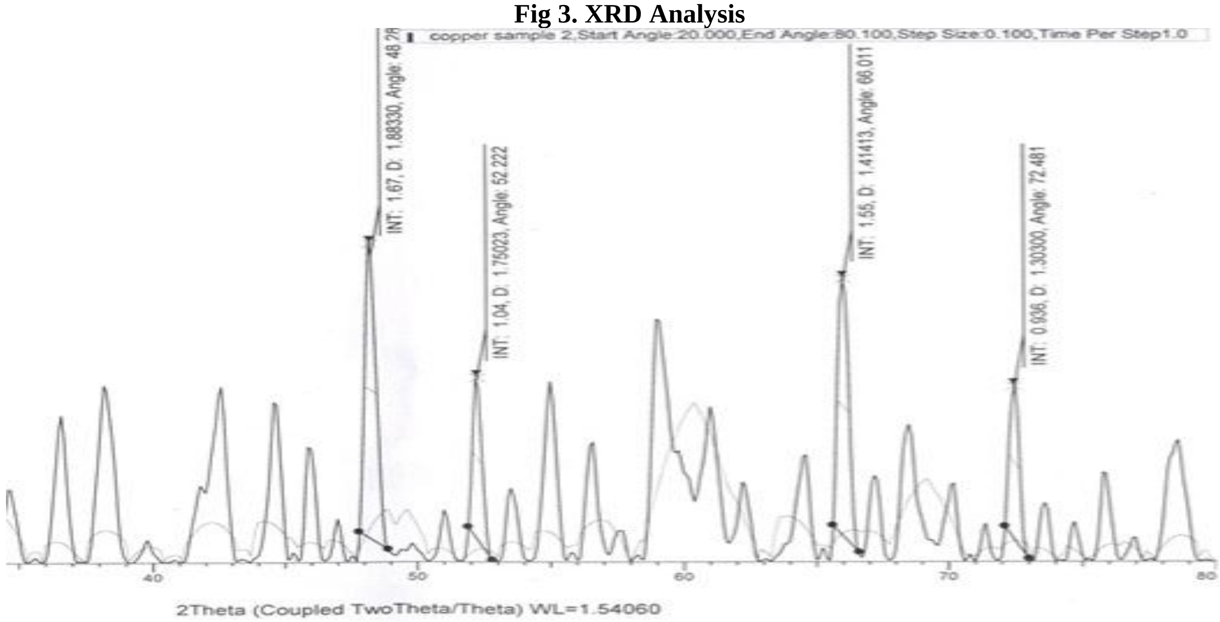
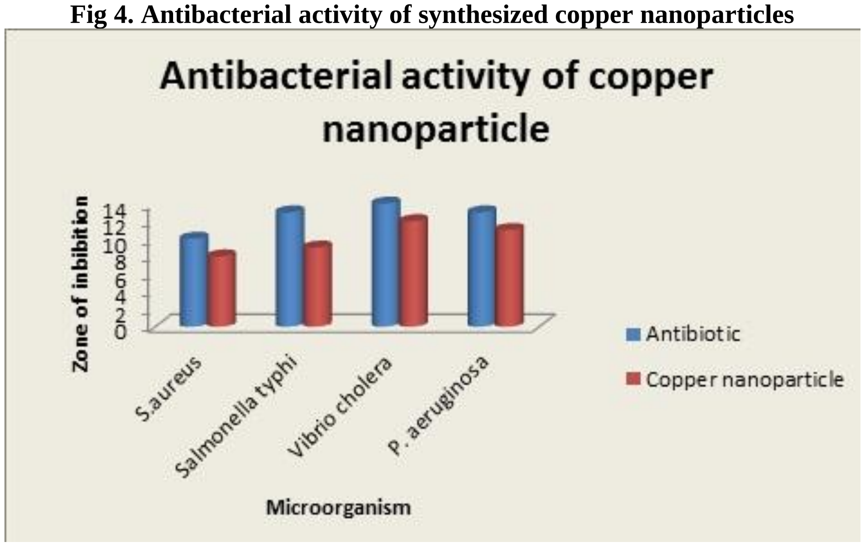


Fig 4. Antibacterial activity of synthesized copper nanoparticles



DISCUSSION AND CONCLUSION

In the present study, the successful synthesis of copper Nano particles was done by reduction method. The synthesized nanoparticles show higher antimicrobial activity against *Vibrio cholera*.

FUTURE IMPORTANCE

The potential application of the nanomaterial's are novel techniques such as therapeutics in cancer, as drug delivery vehicles, smart Nano composites, in bioremediation of industrial effluents, biodegradation of effluents and for water purification.

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