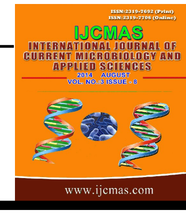
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 9 (2014) pp. 768-774**
http://www.ijcmas.com



Original Research Article

Biosynthesis of Copper Nanoparticles by *Vitis vinifera* Leaf aqueous extract and its Antibacterial Activity

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A B S T R A C T

Keywords

Nano-technology, UV visible Spectro-photometer, FTIR, *Staphylococcus aureus*

Nanotechnology is an emerging field in the area of interdisciplinary research, especially in biotechnology. In this study the copper nanoparticles are synthesized by using a plant leaf extract of Vitis vinifera. The reduction of copper sulphate to copper nanoparticles was confirmed by UV-visible spectrophotometer. An absorption peak at 384nm in UV-visible spectrum corresponds to the Plasmon resonance of copper nanoparticles. The strong band peak at 3431 cm-1 which is the characteristic of N-H stretching vibrations indicates strong hydrogen bonding. Staphylococcus aureus exhibited the highest mean zone of inhibition in copper nanoparticles.

Introduction

A particle has one or more dimensions of the order of 100nm or less" (Paul *et al.*, 2003). Nanotechnology is the field of vast and active area of research. The nanotechnology is rapidly increasing because of their size which brings a change of physiochemical and optical properties of metals. The development of eco-friendly synthesis of nanomaterials is the main aspects of nanotechnology. The cheapest and simplest method for synthesis of nanomaterials is a biological method such as plants, bacteria, fungi which are applicable in many fields such as agriculture, pesticide and pharmaceutical industry (Begam *et al.*, 2009). The biosynthesis of nanoparticles is the intersection of nanotechnology and biotechnology has received increasing attention due to a growing need to develop eco-friendly technologies in material synthesis. Various metal nanoparticles such as silver, gold, copper, platinum, palladium and lead have previously been synthesized using the green synthesis method. The copper nanoparticles have an antifouling, antibacterial activity, catalytic activity which is applicable in gas sensor, wound dressing, super conductors and solar cells (Jung *et al*., 2006). *Vitis vinifera* is a berry known as grape which has been harvested for both medicinal values as well as nutritional values.

A wine is produced from the grape and their seed contain various photochemical content. It can be used to cure skin diseases, eye diseases and the leaves being used to stop bleeding, pain and inflammation of hemorrhoids. Unripe grapes were used for treating sore throats and raisins were used for tuberculosis treatment, thirst and constipation. Ripe grapes were used for treatment of cancer, cholera, smallpox, nausea, kidney and liver diseases. The metal nanoparticles have tremendous application in the area of catalysis, optical, electric, magnetic properties, diagnostics biological probes and display devices (Subbaiya *et al*., 2014).

Materials and Methods

Source

The leaf samples of *Vitis vinifera* were collected from the surrounding areas of Erode and used as a source for experiment. Samples were brought to laboratory in polythene bags and cleaned thoroughly with fresh water to remove adhering debris and associated biota. The plant was cleaned using brush for the removal of the epiphytes with distilled water. After cleaning, leaf was dried.

Preparation of the plant extract

After one week the dried leaves of *Vitis vinifera* were grind into fine powder then 5 gm. of powder sample boiled with 50 ml of sterile distilled water for 5 min in 40°C. Then crude extract was filtered by using Whatmann No.1 filter paper and the filtrates were stored at 4°C for further use.

Synthesis of copper nanoparticles from *Vitis vinifera* extract

The chemical, $CuSO_4$ was purchased from Hi Media Laboratories Pvt. Limited, Mumbai, India. *Vitis vinifera* leaf extract **(**10ml**)** was added into of 40 ml aqueous solution of 1 mM copper sulphate in test tube for reduction into Cu^+ ions and kept for incubation (darkroom) at room temperature. Here the filtrate acts as reducing and stabilizing agent for 1mM of $CuSO_4$. Suitable controls (40ml distilled water + 10 ml Plant extract) in another test tube were maintained throughout the experiments. Reduction of copper sulphate to copper ions was confirmed by the color change from light color to dark brown color. A control setup was also maintained without adding copper sulphate to the plant extract. The formation of copper nanoparticles was also confirmed by spectrophotometric determination.

UV- Vis Spectroscopy Analysis

UV-Vis spectral analysis was done by using UV-Visible Hitachi U-2900 spectrophotometer. The reduction of pure Cu^+ ions was monitored the UV-Vis spectrum of the reaction medium after diluting a small aliquot of the sample into deionized water. 100µl of the sample was pipetted into a test tube and diluted with 10 ml of deionized water and subsequently analyzed at room temperature (Veerasamy *et al*., 2010).

Fourier Transform Infra Red Spectroscopy

To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 5000 rpm for 10 min. The supernatant was again centrifuged at 10000 rpm for 60 min and the pellet was obtained. This is followed by redispersion of the pellet of Cu-NPs into 1 ml of deionized water. Thereafter, the purified suspension was freeze dried to obtain dried powder. Then dried powder was analyzed for FTIR (Shimadzu).

Experimental Microorganisms

Escherichia coli MTCC1692, *Staphylococcus aureus* MTC7443, *Bacillus subtilis* MTCC1133, *Salmonella typhi* MTCC1692 and K*lebsiella pneumonia* MTCC 7407 three different laboratory bacterial strains were used as test organisms. The bacteria were incubated on a nutrient agar slant (Stationary culture) for 24 hrs at 37°C followed by inoculation in Nutrient agar medium.

Preparation of Nutrient Agar Medium

The ingredients of the experimental media dissolved in 800 ml of distilled water except agar and set the pH 7.0 finally made up to 1000 ml and the prepared media, experimental glassware's were sterilized at 121°C, 15 lb for 15 minutes.

Antibacterial Activity

Antibacterial activity was demonstrated using a modification of the method Veerasamy *et al.,* 2010 which is widely used for the antibacterial susceptibility testing. A loop full of bacteria was taken from the stock culture and dissolved in 1 ml of Lb broth kept for incubation 12hrs. The copper nanoparticles synthesized using *Vitis vinifera* leaf extract was tested for antibacterial activity by agar well- diffusion method against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi* and K*lebsiella pneumonia*. The pure cultures of bacteria were swabbed uniformly on the individual plates using sterile cotton swabs on the Nutrient Agar medium. Four wells were made on 6 nm in diameter in the Nutrient Agar medium plates with help of gel puncture using a micropipette. Then add 40µl of synthesized copper nanoparticles solution in well '1' (Centrifuged), Plant extract in '2', Antibiotic in '3' (Chlorompenicol) and sterile distilled water in '4'. Plates were incubated at 37°C for 24 hrs to observe formation of zone of inhibition**.**

Results and Discussion

Synthesis of Copper Nanoparticles

The synthesis of copper nanoparticles from the culture of bacterial strain was performed as shown in Figure1. It is well known that copper nanoparticles exhibit Brown colour in aqueous solution due to excitation of surface Plasmon vibration in copper nanoparticles. As culture filtrate was mixed in to aqueous solution of the copper sulphate, it started to change the colour from watery to Brown due to the reduction of copper ion; which indicated the formation of copper nanoparticles.

UV-Visible Spectroscopy Analysis

It is generally recognized that UV-Visible Spectroscopy could be used to examine size and shape controlled nanoparticles in aqueous suspensions. Absorption spectra of copper nanoparticles formed in the reaction media has absorbance peak at 384nm.

Figure.1 Solutions of copper sulphate before (right) and after (left) exposure to the plant leaf extract of *Vitis vinifera*

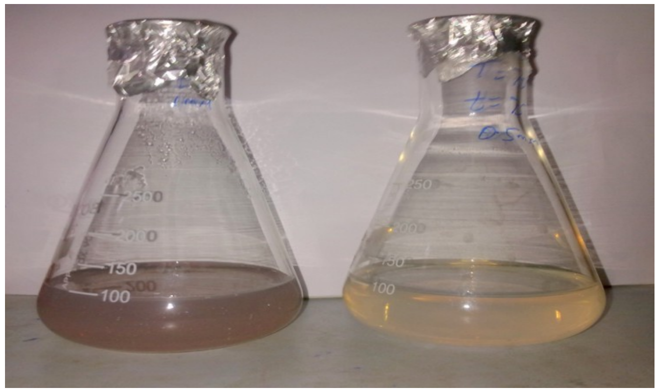


Figure.2 Antibacterial activity of *Staphylococcus aureus*

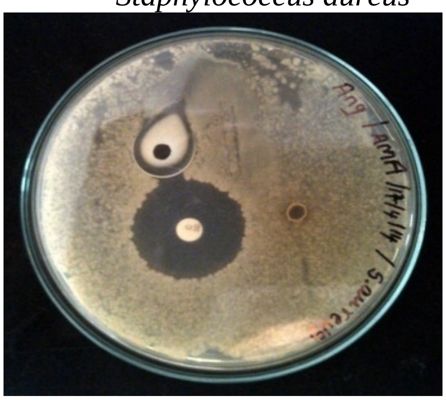


Figure.3 Antibacterial activity of *Escherichia coli*

Figure.4 Antibacterial activity of K*lebsiella pneumonia*

Figure.5 Antibacterial activity of *Salmonella typhi*

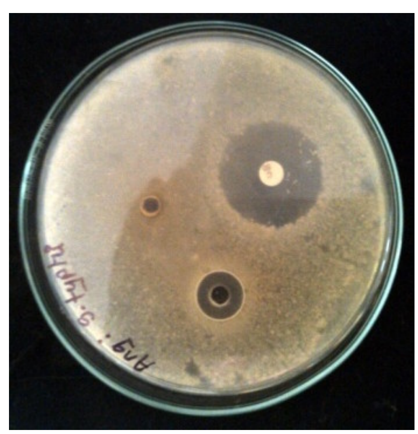


Figure.6 Antibacterial activity of *Bacillus subtilis*

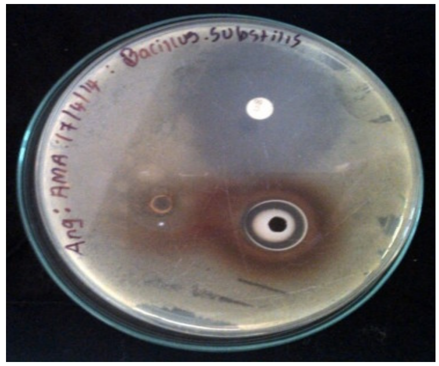
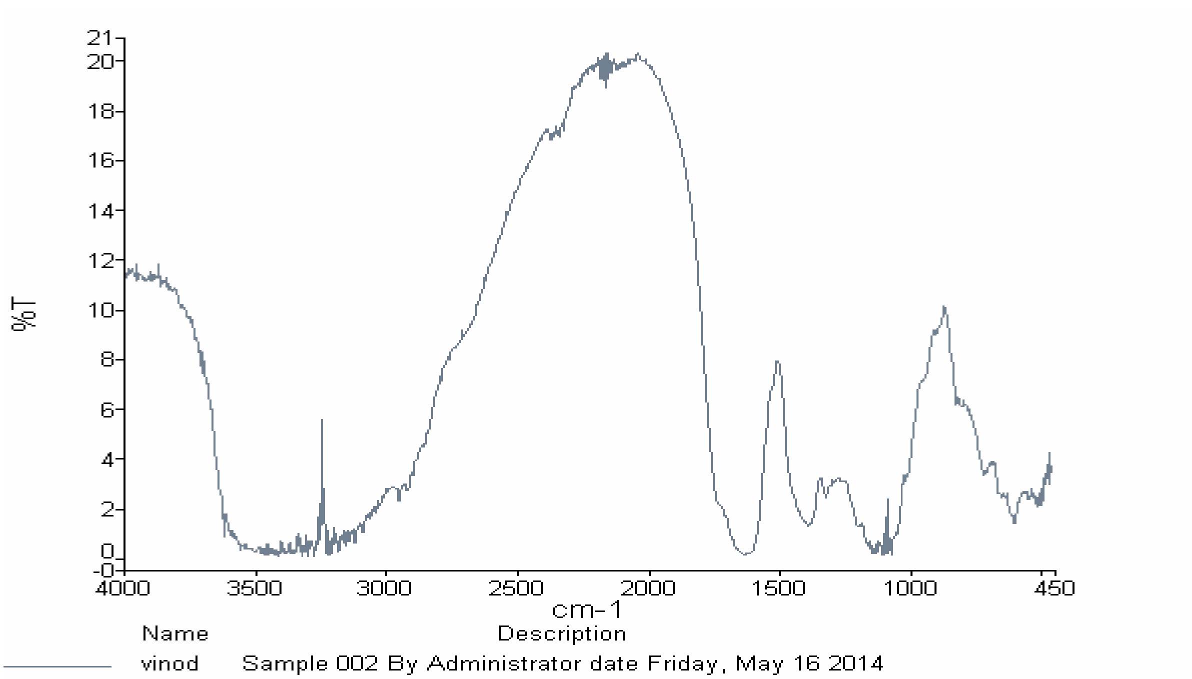


Chart.1 The characteristic FT-IR Spectrum of copper nanoparticles.



Fourier Transform Infra Red Spectroscopy

The characteristic FT-IR Spectrum of copper nanoparticles is illustrated in Chart 1. The strong band peak at 3431 cm^{-1} which is the characteristic of N-H stretching vibrations indicates strong hydrogen bonding. The strong band at 3431 cm^{-1} may result from the N-H stretching vibration. The copper nanoparticle synthesized using *vitis vinifera* showed strong bands at 2520cm^{-1}, 2245 cm^{-1}, 1567cm^{-1} and 1076 cm^{-1}. The overall observation confirms the role of protein in the formation of copper nanoparticle.

Antibacterial activity

The antibacterial effects of biologically synthesized copper nanoparticles have been investigated against *Escherichia coli*, *Staphylococcus aureus*, K*lebsiella* *pneumonia*, *Salmonella typhi* and *Bacillus subtilis*. Observed that a clear zone of growth inhibition was against in *Escherichia coli*, *Staphylococcus aureus*, K*lebsiella pneumonia*, *Salmonella typhi* and *Bacillus subtilis* confirms the antibacterial property of biologically synthesized nanoparticles and higher zones was observed in the well loaded with Chlorompenicol and there is minimum clear zone of inhibition was observed in the well loaded with copper sulphate.

In this susceptibility test, *Staphylococcus aureus* showed (Figure.2) more sensitive in biologically synthesized nanoparticles 18 mm zone of inhibition and followed by *Escherichia coli* (Figure.3) 14mm, and K*lebsiella pneumonia* (Figure 4) 12 mm, *Salmonella typhi* (Figure.5) 8mm, *Bacillus subtilis* (Figure.6) 9mm all the experimental bacteria showed resistant biologically synthesized copper nanoparticles and Chlorompenicol, for the throughout the experiment.

In this study, the positive control of Chlorompenicol has showed the highest antibacterial activity than synthesized copper nanoparticles, and there is no any activity for aqueous plant extract and Sterile distilled water. Among the organisms experimented *Staphylococcus aureus* exhibited the highest mean zone of inhibition in copper nanoparticles and followed by *Escherichia coli*, K*lebsiella pneumonia*, *Bacillus subtilis* and *Salmonella typhi*.

The development of reliable, eco-friendly processes for the synthesis of nanomaterials is an important aspect of nanotechnology today. Biological synthesis process provides a wide range of environmentally acceptable methodology, low cost production and minimum time required. We have studied a simple biotechnological process for synthesis of copper nanoparticles using leaf extract of *Vitis vinifera*. In an antibacterial study the positive control of Chlorompenicol has showed the highest antibacterial activity than synthesized copper nanoparticles, and there is no any activity for aqueous plant extract and Sterile distilled water. Among the organisms experimented *Staphylococcus aureus* exhibited the highest mean zone of inhibition in copper nanoparticles and followed by *Escherichia coli*, K*lebsiella pneumonia*, *Bacillus subtilis* and *Salmonella typhi*.

Acknowledgement

The authors are thankful to, The Management, Head, Department of Biotechnology and Department of Nanotechnology, K.S.Rangasamy College of Technology, Tiruchengode, India for their encouragement and constant support to carry out this work.

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