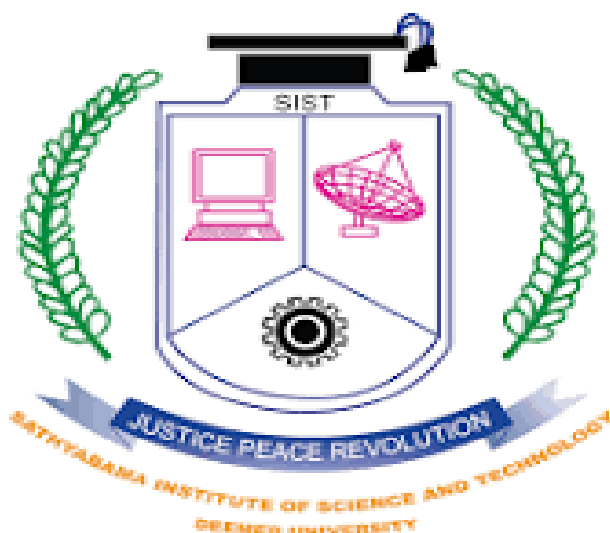


BIOSYNTHESIS AND CHARACTERIZATION OF COPPER NANOPARTICLES USING *PISTACHIO SHELLS*

Submitted in partial fulfilment of the requirement for the award of
Bachelor of Technology degree in Biotechnology

DHANUSH KUMAR .R (41770046)



**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

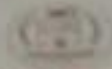
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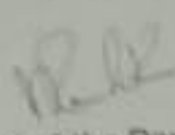
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of Dhanush Kumar H
(41770040) who carried out the project entitled "BIOSYNTHESIS AND
CHARACTERIZATION OF COPPER NANOPARTICLES USING PISTACHIO
SHELLS" done under my supervision from January 2024 to April 2024

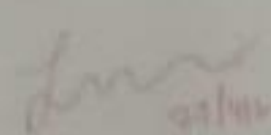

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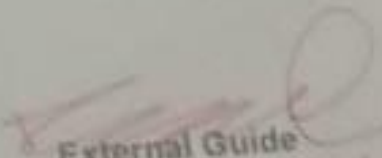

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DECLARATION

I, Dhanush Kumar R hereby declare that the Project Report entitled "BIOSYNTHESIS AND CHARACTERIZATION OF COPPER NANOPARTICLES USING *PISTACHIO SHELLS*" done by me under the guidance of Dr. M. Bavanilatha at Sathyabama Institute of Science and Technology, Chennai, is submitted in partial fulfilment of the requirements for the award of Bachelor of Science degree in Biotechnology.

DATE: 27-4-2024

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Abstract

The minimum size of nanoparticles is 100 nm. Biosynthesis of copper nanoparticles using pistachio shells is an environmentally friendly and sustainable way to create them. Nanoparticles can have a wide range of applications in many fields. The metal is vaporized in a vacuum chamber and then super cooled by a stream of inert gas. The super cooled metal vapour condenses into Nano-meter-sized particles that can be entrained by the inert gas flow and deposited on the substrate or studied in situ. Several different techniques are used for characterization, mostly from material science. Common techniques include scanning electron microscopy (TEM, SEM), atomic force microscopy (AFM), dynamic light scattering (DLS), X-ray photoelectron spectroscopy (XPS), X-ray powder diffraction (XRD), Fourier transform infrared spectroscopy (FTIR). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF), ultraviolet-visible spectroscopy, dual polarization interferometry and nuclear magnetic resonance (NMR). Therefore, this study focused on the biosynthesis and characterization of copper nanoparticles from pistachio shells and their antimicrobial activity studies.

Key words: Green synthesis, copper nanoparticles, Characterization, SEM WITH EDAX, antimicrobial studies.

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ABBREVIATIONS

CU NPs	Copper nanoparticles
E.coli	Escherichia Coli
EDAX	Energy-dispersive X-ray analysis
FE-SEM	Field-Emission Scanning Electron Microscopy
FTIR	Fourier-transform infrared microscopy
MALDI-TOF spectroscopy	Matrix assisted laser desorption/ionization time of flight mass
MIC	Minimal inhibitory concentration
NPs	Nanoparticles
NMR	Nuclear Magnetic Resonance
SEM	Scanning electron microscopy
S.aureus	Staphylococcus aureus
TEM	Transmission electron microscopy
UV-vis	Ultraviolet-visible spectroscopy
XRD	X-Ray Diffraction
XPS	X-ray photoelectron spectroscopy
ZOI	Zone of inhibition

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CHAPTER 1

INTRODUCTION

Nanotechnology is a scientific revolutionary and enhanced due to elaborate research all over the world. Nanoparticles have a minimum dimension of 100nm in size. Nanoparticles are demonstrated by the completely new and enhanced properties. They also offer high surface to volume ratios. These unique particles are divided into three distinct groups: natural, incidental, engineered. This classification is based on the basis of origin, metal nanoparticles, oxides, bimetallic and other inorganic nanoparticles. In the contemporary landscape, the realm of nanoparticles has evolved into a dynamic field of research, capturing the imagination of scientists and engineers alike. (Mott D et al., 2013)

The current state of nanoparticle research is marked by a surge in interdisciplinary efforts, spanning fields such as chemistry, physics, biology, and materials science. Researchers are actively exploring novel synthesis methods, including green and sustainable approaches, to address environmental concerns and promote eco-friendly applications. Over the past few years, considerable interest has been focused on metal nanoparticles due to their potential applications in diverse fields including catalysis, magnetic recording media, or microelectronics. Various methods are now known which enable one to prepare these nanoparticles with controlled size and shape. (Ananth et al., 2015)

The biosynthesis of nanoparticles using natural sources has emerged as an environmentally friendly and sustainable alternative to conventional chemical methods. The pistachio shell, often considered as an agricultural waste, presents an intriguing resource for the green synthesis of copper nanoparticles. Harnessing the inherent reducing and capping agents present in pistachio shells allows for an eco-friendly and cost-effective synthesis route. (Ahamed et al., 2014)

To comprehend the characteristics and properties of the synthesized copper nanoparticles, various sophisticated characterization techniques have been employed. Ultraviolet-visible spectroscopy (UV), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and

assessment of anticancer properties stand as the pillars of this comprehensive investigation. (Abboud et al., 2014)

UV spectroscopy is employed to probe the optical properties of the nanoparticles, providing insights into their size, shape, and stability. XRD analysis aids in elucidating the crystalline nature and crystallographic structure of the nanoparticles. FTIR spectroscopy enables the identification of functional groups on the nanoparticle surface, shedding light on the biomolecules responsible for capping and stabilizing the nanoparticles.

The morphological aspects of the synthesized copper nanoparticles are unveiled through SEM, allowing for a detailed examination of their size, shape, and surface characteristics. Beyond mere physical characterization, this study also ventures into the potential biomedical applications of the copper nanoparticles by assessing their anticancer properties, exploring their role as a promising candidate in the fight against cancer. (Ren G et al., 2009)

In essence, the biosynthesis and characterization of copper nanoparticles using pistachio shells represent a fusion of biotechnology nanotechnology, and biomedical research. The findings of this study not only contribute to the expanding knowledge base in Nano-science but also hold promise for the development of sustainable and multifunctional Nano-materials with diverse applications. (Singh J et al., 2016)

PROPERTIES

Nanoparticles fall in the intermediate size range between bulk materials and individual atoms or molecules. The size of materials approaches the Nano scale (1-100 Nano meters) size-dependent properties start to emerge. The percentage of atoms at the surface becomes significant compared to the bulk. The large surface area relative to the small volume of the nanoparticle interesting and sometimes unexpected properties of nanoparticles therefore largely due to the large surface area of the material, which dominates the contributions made by the small bulk of the material.

An excellent example of this is the absorption of solar radiation in photovoltaic cells, which is much higher in materials composed of nanoparticles than it is in thin

films of continuous sheets of material. In this case the smaller the particles greater than solar absorption.

Other size-dependent property changes include quantum confinement in semiconductor particles, surface Plasmon resonance in some metal particles and superparamagnetism in magnetic materials. Ironically, the changes in physical properties are not always desirable. Ferroelectric materials smaller than 10 nm can switch their magnetization direction using room temperature thermal energy, thus making them unsuitable for memory storage.

Suspensions of nanoparticles are possible since the interaction of the particle surface with the solvent is strong enough to overcome density differences, which otherwise usually result in a material either sinking or floating in a liquid. Nanoparticles also often possess unexpected optical properties as they are small enough to confine their electrons and produce quantum effects. For example gold nanoparticles appear deep red to black in solution.

Clay nanoparticles when incorporated into polymer matrices increase reinforcement, leading to stronger plastics, verifiable by a higher glass transition temperature and other mechanical property tests. These nanoparticles are hard, and impart their properties to the polymer (plastic). Nanoparticles have also been attached to textile fibers in order to create smart and functional clothing.

Metal, dielectric, and semiconductor nanoparticles have been formed, as well as hybrid structures (e.g., core-shell nanoparticles). Nanoparticles made of semiconducting material may also be labeled quantum dots if they are small enough (typically sub 10 nm) that quantization of electronic energy levels occurs. Such Nano-scale particles are used in biomedical applications as drug carriers or imaging agents.

Semi-solid and soft nanoparticles have been manufactured. A prototype nanoparticle of semi-solid nature is the liposome. Various types of liposome nanoparticles are currently used clinically as delivery systems for anticancer drugs and vaccines.

Synthesis

There are several methods for creating nanoparticles, including both attrition and pyrolysis. In attrition, macro or micro scale particles are ground in a ball mill, a planetary ball mill, or other size reducing mechanism. The resulting particles are air classified to recover nanoparticles. In pyrolysis, a vaporous precursor (liquid or gas) is forced through an orifice at high pressure and burned. The resulting solid (a version of soot) is air classified to recover oxide particles from by-product gases. Pyrolysis often results in aggregates and agglomerates rather than singleton primary particles.

Thermal plasma can also deliver the energy necessary to cause evaporation of small micrometer size particles. The thermal plasma temperatures are in the order of 10,000 K, so that solid powder easily evaporates. Nanoparticles are formed upon cooling while exiting the plasma region. The main types of the thermal plasma torches used to produce nanoparticles are dc plasma jet, dc arc plasma and radio frequency (RF) induction plasmas. In the arc plasma reactors, the energy necessary for evaporation and reaction is provided by an electric arc which is formed between the anode and the cathode. For example, silica sand can be vaporized with arc plasma at atmospheric pressure. The resulting mixture of plasma gas and silica vapor can be rapidly cooled by quenching with oxygen, thus ensuring the quality of the fumed silica produced. In RF induction plasma torches, energy coupling to the plasma is accomplished through the electromagnetic field generated by the induction coil. The plasma gas does not come in contact with electrodes, thus eliminating possible sources of contamination and allowing the operation of such plasma torches with a wide range of gases including inert, reducing, oxidizing and other corrosive atmospheres.

The working frequency is typically between 200 kHz and 40 MHz. Laboratory units run at power levels in the order of 30-50 kW while the large scale industrial units have been tested at power levels up to 1 MW. As the residence time of the injected feed droplets in the plasma is very short it is important that the droplet sizes are small enough in order to obtain complete evaporation. The RF plasma method has been used to synthesize different nanoparticle materials, for example

synthesis of various ceramic nanoparticles such as oxides, carbours/carbides and nitrides of Ti and Si.

Inert-gas condensation is frequently used to make nanoparticles from metals with low melting points. The metal is vaporized in a vacuum chamber and then super cooled with an inert gas stream. The super cooled metal vapor condenses in to nanometer-sized particles, which can be entrained in the inert gas stream and deposited on a substrate or studied in situ.

Characterization

Nanoparticle characterization is necessary to establish understanding and control of nanoparticle synthesis and applications. Characterization is done by using a variety of different techniques, mainly drawn from materials science. Common techniques are electron microscopy (TEM,SEM), atomic force microscopy (AFM), dynamic light scattering (DLS), x-ray photoelectron spectroscopy (XPS), powder X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF),ultraviolet-visible spectroscopy, dual polarization interferometry and Nuclear magnetic resonance(NMR).

Therefore, the present investigation was focused on “Biosynthesis and characterization of copper nanoparticles from *Pistachio shells*” and studies on their antimicrobial activity. ”

CHAPTER 2

LITERATURE SURVEY

In a study, Nanotechnology is a set of methods and techniques aimed at obtaining materials with novel functionalities and improved characteristics. Nanotechnology was first postulated by Richard Feynman in 1959 and is considered one of the fastest growing fields of scientific research as well as technology development at world level. The utilization and application of nanoparticles are widely focused on field of chemical, energy, healthcare, and cosmetics (Santos et al., 2015). Nanoparticles exist in organic and inorganic modules constituting ferritins, liposomes, micelles, dendrites and NPs with magnetic properties, metal, semiconductor NPs such as oxides, nitrides and sulphides, respectively (Nabi et al., 2019).

For the study of copper existing as oxide of titanium is recognized as NPs of metal oxides constituting unique optical, thermal, electric, and magnetic properties and these synthesis methods have limitations arising from the use of high energy pressure and temperature as well as from the use of toxic chemicals for synthesizing. Therefore, there is a need for developing environmental and less costly synthesis methods for CUNPs, making it applicable for large scale production. The cost-effective and eco-friendly Cu NPs synthesis is possible through employing green synthesis methods by utilizing biological extracts as reducing agents and can also lead to large scale production of NPs (Edmundson et al., 2014; Chen et al., 1995).

2.2 SYNTHESIS OF COPPER NANOPARTICLE

The Cu nanoparticles were synthesized by chemical reduction process using copper (II) sulphate pent hydrate as precursor salt and starch as capping agent. The preparation method starts with addition of 0.1 M copper (II) sulphate pent hydrate solution into 120 mL of starch (1.2 %) solution with vigorous stirring for 30 min. In the second step, 50 mL of 0.2 M ascorbic acid solution is added to synthesis solution under continuous rapid stirring. Subsequently, 30 mL of 1 M

sodium hydroxide solution was slowly added to the prepared solution with constant stirring and heating at 80 °C for 2 h. The colour of the solution turned yellow to ochre. After the completion of reaction, the solution was taken from the heat and allowed to settle overnight and the supernatant solution was then discarded cautiously. The precipitates were separated from the solution by filtration and washed with deionized water and ethanol for three times to take out the excessive starch bound with the nanoparticles. Ochre colour precipitates obtained are dried at room temperature. After drying, nanoparticles were stored in glass vial for further analysis (Shenmar et al, 2002).

2.3 CHARACTERIZATION OF COPPER NANOPARTICLE

For the study of the copper nanoparticles absorption spectra of the biosynthesized CU NPs were recorded by using JASCO V-670 UV-Vis spectroscopy equipped with a diffuse reflectance attachment for powder samples in between a wavelength scan of 200 and 800 nm. Bio- synthesized CU NPs were characterized by field emission scanning electron microscopy equipped with energy dispersive X-ray spectroscopy (FE-SEM, JEOL-JSM 6500F, made in Japan) (Mukunthan K & Balaji S et al., 2012). The crystalline structure and the average crystalline size of the biosynthesized copper NPs were investigated using an X-ray diffract meter (XRD-7000, Shimadzu Co., Japan) and were recorded with 2θ from 10 to 80 using $\text{CuK}\alpha$ ($\lambda = 1.54056 \text{ \AA}$) radiation operated at 40 kV and 30 mA. The morphology was analysed using high-resolution transmission electron microscopy (HRTEM, Tecnai F20 G2, Philips, Netherlands) at an accelerating voltage of 200 kV.

CUNPs were characterized and recorded using Fourier transform infrared spectroscopy (FTIR, Perkin Elmer 65) to analyse and detect surface functional groups of the bio-synthesized CUNPs nanoparticles at the scanning range of 4000-400 cm^{-1} (R. Shashikant Kuchekar et al., 2017).

Similarly, the optical property of CU NPs with visible spectroscopy is usually used technique in the characterization of nanoparticles. The Ultra-violet (UV) and visible (Vis) commonly known as UV-visible within the 300-800 nm are generally used for characterizing various metal Nano-particles in the size range of 2-100 nm and more (Pal et al., 2007;Gugliotti et al., 2005). The absorption spectrum of UV-

Visible (wave-length versus absorbance) exhibits two peaks at wavelength of 264 nm and 322 nm, that it's unique of CU NPs well in an agreement with their ported results (Maar et al., 2019; Solano et al., 2019). UV-visible spectroscopy has also been used for the confirmation of CU NPs formation in aqueous solution. At different conditions by different scientific studies showed that the reduction of copper ions and the generation of CU NPs are completed after few hours incubation time at room temperature (Schuler et al., 2013; Yeganeh-Faal et al., 2017).

A study conducted in the Fourier transform infrared spectroscopy (FTIR) is a characterization technique used to obtain infrared spectra of absorption, emission, and photo conductivity of solid, liquid, and gas. FT-IR spectroscopy was also employed for all the synthesized CU NPs either by green or other methods to specify the possible functional groups present in the synthesized samples by recording the FTIR spectra's in the range between 4000 and 400 cm^{-1} and It being noticed that CU NPs synthesized by green method showed a similar FTIR spectrum normally, green samples showed absorption band in the range 650 – 700 cm^{-1} , which corresponds to stretching bond. This absorption peak in between the wavenumber range 650-700 cm^{-1} is the characteristic peak of CU NPs anatase. The peaks appearing in between the range of 1000-1300 cm^{-1} can be associated to mainly vibrations which clearly showed O-CU-O bond literature (Irshad et al., 2020; Sethy et al., 2020).

A study characterized in USA, the crystalline phase identification of the prepared nanoparticles in the 2θ range of 20 to 80° was achieved using D8 Advanced X-ray diffract meter from Bruker (Billerica, MA, USA). The machine is equipped with a proportional counter using Cu $K\alpha$ radiation ($\lambda = 1.5405 \text{ \AA}$, nickel filter). The crystallinity and size of crystallites of the synthesized material was often characterized with the help of XRD pattern. The structural information was obtained for the comparison of diffraction pattern of X-rays with the pattern of standardized plane angles of the substance. For the confirmation of crystallinity, the obtained results are matched with the Joint Committee on Powder Diffraction Standards (XRD, JCPDS Card No. 78-2486) (Abisharani et al., 2019; Goutam et al., 2018).

According to X-Ray Diffraction pattern of crystals, this value is linked to the anatase type of CU NPs with the phases (anatase, brookite and rutile). Moreover, this trend is same throughout the research community that anatase form of CU NPs is observed at this specific plane angle. Synthesis method does not affect this property of CU NPs such as the study revealed that both CU NPs either synthesized by sol-gel or green method have the 2θ peak 25.6° . The XRD pattern of CU NPs synthesized by the plant extracts of *T. portulacastrum* and *C. quinoa* compared with sol-gel method and it is confirmed that synthesis method is independent to the crystallinity of CU NPs (Irshad et al, 2020).

In a study of copper nanoparticles, the use of scanning electron microscopy (SEM) made it possible to provide information on the size of CU nanoparticles. The plant synthesized CU nanoparticles are quite poly disperses and they range in size about 120nm. The scale bar is 10 μ M and 20 μ M). The numbers 1, 2, 3, 4, 5 and 6 indicate the points in which the measurement was made. To gain a further insight into the features of CU nanoparticles, the analysis of the sample was performed using EDAX techniques. The nanoparticles are poorly dispersed, with spherical clusters with agglomeration. Agglomeration makes it difficult to study individual nanoparticles. In addition, the presence of CU nanoparticles was confirmed in the sample. Quantification was studied for each of the areas. This confirmed the presence of CU nanoparticles in the sample.

A study examined using the synthesized CU NPs were evaluated for antimicrobial activity against different microorganisms using the disc-diffusion method. Further antimicrobial activity was evaluated by micro dilution method to estimate the minimum inhibitory concentration (MIC) for the synthesized CUNPs. All the tests were done in triplicate. The newly synthesized nanoparticles had higher antimicrobial activity against Gram-positive bacteria than yeast and Gram-negative bacteria, respectively. *P. vulgaris* was the most resistant strain among the tested microorganisms (inhibition zone diameter 7 mm and MIC value 128 μ g/mL) while *S. epidermis* was the most susceptible microorganism (inhibition zone diameter 35 mm and MIC value 8 μ g/mL). (P. Ristivojevic, 2016).

CHAPTER 3

AIM AND SCOPE OF THE PRESENT INVESTIGATION

3.1 Aim

To synthesize and characterize the copper nanoparticles using *Pistachio shells* and to study their anticancer, cytotoxic and anti-inflammatory effects

3.2 Aim and Scope of the Present Investigation

Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Copper nanoparticles exhibit antimicrobial activity. The use of environmentally benign materials like plant leaf extract, bacteria, fungi and enzymes for the synthesis of copper nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. By taking all these views, the present study was designed with the following objectives,

- To synthesize copper nanoparticles using *Pistachio shells*.
- To analyze the synthesized copper nanoparticles using UV-Visible Spectrophotometer.
- To access the synthesized copper nanoparticles using XRD.
- To confirm the chemical compounds of nanoparticles using FTIR.
- To characterize the synthesized copper nanoparticles using SEM and EDAX.
- To study the antimicrobial effect of the synthesized copper nanoparticles against *E.coli* and *S.aureus*.

CHAPTER 4

MATERIALS AND METHODS

The materials used and the methods followed in conduct of various experiments are detailed here.

4.1 Materials:

4.1 Preparation of extracts of Pistachio shells

2.5g of dried *pistachio shells* is weighed and it was crushed without adding water in a pestle and mortar. The content was filtered in a mesh and the filtrate was again passed through what Mann no.1 filter paper (pore size 25 μm). The filtrate then obtained was stored at 4°C for further use.

4.2 Methods:

4.2.1 Preparation of 1mm Copper Sulphate Pentahydrate Solution

24.968g of CuNPs was weighed and it was dissolved in 1000ml of distilled water in 1000ml conical flask. The preparation was mixed and swirled properly. The flask was then covered with aluminium foil in order to prevent the copper sulphate solution from photosensitive reaction.

4.2.2 Synthesis of Copper Nanoparticles

The preparation method starts with addition of 0.1 M copper (II) sulphate pentahydrate solutions into 120 mL of starch solution with stirring for 30 min. 50 mL of 0.2 M *Pistachio shells* solution is added to synthesis solution under continuous rapid stirring. 30 mL of 1 Mm sodium hydroxide solution was slowly added to the prepared solution with constant stirring and heating at 80 °C for 2 hrs. The colour of the solution turned yellow to red. After the completion of reaction, the solution was taken from the heat and allowed to settle overnight and the supernatant solution was then discarded cautiously. The precipitates were separated from the solution by filtration and washed with deionized water and ethanol for three times to take out the excessive starch bound with the

nanoparticles. Red colour precipitates obtained are dried at room temperature. After drying, nanoparticles were stored for further analysis.

4.2.3 Characterization studies

4.2.3.1 Analysis of Synthesized Copper Nanoparticles Using UV-Visible Spectrophotometry

4.2.3.1.1 Aim

To measure the absorbance of synthesized copper nanoparticles under UV-Visible Spectrophotometry.

4.2.3.1.2 Principle

Sources of UV radiation

The production of a continuous ultraviolet (UV) spectrum through the electrical excitation of deuterium or hydrogen at low pressure and process typically involves the formation of an excited molecular species, which subsequently breaks up to release two atomic species and an ultraviolet photon. This process typically involves the formation of an excited molecular species, which subsequently breaks up to release two atomic species and an ultraviolet photon. This can be shown as;



Both deuterium and hydrogen lamps emit radiation in the range 160 - 580 nm. Quartz windows must be used in these lamps, and quartz cuvettes must be used, because glass absorbs radiation of wavelengths less than 480 nm.

Sources of visible radiation

Tungsten filament lamps, also known as incandescent lamps, produce visible light by heating a tungsten wire filament to a high temperature, causing it to glow. The emitted light spans a broad range of wavelengths, typically from around 350 to 2500 Nano meters (nm). The temperature is directly related to the operating voltage. Therefore, the energy emitted is proportional to the fourth power of the operating voltage. Electronic voltage regulators or constant-voltage transformers are employed to ensure a steady and reliable power supply to the lamp.

Tungsten/halogen lamps consist of a tungsten filament enclosed in a small quartz envelope. The envelope is filled with a small amount of a halogen gas, usually iodine or bromine. The tungsten halide compounds are carried to cooler parts of the lamp, such as the envelope walls. When they come into contact with the filament again, the tungsten is re-deposited onto the filament. Tungsten/halogen lamps are known for their efficiency and produce a more consistent and bright light compared to traditional incandescent lamps. They emit light over a broader spectrum, including ultraviolet (UV) radiation.

Wavelength selector (monochromator)

All monochromators contain the following component parts;

- An entrance slit
- A collimating lens
- A dispersing device (usually a prism or a grating)
- A focusing lens
- An exit slit

Polychromatic radiation, which consists of a range of wavelengths, enters the monochromator through an entrance slit. This slit serves to limit the width of the incoming beam and allows only a portion of the radiation to enter the instrument. The incoming beam is then collimated, meaning that the rays of light are made parallel. This is often achieved using lenses or mirrors to ensure that the light is incident on the dispersing element as parallel rays. The collimated beam then strikes a dispersing element, which can be either a diffraction grating or a prism. The exit slit is a narrow aperture that can be adjusted to allow only a specific wavelength or a narrow range of wavelengths to exit the monochromator.

Cuvettes

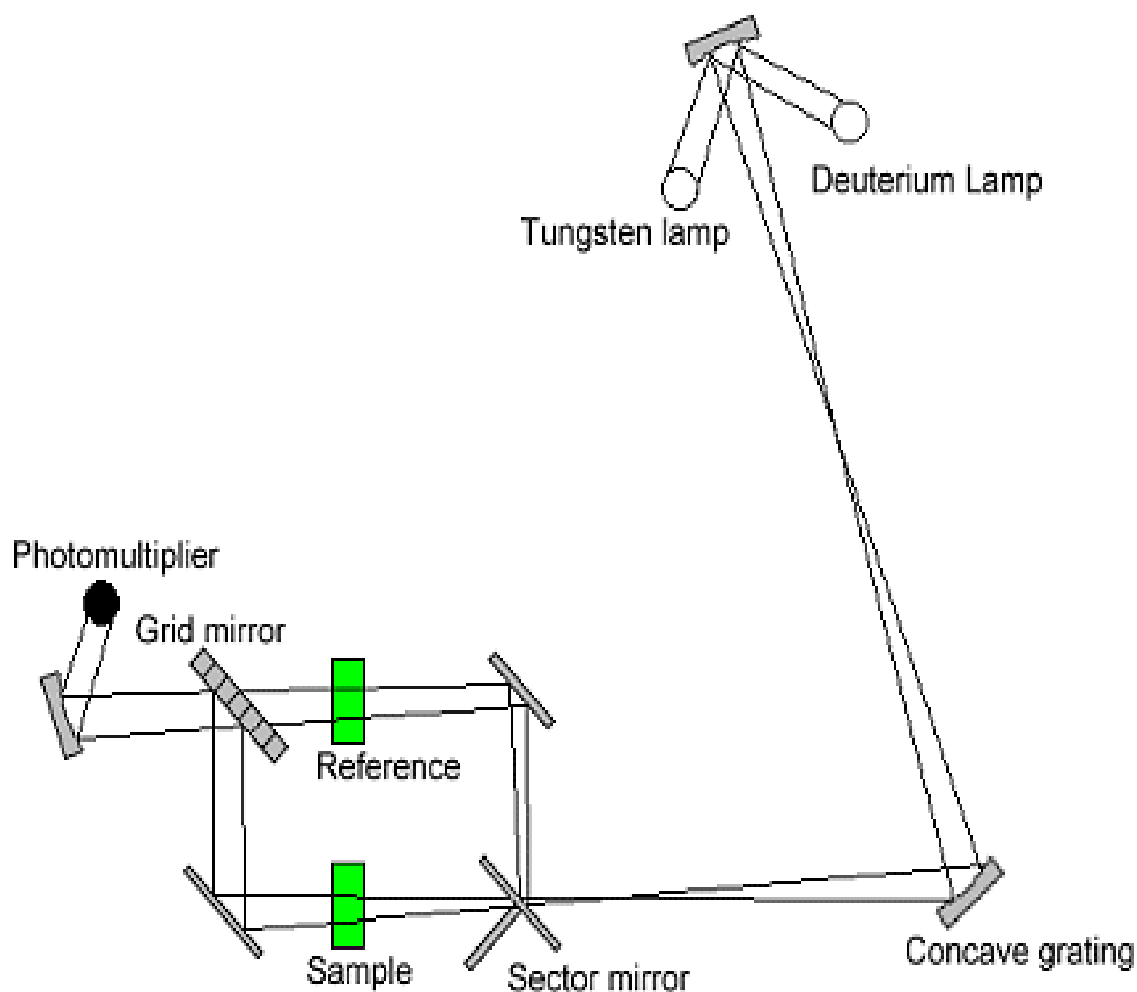
Quartz and fused silica cuvettes not only transmit UV light but are also transparent in the visible region of the spectrum. This makes them versatile for spectroscopic measurements that cover both UV and visible wavelengths. Silicate glasses can be used for cuvettes designed for a broader spectral range, typically between 350 and 2000 nm.

Detectors

This is the entrance point for incoming photons. When photons strike the photocathode, they release electrons through the photoelectric effect. A series of dynodes follows the photocathode. Each dynode is at a higher voltage than the previous one. When an electron strikes a dynode, it releases several secondary electrons. This process is repeated through multiple dynodes, resulting in a cascade of electrons amplifying the signal. The final stage of the PMT is the anode, where the multiplied electrons are collected and produce a measurable electric current.

A photon of radiation enters the photomultiplier tube and strikes the photocathode, causing the emission of electrons through the photoelectric effect. These emitted electrons are accelerated towards the first dynode, which is typically at a higher potential (e.g., 90V) compared to the cathode. The multiplied electrons eventually reach the anode, where they are collected. The resulting current is proportional to the number of electrons produced, which in turn is proportional to the intensity of the incident light. The amplification factor, represented by the ratio of output electrons to input photons, is typically on the order of $10^6 - 10^7$. The amplified current at the anode is then measured and used to quantify the intensity of the incoming light.

Fig 4.1: Working principle of double beam UV- visible Spectrophotometer



Source:

<http://teaching.shu.ac.uk/hwb/chemistry/tutorials/molspec/dbspec2.gif>

Fig 4.1: UV-Visible spectroscopy of copper nanoparticles

Photomultipliers are very sensitive to UV and visible radiation. They have fast response times. Intense light damages photomultipliers; they are limited to measuring low power radiation.

4.2.3.1.3 Procedure

The reduction of pure Cu^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample into distilled water. 1ml of the sample solution was mixed with 2ml of sterile distilled water in a glass quartz cuvette. The contents were pipetted in and out to enhance proper mixing. *Pistachio shells* was of 3ml alone was served as blank. After keeping the blanks in the sample compartment, the baseline correction was made. Out of the two blanks, one blank was replaced with the sample solution and it was scanned from 800nm to 200nm. UV-Vis spectral analysis was done by using double beam UV-Vis spectrophotometer (SpectrascanUV-2700, Thermo Scientific).

4.2.3.2 Characterization of Synthesized Copper Nanoparticles Using SEM and EDAX

4.2.3.2.1 Aim

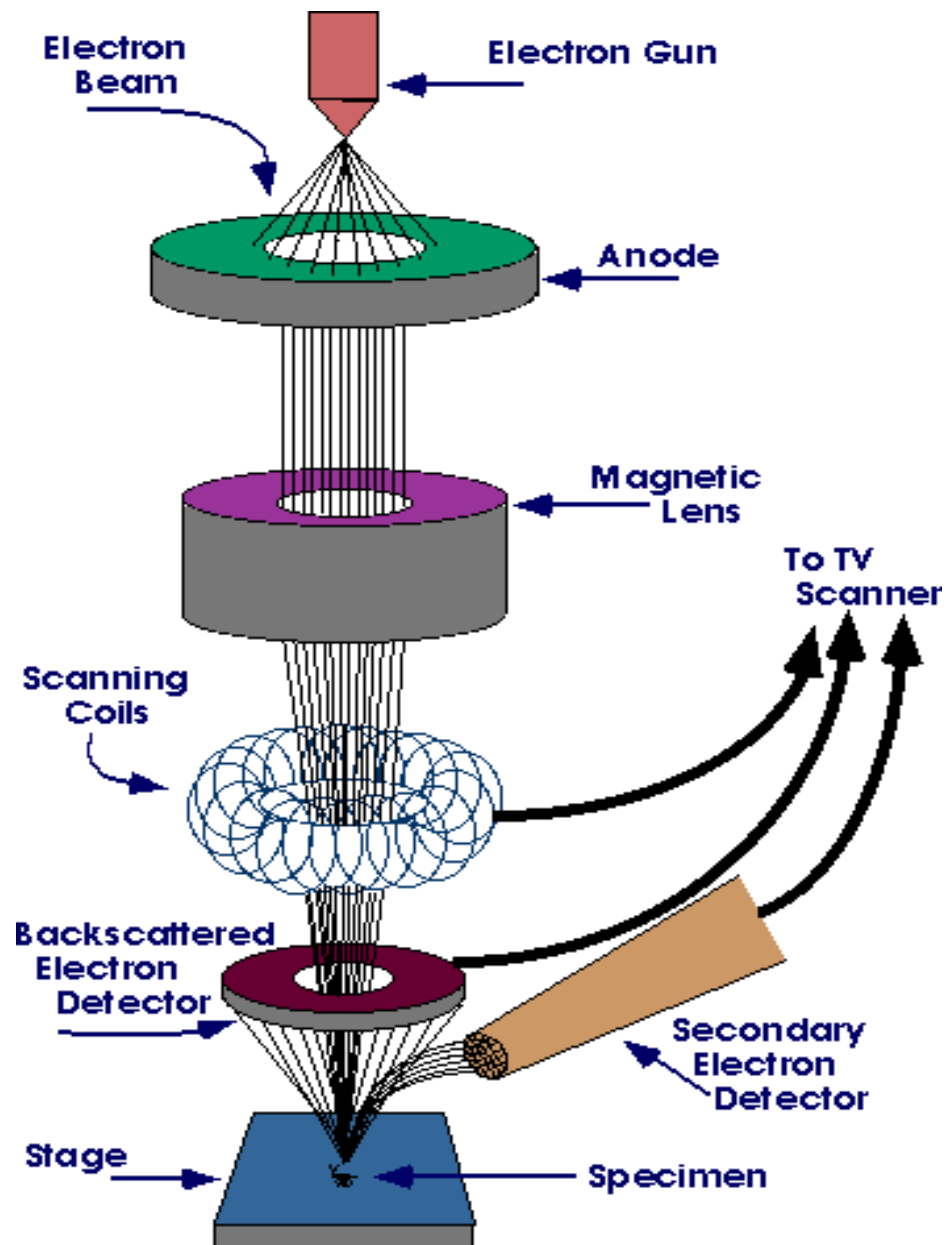
To characterize the synthesized copper nanoparticles using SEM

4.2.3.2.2 Principle

Rutherford elastic scattering, which provides information about the atomic structure and composition, the interaction of high-energy incident electrons with the conduction band electrons can lead to various other processes. Inelastic scattering occurs when the incident electron transfers some of its energy to the sample electrons, causing them to move to higher energy levels. The scattered electron loses a small amount of energy and changes direction. This process contributes to the overall signal detected in the electron microscope and can provide information about the electronic structure of the material. Ionization involves the removal of an electron from an atom in the sample, resulting in the formation of a positively charged ion. This process is particularly relevant when studying the elemental composition of a sample. The detection of ionized species can help identify the types of atoms present and their concentrations. Excitation occurs when the incident electron imparts energy to an atom in the sample, causing one of its electrons to move to a higher energy level without being completely removed. The subsequent relaxation of the excited electron back to its

original state may lead to the emission of characteristic X-rays, which can be detected to obtain elemental information about the sample.

Fig 4.2: Working principle of SEM



Source: <http://www.purdue.edu/rem/rs/graphics/sem2.gif>

Fig 4.2: Working principle of SEM

Secondary electrons have a limited range in the sample, typically within Nano-meters of the surface. This characteristic contributes to the high resolution of topographical images in SEM, as only those secondary electrons produced near the surface can escape and be detected. SEM is widely used for obtaining high-resolution topographical images due to its ability to capture surface details at the Nano-scale. High-energy primary electrons in SEM can cause impact ionization in semiconducting samples, promoting valence band electrons to the conduction band. The subsequent recombination of electron-hole pairs can be studied, providing information about the material's properties. The recombination of electron-hole pairs may result in the emission of visible or near-visible light. Spectroscopy of this emitted light allows researchers to understand the recombination processes, and scanning the electron beam enables the mapping of spatial variations in luminescence. A high vacuum is required for generating a focused electron beam. Therefore, SEM instruments typically house the beam, specimen, and detector in a vacuum enclosure to maintain optimal conditions for electron beam focusing. The useful magnification in SEM is defined by the size of the focused spot projected on the specimen. This magnification is ultimately limited by the diffraction of the focused electron beam. Electronic displacement of the beam allows the analysis of multiple particle fields without moving the stage. Stage motion is reserved for larger field displacements.

4.2.3.2.3 Procedure

Scanning Electron Microscopic (SEM) analysis was done for the synthesized copper nanoparticle from *Pistachio shells* in Centre for Advanced studies, Sathyabama University, Chennai using SEM machine.

4.2.3.3. Characterization of Synthesized Copper Nanoparticles Using EDAX

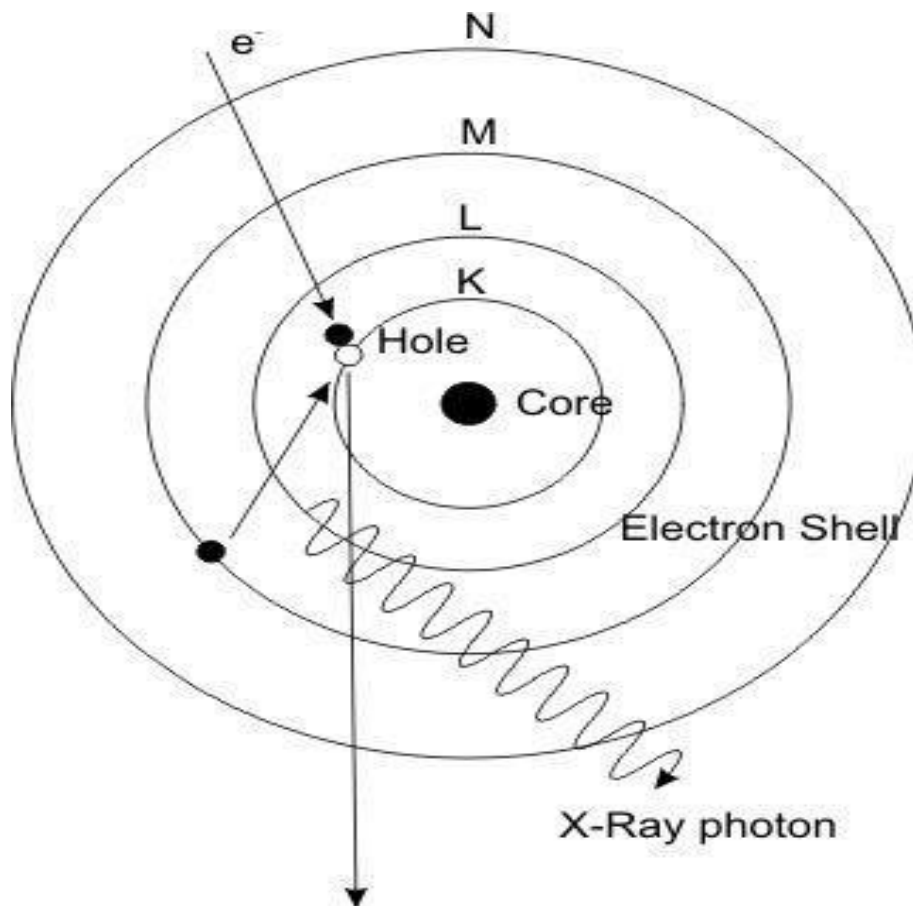
4.2.3.3.1 Aim

To characterize the synthesized copper nanoparticles using EDAX

4.2.3.3.2 Principle

An outer-shell electron then fills the vacancy created in the inner shell. This transition releases energy. The more relevant pathway here is the emission of X-rays. The energy released during the transition of an outer-shell electron filling an inner-shell vacancy can be in the form of X-rays. EDAX is a technique used to analyze the X-ray spectrum produced during electron interactions with a sample. EDAX is often coupled with SEM, enabling the simultaneous acquisition of high-resolution images and elemental information.

Fig 4.3: Working principle of EDAX



Source - <http://www.globalsino.com/micro/1/images/micro9999.jpg>

Fig 4.3: Working principle of EDAX

The composition of the material can be deduced. This can be used to provide quantitative information about the elements present at a given point on the sample, or alternatively it is possible to map the abundance of a particular element as a function of position.

4.2.3.3.3 Procedure

EDAX analysis was done for the synthesized copper nanoparticle from *Pistachio shells* in the Centre for Advanced Studies, Sathyabama University, and Chennai.

4.2.3.4. Characterization of Synthesized Copper Nanoparticles Using FTIR

4.2.3.4.1 Aim

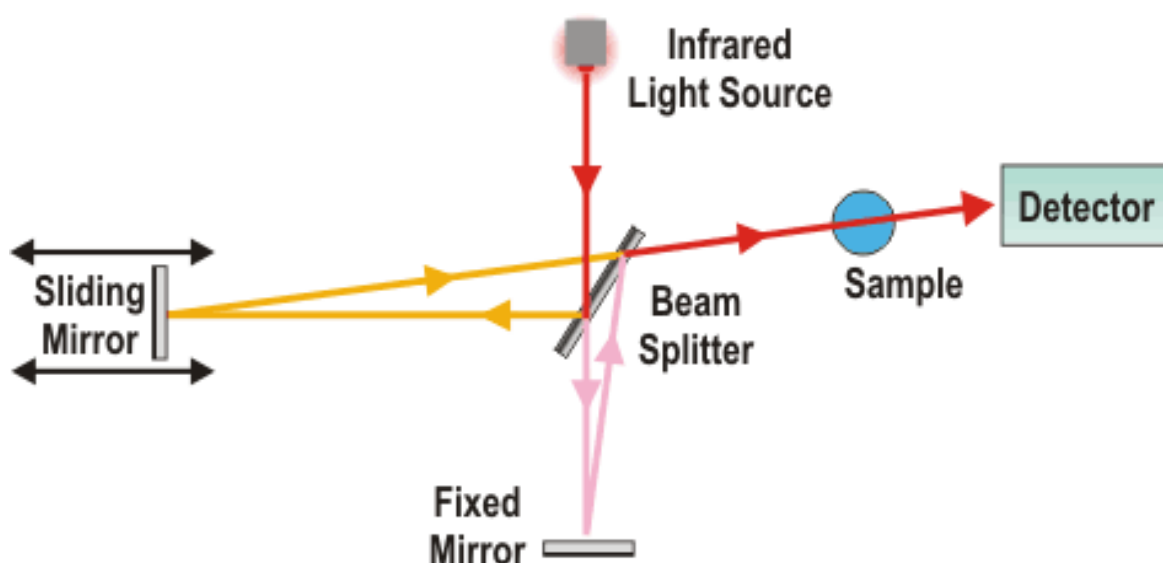
To characterize the synthesized copper nanoparticles using FTIR

4.2.3.4.2 Principle

The spectroscopy is based on the idea that the interference of radiation between the two beams yields an interferogram. The latter is a signal produced as a function of the path length change between the two beams reflected from two mirrors (resides in the interferometer block). The two domains of distance and frequency are interconvertible by the mathematical method of Fourier transformation hence the name Fourier transform infrared spectroscopy.

The main difference between an FTIR spectrometer and a dispersive IR spectrometer is the interferometer. The interferometer is the core of FTIR spectrometers and is used to split one beam of light into two creating the different paths of the two beams. Then it recombines the two beams and conducts them into the detector where the differences in the intensity of these two beams are measured as a function of the difference in the paths.

Fig4.4: Working principle of FTIR



Source:<https://www.tribonet.org/wiki/fourier-transform-infrared-spectroscopy/>

Fig 4.4 Working principle of FTIR

4.2.3.4.3 Procedure

FTIR analysis was done for the synthesized copper nanoparticles from *Pistachio shells* in the Centre for Advanced Studies, VIT University, and Vellore.

4.2.3.5. Characterization of Synthesized Copper Nanoparticles Using XRD

4.2.3.5.1 Aim

To characterize the synthesized copper nanoparticles using XRD

4.2.3.5.2 Principle

X-ray diffraction is based on constructive interference of monochromatic X-rays and a crystalline sample. These X-rays are generated by a cathode ray tube,

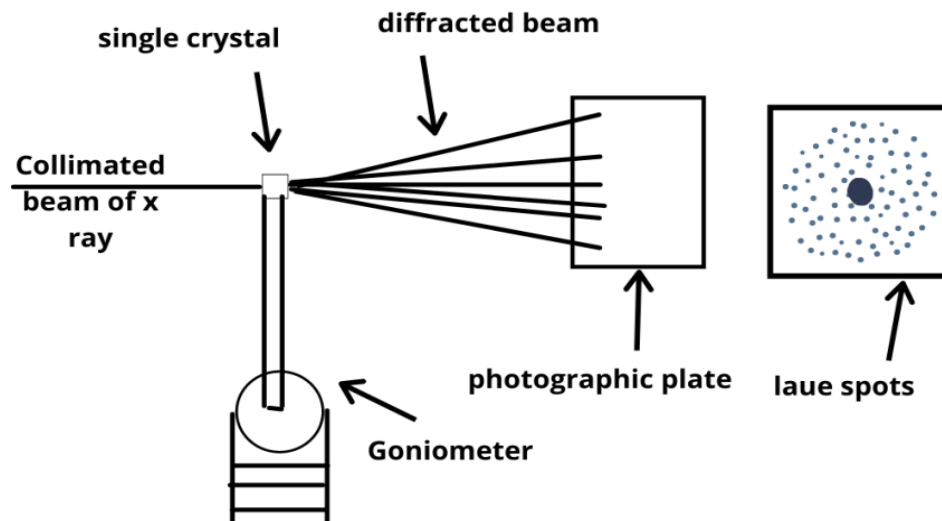
filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample. The interaction of the incident rays with the sample produces constructive interference (and a diffracted ray) when conditions satisfy

Bragg's Law,

$$(N\lambda = 2d \sin \theta)$$

This law relates the wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample. These diffracted X-rays are then detected, processed and counted. By scanning the sample through a range of 2θ angles, all possible diffraction directions of the lattice should be attained due to the random orientation of the powdered material. Conversion of the diffraction peaks to d-spacing allows identification of the mineral because each mineral has a set of unique d-spacing. Typically, this is achieved by comparison of d-spacing with standard reference patterns.

Fig 4.5: Working principle of XRD



Sources: <https://physicswave.com/x-ray-diffraction-analysis-principle-instrument-and-applications/>

Fig 4.5 Working principle of XRD

4.2.3.5.3 Procedure

XRD analysis was done for the synthesized copper nanoparticles from *Pistachio shells* in the Centre for Advanced Sciences, VIT University, and Vellore.

4.2.4 Antimicrobial Studies

The antibacterial assays were done on *Escherichia coli* and *staphylococcus aureus* by diffusion method. Briefly, Nutrient broth/agar medium was used to cultivate bacteria. Fresh overnight cultures of inoculum (100 µl) of each culture were spread on to nutrient agar plates. Six wells, one at the centre and five around the centre well were punched using the cork borer. In the centre well, standard erythromycin antibiotic disc of 15µg was placed and the surrounding wells were loaded with 10 µl, 20 µl, 30 µl, 40 µl and 50 µl of the synthesized nanoparticle solutions from *Pistachio shells*. The plates were then incubated in the incubator at 37°C for 24 hours and then they were observed visually for the formation of ZOI.

The antibacterial activity of sample was carried out by the agar well diffusion method. The pathogens used in this study were (*staphylococcus aureus*, *E.coli*). Briefly, the freshly cultured pathogens were smeared on Nutrient agar medium. Wells of 4 mm diameter were punched aseptically with a sterile cork borer and further loaded with different concentration (25, 50, 75, 100 µg/ml) of sample and 20 µl of gentamycin (positive control; 10µg). The plates were then incubated for 24 hr at 37°C, and the zone of inhibition (ZOI; mm) appearing around the wells was measured.

CHAPTER 5

RESULT AND DISCUSSION

Following are the results obtained from the present investigation

5.1 Synthesis of Copper Nanoparticles

Fig 5.1: Synthesis of copper nanoparticles from *Pistachio shells*

Pistachio shells were mixed with 1mM copper sulfate solution (1:9 ratios) and they were incubated under sunlight for 4 minutes. As the *Pistachio shells* extract was mixed in the aqueous solution of the copper ion complex, it started to change colorless to dark brown due to reduction of copper ion (Plate 1), which indicated the formation of copper nanoparticles in the reaction mixture. The change of color is due to the excitation of surface Plasmon vibrations in copper nanoparticles.



Fig 5.1: Synthesis of copper nanoparticle using *Pistachio shells*

Tube 1	-	<i>pistachio shells</i> preparation
Tube 2	-	1mM Copper Sulphate solution
Tube 3	-	1ml <i>pistachio shells</i> filtrate + 9ml CuSo ₄ (after four minutes under sunlight)

5.2 Characterization studies

5.2.1 Analysis of Synthesized Copper Nanoparticles Using UV-Visible Spectrophotometer

It is generally recognized that UV-Vis spectroscopy could be used to examine size- and shape-controlled nanoparticles in aqueous suspensions. Double beam UV-Vis spectrophotometer (SpectrascanUV-2700, Thermo Scientific) was used to analyse the samples.

Pistachio shells extract were acted as blank. Small aliquot was prepared by mixing the sample solution to sterile distilled water. It was then scanned under UV from 800nm to 200nm. Graph 1 shows the UV-Vis spectra recorded from the reaction mixture after 2 hours. Absorption spectra of copper nanoparticles formed in the reaction media obtained from *Pistachio shells* has absorbance peak at 420 nm. The broadening of the peak indicated that the copper nanoparticles synthesized from *Pistachio shells* are poly dispersed.

Fig 5.2: Absorption peak of synthesized copper nanoparticles from *Pistachio shells* under UV-Visible double beam spectrophotometer

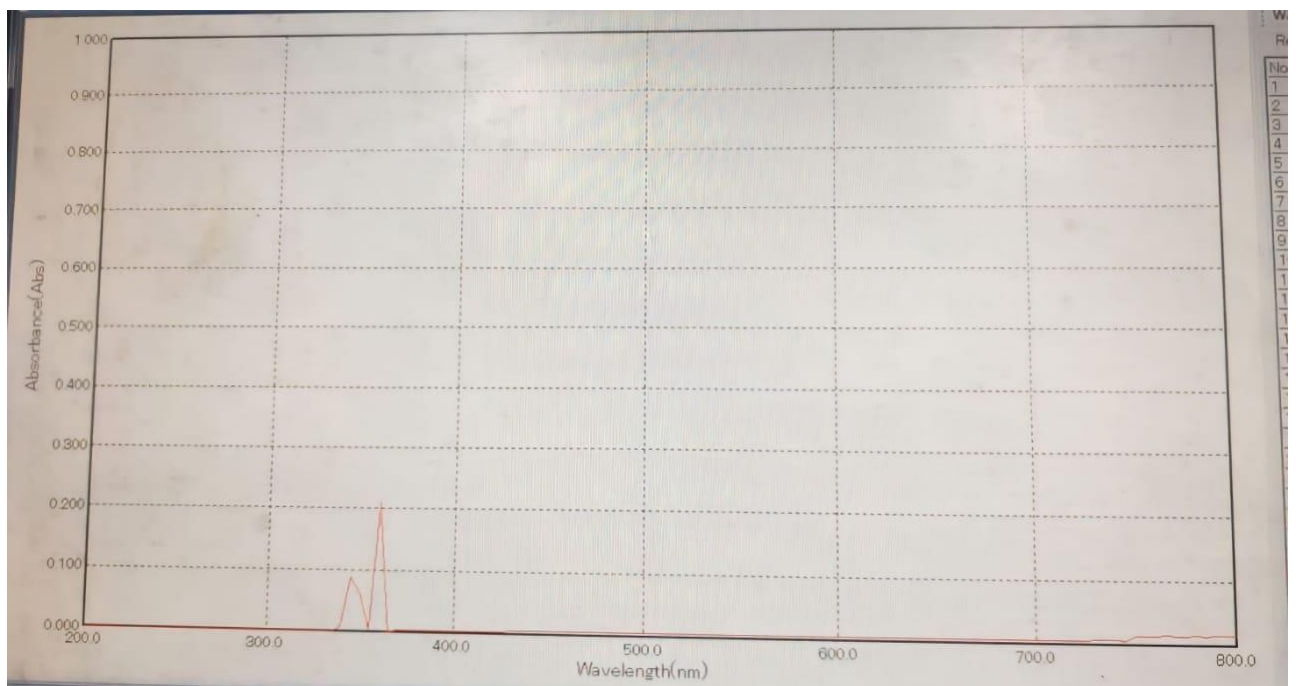
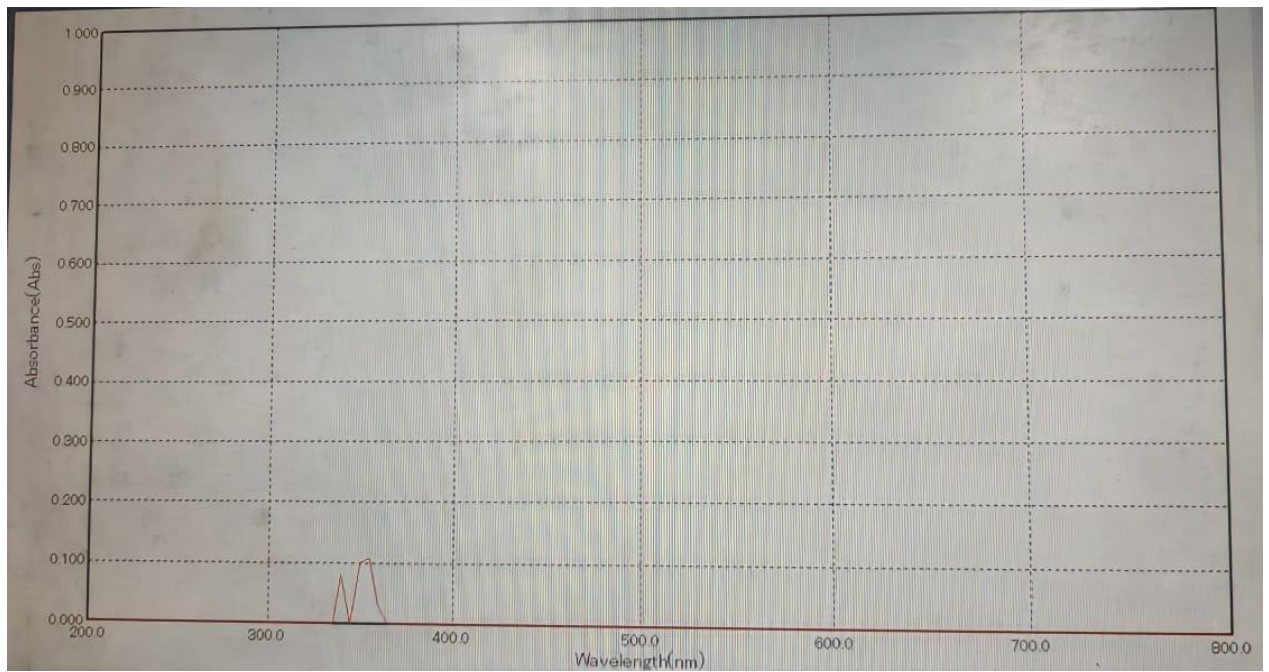


Fig 5.2: UV-visible spectroscopy of copper nanoparticles

5.2.2 Characterization of Synthesized Copper Nanoparticles Using SEM

The biosynthesised copper nanostructure by employing *Pistachio shells* was further confirmed by structural view under the scanning electron microscope. The useful magnification in SEM is defined by the size of the focused spot projected on the specimen. This magnification is ultimately limited by the diffraction of the focused electron beam.

Fig 5.3: SEM image of copper nanoparticles synthesized from *Pistachio shells*

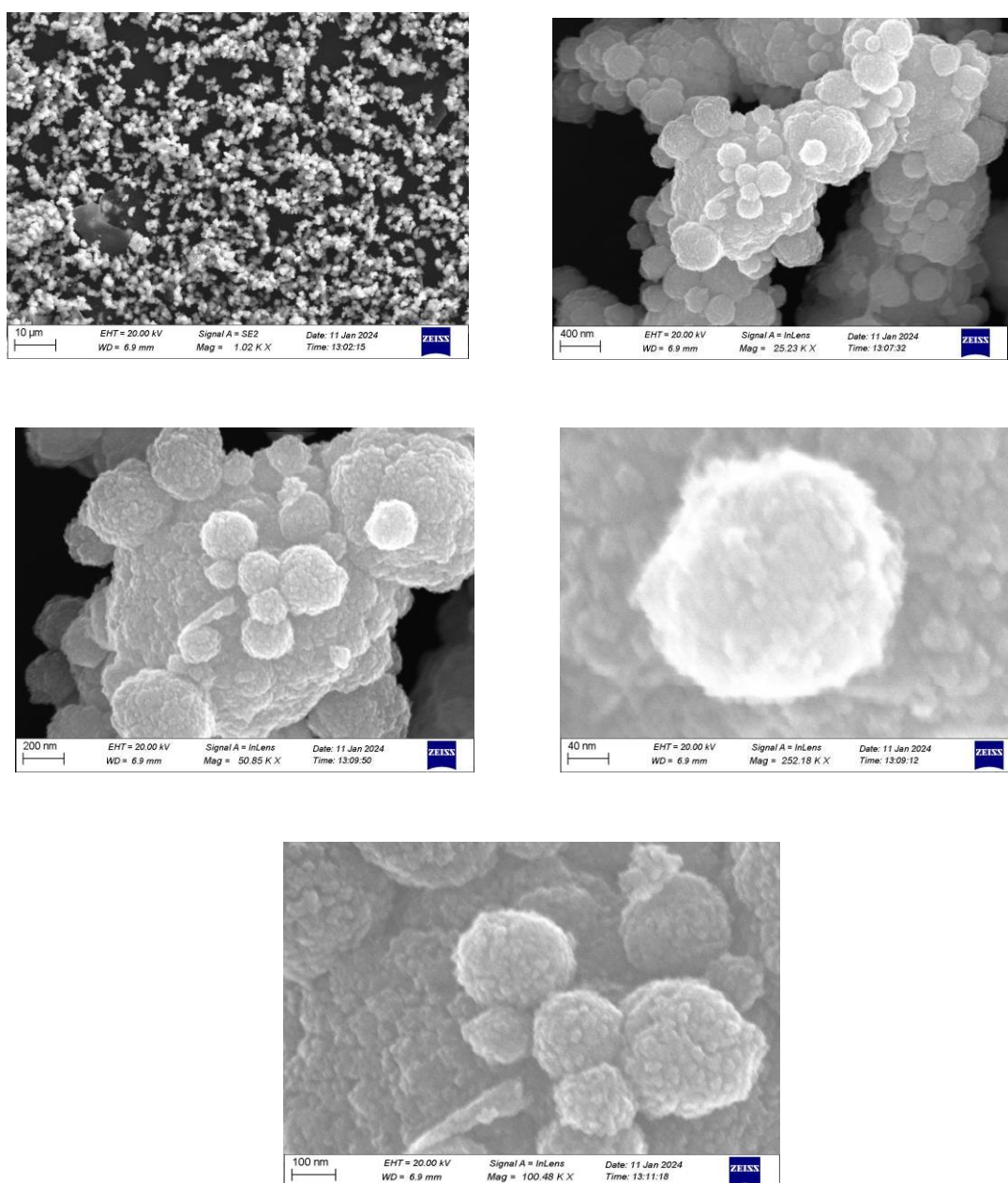


Fig 5.3: FESEM images of copper nanoparticles

5.2.3 Characterization of Synthesized Copper Nanoparticles and EDAX

The biosynthesised copper nanostructure by employing *Pistachio shells* was further confirmed by structural view under the scanning electron microscope and the possible chemical compounds in each of the sample was identified using EDAX. Using EDAX, the percentage of copper present in the synthesized copper nanoparticle solution from *Pistachio shells* is 54.12%.

Fig 5.4: EDAX image of copper nanoparticles synthesized from *Pistachio shells*

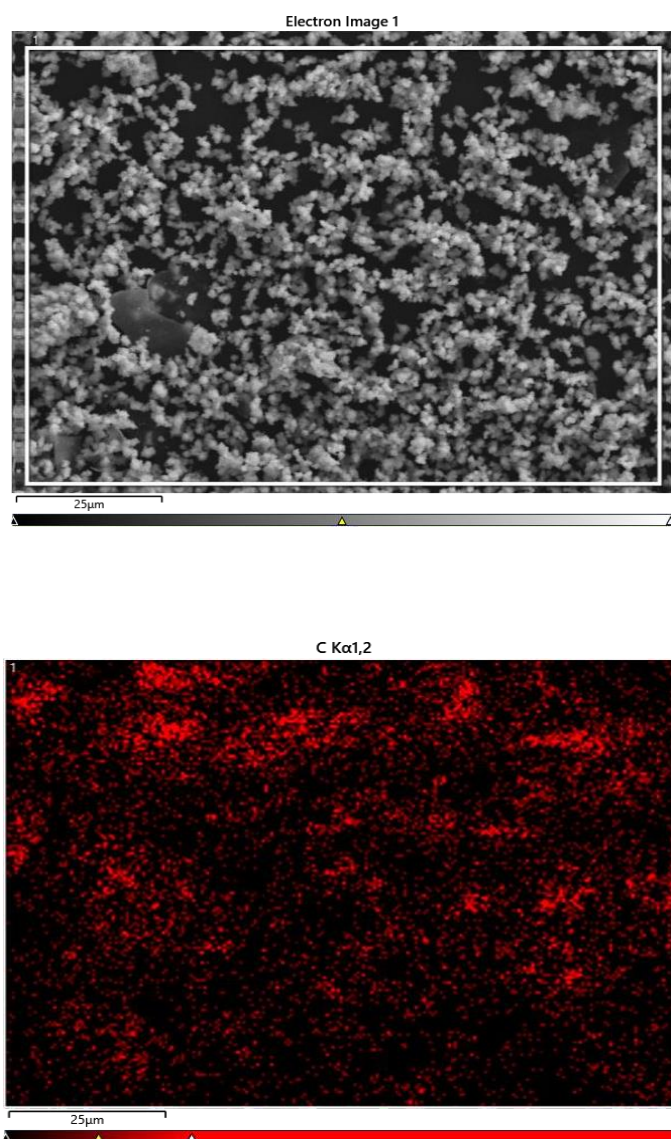


Fig 5.4: EDAX images of copper nanoparticles

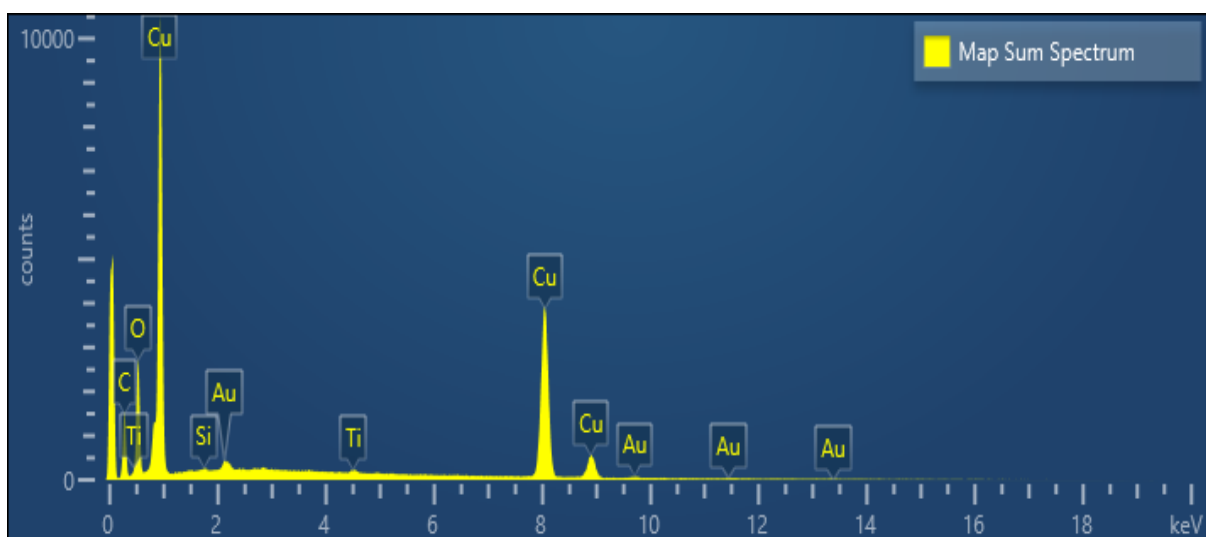
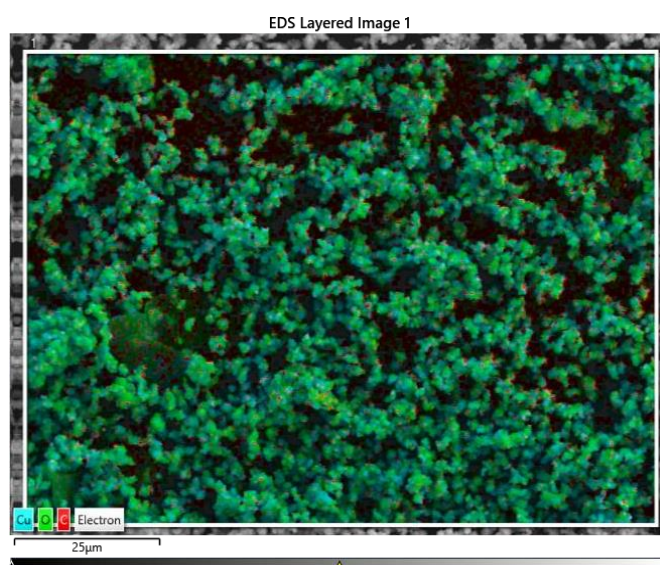


Table 5.1: EDAX analysis of chemical Compounds present in Copper Nanoparticles

Map Sum Spectrum				
Element	Line Type	Weight %	Weight % Sigma	Atomic %
C	K series	32.04	0.43	59.24
O	K series	16.59	0.23	23.03
Si	K series	0.10	0.03	0.08
Ti	K series	0.24	0.04	0.11
Cu	K series	49.79	0.36	17.40
Au	M series	1.23	0.12	0.14
Total		100.00		100.00



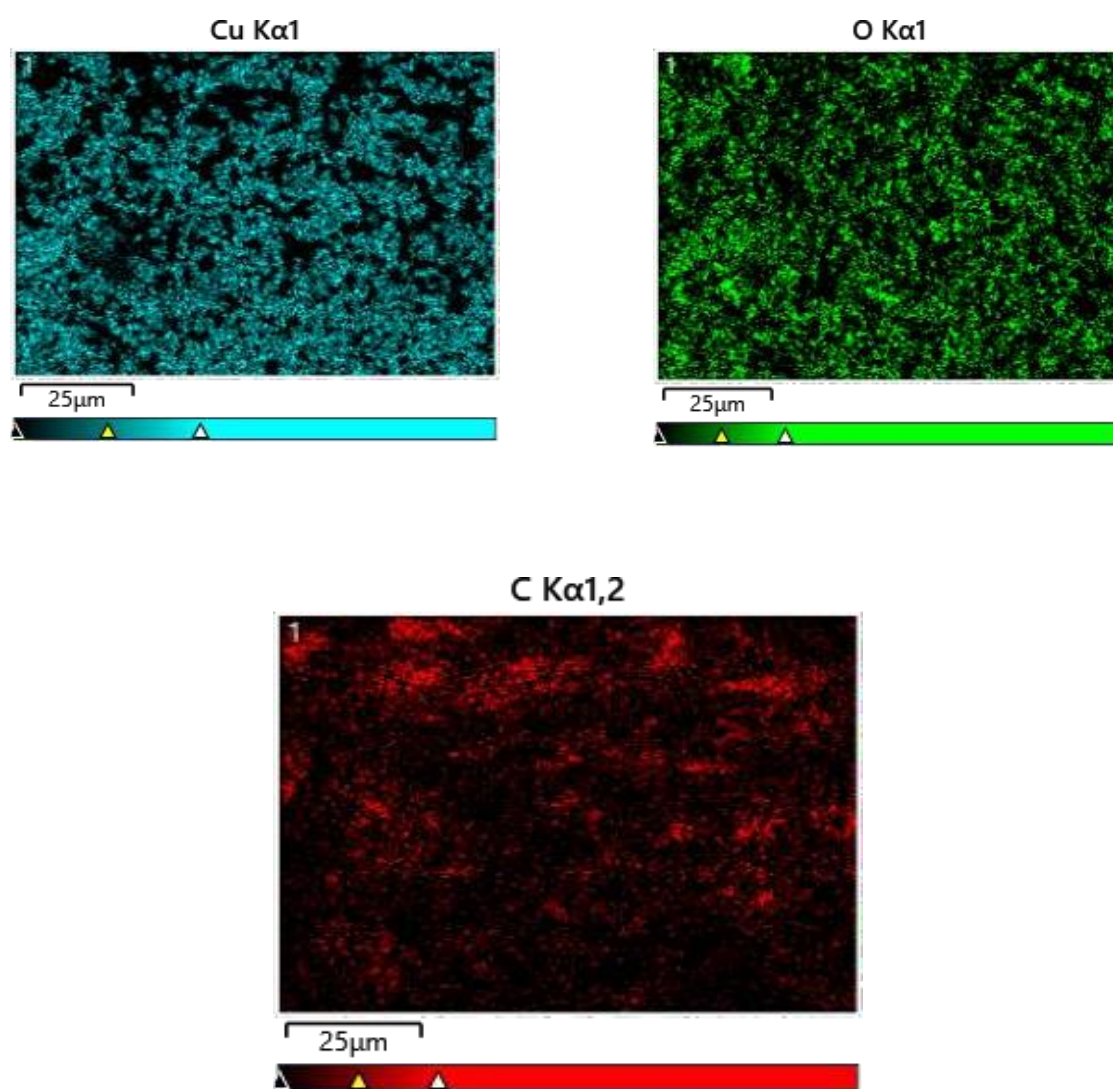


Fig 5.5: ED's electron images of copper nanoparticles

5.2.4 Characterization of synthesized copper nanoparticle using FTIR

To characterize the synthesized copper nanoparticle by using plant extract of *Pistachio shells* and confirmed under crystallographic structure of the material and the possible compounds in each of the sample was identified using FTIR.

Fig 5.6: Synthesized copper nanoparticles from *Pistachio shells* under FTIR

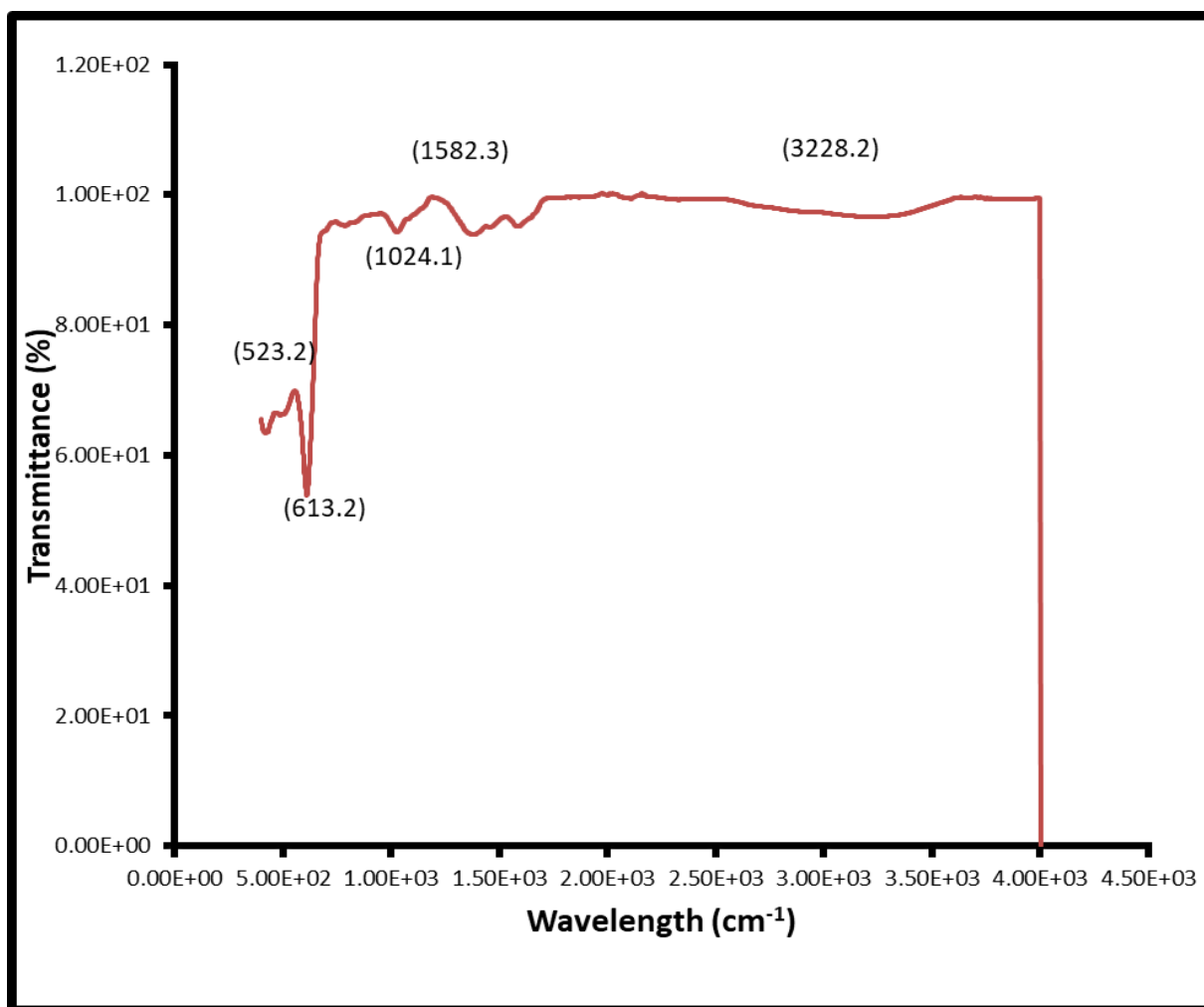


Fig 5.6: FTIR analysis of copper nanoparticles

5.2.5 Characterization of synthesized copper nanoparticle using XRD

To characterize the synthesized copper nanoparticle by using plant extract of *Pistachio shells* and confirmed under crystallographic structure of the material and the possible compounds in each of the sample/ was identified using XRD.

Fig 5.7: Synthesized copper nanoparticles from *Pistachio shells* using XRD

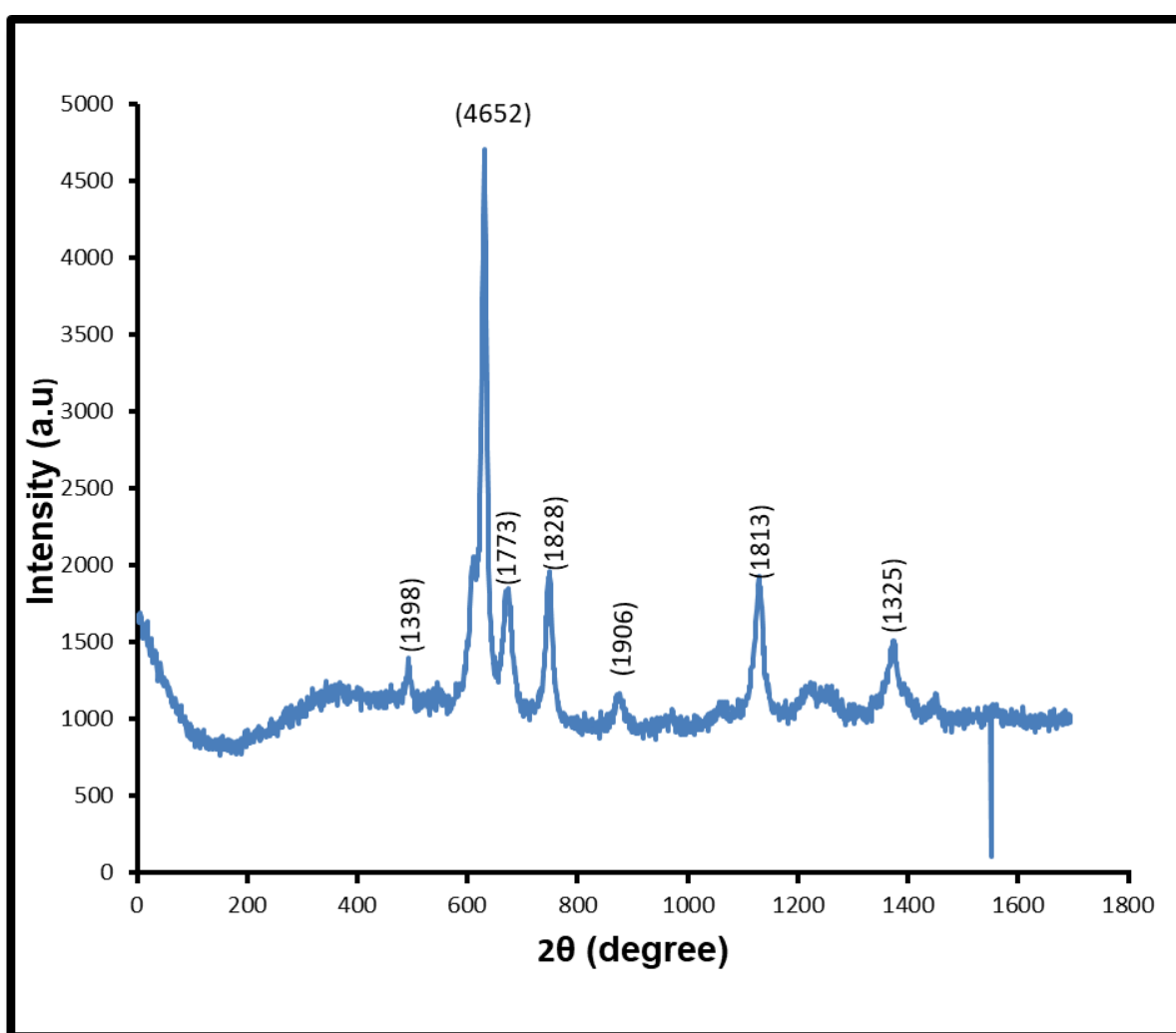


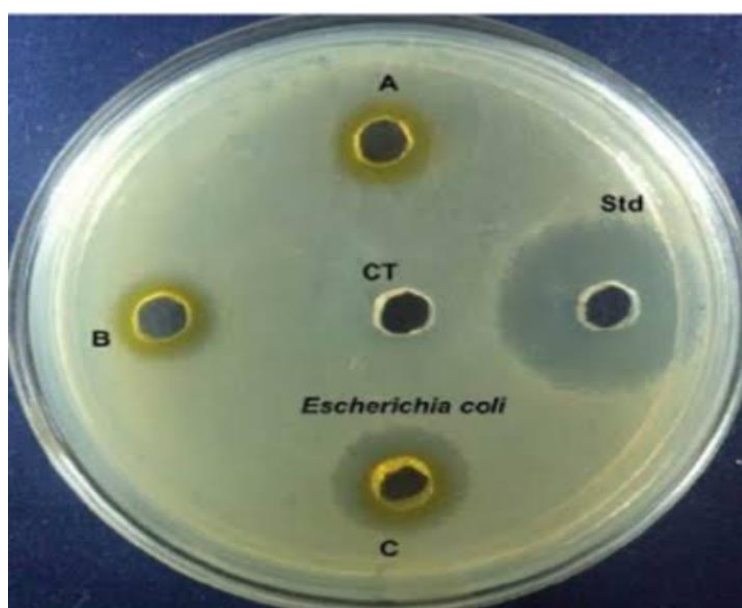
Fig 5.7: XRD pattern of copper nanoparticles

5.3 Antimicrobial Studies

Further, the nanoparticles synthesized by green route are found toxic against *E.coli* and *S.aureus* at a concentration of 100 μ l (Plates 1 and 2). Copper nanoparticles exhibited antibacterial activity against *E. coli* and *Staphylococcus aureus* it showed a clear inhibition zone and they were compared with the standard antibiotics like gentamycin disc of 24 μ g.

Fig 5.8: Antimicrobial effects of copper nanoparticles from *Pistachio shells* on *Escherichia coli*

The control, gentamycin disc had formed ZOI of 24 mm diameter against *E.coli*, Whereas the synthesized copper nanoparticles from Pistachio shells for 25 μ l, 50 μ l, 75 μ l, and 100 μ l was showing ZOI of 0mm, 0mm, 9mm, and 12mm respectively. When compared to the control, nanoparticles are showing better zone of inhibition with 75 μ l and 100 μ l concentration against *E.coli*.

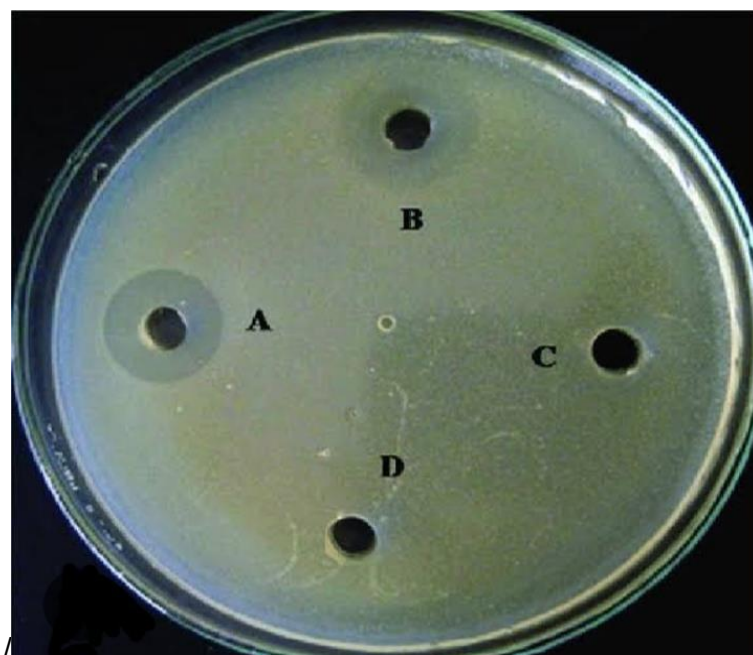


Escherichia coli

Fig 5.8: Antimicrobial effect of copper nanoparticles against from *E.coli*

Fig 5.9: Antimicrobial effects of copper nanoparticles from *Pistachio shells* on *Staphylococcus aureus*

The control, gentamycin disc had formed ZOI of 24 mm diameter against *Staphylococcus aureus*, Whereas the synthesized copper nanoparticles from Pistachio shells for 25 μ l, 50 μ l, 75 μ l, and 100 μ l was showing ZOI of 0mm, 0mm, 10mm, and 14mm respectively. When compared to the control, nanoparticles are showing better zone of inhibition with 75 μ l and 100 μ l concentration against *Staphylococcus aureus*.



Staphylococcus aureus

Fig 5.9: Antimicrobial effect of copper nanoparticles against from *S. aureus*

Table 2: Antimicrobial effects on Copper Nanoparticles against strains of microorganisms

MICROORGANISM	25µl	50µl	75µl	100µl	+VE
E.Coli	0	0	10	12	24
Staphylococcus aureus	0	0	9	14	15

CHAPTER 6

SUMMARY AND CONCLUSIONS

Following are the highlights emanated from the investigation.

- The copper nanoparticles were synthesized from *Pistachio shells* and the synthesized copper nanoparticles were detected by observing the change of color from reddish yellow to dark brown.
- While observing the synthesized copper nanoparticles solutions under UV-Visible double beam Spectrophotometer, there was broadening of the peak for the sample at 460nm. This says that all the synthesized nanoparticles are not of uniform size. They are poly dispersed copper nanoparticles.
- The copper nanoparticles synthesized from *Pistachio shells* was characterized using SEM in the Centre for Advanced sciences, Sathyabama University, Chennai and the size of copper nanoparticles ranged between 43nm and 58nm. Using EDAX, the percentage of copper present in the synthesized copper nanoparticle solution from *Pistachio shells* is 68.12%.
- The copper nanoparticles synthesized from *Pistachio shells* was characterized using FTIR AND XRD in the centre for advances sciences, VIT Vellore, Vellore and to characterize the synthesized copper nanoparticle by using plant extract of *Pistachio shells* and confirmed under functional group characterized and crystallographic structure of the material and the possible compounds in each of the sample/ was identified using FTIR AND XRD.
- The copper nanoparticles synthesized from *Pistachio shells* when treated against *E.coli* and *S.aureus* was showing the maximum ZOI as 9mm, 12mm, 12mm and 14mm respectively. The concentration present in 75µl and 100µl each of the sample loaded is 1.58 µg. Out of this, the copper

nanoparticles synthesized was effective against *S.aureus* and *E.coli* are less effective.

- Without adding any chemicals or applying any voltage and pressure, the copper nanoparticles had been synthesized from pistachio shells. These biological synthesized nanoparticles were confirmed by measuring the absorbance under UV-Visible Spectrophotometer and the copper nanoparticles were also characterized using SEM and EDAX.
- The antimicrobial studies of the synthesized copper nanoparticles are promising against *S.aureus* and *E.coli* and also less effect was observed, when compared with the control, gentamycin. Therefore, the present investigation concludes that the green synthesized copper nanoparticles are one of the potent tools as antimicrobial and antitumor agent.

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