Synthesis, characterization and antibacterial study of copper oxide nanoparticles

Submitted in partial fulfillment of the requirement for the degree of

BACHELOR OF TECHNOLOGY

IN

DEPARTMENT OF BIOTECHNOLOGY AND BIOINFORMATICS

By

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DECLARATION

We hereby declare that the work reported in this B.tech thesis entitled **"Synthesis, characterization and antibacterial study of copper oxide nanoparticles"** submitted at **Jaypee University of Information Technology, Waknaghat India,** is an authentic record of our work that was carried out under the supervision of **Dr. Gopal Singh Bisht**. I have not submitted this work elsewhere for any other degree or diploma.

…… …… ……

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Date: ……..

SUPERVISOR'S CERTIFICATE

This is to certify that the work reported in the B.tech. thesis entitled "**Synthesis, characterization and antibacterial study of copper oxide nanoparticles**", submitted by **Samriti (141801) and Akshita Gupta(141822)** at **Jaypee University of Information Technology, Waknaghat , India** is a authentic record of their original work carried out under my supervision. This work has not been submitted elsewhere.

……..

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Acknowledgment

We would like to thank everyone from the bottom of our heart who have helped us to complete our project on time either directly or indirectly.

We would like to give a sincere vote of thanks to our guide and mentor **Dr. Gopal Singh Bisht** without whose help, guidance and constant support we won't have been able to complete our project. His constant motivation helped us to complete our project.

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Samriti (141801)

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LIST OF ACRONYMS & ABBREVIATIONS

µl	Micro liter
AgNPs	Silver Nanoparticles
$AuCl_4$	Gold Chloride
CuO	Copper oxide
CuO NPs	Copper oxide Nanoparticles
EDX	Energy dispersive x-ray analysis
FTIR	Fourier transform infrared spectroscopy
LB	Luria-Bertani
MBC	minimum bactericidal concentration
MeNP	Metallic Nanoparticles
MIC	Minimum Inhibitory Concentration
Nm	Nanometer
NP	Nanoparticles
TEM	Transmission electron microscopy
UV-vis	ultra violet-visible
XRD	X-ray diffraction analysis

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CHAPTER 1

INTRODUCTION

1. Introduction

1.1 Nanotechnology

Nanotechnology: In Greek 'Nano is acquired from 'vaos' which mean small. In 1959, Dr. Richard Feynman was given a lecture on "There's Plenty of Room at the Bottom,". This Lecture implement motivation from the field of nanotechology. In 1974 Taniguchi had been originate the word nanotechnology. In 1981 Gerd Binnig and Heinrich Rohrer development of scanning tunneling microscope which are used to see single atoms and band and in 1985 buckyball (c60) was discovered which advance to the development of nanotechnology.

In current years, remarkable development has been made in the emergening field of nanotechnology. Thus, nanotechnology finds an extensive application in manufacturing a great variety of nanoparticles that have a vast use in today's era [1]. Nanotechnology incorporates the synthesis, manipulation and use of the particles in the nanoscale range i.e. 1-100mn. There are many differences that the particles possess at this size range, which are not visible at higher range of size. These small particles also possess large surface:volume ratio, which is a vital feature that is responsible for their use in many industries [2]. In recent years, convergence between nanotechnology' and biology has created the new field of nanobiotechnology that employs the use of biological entities in many biophysical' & biochemical' processes. The synthesis using biological entities through 'nano-biotechnology have a considerable probability to increase synthesis of the nanoparticles with the least or no use of harmful, toxic, & costly chemicals which are commonly' employed in conventional' physical' & chemical processes'[3]. Different methods have proved the synthesis of nanoparticles by precipitation method like co-precipitation and chemical precipitation ,reverse-co-precipitation method and many more[4].Nanoparticles can also be obtained by biological synthesis materials through green chemistry technique. Many strategies are made involved in green synthesis are being focused[5].

1.2 Metallic Nanoparticles

Plants can be used in the syntheses of metallic nanoparticles (MeNPs) by degradation of the metallic ions occupied as soluble salts. Plant metabolites are the possible likely reason for the plant mediated synthesis of metallic nanoparticles. The synthesis of MeNPs can be done by the following steps: "**Induction phase"** include quick degradation of metallic seeds. In **Growth phase** these reactive and unstable crystals quickly accumulate and these get transformed into large accumulates. In **termination phase** the size and shape of these accumulates become approving. In particular, the role of plant metabolism in the synthesis of MeNPs is still not properly known [6].

1.2.1 Important metallic nanoparticles

Gold Nanoparticles:

The range of GNP use in modern medical and biology studies is extremely wide. Gold nanoparticles are used variety of sensors, electronic chips, therapeutic agent delivery (the therapeutic agents can be coated on the particles and can be used as a method of delivery) etc. biomarkers could be detected by the use of gold nanoparticles [7].

Silver Nanoparticles:

Among several nanoparticles that have biomedical applications silver nanoparticles are one of the most important and interesting metallic nanoparticles [2]. Due to their essential therapeutic property silver nanoparticles a wide use in the field of medicine [7]. Though many metallic nanoparticle have been used for many purposes, AgNPs have been concentrated on its applications in diagnosing and treating the cancer [2]

Ceramic Nanoparticles:

Main components of ceramic nanoparticles carbide , oxides , phosphates and carbonates of metals and metalloids such as calcium , titanium , silicon and many more are the main. These nanoparticles find an extensive variety of applications due to many constructive properties, like high resistance against heat and shows no reaction against chemicals. Out of many uses of ceramic nanoparticles, it finds its utmost use in

the field of biomedical. The ceramic nanoparticles are an outstanding carriers for drugs , imaging agents, proteins , genes etc, in the field of biomedicine [8].

Copper oxide nanoparticles:

CuONPs finds gigantic properties like antimicrobial potential against many pathogenic and non pathogenic bacteria [9]. Their antimicrobial and Biocide property have attracted a lot of interest in now days and this property are helpful for biomedical operation. Toxicity of CuO NPs naturally increases in case of differentiated cells rather than non-differentiated cells. Extensive antimicrobial activity of CuO NPs observed against *Bacillus subtilis*

Iron Nanoparticles:

Currently, due the distinctive properties iron nanoparticles (FeNPs) have attracted the eye of many researchers. The reactivity of iron is significant in many applications (chiefly, rusting). Crushed iron nanoparticles are known to ignite on exposure to air, due to this, iron nanoparticles are still not completely explored. This tremendous reactivity has conventionally made the NPs difficult to explore & not convenient for practical use. However, FeNPs have a huge deal to offer for nanoparticles, consisting quite strong catalytic and magnetic properties.

1.3Copper oxide nanoparticles:

Copper oxide nanoparticles are brick red color. The chemical indication is CuO. Density of copper oxide nanoparticles is 6.31 g/cm^3 and molar mass is 79.55 g/mol. Catalysis, protective, diagnostic, therapeutic, cosmetic, food industry and microchip are extensively used by these nanoparticles. Copper oxide are also used as natural farming, Paint industry and wood consumption. The action of infective microbes is conflict by CuONPs. The catalytic and selectivity activity of these nanoparticles are more than that of simple copper oxide particles. At high temperature when copper oxide nanoparticles accommodate hydrogen or carbon monoxide they can be diminished to metallic copper. Their antimicrobial and Biocide property have attracted a lot of interest in now days and this property are helpful for biomedical operation. The optical and magnetic properties of copper oxide are utilized for various applications such as super capacitors, magnetic storage media, sensors, near-infrastructure filters.

CuO nanoparticles toxicity: Toxicity of CuO nanoparticles are altered by different aspects such as size, surface charge, Dissolution. Larger nanoparticles are less toxic than small nanoparticles. Nanoparticles toxicity is increased by positive charge and this charge helps to deals between cell and nanoparticles. In dissolution nanoparticles of copper oxide depends on ph. of the solution and temperature. Toxicity of CuO NPs naturally increase in case of differentiated cells rather than non-differentiated cells. CuO NPs are able to generate cell death, alike at little dose CuO NPs develop cytotoxicity.

Antibacterial action: The antimicrobial action depends on the magnitude and surface property of CuO NPs. Large surface area with small particles having a superior antibacterial activity in comparisons with large particles. In time dependent aspect CuO NPs prohibit the proliferation of *P. aeruginosa, S. aureus and E. coli*. Extensive antimicrobial activity of CuO NPs observed against Bacillus subtilis.

CuO NPs function:

- They are primarily used as antimicrobial agents.
- They can prevent skin irritation.
- It prohibits biofilm formation.
- CuO NPs having wound healing property in which they are used to treat burns and skin damage
- In food packaging industries CuO NPs are used as anti-fouling agents.

1.4 Methods of synthesis of Nanoparticles

1.4.1 Top-Down Approach

Synthesis of metallic nanoparticles can be done using traditional methods that utilizes physical and chemical methods with a top down method. However, such methods are expensive and have a low production rate; moreover, they are harmful as the chemicals used are often poisonous and not easily disposable due to environmental issues [10].

1.4.2Bottom-Up Approach

'Bottom-up' approach is a new area of research is the field of biosynthesis of nanomaterials. Many microorganisms are known to synthesis MeNPs at room temperature and pressure which does not require any toxic chemicals and in-turn does not produce any harmful by-products [10].

1.4.3 Plant mediated method:

The synthesis of MeNPs can be done in the following steps: "**Induction phase"** include quick degradation of metallic seeds. In **Growth phase** these reactive and unstable crystals quickly accumulate and then get transformed into large aggregates. In termination phase, the size & shape of these aggregates become energetically favorable. In particular, the role of plant metabolism in the synthesis of MeNPs is still not properly [11].

Gel Combustion Method:

In this method, molar ratio of 1:1 reducing agent and cupric salt were dissolved in distilled water. High stirring was given to formed solution, so that the solution is converted to a gel. The gel was heated at high temperature to obtain powder. This powder was additionally studied at variable temperature to obtain different size of NPs [12]

1.5 Green Synthesis

Green synthesis has vital application due its environmental effective technique. Now-a-days, the synthesis of nanoparticles using green chemistry has been given a new term "**Green Nano synthesis"**. A vital advantage of this technique is that hazardous substances are generally not used or are used in very less proportion. Plant – mediated Ag nanoparticles and silver nanoparticles using the plant extract is reported by some researchers. The biological method to synthesis the nanoparticles is comparatively simple, cheap, a friendly method than the traditional chemical method used for synthesis of the nanoparticles. Green synthesis is leading methods because of cost effectiveness & environmental approach. These were these reasons, that we included green synthesis method in our study.

1.6 ***HYPERICUM PERFORATUM:***

Fig 1.0 (*Hypericum perforatum* in the campus)

1.6.1 Biological Classification

Table 1: Biological classification

Kingdom	Plantae
Clade	Angiosperm
Clade	Eudicots
Clade	Rosids
Order	Malpighiales
Family	Hypericaceae
Genus	Hypericum
Species	H.Perforatum
Biological name	Hypericum perforatum

St John's wort, Saint John's wort are the typical term of *Hypericum perforatum*. Europe and Asia are the endemic part for St John's wort. The branches are vertical, lengthen to 1m long and branched in uppermost area and the petals are sprinkled luminous spots of glandular material and green-yellow in color. St John's Wort's sepals are sharp along black glandulous spots. Reddish-purple color is made when we mash the seed husk and flower bloom [13].

1.6.2 Chemicals components:

Some of chemicals are involved in Hypericum perforatum such as Flavanoids, Phenolic acids, Naphthodianthrones, Tannins, Volatile oils, Alkanols, Vitamins [14].

1.6.3 Uses:

(1) The plant is helpful for treatment of serotonin disorder when it is mixed with antidepressants drugs.
(2) This plant is used for cure cut, blister and burns.
(3) Hyperforin and Hypericin involve antimicrobial, anti-inflammatory antioxidant and anticancer action [15].
(4) St John's wort leaves extract are used for protection of skin to cosmic rays. They are also used in skin disorder such atopic dermatitis, herpes disease and white skin cancer.
(5) In keratinocytes St John's wort trigger growth and differentiation [16].

CHAPTER 2

REVIEW OF LITERATURE

Gardea-Torresdey *et al* (2007), reported the first ever experimental proof of the synthesis of metallic nanoparticles (MeNPs) from the living vascular plants. It was observed plant *Medicago sativa* (alfalfa) with gold chloride ($AuCl_4$). The second reported species from which the MeNPs were synthesized was *Brassica juncea* (Indian mustard) [17]. Several plant species also have been studied for the production MeNPs other than alfalfa and Indian mustard [18].

Palaniselvam Kuppusamy *et.al* (2014), reported that silver nanoparticles delivered from plant have imposed major impact on treatment and diagnosis of a variety diseases with restricted side effects. The AgNPs derived from plant have wide range of pharmacological applications. Silver nanoparticles were studied for various pharmacological activities like were Antibacterial activity, anti cancerous activity, ant-inflammatory activity and anti fungal activity [19].

Alanazi FK *et. al* (2010), illustrated the properties of nanogold. They also reported chemical physical and biological method to prepare nanogold. They explained that most important application of nanogold are its catalytic and biomedical applications in the industry. They also evaluated the therapeutic and diagnostic & biosensing application of gold nanoparticles [20]

Volland M *et.al* 2018, reported that synthesis method can manipulate characteristics and behavior of the particles, as well as the toxicity of CuONPs. Results further verified that under the controlled conditions aggregating behavior influences the toxicity of CuO. Lastly, the work reported a range of differences in the dissolution and aggregation kinetics of CuO particles under environmental (marine) and cell culture contact conditions that need thought when extrapolating in vitro findings [21].

Hassan MS *et.al.* 2012, reported CuO synthesis using (XRD), (EDX), ultra violet-visible (UV-Vis) spectroscopy & transmission electron microscopy (TEM) technique .these technique proved the size of copper oxide nanocrystal i.e~6 nm. Study of antibacterial was complete against E.coli bacteria and CuO nanocrystal range in minimum inhibitory concentration is about 2.5ug/ml .Study of TEM proved that nanocrystal of CuO cause disruption to the cell envelop leading to cell death [22].

Kattumuri V. *et.al* 2007, confirmed that naturally occurring Gum arabic can be used in the synthesize of willingly biocompatible AuNPs for therapeutic and diagnostic applications in nanomedicine [23].

Rao CN *et.al* 2002, reported that the size determines the chemical reactivity of metallic nanoparticles not only due to the huge surface area but also a result of the considerably different electronic structure of the small nanocrystals. Size is also considered in the assembly of nanoparticles into crystalline arrays[24].

Wiegand I *et.al* 2008, reported susceptibilities of bacteria to drugs and they also assessed the activity of new antimicrobial agents MIC is used. Agar dilution was used to check the antimicrobial activity, the drug was inserted into the agar plate at varied concentrations the swabbing of microbial cells was done. The MIC value was read after the incubation period of the plate. In broth dilution, which was determined in 96-well plate, the bacterial cells were grown in a liquid growth which is done in the presence of antimicrobial agent at variable concentrations. Growth of the bacterial cells was assessed after the incubation time and then the MIC value is taken [25].

Das D *et.al* 2013, reported that thermal decomposition method was used ton synthesized copper oxide NPs. The synthesized nanoparticles were characterized by UV-visible spectroscopy, TEM and XRD analysis. Through characterization it was confirmed that the nanoparticles were spherical in shape with a size of 30nm appx. The synthesized nanoparticles were tested for their antioxidant property. CuONP showed proficient antioxidant activity against *E.coli* and *P.aerugenosa* [26]

Ren G *et.al* 2009, reported that CuO nanoparticles synthesized by thermal plasma technique contained the traces of CuNP & Cu2O NP. The size of the particles were demonstrated by TEM which came in range of 25-90nm. The capability of CuONP to decrease bacterial populations to zero was improved in the presence of sub-MBC concentrations of silver nanoparticles. CuO nanoparticles aggregated polymers propose release of ions may be required for optimum killing [27].

Ameer Azam *et.al* 2012, used gel combustion method to synthesize copper oxide nanoparticles. Through their studies crystallite size of 20 nm was observed when nanoparticles annealed at 400°C. All CuO nanoparticles showed anti-bacterial

activity against both Gram-positive and Gram-negative bacterias. Size dependency was observed in the anti bacterial activity of copper oxide nanoparticles [12].

Yoon KY *et.al* 2007, reported that to assess the antimicrobial characteristics of silver (Ag) and copper (Cu) nanoparticles against *Escherichia coli* and *Bacillus subtilis* nanoparticle susceptibility constant was used. They reported that copper nanoparticles of size 100 nm showed the highest susceptibility against *Bacillus subtilis* whereas the silver nanoparticles of size 40 nm showed lowest susceptibility against *E.coli* [28].

Ruparelia JP *et.al* 2008, synthesized silver and copper nanoparticles of an average size of 3 nm and 9 nm, respectively(as determined through TEM). The anti microbial activity of cooper and silver nanoparticles was checked against *E. coli*, *Bacillus subtilis and Staphylococcus aureus*. The anti microbial potential of silver and copper were checked on the basis of diameter of zone of Inhibition through disk diffusion method, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of nanoparticles dispersed in batch cultures . Anti microbial potential of the nanoparticles varied depending on the species . According to disk diffusion method silver nanoparticles showed greater effectiveness against *E. coli and S. aureus* as compared to copper nanoparticles . Whereas, B. subtilis depicted the highest sensitivity to nanoparticles than other strains and was more unfavorably affected by the copper nanoparticles [29].

Beattie IR *et.al* 2011, prepared silver and gold nanoparticles from plant. The size of the particles came around 2-100 nm and were spherical in shape. They reported that the chloroplasts which contained high reducing sugar (glucose and fructose) content were the sites of the most abundant reduction of metal salts to nanoparticles. It was proposed by them that these sugars are responsible for reducing the metal salts and the amount of reducing sugar determines the quantity of the nanoparticles synthesized [30].

Ericka Rodriguez-Leon *et.al* 2011, used extracts of *Rumex hymenosepalus* they synthesized silver nanoparticles. They used antioxidant molecules as reducing agent, which is rich in this plant.. Characterization of the nanoparticles was done by ultraviolet-visible spectroscopy and transmission electron microscopy . The diameter of

the synthesized nanoparticles came to be in the range of 2 to 40 nm. They reported that two kinds of crystal structures are obtained: face-centered cubic and hexagonal which was confirmed by High-resolution transmission electron microscopy and fast Fourier transform analysis [11].

Alt V *et.al* 2004, reported about in vitro antibacterial activity against multiresistant bacteria and in vitro cytotoxicity of NanoSilver. They reported the size in the range of 5-50cm. In vitro antibacterial activity against S. epidermidis, methicillin-resistant S. aureus (MRSA) and methicillin-resistant S. epidermidis (MRSE) was studied using micro plate proliferation tests . Qualitative on-growth of human osteoblasts and quantitative elution testing was done to study in vitro cytotoxicity. They reported that NanoSilver was free of in vitro cytotoxicity and also showed high effectiveness against multiresistant bacteria [31].

Cruz D *et.al* 2010, synthesized silver nanoparticles (AgNPs) by simple biological method using *Lippia citriodora* leaves aqueous extract as reducing agent. The reduction of silver ions to AgNPs was confirmed by Transmission electron microscopy (TEM), energy-dispersive spectroscopy (EDX), X-ray diffraction (XRD) and UV- visible absorption spectroscopy (UV-vis). Spherical crystalline, stable AgNPs with an average size of the range 15-30nm were obtained. The main compounds found were isoverbascoside, verbascoside, luteonin-7-O-diglucoronide & chrysoeriol-7-O-diglucoronide . The data that was obtained suggests that isoverbascoside is responsible for silver ions reduction and also act as capping agents of the nanoparticles [32].

Suriyakalaa U *et.al* 2013, fabricated nanoparticles at varying temperatures and they were characterized by UV-vis spectroscopy, TEM, FTIR, EDX and ICP-OES. UV-visible spectroscopic readings indicated a major peak at 423 nm. The biologically synthesized nanoparticles were in the size range of 13-27nm that was confirmed by TEM.90.1% of purity of AgNPs was reported that was indicated by EDX [33].

Suman TY *et al* 2013, synthesized silver nanoparticles from the root of Morinda *citrifolia* . The method did not involve any toxic chemical, the method used in the synthesis of the nanoparticles was totally environmental friendly. UV-vis absorption indicated a clear peak at 413nm which clearly revealed the formation of silver nanoparticles. The synthesized silver nanoparticles were capped with plant compounds

and this was confirmed through Fourier transmission infra red spectroscopy (FTIR). Field emission-scanning electron microscopy (FE-SEM) and Transmission electron microscopy (TEM) indicated that the particles were of spherical shape and the size was in the range of 30-55 nm. The crystalline nature of the nanoparticles was indicated by XRD pattern. Silver nanoparticles also proved to show good cytotoxic effect on HeLa cell [34].

Sondi I *et.al* 2003, reported the antimicrobial activity of AgNPs (silver nanoparticles) against gram-negative bacteria. Luria-Bertani (LB) medium solid agar plates were used for bacteriological tests. AgNPs were characterized using Scanning and transmission electron microscopy (SEM and TEM) [35].

CHAPTER 3

MATERIALS AND METHODS

3 Materials used:

3.1 Chemicals used:

- Fehling's solution 1 (Loba chemie, India)
- Fehling's solution 2 (Loba chemie, India).

3.2 Microbiological Culture media

- Mueller Hinton broth,
- Luria broth,
- Luria agar

3.3 Microbial strains used:

Microbial strains were taken from the microbial repository in Jaypee University of Information Technology Waknaghat.

- *E. coli DHα*
- *B. subtilis*

3.3 Plant Material:

Hypericum perforatum leaves were collected from the university campus itself, the authentication of the leaves was done at Dr Y. S. Parmar University , Nauni.

3.4 Preparation of pant extract

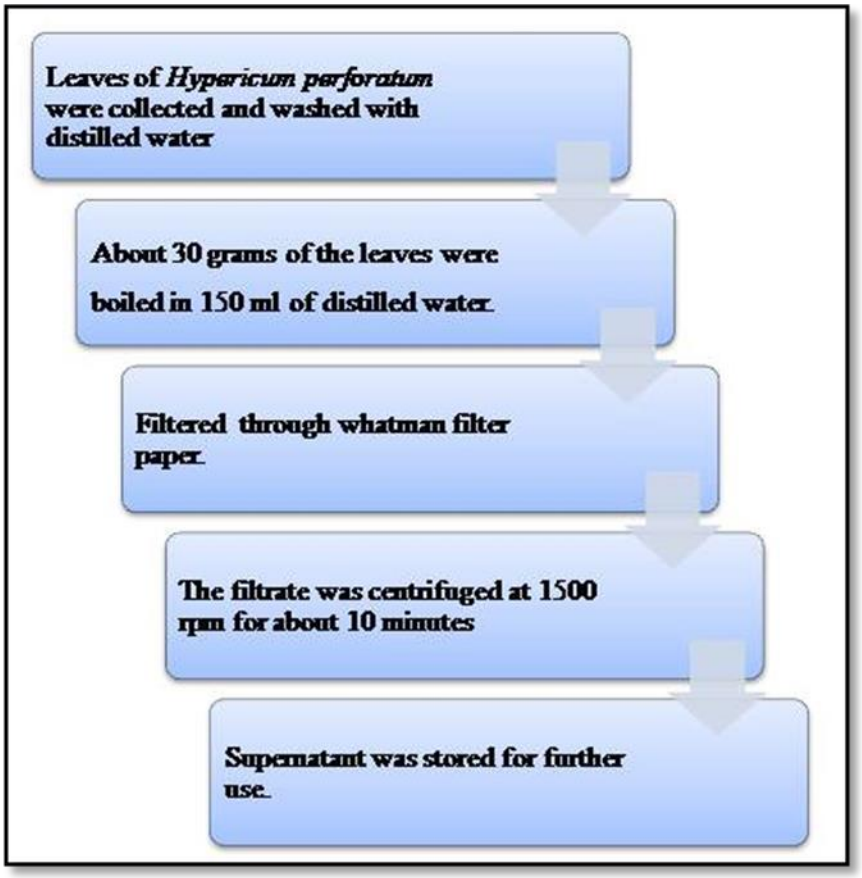

Fig 3.0 (Synthesis of plant extract)

Fig 3.1 (Plant extract Synthesized)

3.5 Green synthesis of copper oxide nanoparticles

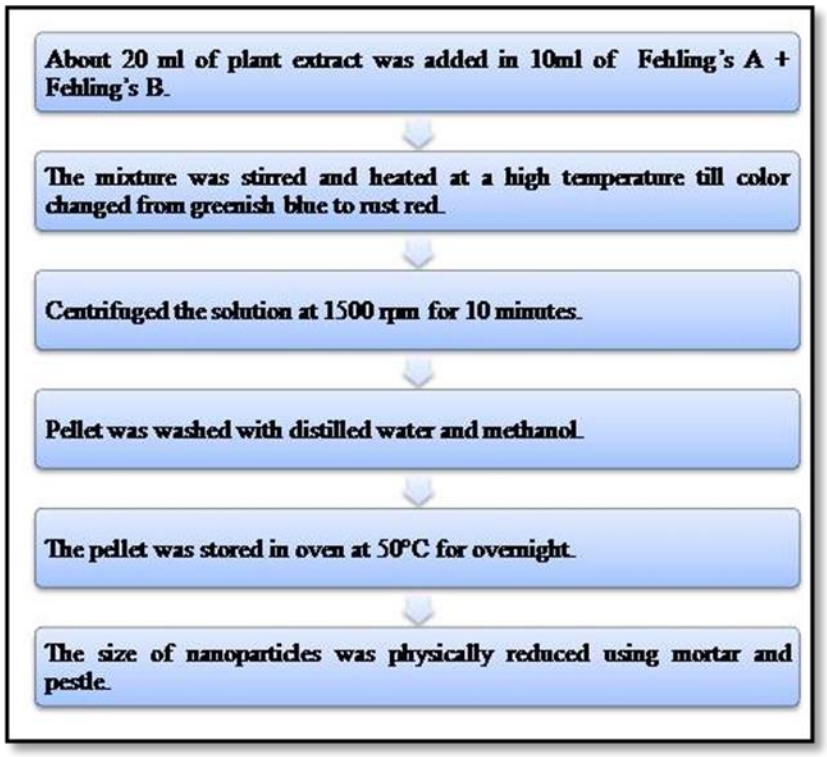

Fig 3.2 (Green synthesis ofCuONP)

Fig 3.3 (Color change to rust green)

Fig 3.4 (Copper oxide nanoparticle solution)

3.6 Characterization of CuO nanoparticles

Synthesized Cu_2O nanoparticles were characterized by UV spectrophotometer, FTIR spectrophotometer and XRD diffractometer, photoluminescence spectroscopy.

3.6.1 UV-vis spectroscopy

Ultraviolet-visible spectroscopy (UV-Vis spectroscopy) refers to absorption of light in the ultraviolet-visible spectral region. UV-vis spectrophotometer is used to determine the quantitative concentrations of the absorber in the solutions of transition metal ions and highly conjugated organic compounds. UV-vis spectroscopy is used to find out the concentration of the absorber in a solution.

3.6.2 FTIR spectroscopy

Fourier-transform infrared spectroscopy come from the fact that they are appropriate to convert organic data into actual spectrum [2]. The components of FTIR is source, interferometer, sample compartment, detector, amplifier, A/D convertor, and computer machine. Source develop radiations through interferometer these radiations are passes the sample and arrive at detector [36]. With the help of amplifier & analogue-to-digital converter signal is amplified and change to digital signal. Finally the signal is passed to a computer machine where fourier transform is executed. Qualitative study of matter is used by this technique .Different functional group present in nanoparticles are identified with the help of ftir [37]. At different wave number every functional group acquire one or more distinctive peak and one specific frequency range multiple functional groups absorb but functional group are arise to distinctive absorptions [38]. In FTIR vibrations, chemical bond movement, bending and stretching can be detected. FTIR spectra shows n number of peaks in copper oxide whereas these peaks are absent in plant extract.

3.6.3 X-ray powder diffraction (XRD)

X-ray powder diffraction is commonly used to determine the crystalline nature of the particles. The basic principal of XRD is constructive interference of monochromatic X-rays and a crystalline sample. X-ray powder diffraction is most commonly used to identify the unknown crystalline materials, characterization of crystalline materials and determination of unit cell dimensions.

3.6.4 Photo luminescence

It is non-destructive technique of penetrating the electronic arrangement of substances. Photo-excitation occurs when sample materials 'absorb light and release out excess of energy when exposed to light. By the emission of light, energy can be consumed by the sample and in instance of photo excitation this luminescence is known as Photoluminescence [39]. When they were excited by electromagnetic radiation source, they exhibited by semiconductor substances. The optical property of molecules and semiconductor are characterized by this technique. Optical device like laser, sensor, medical purpose, sensors, scintillators has been extensively used by photoluminescence of nanomaterials [40].

3.7 Antimicrobial Activity

The synthesized nanoparticles were checked for their antimicrobial potential against *E.coli* DH α and *Bacillus subtilis* using well diffusion method.

- *10* µl of initial inoculum of *E.coli* DH α and *Bacillus subtilis* which were taken from the JUIT microbial repository were grown overnight in MH broth.
- The Luria agar plates were swabbed with *E.coli* DH α and *Bacillus subtilis.*
- The surface of the plate was allowed to dry before punching the wells in the plate with the puncher.
- 20 µl of the standard was loaded in one of the wells.

- 25 µl, 50µl and 100µl of the nanoparticle sample was loaded in the remaining wells .
- The plate was incubated at 37°C for 24 hrs.
- The plate was observed after 24hrs and the zone of inhibition was measured.

CHAPTER 4

RESULTS

4.1 **Production of the nanoparticles**

3.5 mg of Copper oxide nanoparticles were successfully prepared using *Hypericum perforatum* leaf extract and Fehling's A and Fehling's B solution.

Fig4.1

4.2 Characterization of copper oxide nanoparticles

4. 1Uv-vis spectroscopy

UV spectra confirmed the formation of copper oxide nanoparticles. The maximum absorption came to be around 220 nm the results in accordance with studies performed by Arnim Henglein [43].

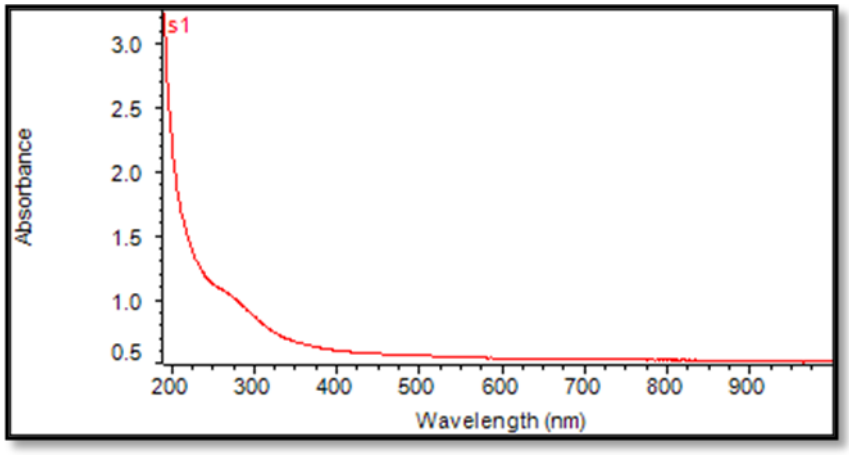

Fig 4.2.1 (UV spectra)

4.2.2 Fourier Transmission Infra-red Spectroscopy

FTIR spectra of plant extract shows broad peak around 3200 to 3600 cm^{-1} due to hydroxyl group whereas these peaks are absent in FTIR spectra of CuO. FTIR spectra of plant extract show IR stretching frequency (1720-1660cm^{-1}; carbonyl bond C-O stretching), (1350-1000cm^{-1}; amine bond C-N stretching) and (800-860cm^{-1}; aromatic C-H stretching). Copper oxide generally gives peaks between 400 to 700 cm^{-1} and in our study also we found CuO stretching vibrations at 665 cm^{-1}. This confirms the formation of CuONPs.

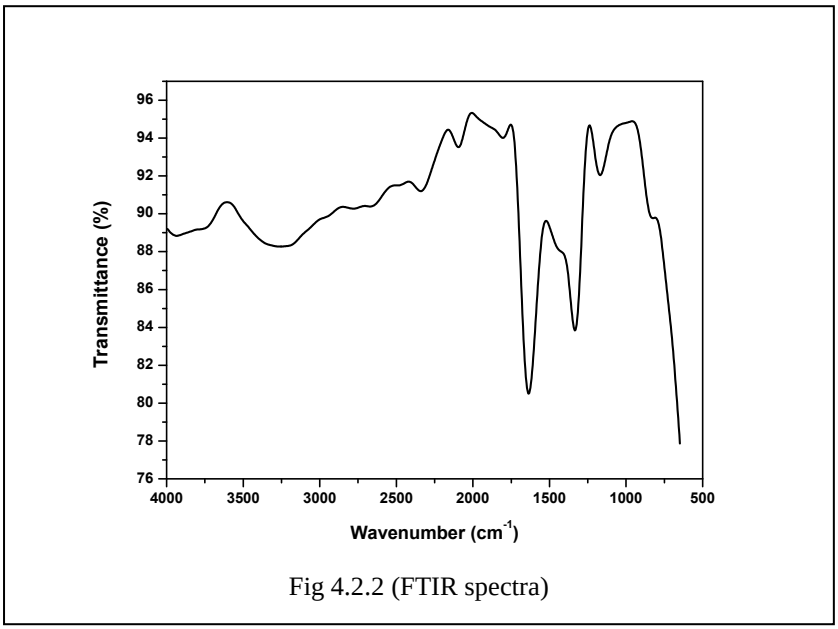

Fig 4.2.2 (FTIR spectra)

4.2.3 X ray powder diffraction

4.2.3a Purity of Sample: Amrut. S. Lanje *et.al* reported that sharp peaks indicate that the nanoparticles are pure and there is no trace of impurity in the sample.[41]. XRD spectra our sample also gave a sharp peak around 36.5° of high intensity, this shows that prepared sample of CuO is pure.

4.2.3b Crystalline size of particles: Scherrer formula was used to calculate the crystalline size of the synthesized nanoparticles using Tiny tools Crystallite size calculator; average crystallite size of particles was found to be 63.82nm.

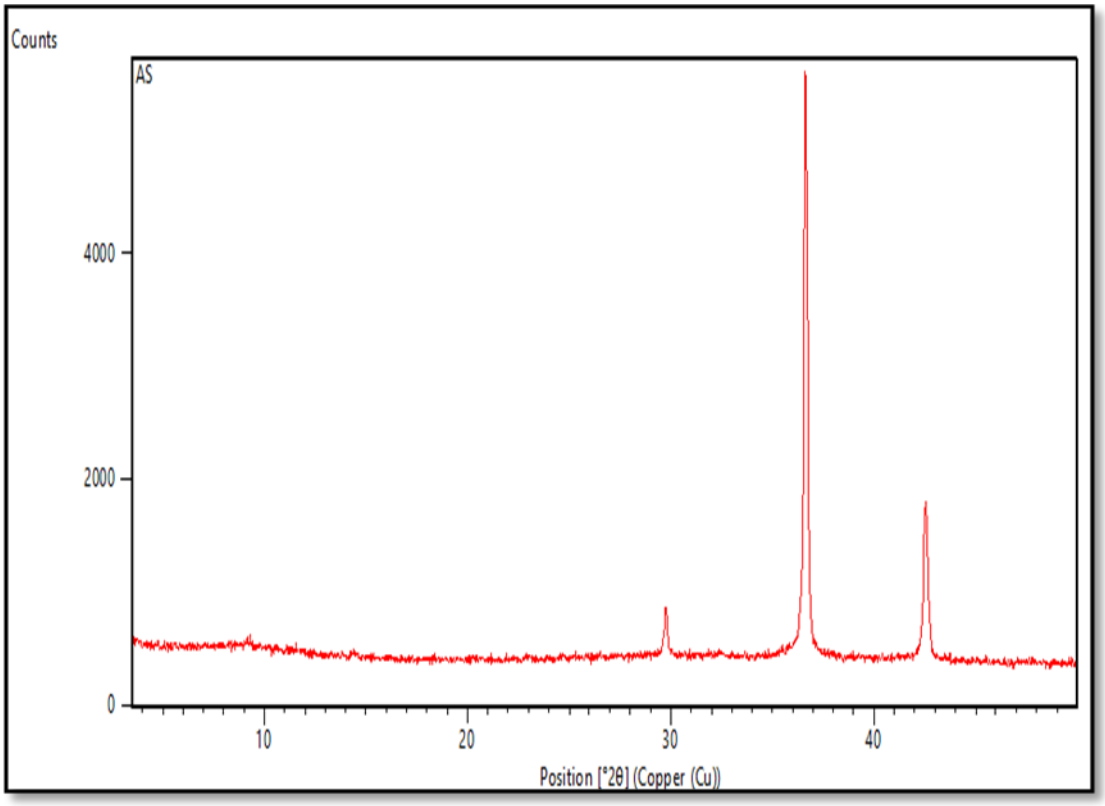

Fig 4.2.3 (XRD Spectra)

4.2.4 Photo-luminescence(PL)

The PL spectrum was plotted using origin software. The photo luminescence spectrum shows the emission peak around 427 nm. This shows that prepared CuONPs have photo luminescent property. The results are in accordance with the results reported by Y.Suresh [41].

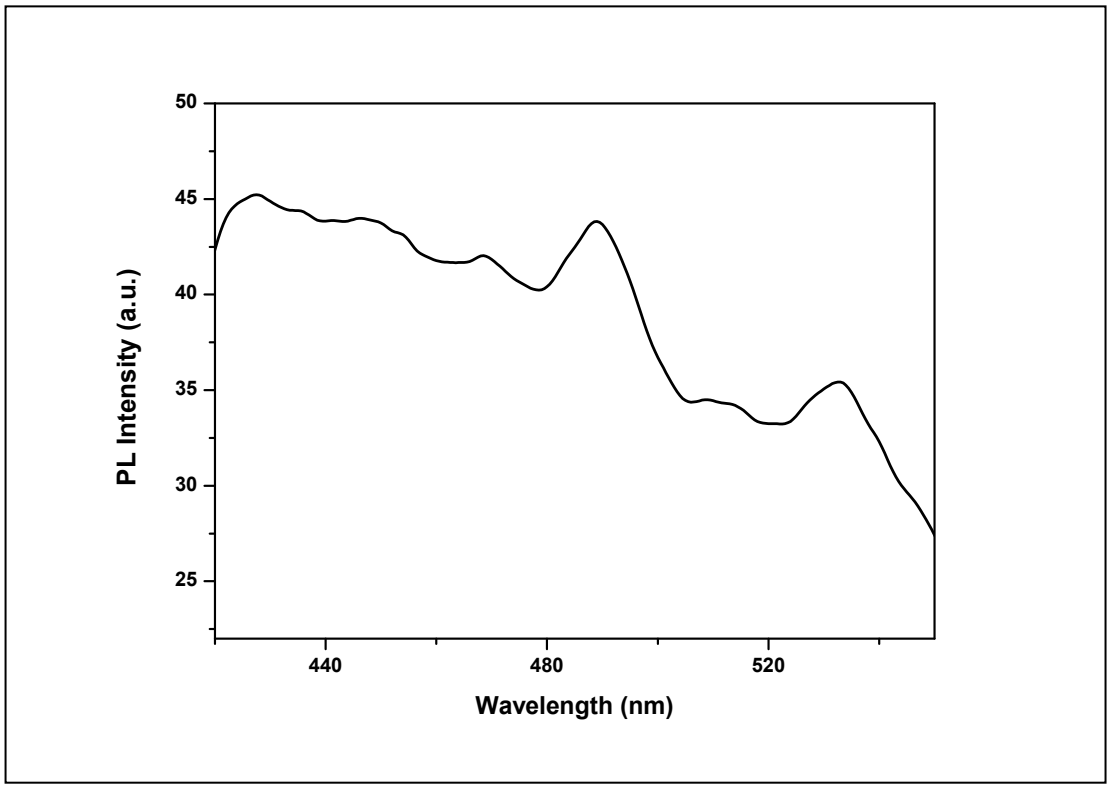

Fig 4.2.4 Photo luminescence spectra

4.3 Antimicrobial Activity

4.3.1 Antimicrobial potential against gram positive bacteria

The results of antimicrobial activity by well diffusion method against *Bacillus subtillis,* are depicted in figure 4.3.1a, 4.3.1b and table 2. From the results it was observed that zone of inhibition increased as the concentration of CuONP increased.

Fig 4.3.1a *B. subtilis at 24 hrs*

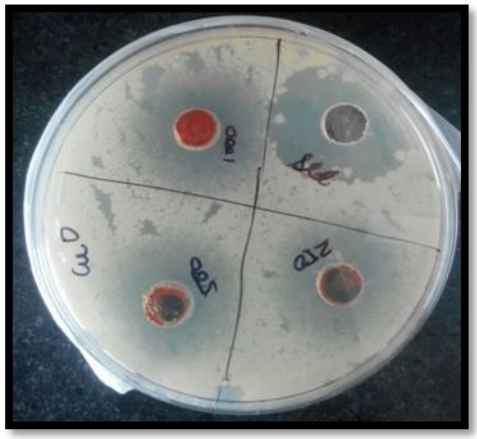

Fig 4.3.1b *B. subtilis at 48 hrs*

Table 2: Zone of inhibition in *Bacillus subtilis*

Sample	Zone of inhibition
Drug	1.1cm
100 µg	1cm
50 µg	0.9cm
25 µg	0.9cm

4.3.2 Antimicrobial Activity against gram negative bacteria

The results of antimicrobial activity by well diffusion method against *Bacillus subtillis*, are depicted in figure 4.3.2a and table 3. From the results it was observed that zone of inhibition increased as the concentration of CuONP increased.

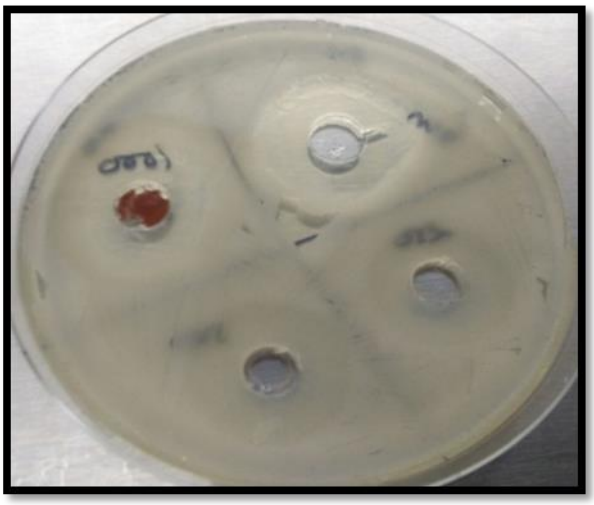

Fig 4.3.2 Antimicrobial activity against *E.coli DH 5α*

Table 3: Zone of inhibition of *E.coli DH 5α*

Sample	Zone of inhibition
Drug	1cm
100 µg	0.9cm
50 µg	0.7cm
25 µg	0.7cm

CHAPTER 5

CONCLUSION

Conclusion

Copper oxide nanoparticles were successfully synthesized from the plant extract of ***Hypericum perforatum*** using the bio reduction- method. The method used was highly economical and environmental friendly as no harmful chemicals were used in the preparation of the nanoparticles.

The presence of high amount of polyphenols in ***Hypericum perforatum*** like flavanoids (flavones, flavanols, isoflavones, flavanonoes etc), phenolic acid (Hydroxybenzoic acids, hydrocinnamic acid) , lignans etc might be the reason for the reduction of copper ions to copper oxide nanoparticles [42] .

The nanoparticles were characterized using UV-vis spectroscopy. FTIR, XRD and photo-luminescence the characterization results clarified the formation copper oxide nanoparticles. UV-vis spectroscopy confirmed the formation of copper oxide. FTIR confirmed the role of plant extract in reduction. Purity and nano size was confirmed by XRD analysis. Photo luminescence of the particles showed its optical properties.

Antimicrobial activity that was performed against *E.coli* DH*5α (*gram –ve) and *B. subtilis* (gram+ve), proved that copper oxide nanoparticles can be used against the microbes. This activity confirmed that copper oxide nanoparticles could be used in food packaging industry.

Chapter 6

Significance of the Project

In current years a remarkable development has been made in the field of nanotechnology. The use of nanoparticles has been amplified in the field of drug delivery therapy, diagnosis etc. They have great applications in medical and commercial industry; various conventional methods that are used in production of nanoparticles require toxic and costly chemicals. That is why production of cost effective and environmental nanoparticles is the need of hour. Therefore, to overcome these problems, new biological methods were utilized in our project. In current study we have synthesized nanoparticles via green synthesis and explored its antimicrobial potential.The copper oxide nanoparticles showed good antimicrobial property, this might have occurred due to higher surface area of nanoparticles, as top down approach was utilized to reduce their size.

In our project, we are reporting for the first time the use of *Hypericum perforatum* for the synthesis of copper oxide nanoparticles. This will encourage the utilization of plants and other biological methods for the synthesis of nanoparticles. It is one of the non toxic, cost effective and environmental friendly methods. By exploring the anti-microbial potential of the nanoparticles that were synthesized, it can be suggested that they could be used to treat antibiotic resistant infections and drug delivery problems in future.

CHAPTER 7

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