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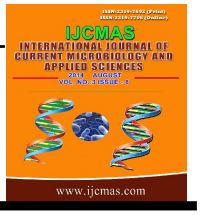

Original Research Article

Synthesis of Copper Nanoparticles from *Nerium oleander* Leaf aqueous extract and its Antibacterial Activity

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Keywords

Copper Nanoparticles, *Nerium oleander*, Antibacterial Activity.

A B S T R A C T

Copper metal ions and their derivatives has been used for different Medical purposes like to prevent infection, leg ulcers etc., The advancement of Nanotechnology, the biogenic synthesis of Nanoparticles have an emerging application towards the Medical field now a days. The copper Nanoparticles is achieved due to the reduction of Copper Sulphate while the aqueous leaf extract of *Nerium oleander* act as a reducing agent. The synthesized Nanoparticles is characterized through the UV-Vis Spectrophotometer and FT-IR. The antibacterial activity of copper nanoparticles was studied against disease causing five bacterial pathogen like *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi and Bacillus subtilis.*

Introduction

Nanotechnology and Nanoparticles based product and application are increased now a days due to the biological effectiveness. However, it is well known that inorganic nanomaterials are good antimicrobial agents. Current research in bactericidal nanomaterials has opened a new era in pharmaceutical industries (Vyom Parashar *et al.*, 2009) Among the various nanoparticles, metal nanoparticles assume special importance because they are easier and cheaper to synthesize and are the most promising in applications.(Pillai Raji K. *et al.*, 2012)

In this study, the green synthesis method is used to produce the Nanoparticles, it is cost effective and main advantage is eco-friendly approach compared to other methods like Laser ablation, arc discharge etc., Some specific plant parts or whole plant specially angiospermic plants are used for the great synthesis of nano particle (Amal Kumar Mondal *et al.*, 2011). Many of them reported

that plant like *Musa sapientum (*Dineshkumar B *et al.,* 2012*) Clerodendrum inerme (*Arshad Farooqui .MD *et al.,* 2010*) Coriandrum sativum (*Sathyavathi. R *et al.,* 2010*) Cleome Viscosa (*Yamini SudhaLakshmi.G *et al.,* 2011*) Saururus Chinenis* (Nagajyoti.P.C *et al.,* 2011*)* are used for the synthesis of Nanoparticles.

Nerium oleander is an evergreen shrub or small tree in the dogbane family Apocynaceae, toxic in all its parts. It is the only species currently classified in the genus *Nerium* (Subbaiya.R *et al.***,** 2014). Herein the leaf extract of *Nerium oleander* is used for the synthesis of Nanoparticles.

Materials and Methods

Collection of Plant Leaf

Nerium oleander leaf was collected from Moolapalayam, Erode District. The collected leaf was tightly packed with Polyethene bag and then transfer to the laboratory. Then it was washed with distilled water twice and kept under room temperature for two weeks in dark condition. Then it was make into powder using blender

Preparation of Plant leaf Extract

The powder of Nerium Oleander leaf was weighed 5g and dissolved in 100ml of distilled water and boiled for 20 min at 50□C. The extract is filtered by Whatmann No1 filter Paper. Then the filtrate is stored in a tight seal pack under 4□C for further use.

Synthesis of Copper Nanoparticles

For a reaction mixture 80ml of 1mM cuso4 and 20ml of Plant leaf Extract was added. Blank is prepared by addition of 80ml of distilled water to 20ml of plant leaf. The reduction of Cu+ was indicated by color change from light color to dark color.

Characterization of Copper Nanoparticles

UV-Spectrophotometer analysis

The synthesized copper Nanoparticles were characterized through UV-Vis spectrophotometer HITACHI U2300. The reduction of copper Nanoparticles was Monitored by UV-spectrophotometer range of absorbance from 250-480nm.

FT-IR analysis

Then the sample mixture is poured into a petridish and kept in a hot air oven until its getting dried off, after that the dried sample is scrubbed, powder form of sample is stored in a sterile eppendroff. Then it is used for the FT-IR analysis from region of 400-4000cm-1 of Cu-Nps from *Nerium oleander* leaf extract.

Antibacterial activity

A five different bacteria (*Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi and Bacillus subtilis*) was taken from the stock culture and dissolved in 25 ml of Nutrient broth kept for incubation 12 hrs. The synthesized copper nanoparticles using *Nerium oleander* leaf extract was tested for antibacterial activity by agar well - diffusion method against *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi* and *Bacillus subtilis*. Then incubated cultures of bacteria were swabbed uniformly on the individual plates using sterile cotton swabs on the Muller Hinton Agar. Three wells were made on 6 mm in diameter on Muller Hinton Agar plates with help of gel puncture using a micropipette, 35µl of

synthesized copper nanoparticle 'A' and Antibiotic 'B' (Gentamycin), Plates were incubated at 37°C for 24 hrs to observe the formation of zone of inhibition.

Results and Discussion

Synthesis of Cu-Nps (Visual Inspection)

After 28 hrs of reaction, The reaction mixture colour change from Light to dark colour, that can be given by below Figure 1, the reduction of Cu+ ions it exhibit the dark colour due to the excitation of Surface Plasmon vibration in a metal nanoparticles.

Characterization of Copper Nanoparticles

UV-Spectrophotometer

The reduction of Cu+ ions was monitored by UV-Vis Spectrophotometer for the metal ions stability. The characterization of copper Nanoparticles by UV-Spectrophotometer from the range 250-450. In these the broad peak was obtained at 325-370 was observed and the graph was represented in Figure 2.

FT-IR Results

The FT-IR Characterization is used to find the molecules and their functional group present in the synthesized copper Nanoparticles. The below Figure 3 represent the FT-IR spectra peaks at 3913.68, 3829.92, 3674.27, 3590.48, 3430.22, 3254.34, 3247.35, 2924.64, 2747.63, 2668.61, 1639.09, 1317.80, 779.69, 1417.70, 1383.92, 1112.67, 617.84, 496.23cm^{-}1. The FTIR spectra revealed the presence of different functional groups like Alcohol(O-H stretch H-bonded,free), Alkane (C-H stretch, -C-H bending) Alkene(=C-H bending, C=C stretch) Amine(C-N, stretch) Nitro compounds (N-O stretch) Acid(O-H,stretch) Ester(C-O, stretch). These functional group plays an very important role in these copper nanoparticles synthesis.

Figure.1 Synthesis of copper Nanoparticles exhibits light colour to dark colour

Figure.2 UV-Spectrophotometer Results for Synthesized Nanoparticles

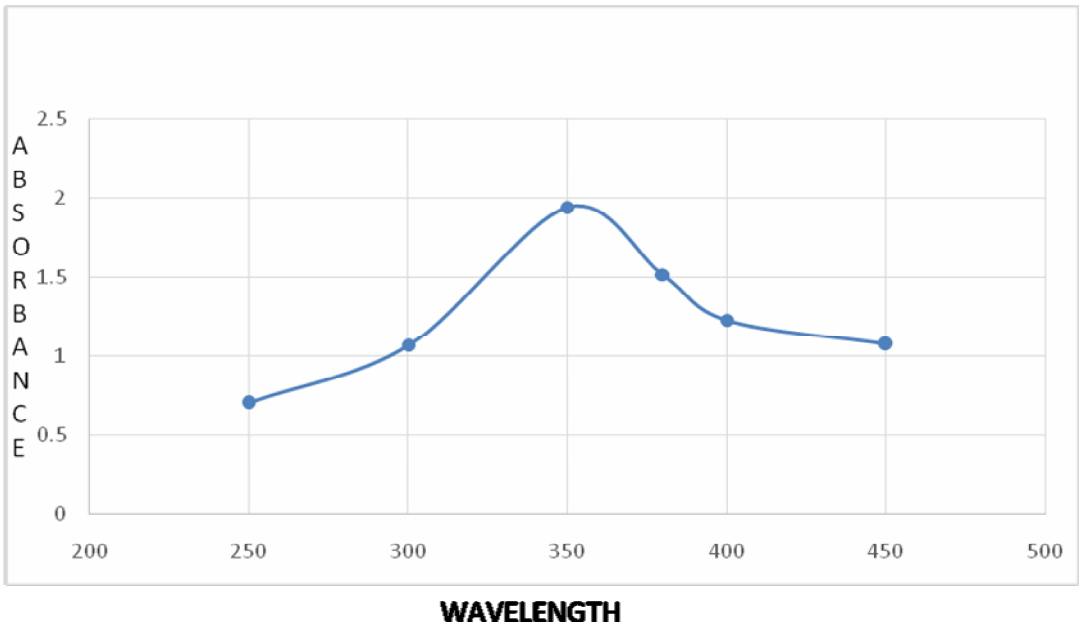
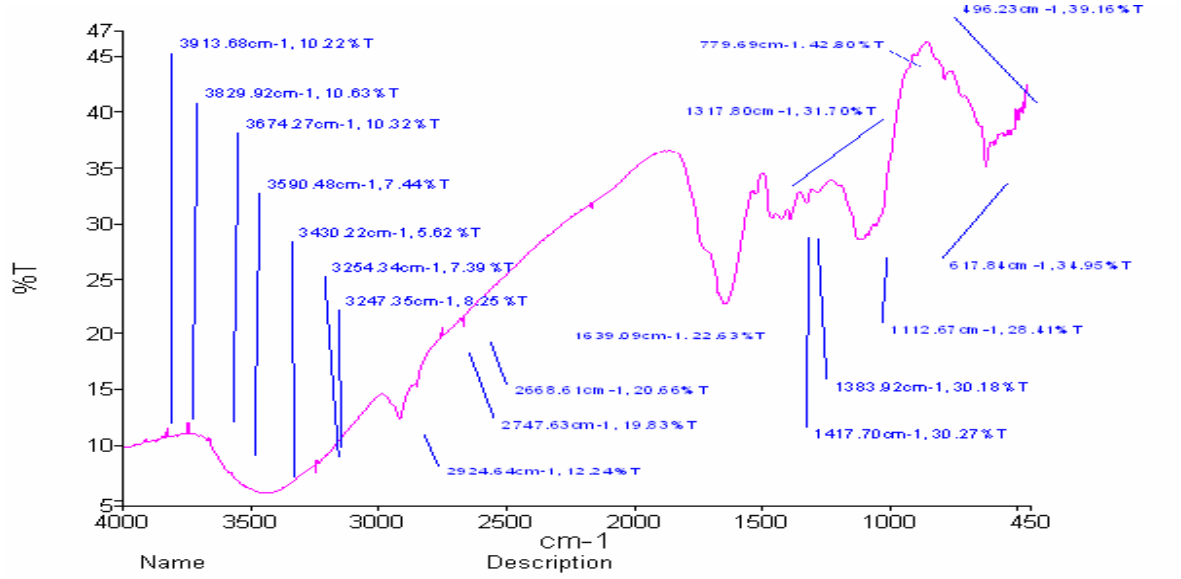


Figure.3 FT-IR Analysis



Antibacterial Activity

The copper Nanoparticles pretence to have an good bactericidal activity so in these work the five different organism like *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi* and *Bacillus subtilis.* In these Gentamycin is acted as a Positive Control, the maximum zone of inhibition on *Staphylococcus aureus* (21mm) and followed by *Salmonella typhi* (20mm), *Klebsiella pneumonia* (18mm) *Bacillus subtilus* (15mm) *Escherichia coli* (10mm). Then maximum zone of inhibition of copper nanoparticles on *Salmonella typhi*

(18mm) and followed by *Bacillus subtilus* (14mm), *staphylococcus aureus* (13mm), *Klebsiella pneumoniae* (10mm), *Escherichia coli* (10mm).

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