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GREEN SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF COPPER OXIDE NANOPARTICLE

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ABSTRACT

An investigation has been carried out to synthesize copper oxide nanoparticle using leaf extract of *Jasminum fluminense* an eco-friendly approach. The green synthesized copper oxide nanoparticle was characterized using UV- Spectroscopy, FTIR analysis, SEM and TEM. The results of the UV- Spectroscopy study revealed that the absorption spectra of copper oxide nanoparticle formed in the reaction media has absorbance peak at 430nm. The results of FTIR spectrum of copper oxide nanoparticle revealed that copper oxide nanoparticle was surrounded by different organic molecules such as alcohols, ketones, aldehydes, carboxylic acid etc, which act as reducing and capping agents that provide stability to nanoparticle. The results of the SEM showed that the diameter of green synthesized copper oxide nanoparticle ranged from 30 nm – 60 nm in diameter and the shape was found to be in the form of spherical, cylindrical and flakes with agglomerates were also observed. The results of TEM showed the formation of well distributed spherical and cylindrical shapes with size ranges from 100 nm to 500 nm in magnification. Copper oxide nanoparticle showed antimicrobial activity against microbial pathogens used in the study except *Aspergillus niger*.

Keywords: Green synthesis, copper oxide nanoparticle, *Jasminum fluminense*, leaf extract, characteristics, antimicrobial activity

1.0 INTRODUCTION

Nanotechnology is mainly concerned with synthesis of nanoparticles of variable sizes, shapes, chemical compositions and controlled dispersity with their potential use for human benefits. Development of green nanotechnology is generating interest of researchers towards eco-friendly biosynthesis of nanoparticles **[1]**. Metal nanoparticles possess fascinating ultraviolet-visible sensitivity, electrical, catalytic, thermal and antibacterial properties, quantum effects, and large surface-to-volume ratio **[2]**. The synthesis of metal nanoparticle can be broadly and traditionally categorized into physical, chemical and biological methods. Physical and chemical methods involve very costly instruments, chemical and laboratory set up as well as the use of extremely toxic and hazardous chemicals which may pose environmental and biological risks. However biological method of synthesis of nanoparticles using microorganisms, fungi, enzymes, plant and plant extracts are eco-friendly and much reliable alternatives to physical and chemical method for synthesis of nanoparticles. Biogenic synthesis of metal nanoparticles is frequently achieved by the activity of bioactive components present in plant extracts such as phenols, flavonoids, amines, alcohols, proteins, aldehyde etc. *Jasminum fluminense* (Nithya malli) is an evergreen woody and twining vine in the family *oleaceae*. *Jasminum* plants have long been used for their therapeutic and medicinal properties. It plays an essential role in ayurvedic medicine. It is known traditionally in ayurveda for treating intestinal worms.

Among various metal nanoparticles copper nanoparticlehas been of great interest due to their excellent physical and chemical properties and low cost of preparation **[3]**. Copper nanoparticle has wide applications as heat transfer system, antimicrobial materials, super strong materials, sensors and catalyst **[3]**.

It can be used as an anti-bactericidal agent to coat hospital equipment **[4]**. Copper nanoparticle have prospective applications in optics, in manufacture of lubricants, nanofluids, conductive films **[5]**.

Hence based upon the above views the present study was carried out to synthesize copper oxide nanoparticle using the leaf extract of *Jasminum fluminense*, to characterize green synthesized copper oxide nanoparticle using UV-Spectroscopy, FTIR, SEM, TEM and to determine antimicrobial activity of green synthesized copper oxide nanoparticle against *Escherichia coli, Staphylococcus aureus, Aspergillus flavus and Aspergillus niger*.

2.0 MATERIALS AND METHODS

2.1 Materials

Jasminum fluminense were collected from local nursery located in Chennai, Tamil Nadu, India. Copper sulphate with 90% purity was obtained from Himedia and the distilled water was used throughout the experiments for the synthesis of copper oxide nanoparticle.

2.2 Preparation of aqueous decoctions using *Jasminum fluminense* leaves

The preparation of aqueous decoctions using *Jasminum fluminense* leaves to synthesize copper oxide nanoparticle was carried out by following the procedure of **[6]**. It was prepared by boiling 30gms of *Jasminum fluminense* leaves in 100ml of sterile distilled water taken in conical flask for 30 minutes and then contents in the flask was cooled, followed by filtration of the contents and stored at 4˚C until further use **(Figure 1a, 1b, 1c)**.

Figure 1a: Fresh leaves of *Jasminum fluminense*

Figure 1b: Boiled leaves of *Jasminum fluminense*

Figure 1c: Aqueous extract of *Jasminum fluminense* leaves

2.3 Green synthesis of Copper oxide nanoparticle:

Preparation of copper sulphate solution for green synthesis of Copper oxide nanoparticle was carried out by following the procedure of **[7]**. 1Mm of Copper sulphate was **(Figure 1d)** dissolved in 80ml of already prepared *Jasminum fluminense* leaves extract and was mixed homogenously. The mixed solution **(Figure 1e)** was dried at 60˚C overnight to yield pale-green coloured copper oxide nanoparticle, which were finally calcined at 100˚C for 1 hr and preserved in air-tight vessels for further studies.

Figure 1d: Copper sulphate solution for synthesis of copper oxide nanoparticle

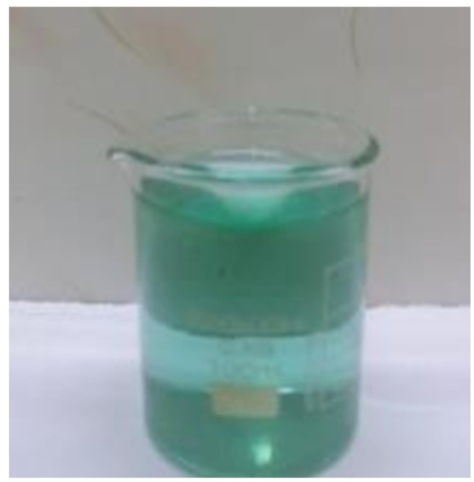

Figure 1e: Copper sulphate and aqueous extract of *Jasminum fluminense*

2.4 Characterization of green synthesized copper oxide nanoparticle:

The formation of green synthesized copper oxide nanoparticle was confirmed by UV-visible spectroscopy using Jasco V-550 spectrophotometer. Wavelength range of copper oxide nanoparticle was analysed with UV spectrophotometer. FTIR Spectroscopy was used to investigate the interactions between different species and changes in chemical compositions of the mixtures. The FTIR spectra of green synthesized copper oxide nanoparticle were recorded in SHIMADZU-8400 spectrometer using KBR pellet method SEM machine was employed to study the morphology of green synthesized copper oxide nanoparticle. The experiment was performed at an accelerating voltage of 30 kV. The slide was coated with platinum and after the platinum coating, the SEM image was taken. Transmission Electron Microscope (TEM) analysis was done using Philips (technai 10). Thin films of sample were prepared on a carbon coated copper grid by just dropping a very small amount of sample on the grid, extra solution was removed using a blotting paper and then the film on the TEM grid were allowed to dry by putting it under incubator. The images were obtained by technai, Twin 200KV and a bias voltage of 200kV was used to analyze samples.

2.5 Antimicrobial activity of green synthesized copper oxide nanoparticle:

Antibacterial activity of green synthesized copper oxide nanoparticle against bacterial isolates – *Escherichia coli* and *Staphylococcus aureus* was determined using Muller-Hinton Agar (MHA) medium by following agar well diffusion method of [8]. All the microbes used in the study were collected from a hospital located in Chennai. Samples were transported to the laboratory for further processing in an ice box.

The Muller Hinton Agar medium was weighed dissolved in 100ml of distilled water 1gm of agar was added to it and sterilized. After sterilization, the medium was poured into sterile petriplates and allowed to solidify for 1hr. After the solidification, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension (Bacterial suspension was prepared by sub culturing the bacterial isolates in Muller Hinton broth). Wells were made and loaded with 20 µl sample of respective concentrations (50 µg/ml, 100 µg /ml, 150 µg /ml and 200/ µg ml) of green synthesized copper oxide nanoparticle. Distilled water was used as a control. These plates were incubated for 24 hrs at 37ºC. Antibacterial activity of copper oxide nanoparticle was determined by formation of zone of inhibition on the medium in the petri plates around bacterial isolates.

Antifungal activity of green synthesized copper oxide nanoparticle against fungal isolates – *Aspergillus flavus* and *Aspergillus niger* were determined using Sabouraud Dextrose Agar (SDA) medium by following agar well diffusion method of **[8]**. 5gms of Sabouraud Dextrose Agar (SDA) medium was weighed and dissolved in 100ml of distilled water and 1gm of agar was added to it and sterilized. After sterilization, the medium was poured into sterile petriplates and allowed to solidify for 1hr. After the solidification, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension (Fungal suspension was prepared by sub culturing the fungal isolates in Sabouraud dextrose broth). Wells were made and loaded with 20 µl sample of respective concentrations (50 µg/ml, 100 µg /ml, 150 µg /ml and 200/ µg ml) of green synthesized copper oxide nanoparticle. Distilled water was used as a control. These plates were incubated for 24 hrs at 37ºC. Antifungal activity of copper oxide nanoparticle was determined by zone of inhibition formation on the medium in the petri plates around fungal isolates.

3.0 RESULTS AND DISSCUSSION

3.1 UV-Visible spectroscopy

UV-Visible spectroscopy is a very useful technique for studying metal nanoparticle because the peak positions and shapes are sensitive to particle size. The results of UV analysis of green synthesized copper oxide nanoparticle was presented in the **Figure 2**. The results of the study revealed that the absorption spectra of copper oxide nanoparticle formed in the reaction media has absorbance peak at 430nm. Metals have free electrons, the collective oscillations of all the free conduction band electrons which are excited by the incident electromagnetic radiations corresponds to the surface Plasmon absorption in copper oxide nanoparticle. This surface Plasmon absorption at 430nm specifies the formation of copper oxide nanoparticle **[9]**.

3.2 Fourier Transform Infra-Red (FTIR) Spectroscopy Analysis

FTIR Spectroscopy was used to investigate the interactions between different species and changes in chemical compositions of the mixtures. FTIR measurements of the green synthesized copper oxide nanoparticle was carried out to identify the possible biomolecules responsible for the reduction, capping and efficient stabilization of the bio-reduced copper oxide nanoparticle. The results of FTIR analysis of green synthesized copper oxide nanoparticle was depicted in **(Figure 3)**. The results of the study revealed the presence of functional groups in the green synthesized copper oxide nanoparticle. The copper oxide nanoparticle displays a number of absorption peaks, reflecting its complex nature. The peaks at 418.00cm-1, 436.00cm-1, 618.00cm-1, 774.00cm-1 may be due to the presence of chemical bonding, crystal structure and relative intensities of carbonates. The peak at 1632.00 cm-1 denotes the OH group, 1402.00 cm1 reveals the C=C group and 1122.0 cm-1 indicates the presence of C-O group as well as the bands at 3839.00cm-1, 3763.00cm-1 and 3143.00cm-1 attributes to aliphatic C-H stretching and bending modes. The bands at 2368.00 cm-1, 2109.00 cm-1 and 2030.00cm-1 may be due to N-H group of amines. Thus the results of FTIR spectrum of copper oxide nanoparticle suggested that copper oxide nanoparticle was surrounded by different organic molecules such as alcohols, ketones, aldehydes, carboxylic acid etc. that act as reducing and capping agents which provide stability to nanoparticle. The result of the study is in accordance with the report of **[11]**.

3.3 Scanning Electron Microscope (SEM)

The surface morphology and size of the copper oxide nanoparticle was obtained by Scanning Electron Microscopy (SEM). It is obtained by scanning it with a high energy beam of electrons in vacuum chamber. When the beam of electrons strikes the surface of the specimen and interacts with atoms of the sample, signals in the form of secondary electrons are generated that contain information about sample's surface morphology **[10]**. The results of the SEM studies of green synthesized copper oxide nanoparticle was shown in the **(Figure 4)**. The results of the study revealed that the size of green synthesized copper oxide nanoparticle ranged from 30 nm – 60 nm in diameter, the shape was found to be in the form of spherical, cylindrical and flakes with agglomerates were also observed.

3.4 Transmission Electron Microscope (TEM):

The result of TEM micrographs of green synthesized copper oxide nanoparticle using aqueous leaves extract of *Jasminum fluminense* was depicted in **Figure 5**. The result shows the formation of well distributed spherical and cylindrical shapes with size ranges from 100 nm to 500 nm in magnification. The TEM images of copper oxide nanoparticle show that the average size of the Copper oxide nanoparticle range under 100nm, which is in accordance with the work of **[12]**.

3.5 Antimicrobial studies:

The results of antibacterial activity of copper oxide nanoparticle was depicted in **Figures (6a, 6b and in Table 1a)**. The results of the study revealed that the maximum zone of inhibition was recorded in *Escherichia coli* which were found in 200µg/ml of concentration (17.66±1.52) and minimum zone of inhibition was found in 100µg/ml of concentration (11.33±0.57). The maximum zone of inhibition in *Staphylococcus aureus* was recorded in 200µg/ml of concentration (28.16±0.76) and minimum zone of inhibition was found in 50µg/ml of concentration (19.66±2.51). Highest zone of inhibition in both the bacterial species were observed in highest concentration of copper oxide nanoparticle. Zone of inhibition formation were observed in both the bacterial species. Maximum zone of inhibition formation was recorded in *Staphylococcus aureus* than in *Escherichia coli.* This is in agreement with the work of **[13]**.

The results of antifungal activity of green synthesized Copper oxide nanoparticle against the fungal isolates *Aspergillus flavus* and *Aspergillus niger* were depicted in the **(Figures 6c, 6d and Table 1b)**. The results of the study revealed that the maximum zone of inhibition formation in *Aspergillus flavus* was found at 200µg/ml of concentration (44.33±3.05) and minimum zone of inhibition was recorded in 50µg/ml of concentration of copper oxide nanoparticle (26.66±1.52).

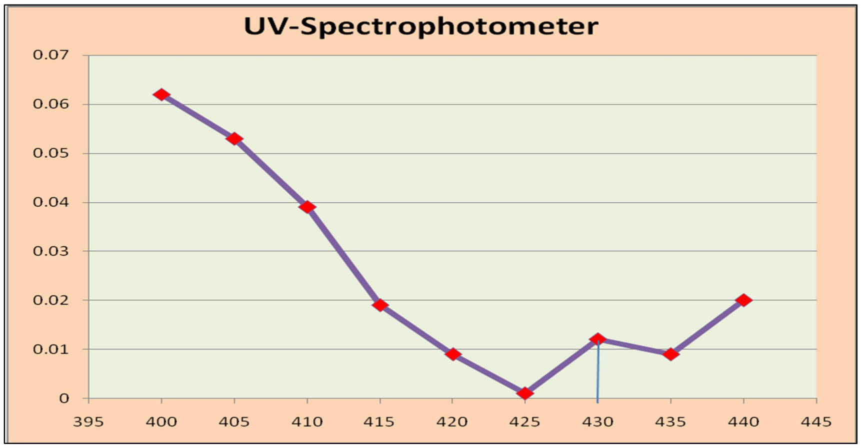

Figure 2: UV- Spectrophotometric analysis of Copper oxide nanoparticle

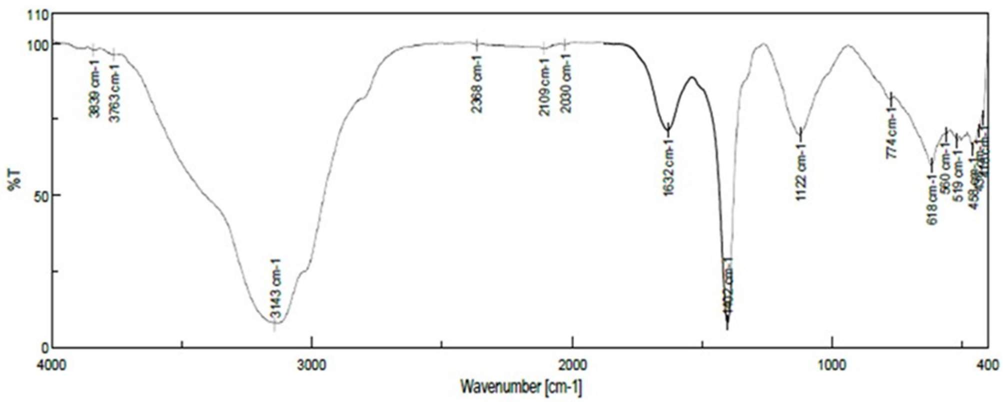

Figure 3: Fourier Transform Infra-Red Spectroscopic analysis of Copper oxide nanoparticle

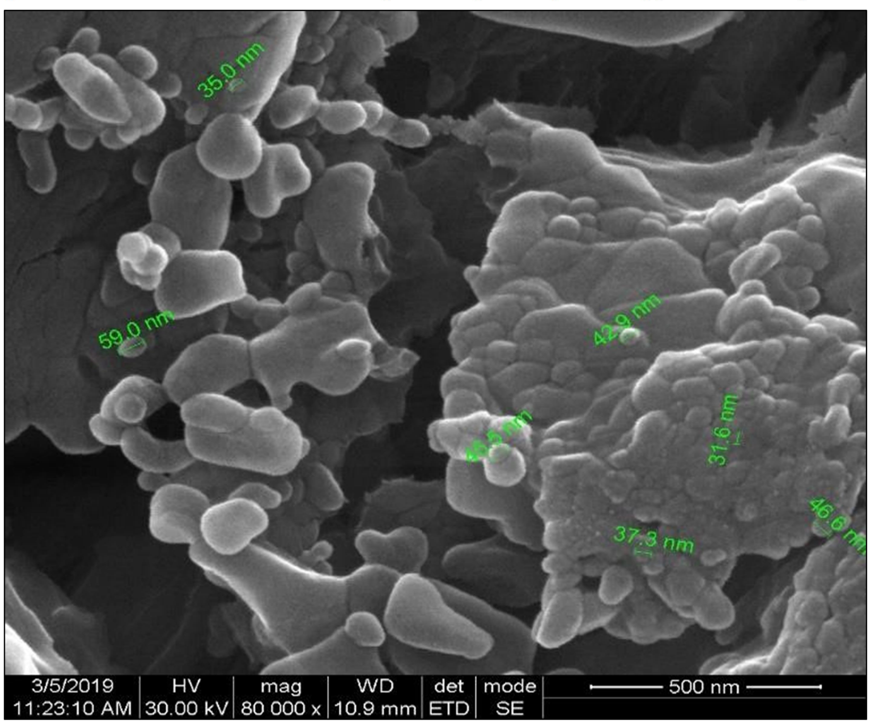

Figure 4: SEM micrographic analysis of green synthesized Copper oxide nanoparticle

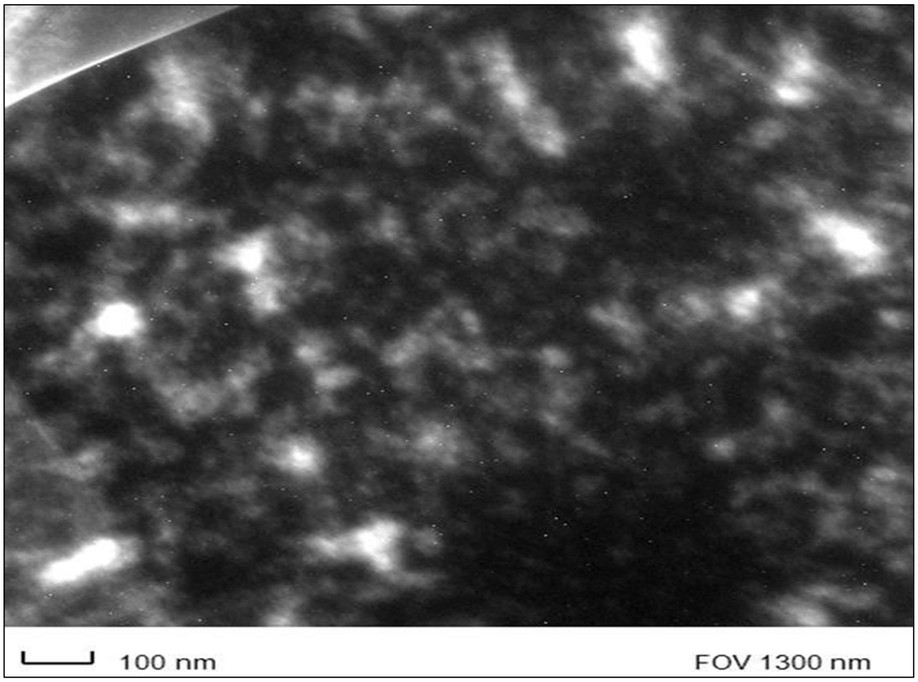

Figure 5: TEM micrographic analysis of green synthesized Copper oxide nanoparticle

Figure 6a: Zone of inhibition formation of green synthesized Copper oxide nanoparticle against *E.coli*

Figure 6b: Zone of inhibition formation of green synthesized Copper oxide nanoparticle against *Staphylococcus aureus*

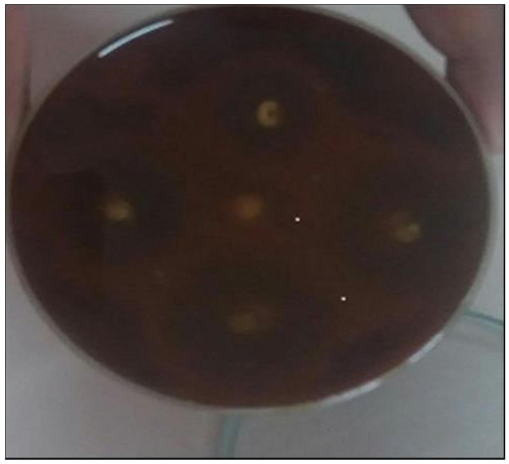

Figure 6c: Zone of inhibition formation of green synthesized Copper oxide nanoparticle against *Aspergillus flavus*

Figure 6d: Zone of inhibition formation of green synthesized Copper oxide nanoparticle against *Aspergillus niger*

Table 1a: Statistical data of antibacterial activity of green synthesized copper oxide nanoparticle against bacterial isolates

Name of the microorganisms	Concentration of greensynthesized copperoxide nanoparticle µg/ml	Mean±standard deviation (SD)	Chi-Square test
Escherichia coli	**50**	**0**	**0.05**
	100	**11.33±0.57**	
	150	**14±1**	
	200	**17.66±1.52**	
Staphylococcus aureus	**50**	**19.66±2.51**	**0.05**
	100	**22.33±1.52**	
	150	**24.66±0.57**	
	200	**28.16±0.76**	

The values are significant at 0.05

Table 1b: Statistical data of antifungal activity of green synthesized copper oxide nanoparticle against fungal isolates

Name of the microorganisms	Concentration of green synthesized copperoxide nanoparticle µg/ml	Mean±standard deviation (SD)	Chi-Square test
Aspergillus flavus	**50**	**26.66±1.52**	**0.05**
	100	**35±2**	
	150	**42±1**	
	200	**44.33±3.05**	

The values are significant at % level 0.05

4.0 CONCLUSION

Thus from the results of the present study, it can be concluded that green synthesized copper oxide nanoparticle could be successfully used as an antibacterial and antifungal agent replacing the conventional drugs which leads to resistance in microbes. Thus green synthesized copper oxide nanoparticle using *Jasminum fluminense* leaves extract is cost effective and eco-friendly when compared to the physical or chemical methods of nano particle synthesis which are expensive and time consuming.

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