

BIOSYNTHESIS OF COPPER NANOPARTICLES WITH PLANT EXTRACTS

USING *PARKINSONIA ACULEATA*

PROJECT WORK

Submitted in partial fulfilment of the requirements for the award of Bachelor of Science

in Microbiology

By

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DEPARTMENT OF BIOTECHNOLOGY

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY (DEEMED

TO BE UNIVERSITY)

CATEGORY – 1 UNIVERSITY BY UGC

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of Praveena E (41300029) Nivetha E (41300025) Who carried out of project "**BIOSYNTHESIS OF COPPER NANOPARTICLES WITH PLANT EXTRACTS USING *PARKINSONIA ACULEATA***" under my supervision from September 2023 to April 2024.

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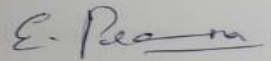
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DECLARATION

We Nivetha E (41300029), Praveena E (41300025) hereby declare that the project report entitled "**BIOSYNTHESIS OF COPPER NANOPARTICLE WITH PLANT EXTRACTS USING *PARKINSONIA ACULEATA***" done by me under guidance of Dr. R. THYAGARAJAN & DR. SAQIB HASSAN and Dr. M. BAVANILATHA at the Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai, is submitted in partial fulfilment of the requirements for the award of a Bachelor of Science degree in Microbiology.

DATE : 25/4/24

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ABSTRACT

Nanotechnology is one of the most dynamic research areas and the fastest-growing market. Developing eco-friendly products using available resources to acquire maximum production, better yield, and stability is a great challenge for nanotechnology. In this study, copper nanoparticles (CuNPs) were synthesized via the green method using plant extract of the medical plant *Parkinsonia aculeata* as a reducing and capping agent and used to investigate the influence of microorganisms. The maximum production of CuNPs was noted at 80 °C after 4h of reaction time. The formation of nanoparticles was confirmed through a UV-spectrophotometer, and the product showed an absorbance peak in the 567 nm range. The functional groups were observed using the FTIR technique absorbance peak 3450-1097cm⁻¹, such as isocyanic acid attached to stabilize the nanoparticles., Scanning Electron Microscopy (SEM), revealed the synthesis copper particle's structures are spherical and their size is less than 60 nm., and X-ray diffractometer (XRD) analysis in the peak range of 12.12 – 26.09. In tests with a few drug-resistant pathogenic bacteria and fungus species, CuNPs showed encouraging antimicrobial efficacy. CuNPs had a significant antioxidant capacity of 83.81% at 200 g/m⁻¹. Green synthesized CuNPs are cost-effective and nontoxic and can be applied in agriculture, biomedical, and other fields. Another test Anticancer test. It acts as a medicine for joint pain, sugar control, facial products, taking as a food of the seed for energy purposes.

KEYWORDS: Copper Nanoparticles – green synthesis, characterizations, antimicrobial and anticancer, Staining.

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LIST OF ABBREVIATION

CuNPs	Copper Nanoparticle
SEM	Scanning Electron Microscopy
EDS	Energy Dispersive X - Ray Spectroscopy
FTIR	Fourier Transmission Infrared Spectroscopy
XRD	X-Ray Diffraction
EDX	Energy Dispersive X – Ray
S. TYPHI	<i>Salmonella Typhi</i>
E. COLI	<i>Escherichia Coli</i>
RSM	Response Surface Methodology
K PNEUMONIA	<i>Klebsiella Pneumonia</i>
PH	Potential of Hydrogen
DNA	Deoxyribonucleic Acid
UPLC	Ultra Performance Liquid Chromatography
ESI / MS	Electrospray Ionization Mass Spectrometry
UV	Ultraviolet Xray
TLC	Total Leucocyte Count
GLC	Gas Liquid Chromatography
HEPA	High Efficiency Particulate Air
DDW	Deuterium Depleted Water
BS	<i>Bacillus Subtills</i>
PA	<i>Pseudomonas aeruginosa</i>

DMEM	Dulbecco's Modified Eagle Medium
FBS	Fetal Bovine Serum
MTT	3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide
PBS	Phosphate Buffer Saline
DMSO	Dimethyl Sulfoxide
DAPI	Diamino-2-Phenylindole

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CHAPTER 1.

INTRODUCTION

1.1 NANOPARTICLES

Nanoparticles are incredibly small particles, typically ranging in size from 1 to 100 nanometers. They can be engineered from various materials, including metals, polymers, ceramics, and semiconductors. Due to their small size, nanoparticles exhibit unique properties that differ from their bulk counterparts. These properties make them highly versatile and useful in a wide range of applications

Medicine: Nanoparticles are used in drug delivery systems to improve the efficacy and targeted delivery of medications. They can also be used for imaging and diagnostics, such as in contrast agents for MRI or fluorescent markers for microscopy.

Electronics: Nanoparticles are utilized in the development of electronic devices, such as nanoscale transistors, sensors, and memory devices. They can also enhance the performance of conductive materials and improve the efficiency of solar cells.

Catalysis: Nanoparticles serve as catalysts in various chemical reactions due to their high surface area-to-volume ratio, which provides more active sites for reactions to occur. They are used in industries ranging from petroleum refining to environmental remediation.

Cosmetics: Nanoparticles are employed in cosmetics and personal care products for their ability to improve product texture, enhance UV protection, and provide better delivery of active ingredients.

Food and Agriculture: Nanoparticles are utilized in food packaging to improve shelf life and safety. They can also be used as sensors to detect pathogens or spoilage in food products. In agriculture, nanoparticles are investigated for applications such as crop protection, nutrient delivery, and soil remediation.

Environmental Remediation: Nanoparticles are explored for their potential to clean up contaminated soil and water by adsorbing or degrading pollutants.

Nanoparticles, the tiny building blocks of the nanotechnology revolution, have captured the imagination of scientists, engineers, and innovators worldwide. These minuscule structures, typically ranging from 1 to 100 nanometers in size, possess remarkable properties that differ from those of bulk materials. At this scale, quantum effects dominate, giving rise to unique optical, electronic, magnetic, and catalytic behaviors.

The synthesis and manipulation of nanoparticles have opened up a vast array of possibilities across numerous fields, from medicine and electronics to energy and environmental science.

In medicine, nanoparticles are revolutionizing drug delivery systems, enabling targeted therapy for diseases such as cancer while minimizing side effects. In electronics, they are paving the way for smaller, faster, and more efficient devices, from nanoscale transistors to quantum dots for displays.

The versatile nature of nanoparticles extends beyond traditional disciplines, finding applications in areas such as environmental remediation, where they can clean up contaminated soil and water, and agriculture, where they enhance crop protection and nutrient delivery.

Moreover, their incorporation into cosmetics, textiles, and food packaging is driving innovation in consumer products, offering improved performance and sustainability.

However, along with their immense potential come challenges and concerns regarding their safety, environmental impact, and ethical implications. As researchers delve deeper into understanding the interactions between nanoparticles and biological systems, efforts are underway to ensure responsible development and deployment of nanotechnology.

In this era of rapid technological advancement, nanoparticles stand at the forefront of innovation, promising groundbreaking solutions to some of humanity's most pressing challenges. As our understanding of their properties grows, so too does their potential to transform industries, improve lives, and shape the future of science and technology.

1.2. USES OF NANOPARTICLES

Nanoparticles have a wide range of uses across various fields due to their unique properties. Here are some of the key applications:

Drug Delivery: Nanoparticles can be engineered to encapsulate drugs and deliver them to specific targets in the body, such as tumors or diseased cells. This targeted delivery reduces side effects and improves the efficacy of treatments.

Medical Imaging: Nanoparticles are used as contrast agents in medical imaging techniques like MRI, CT scans, and ultrasound. They enhance the visibility of tissues and organs, aiding in diagnosis and treatment planning.

Cancer Therapy: Nanoparticles can be designed to selectively target cancer cells, delivering therapeutic agents such as chemotherapy drugs or heat for localized tumor destruction while minimizing damage to healthy tissue.

Cosmetics: Nanoparticles are used in sunscreen formulations to provide better UV protection without leaving a white residue on the skin. They also improve the texture and performance of cosmetics and skincare products.

Electronics: Nanoparticles play a crucial role in the development of electronics, including conductive inks for printed electronics, nanoscale transistors, and quantum dots for displays and lighting applications.

Water Purification: Nanoparticles are employed in water treatment technologies to remove contaminants, such as heavy metals, organic pollutants, and pathogens, from wastewater and drinking water sources.

Food Packaging: Nanoparticles are incorporated into food packaging materials to improve barrier properties, extend shelf life, and provide antimicrobial effects, thus enhancing food safety and reducing food waste.

Energy Storage and Conversion: Nanoparticles are used in batteries, fuel cells, and supercapacitors to improve energy storage capacity, charge-discharge rates, and overall efficiency.

Textiles: Nanoparticles are integrated into fabrics to impart desirable properties such as stain resistance, UV protection, antimicrobial activity, and moisture management.

These are just a few examples of the diverse applications of nanoparticles, and ongoing research continues to explore new ways to harness their unique properties for various technological and biomedical advancements.

1.3 SYNTHESIS OF NANOPARTICLES

The synthesis of nanoparticles involves various methods depending on the desired properties, size, shape, and material of the nanoparticles. Here are some common synthesis techniques

Chemical Precipitation: This method involves mixing precursor chemicals in a solvent and then adding a reducing agent or a precipitating agent to induce the formation of nanoparticles.

The size and properties of the nanoparticles can be controlled by adjusting reaction parameters such as temperature, pH, and reaction time.

Sol-Gel Method: This technique involves the hydrolysis and condensation of metal alkoxides or metal salts in a solution to form a colloidal suspension, followed by controlled drying and annealing to obtain nanoparticles in a solid matrix.

Microemulsion: In this method, nanoparticles are synthesized within a water-in-oil or oil-in-water microemulsion system, where surfactant molecules stabilize nanoscale droplets of one phase dispersed in the other phase. The nanoparticles form as the precursors react within these confined spaces.

Hydrothermal Synthesis: This method involves the reaction of precursor chemicals in a high-temperature and high-pressure aqueous solution. The controlled heating and cooling cycles promote the formation of nanoparticles with uniform size and crystallinity.

Green Synthesis: This approach utilizes natural sources such as plant extracts, microorganisms, or biomolecules as reducing and stabilizing agents to synthesize nanoparticles. Green synthesis methods are environmentally friendly and often involve milder reaction conditions.

Electrochemical Deposition: Nanoparticles can be electrodeposited onto conductive substrates by applying an electric current to a solution containing metal ions. The size and morphology of the nanoparticles can be controlled by adjusting deposition parameters such as voltage, current density, and deposition time.

Laser Ablation: In this technique, a high-energy laser beam is focused on a target material immersed in a liquid medium. The intense laser pulse vaporizes the target material, leading to the formation of nanoparticles in the liquid phase.

Template-Assisted Synthesis: Templates such as porous membranes or self-assembled monolayers can be used to control the size, shape, and arrangement of nanoparticles during their synthesis.

Gas-Phase Synthesis: Nanoparticles can be synthesized in the gas phase by techniques such as laser ablation, chemical vapor deposition, or gas-phase condensation. These methods are particularly suitable for producing nanoparticles with precise control over size and composition.

Each synthesis method has its advantages and limitations, and the choice of technique depends on the specific requirements of the desired nanoparticles and the intended applications.

Additionally, rigorous characterization techniques such as electron microscopy, X-ray diffraction, and spectroscopy are essential for evaluating the properties and quality of synthesized nanoparticles.

1.4. CHARACTERIZATION OF NANOPARTICLES

Characterization of nanoparticles is crucial for understanding their properties, structure, size, shape, composition, and surface chemistry, which ultimately influence their behavior and performance in various applications. Here are some common techniques used for nanoparticle characterization:

Transmission Electron Microscopy (TEM): TEM allows for high-resolution imaging of nanoparticles, providing information about their size, shape, and morphology at the nanoscale. It is particularly useful for studying individual nanoparticles and their aggregation behavior.

Scanning Electron Microscopy (SEM): SEM provides detailed surface morphology and topography of nanoparticles by scanning a focused electron beam across the sample surface. It can also provide information about particle size distribution and shape.

Dynamic Light Scattering (DLS): DLS measures the Brownian motion of nanoparticles in solution to determine their hydrodynamic size distribution. This technique is useful for analyzing the size distribution and stability of nanoparticles in liquid dispersions.

X-ray Diffraction (XRD): XRD is used to determine the crystalline structure of nanoparticles by analyzing the diffraction pattern of X-rays scattered by the crystal lattice. It provides information about the phase composition, crystal structure, and crystallite size of nanoparticles.

Fourier Transform Infrared Spectroscopy (FTIR): FTIR spectroscopy is employed to analyze the chemical composition and surface functional groups of nanoparticles by measuring the absorption and transmission of infrared light. It is useful for studying surface modifications and interactions with ligands or biomolecules.

UV-Visible Spectroscopy: UV-Visible spectroscopy is used to characterize the optical properties of nanoparticles, including absorption, scattering, and extinction spectra. It provides information about the size, shape, and concentration of nanoparticles in solution.

Atomic Force Microscopy (AFM): AFM images the surface topography of nanoparticles with high resolution by scanning a sharp probe tip over the sample surface. It can provide information about particle size, shape, and surface roughness.

Zeta Potential Measurement: Zeta potential measurement determines the electrostatic charge on the surface of nanoparticles in solution, which influences their stability, dispersibility, and interaction with other molecules or surfaces.

Thermogravimetric Analysis (TGA): TGA measures the weight loss of nanoparticles as a function of temperature, providing information about their thermal stability, composition, and presence of surface coatings or contaminants.

Surface Area Analysis: Techniques such as Brunauer-Emmett-Teller (BET) analysis are used to measure the specific surface area of nanoparticles, which is important for understanding their reactivity, adsorption capacity, and surface properties.

By combining these characterization techniques, researchers can gain comprehensive insights into the properties and behavior of nanoparticles, enabling the optimization of their synthesis processes and the development of novel nanomaterials for various applications.

The history of nanoparticles traces back to ancient times when artisans unknowingly manipulated materials at the nanoscale through techniques like pottery making and glass coloring. However, the scientific exploration of nanoparticles began in earnest in the late 19th and early 20th centuries with the development of modern chemistry and physics. Here are some key milestones in the history of nanoparticles:

Early Observations (Late 19th Century): Scientists such as Michael Faraday observed the vibrant colors of gold colloids and recognized that their unique properties were due to their small size, though the term "nanoparticle" had not yet been coined.

Quantum Mechanics (Early 20th Century): The emergence of quantum mechanics in the early 20th century provided theoretical insights into the behavior of materials at the atomic and subatomic levels, laying the foundation for understanding nanoparticles' properties.

Electron Microscopy (1930s): The invention of electron microscopy in the 1930s enabled scientists to visualize nanoparticles at unprecedented resolutions, leading to discoveries about their size, shape, and structure.

Nanoparticles in Colloidal Science (1940s-1950s): Colloidal science emerged as a field of study focused on understanding the behavior of particles dispersed in a medium. Researchers investigated the synthesis and properties of nanoparticles dispersed in liquids, leading to applications in catalysis, coatings, and biomedical research.

Nanoparticles in Electronics (1960s-1970s): The semiconductor industry began using nanoparticles in the fabrication of electronic components such as transistors and integrated circuits. Nanoparticles of materials like silicon and gold were incorporated into electronic devices to enhance performance and miniaturization.

Nanotechnology Revolution (1980s-1990s): The term "nanotechnology" gained prominence in the 1980s, with pioneers like Richard Feynman envisioning the ability to manipulate matter at the atomic and molecular levels. Breakthroughs in nanofabrication techniques, such as scanning tunneling microscopy and molecular beam epitaxy, enabled precise control over nanoparticle synthesis and assembly.

Biomedical Applications (1990s-present): Nanoparticles began to find widespread applications in medicine, particularly in drug delivery, imaging, and diagnostics. Researchers developed nanoparticles capable of targeting specific cells or tissues, revolutionizing treatments for cancer and other diseases.

Environmental and Energy Applications (2000s-present): Nanoparticles have been explored for various environmental and energy-related applications, including pollution remediation, renewable energy generation, and energy storage. Nanomaterials such as quantum dots, carbon nanotubes, and nanocomposites offer unique properties for addressing environmental and sustainability challenges.

Safety and Regulation (2000s-present): Concerns about the potential environmental and health impacts of nanoparticles led to increased research into their safety and regulatory oversight. Organizations worldwide have developed guidelines and regulations to ensure the responsible development and use of nanotechnology.

Throughout history, the study and application of nanoparticles have evolved from early observations to a multidisciplinary field at the forefront of science and technology. With ongoing research and innovation, nanoparticles continue to drive advancements in diverse areas, promising transformative solutions to complex challenges in the 21st century and beyond.

1.6. COPPER NANOPARTICLES

However, there are several studies on CuNPs synthesis using different plant extract have been reported but the study of the application of CuNPs on the treatment of dye effluent is limited. The agriculture sector exploits different kinds of pesticides, herbicides, and antimicrobial substances to control plant diseases. These substances are responsible for soil pollution as well as biomagnification in living organisms. Despite the photocatalytic activity, CuNPs attracted more attention due to their nontoxic, antimicrobial efficacy in controlling plant diseases.

An extensive literature survey revealed that the antifungal activity of CuNPs mostly tested against human pathogenic fungi. The least study conducted on CuNPs antifungal activity on plant pathogenic fungi, so, there is a crucial need for more assessment and evaluation in this field. Nowadays nanomaterials are of huge interest due to a wide range of applications in chemical, biological, and environmental sciences Copper nanoparticles were biologically synthesized using plant leaf extract as reducing agent.

On treatment of aqueous solution of *Parkinsonia aculeata* leaf extract, stable copper nanoparticles were formed. Traditionally herbal medicinal plants are used from very long period and trace its roots to ancient civilizations.

They are important as medicines before the availability of synthetic drugs. Venerable herbal infusion and decoction were household medicaments for common ailments. *Parkinsonia aculeata* is a tree from the family Fabaceae. It is a spiny deciduous tree which can grow up to 4 to 10 meters in height. It remains green throughout the year.

The bark, branches and the twigs are yellow-green or blue-green in colour. *Parkinsonia aculeata* is well-known as a medicinal plant. Belongs to the family Fabaceae (pea family). This plant plays an essential role in the human life.

In response to the global need for information on medicinal plants, the present review compiles all relevant information on the traditional, therapeutic, biological chemical and all details of *Parkinsonia aculeata*.

Commonly known as *Parkinsonia*, and Ram babul, the leaves and barks of the plant have crucial therapeutic values on a lot of diseases. like it has anti-microbial activity, anti-bacterial activity, and antioxidant, and also used as anti-healing, anti-diabetic, anti-convulsant, and anti-cancer activity.

The ethanolic extract of leaves of *Parkinsonia aculeata* has a helpful effect in the treatment of piles or haemorrhoids. The use of herbs has conventional medicinal value and the products used for the treatment of many ailments has been generally considered therapeutically effective.

Parkinsonia aculeata plant contains substances, chemicals or phytoconstituents like alkaloids, flavonoids, glycosides, saponins, tannins, phenolic compounds, gums, reducing sugars, etc. which are used for therapeutic purposes. It was used in the Indian traditional system of medicine for the empirical treatment of piles or haemorrhoids, and microbial infection.

In addition to these properties, *Parkinsonia aculeata* has a wide range of pharmacological activities including piles, Antimalarial and antimicrobial properties. *Parkinsonia aculeata* is a Shrubby tree, a smooth yellow greenish Bark, in every season the tree is fully greenery, with branches armed with strong spines of Stipule or leaf rachis origin.

It is often branching near the ground with a very open crown of spreading branches and very thin dropping foliage.

1.7 OVERVIEW OF *PARKINSONIA ACULEATA*

Medicinal herbs are the natural blessing for mankind to have a healthy and disease- free style of living.

India is a unique nation of pharma culture in the universe where the medicinal plant is included in the component of the classical convention for complementing the quality of life. *Parkinsonia aculeata* plant that belongs to the family Fabaceae and it is a shrubby tree, which grows up to the height of 4-10 m.

Presently *Parkinsonia aculeata* can be found in most tropical, sub-tropical and Mediterranean countries. In this article, the pharmacological significance of *Parkinsonia aculeata* is precisely covered with different medicinal activities.

The Phytoconstituent evaluation of the leaves, flowers, seeds and stems of *Parkinsonia aculeata* has exposed the presence of glycosides, flavonoids, alkaloids, saponins, tannins, phenolic compounds, gums, and reducing sugars. *Parkinsonia aculeata* shows several health benefits because of the presence of a large number of bioactive phytochemicals. Habitually *Parkinsonia aculeata* is also known to the medicinal shrub for its beneficial effects as antimicrobial, anti-cancer, anticonvulsant, analgesic, antipyretic and anti-inflammatory, antidiabetic, anti-spermatogenic and antimalarial activities.

A few plant species that have health-promoting effects and have been used as traditional foods and herbal remedies, though limited information has been documented on their specific biological properties.

Moreover, medicinal plants are low-cost and tend to have fewer side effects than synthetic drugs. In this review, the introduction about plants, the method of extraction of various parts of plants, bioactive constituents, and pharmacological significance with results and discussion are succinctly explained.

1.8. CHARACTERIZATION OF NANOPARTICLE

The synthesized nanoparticles were characterized with inductively energy dispersive X-ray spectroscopy (EDS), scanning electron microscopy (SEM), and Electron microscopy analysis of copper nanoparticles indicating that they ranged in size from 40 to 100 nm. Antibacterial tests were carried out by counting viable *Escherichia coli* cells after 24 h growth in shake flasks containing latex foams coated with copper nanoparticles. As a result, foams coated with biologically synthesized copper nanoparticles showed higher antibacterial activity compared with foams untreated. As possible eco-friendly alternatives to chemical and physical methods, biologically synthesized nanoparticles using plant extracts may have applications in various human body-contacting areas. Scanning Electron Microscopy (SEM) and Energy- Dispersive X-ray (EDX) Spectroscopy

For examination and analysis of micro and nanoparticle imaging characterization of solid objects for particle size X-ray diffraction analysis is used - to analyze the physical Properties such as Phase composition, structure, and orientation powder Fourier transform infrared spectroscopy [FTIR] – is used to identify the organic POLYMERIC and inorganic materials. Antibacterial test, Anticancer test.

1.8.1 Energy- dispersive X-ray spectroscopy, scanning electron microscopy

Energy-dispersive X-ray spectroscopy (abbreviated as both EDS and EDX analysis) is a powerful analytical technique used to identify and quantify the elemental composition of materials. It is a non-destructive method that is commonly used in conjunction with scanning electron microscopy (SEM).

1.8.2 X-ray diffraction

Is a versatile analytical technique used to analyse physical properties such as phase composition, crystal structure and orientation of powder, solid and liquid samples.

It is a non-destructive technique, meaning that the sample does not need to be changed or damaged to be analysed and it is used in a wide variety of industries and fields to study the physical properties of materials.

183 ***Fourier transform infrared spectroscopy***

Identify unknown materials in solid, liquid, or gaseous form Understand the total composition of a complex compound, including additives Identify and quantify surface contaminants present on a material Understand the chemical composition of a potential contaminant Test the quality, durability, or vulnerability of a wide range of products and substances.

184 ***Antib***

The ethanolic leaf extracts of *Parkinsonia aculeata* against *S. typhi* shows the antimicrobial activity. The antibacterial activity of *Parkinsonia aculeata* ethanolic extracts was tested against *E. coli*, *S. typhi*, *K. pneumonia* bacteria. The results indicated that the three concentrations were effective against tested bacteria. Besides, the effects of pH caused a significant variation in the zone of inhibition.

The maximum antibacterial activity has been checked for ethanolic extract against three bacterial strains.

The optimization or maximum antibacterial activity has been checked for ethanolic extracts against bacterial species. After the analysis, the final parameters was determined, and it was concluded that temperature, concentration and pH were significant among the three factors studied against the *E. coli*, *S. typhi*, and *K. pneumonia*.

By using Response Surface Methodology (RSM). Hence, it is suggested from this review work that *Parkinsonia aculeata* when used in proper PH, Temperature, and Concentration, can be working to treat profuse bacterial inflammations in the biological systems.

Cancer is the uncontrollable programmed growth of abnormal cells in the body. It can occur in any part of the body which affects the normal mechanism of a working cell. There are different types of cancers which are the leading cause of death. Tobacco is the major cause of cancer, which is responsible for patients' cancer deaths. The cancer treatment includes many treatments such as surgery, chemotherapy, and radiation therapy. All these treatments have painful side effects. [9] Such as chemical changes in the body, weight loss, difficulty in breathing, fatigue, and hair loss. The plant *Parkinsonia aculeata* has the source of providing the latest chemoprotective potential against cancer.

The ethanolic leaves extract using the Soxhlet extraction apparatus of *Parkinsonia aculeata* contains parkinsonin-A, parkinsonin-B, and parkintin, have shown that some flavonoids modulate the metabolism and can contribute to cancer prevention. In the In-vitro antimutagenic and DNA protecting potential of methanol, hexane, n- butanol and aqueous extract of *Parkinsonia aculeata*. It was evaluated by two different assays, the Ames assay and DNA nicking assay.

The study found that all the extracts inhibited DNA damage by the hydroxyl radicals. The study found that *Parkinsonia aculeata* effectively acts as antimutagenic against 4-Nitro-o-phenylenediamine sodium azide and 2-Aminofluorene in the strains of *Salmonella typhimurium*.

1.8.6 Staining cells

DAPI staining is a widely used technique in cell biology to visualize nuclei. It involves using a fluorescent dye called DAPI (4',6-diamidino-2-phenylindole), which binds specifically to DNA. When exposed to ultraviolet light, DAPI emits blue fluorescence, allowing researchers to observe the location and distribution of cell nuclei.

CHAPTER 2

LITERATURE SURVEY.

Hanan M. 2015 - Antimicrobial and antioxidant activities of *Parkinsonia aculeata* and chemical composition of their essential oil. Traditionally herbal medicinal plants are used from a very long period and trace their roots to ancient civilizations. They are important as medicines before the availability of synthetic drugs.

Wafaa H. 2018- Polyphenolic constituents and chromatographic fingerprint of the aqueous fraction obtained from the ethanolic extract of the aerial parts of *Parkinsonia aculeata* L. growing in Saudi Arabia were investigated for the first time using UPLC-ESI/MS/MS in negative mode. Forty compounds were tentatively identified including sixteen C-flavone glycosides, twenty-two O-flavone glycosides, and two polyethoxylated flavonoids.

Mohammed, SY. 2017- *Parkinsonia aculeata* is a small tree that can be found in the northern part of Nigeria. Its leaves are locally used to treat diarrhoea in some villages of Sokoto, however, there is little or no information on its phytochemical constituents. This paper, therefore, reports the findings of the preliminary phytochemical investigation of the leaf extracts

Raka Kamal- Rotenoids the group of naturally occurring insecticidal substances, ketonic in nature were analysed from various plant parts of *Parkinsonia aculeata* L. Three rotenoids viz rotenone, ellipt one and deguelin were identified using TLC, GLC, mp, mmp, IR, and UV studies, which were comparable to that of their respective standard compounds. In different parts of the plant the rotenoid recovery has been observed as per the order: root> stem>leaves> pods>seeds, being maximum in the root and minimum in the seed.

The different doses (1000 $\mu\text{g mL}^{-1}$, 500 $\mu\text{g mL}^{-1}$, 250 $\mu\text{g mL}^{-1}$, 125 $\mu\text{g mL}^{-1}$, 62 $\mu\text{g mL}^{-1}$, 31 $\mu\text{g mL}^{-1}$) of isolated rotenoids from roots were subjected to in vitro and amoebic activity along with standard rotenone and derris resin for different time intervals (24h

48h 72h). The dose level of 500 µg/ml L-1 was found most active as compared to 250 µg/ml L⁻¹ of standards. However, the standard antiamoebic drug metronidazole was effective at 8 µg/ml dose.

Leite AC, 2010- This paper reports the characterization of the antidiabetic role of a hydroethanolic extract from *Parkinsonia* aerial parts (HEPA), in normal and alloxan-induced diabetic rats, treated with HEPA (125 and 250 mg/kg; p.o.). Oral glucose tolerance test, acute oral toxicity test and preliminary phytochemical analyses were performed. The diabetic rats treated with HEPA showed a significant reduction in serum and urinary glucose, urinary urea and triglyceride levels, as compared to the diabetic untreated group.

Sharma S, 2014- preliminary phytochemical screening and in vitro antioxidant activities of *Parkinsonia aculeata*. Butanol and hexane leaves extracts of *Parkinsonia aculeata* L. (Fabaceae) were assessed for its antioxidant potential by in vitro methods. Phytochemical analysis and antioxidant activity of plant extracts were studied using different in vitro assays.

Rehwani H. 2007- To evaluate the effect of *Parkinsonia aculeata* Linn. (Caesalpiniaceae) on male reproductive function and fertility, Cell population dynamics, Leydig cells, *Parkinsonia aculeata*, Sertoli cell, sialic acid, sperm motility. Although *Parkinsonia aculeata* is regarded as one of the worst weeds, the alcohol extract of its bark exhibited CNS-depressant activity in mice, whereas aqueous extract showed cholinomimetic activity (Rao et al., 1979).

Hashempur MH 2017 Hemorrhoids are one of the most common gastrointestinal diseases. There are several therapeutic options associated with some complications. Therefore, researchers look for traditional medicines as a potential resource for the introduction of new natural drugs. The current study reports an evidence-based review of herbal remedies for haemorrhoids in traditional Persian medicine. A comprehensive survey about haemorrhoids on the most important manuscripts of traditional Persian medicine was done.

Then, scientific data banks were searched for possible related properties of each herb in conventional medicine. We reported some historical aspects of traditional Persian medicine views on classification, examination, and predisposing factors of hemorrhoids. In addition, we have reported 105 medicinal plants belonging to 51 families. More than half of the reported herbs exhibited anti-inflammatory and analgesic effects. Although a lack of human studies regarding the mentioned herbs is noted, positive results from experimental findings can be considered for new drug discovery supported by traditional and medieval experience.

Gupta MK,2011- The present study was aimed at evaluation of the analgesic, anti-inflammatory and antipyretic activity of total alcoholic and aqueous extract of leaves of *Parkinsonia aculeata* Linn in mice and rats.

The alcoholic extract of *P. aculeata* Linn. leaves at a dose of 200 mg/kg body weight has shown significant analgesic, antipyretic and anti-inflammatory activity as compared to aqueous extract. The result of hot plate indicated that the total alcoholic extract shows a significant increase ($P<0.01$) in reaction time at a 3, 4 and 6 hours comparable to the reference drug Pentazocine but lesser ($P<0.05$) at 2 hr.

A.K.Sharma, 2018- *Parkinsonia aculeata* (family – Caesalpiniaceae) is a large spinous shrub found in warmer regions of the world.

It is a well-known medicinal plant that has significant and promising biological activities such as antioxidant, anti-inflammatory, antidiabetic, ant spermatogenic, *etc.*

The phytochemical investigations of the leaves, flowers, and stem have revealed the presence of various glycosides, flavonoids, reducing sugars, alkaloids, sterols, tannins, volatile oils, and saponins.

The various biological activities and potent phytoconstituents of the plant indicate its therapeutic potential efficacy. The present study shows the detailed investigation of the methanolic stem bark extract of the title plant.

Tamires Meira Menezes ,2020- Evaluation of acute oral toxicity, embryotoxicity and cytotoxicity of the polar fraction of *Parkinsonia aculeata* aerial parts extract.

Tamires meira Menezes ,2020, Non-clinical repeated dose 28-day oral toxicity, reproductive toxicity and cytotoxicity studies of the polar fraction of *Parkinsonia aculeata* aerial parts extract.

Maria Bs Maia, 2021- Polar fraction from Parkinsonia aculeata aerial parts extract improves imbalanced metabolic profile and reduces proinflammatory interleukin levels in white adipose tissue in obese rats induced by western diet.

CHAPTER 3

AIM AND OBJECTIVES

3.1 AIM:

Preparation of copper nanoparticles by biosynthetic methods and characterization from plant extract of [Parkinsonia aculeata]

3.2 OBJECTIVES:

SEM and EDX for examination and analysis of micro and nanoparticles imaging characterization of solid objects for particle size.

XRD is used for - to analyze the physical Properties such as Phase composition, structure, and orientation powder

FTIR –is used to identify the organic polymeric and inorganic materials.

Antibacterial test is used for copper nanoparticles have garnered significant attention due to their unique properties and potential applications. One area of interest is their antibacterial behaviour

Anticancer test is used for copper has been shown to be cytotoxic to cancer cells. It can regulate protein activity, metabolism, and inflammation within cells.

CHAPTER 4

MATERIALS AND METHODS

4.1 MATERIALS REQUIRED:

- Plant extract (*Parkinsonia aculeata*)
- Water
- Whatman Filter paper
- Magnetic stirrer
- Copper sulphate 2 pentahydrate
- Starch
- Sodium hydroxide
- Centrifuge
- Ethanol
- Hot air oven for powdering
- Petri plates
- Micropipettes
- Conical flasks
- Centrifuge tubes
- Micropipettes tips
- Microcentrifuge tubes
- Weighing balance
- Cotton
- Parafilm
- Autoclave
- Laminar air flow

4.2. METHODOLOGY:

4.2.1 Synthesis of copper nanoparticle

- Collected leaves were rinsed with tap water to remove dust particles. Further, leaves were rinsed with double distilled water (DDW) and shade dried for 1 week to remove the moisture content.
- The dried leaves were powdered in grinder mixer and powder stored in dark at ambient temperature. To prepare the plant extract, 2 gm of dried leaf powder was added in 100 mL deionized water in 200 mL flask, mixed well on a magnetic stirrer with hot plate at 60 °C for 20 min. The prepared extract was filtered using Whatman filter paper with size 11 µm followed by vacuum filtration using cellulose nitrate membrane. The filtrate was used immediately or stored at 4 °C for further use.
- The Cu nanoparticles were synthesized by chemical reduction process using copper (II) sulphate pentahydrate as precursor salt and starch as capping agent. The preparation method starts with addition of 0.1 M copper (II) sulphate pentahydrate 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 degree C for 2 h.
- The colour of the solution turned yellow to ocher. 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. Ocher colour precipitates obtained are dried at room temperature. After drying, nanoparticles were stored in glass vial for further analysis (fig 4.2)



fig.4.2.1 Synthesis of copper nanoparticle

4.3 CHARACTERIZATION OF COPPER NANOPARTICLE

4.3.1 Sem and EDX analysis results

Scanning Electron Microscopy (SEM):

- SEM provides high-resolution images of a sample's surface by scanning a focused electron beam across it.
- It detects secondary or backscattered electrons, revealing topographic information about the sample.

Energy Dispersive X-ray Spectroscopy (EDX):

- EDX, also known as EDS (Energy Dispersive Spectroscopy) or EDA (Energy Dispersive X-ray Analysis), enhances SEM by providing additional insights into a sample's composition.

4.3.2 XRD analysis

We use XRD test in copper nanoparticles for to synthesized nanoparticle was to identify the crystal structure of nanoparticle and showed its peak at 36.34° to 73.57°

XRD provides useful information on the crystal phase, lattice constant, and average particle size of nanoparticle

In the case of bimetallic nanoparticles, XRD is important to confirm whether the bimetallic nanoparticles adopt alloy structure

This technique will measure the internal stress, the size and shape of small crystalline areas, and measure the average spacings between the layers of rows of atoms in samples. It can determine their minerology.

4.3.3 FTIR analysis

Is a very versatile tool for surface characterization of nanoparticles. Under specific conditions, the NPs surface chemicals composition can be determined, and additionally, the reactive surface sites responsible for the surface reactivity can be identified.

FTIR is used for to identify and classify probable biomolecules that can be reliable for capping, leading to proficient stabilization of the gold and silver nanoparticle.

It allows new gases and gas ranges to be added easily to the analysis without any hardware changes to without changing the analyser

4.3.4 Antibacterial activity

The antibacterial property of copper nanoparticles was attributed mainly to adhesion with bacteria because of their opposite electrical charges, resulting in reduction reaction at the bacterial cell wall.

NPs in particular have demonstrated broad spectrum antibacterial properties against both Gram positive and Gram-negative bacteria. Its effectively impede biofilm formation

Antibacterial activity refers to chemicals that kill or limit the growth of bacteria on a local level while remaining non-toxic to surrounding tissue.

4.3.5 Anticancer activity

The anticancer activity can be increased by increasing the concentration of solution. Copper complexes include all types of cell death by influencing various death related genes and proteins.

Reactive oxygen species (ROS) mediated apoptosis being the most studied among them. Up and down regulation of proteins, immunological interventions, inhibition of transcription, site specific cytotoxicity.

Copper is cytotoxic to cancer cells, has anti angiogenic, cell cycle growth inhibiting And energy metabolism regulating effects in anticancer therapy

4.3.6 Staining cells

DAPI staining is generally used to stain fixed cells since the dye is cell impermeant although the stain will enter live cells when used at higher concentrations. For live cells staining.

DAPI live cell staining will also label dead cells unless used with a counterstain.

DAPI associates with the minor groove of double stranded DNA with a preference for the adenine - thymine clusters.

DAPI has poor solubility in water.

CHAPTER 5

RESULTS AND DISCUSSION

The visual displays of size and shape of synthesized CuNPs as depicted by SEM micrographs are shown in figure, the SEM images also demonstrated the non-homogeneity of the particles in terms of their shape and size. All the possible spherical and irregular shapes such as truncated hexagonal, cylindrical, triangular, and prismatic shapes of CuNPs with varying particle sizes were found in the micrographs.

The average grain size of CuNPs was found in the range of 10-50 nm. It seems particles were found to agglomerate moderately due to the high surface area resulting in the formation of medium sized nanoparticles.

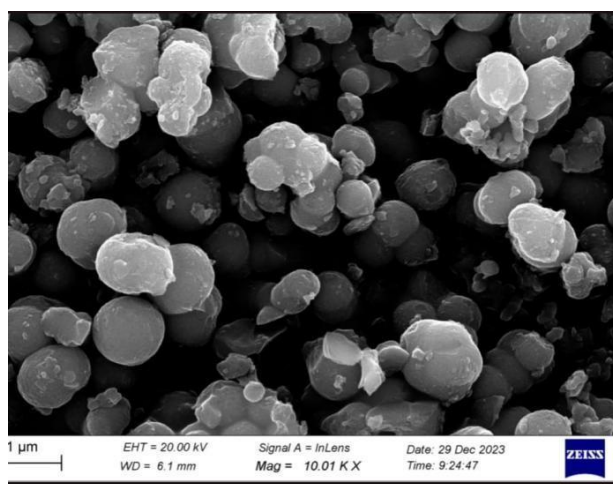


Fig 5.1.1: SEM image of copper nanoparticle at 10.01kx magnification

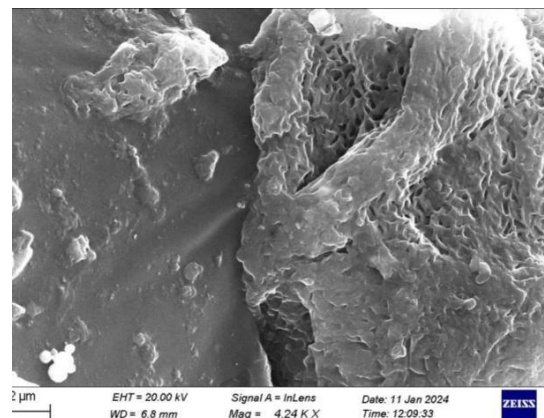


Fig 5.1.2: SEM image of copper nanoparticle at 4.24kx magnification

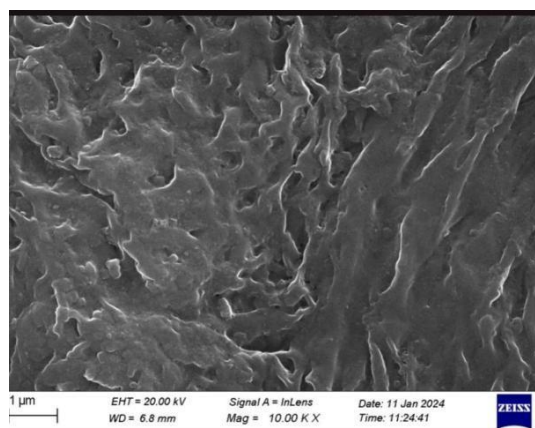


Fig 5.1.3: SEM image of copper nanoparticle at 10.00kx magnification

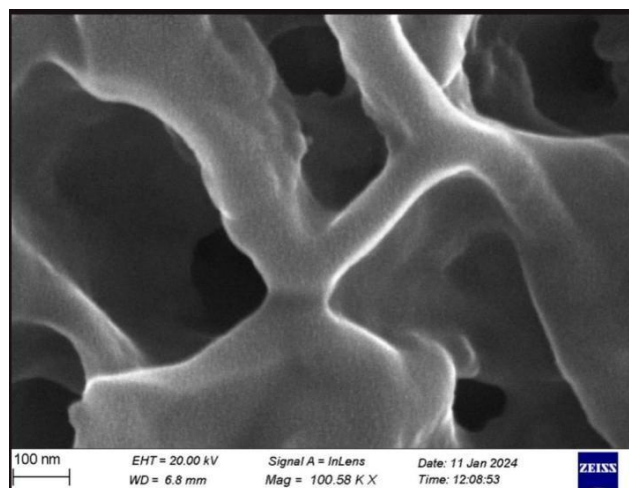


Fig 5.1.4: SEM image of copper nanoparticle at 100.58 kx magnification

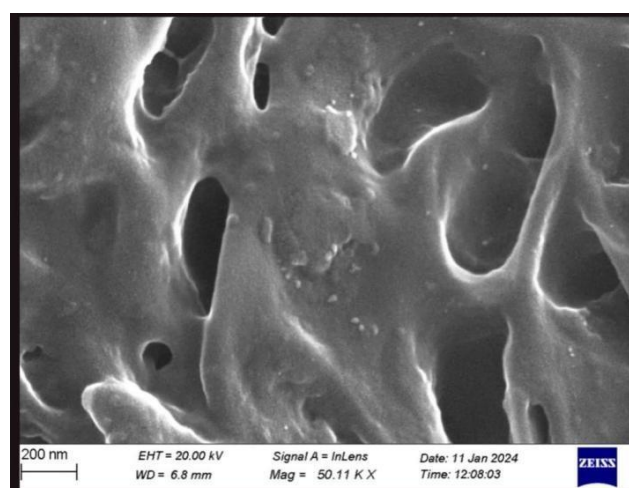


Fig5.1.5: SEM image of copper nanoparticle at 50.11 kx magnification

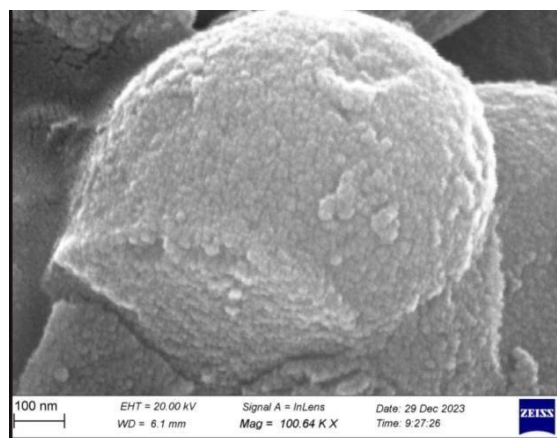


Fig 5.1.6: SEM image of copper nanoparticle at 100.64 kx magnification

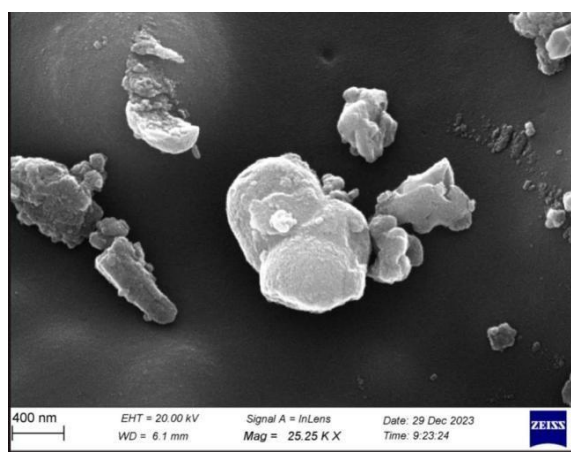


Fig 5.1.7: SEM image of copper nanoparticle at 25.25kx magnification

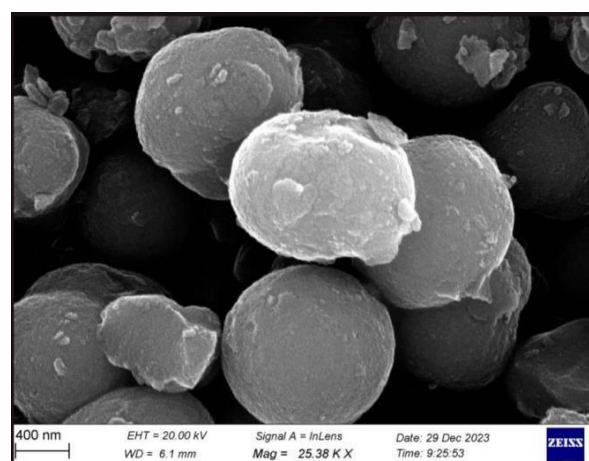


Fig 5.1.8: SEM image of copper nanoparticles at 25.38 kx magnification

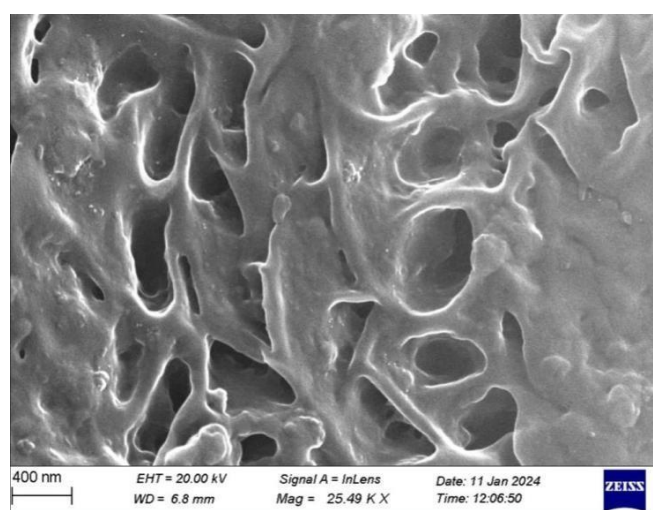


Fig5.1.9 SEM image of copper nanoparticles at 25.49 kx magnification

5.1.10 EDX analysis:

The chemical composition of the NPs was studied by EDAX analysis. Nanoparticles are a key ingredient in many industrialised products such as paints, plastics, metals, cosmetics and even food. Characterising the key parameters of nanoparticles (size, shape, concentration, porosity...etc) is essential to understand their behaviour, performance, and risk to the environment and human health. In addition, their presence needs to be controlled and well labelled on products.

One of the most used techniques to characterise nanoparticles is scanning electron microscopy (SEM) with Energy Dispersive Spectrometry (EDS). Due to their size (1-100nm), they cannot be seen using optical microscopes, hence the need for such techniques. High resolution imaging in SEM allows the imaging of the particles, and EDS gives elemental and chemical information about the nanoparticles.

The size of nanoparticles and their low emission of X-rays make their analysis challenging. Many aspects need to be considered to have a successful analysis

Present in Carbon, oxygen, sodium, chlorine – Electron

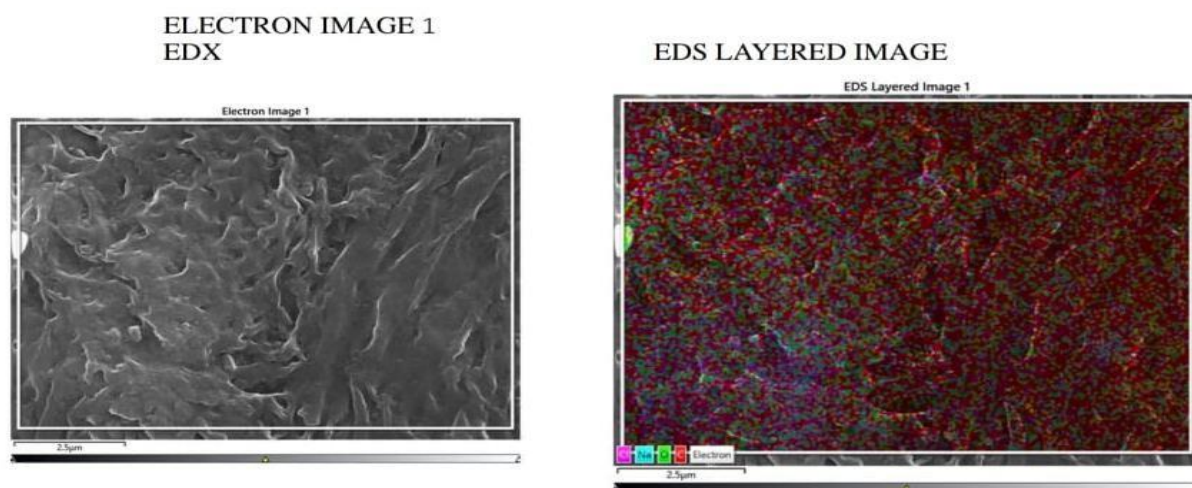


FIG. 5.1.10 EDS ELECTRON LAYERED IMAGE

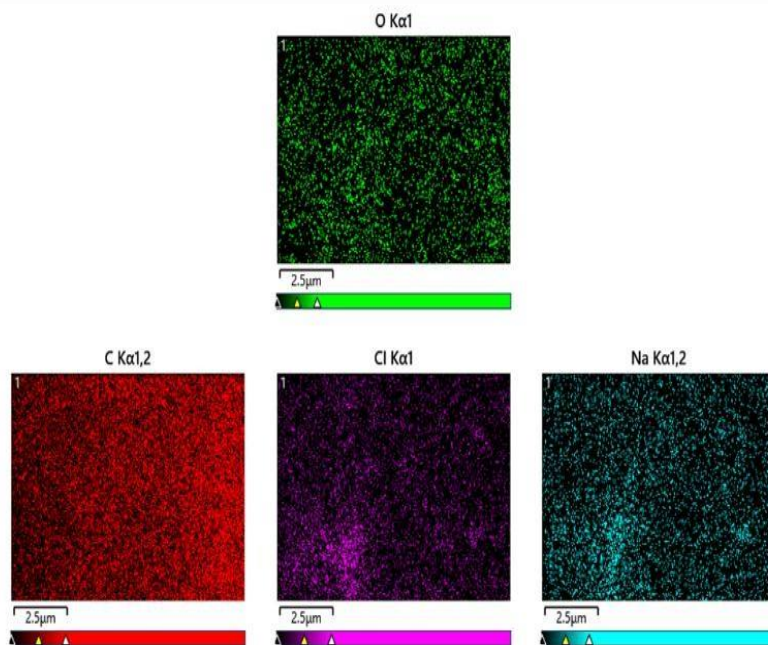


FIG 5.1.11 EDS ELECTRON IMAGE

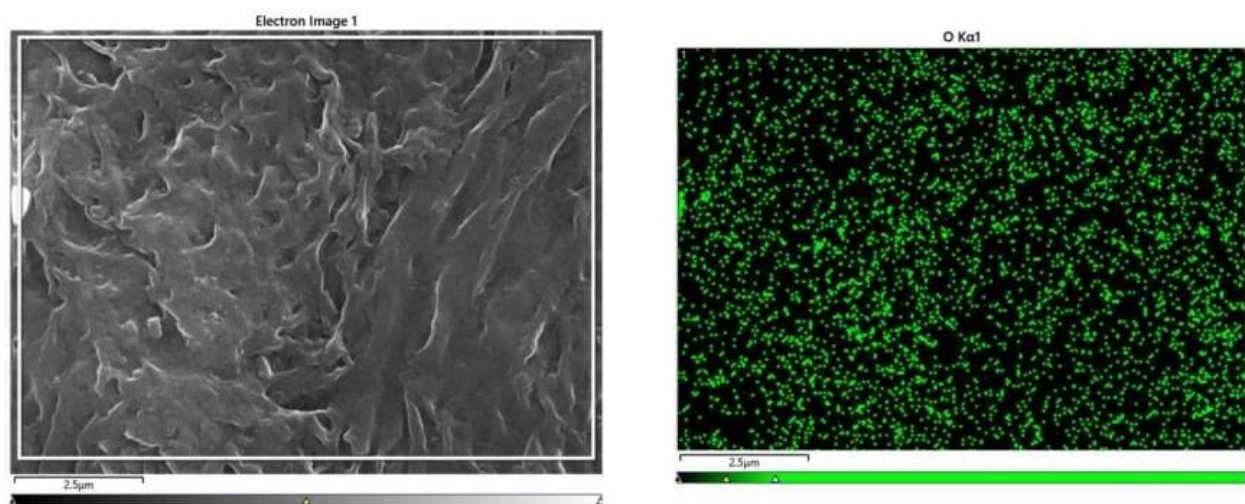
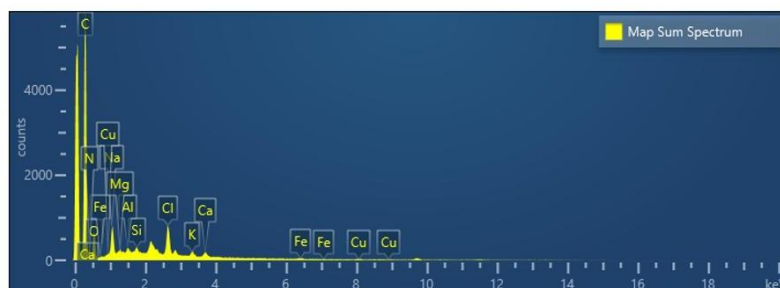


Fig5.1.12
ELECTRON IMAGE

TABLE 5.1.13

Graph And Table for EDS

Map Sum Spectrum				
Element	Line Type	Weight %	Weight % Sigma	Atomic %
C	K series	69.89	1.24	75.99
N	K series	12.23	1.48	11.41
O	K series	12.63	0.40	10.31
Na	K series	1.97	0.08	1.12
Mg	K series	0.11	0.03	0.06
Al	K series	0.21	0.03	0.10
Si	K series	0.19	0.03	0.09
Cl	K series	1.63	0.05	0.60
K	K series	0.32	0.03	0.11
Ca	K series	0.35	0.03	0.11
Fe	K series	0.18	0.05	0.04
Cu	K series	0.29	0.07	0.06
Total		100.00		100.00

5.2. XRD

X-ray diffraction analysis shows the presence of three prominent peaks at 2θ values in the range of 20° to 80° at 43.6° , 50.8° , and 73.5° . These 2θ values present (111), (200), and (220) crystallographic planes from the JCPDS database, respectively. The XRD pattern confirmed the high crystallinity nature of CuNPs.

The peaks can be indexed to be typical monoclinic structures of Cu NPs. The average crystallite size was calculated using the Scherrer equation and was found to be 31.58 nm. Other researchers have confirmed the green synthesis of Cu NPs in this size range. 22.4 nm of Cu NPs were synthesized using leaf extract.

The average size of Cu NPs was calculated as 29.7 nm in a green synthesis using *Curcuma longa* root extracts. The d-spacing and lattice constant parameters were determined using Bragg's law to be 0.2 nm and 0.34 nm, respectively. These results correspond to previous study.

Constructive interference of monochromic X-rays and a crystalline sample. To analyze xrd results in measure intensity of peak from the graph. To identify and study material structures

PEAK RANGE:

size around 50nm peaks

around 560-570

XRD peak 36.34

DIFFRACTION PEAKS:

43.28- 74.81 (Fig 5.2.1)

Match! Phase Analysis Report

Sathyabama Institute of Science and Technology

Sample: XRD

Sample Data

File name	XRD.dat
File path	E:/XRD Data/2024/1. Jan/5.1.24/Nivetha - Sathyabama/XRD
Data collected	05/01/2024 09:57:05
Data range	4.170° - 116.650°
Original data range	0.124° - 118.067°
Number of points	3810
Orig. number of points	3995
Step size	0.030
Rietveld refinement converged	No
Alpha2 subtracted	No
Background subtr.	No
Data smoothed	No
Radiation	X-rays
Wavelength	1.540560 Å

Peak List

No.	2theta [°]	d [Å]	INT (peak height)	Counts (peak area)	FWHM
1	22.92	3.8767	12.12	20.56	0.4190
2	29.60	3.0159	62.46	143.46	0.5671
3	36.48	2.4613	1000.00	2758.29	0.6810
4	42.38	2.1309	294.27	995.86	0.8356
5	52.63	1.7376	19.23	48.33	0.6205
6	60.90	1.5200	94.99	295.40	0.7678
7	73.74	1.2838	120.98	763.26	1.5576
8	77.43	1.2315	30.43	200.66	1.6280
9	103.89	0.9782	26.09	369.41	3.4652

Integrated Profile Areas

Based on calculated profile

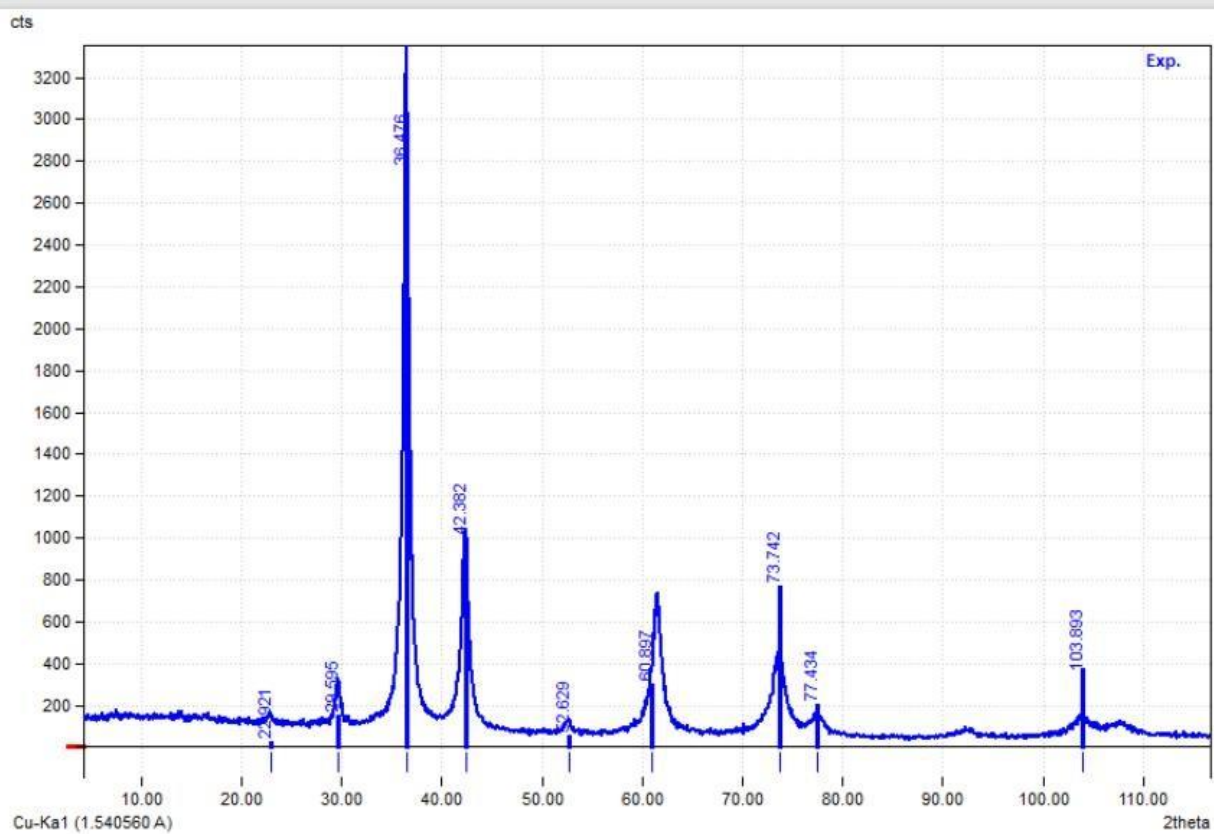
Profile area	Counts	Amount
Overall diffraction profile	540492	100.00%
Background radiation	295335	54.64%
Diffraction peaks	245157	45.36%
Peak area belonging to selected phases	0	0.00%
Unidentified peak area	245157	45.36%

Peak Residuals

Peak data	Counts	Amount
Overall peak intensity	5595	100.00%
Peak intensity belonging to selected phases	679	12.13%
Unidentified peak intensity	4916	87.87%

Diffraction Pattern Graphics

Fig 5.2.1 XRD



PDF-2 Database Copyright International Centre for Diffraction Data (ICDD)
Match! Copyright © 2003-2023 CRYSTAL IMPACT, Bonn, Germany

Graph for XRD 5.2.2

5.3. FTIR

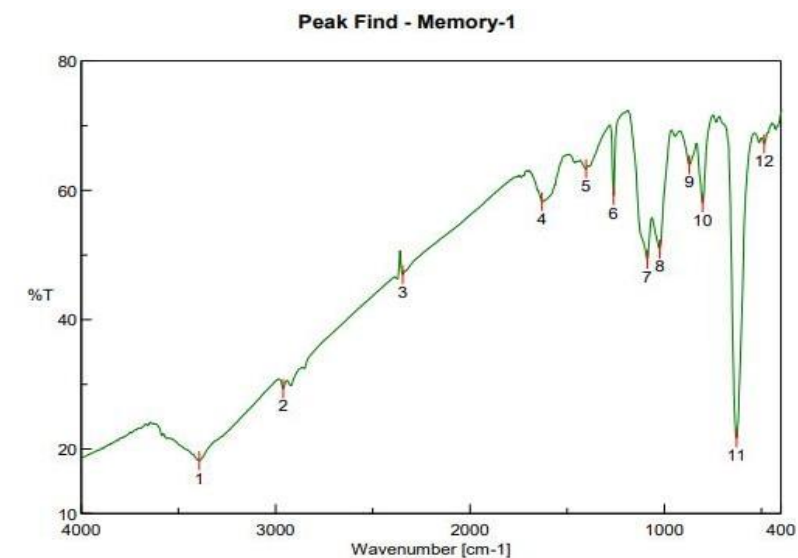
The infrared spectra of copper oxide nanorods are shown in Figure 5.3. The peak observed at $2000 - 400\text{ cm}^{-1}$ is a band associated with the stretching vibration of Cu–O bonds. The absorption bands within 1350 cm^{-1} and 1650 cm^{-1} are indications of atmospheric CO_2 .

Spectra bands occurring between 2800 and 3500 cm^{-1} correspond to O–H bonds. O–H bonds with very low bands are due to the minute presence of a moisture content.

The FTIR confirms that copper oxide was synthesised and confirmed by the presence of a Cu–O bond. The FTIR spectra analysis of zinc oxide is shown in the figure. The absorption band at 863 cm^{-1} is an indication of Zn–O vibration.

(FTIR) is a powerful technique employed to obtain an infrared spectrum of absorption or emission from solids, liquids, or gases.

- It is widely used in fields like chemistry and pharmaceuticals.
- For instance, it can confirm the identity of known compounds or detect unknown compounds
- The Far-IR region lies between 10 cm^{-1} and 700 cm^{-1} .



[Comments]
Sample name
Comment
User
Division
Company

Sathyabama Institute of Science and Technology

[Detailed Information]

Creation date 30-01-2024 10:21
Data array type Linear data array
Horizontal axis Wavenumber [cm-1]
Vertical axis %T
Start 349.053 cm-1
End 7800.65 cm-1
Data interval 0.964233 cm-1
Data points 7729

[Measurement Information]

Model Name FT/IR-6600typeA
Serial Number B049361790
Measurement Date 30-01-2024 10:21
Light Source Standard
Detector TGS
Accumulation 16
Resolution 4 cm-1
Zero Filling On
Apodization Cosine
Gain Auto (2)
Aperture Auto (7.1 mm)
Scanning Speed Auto (2 mm/sec)
Filter Auto (10000 Hz)

[Result of Peak Picking]

No.	Position	Intensity
1	3393.14	18.1931
2	2961.16	29.3378
3	2345.98	46.9432
5	1402	63.3285

[Result of Peak Picking]

No.	Position	Intensity
7	1087.66	49.3361
9	870.703	63.9411
11	628.68	21.7009

Fig 5.3.1 FTIR:

5.3 ANTIBACTERIAL ACTIVITY:

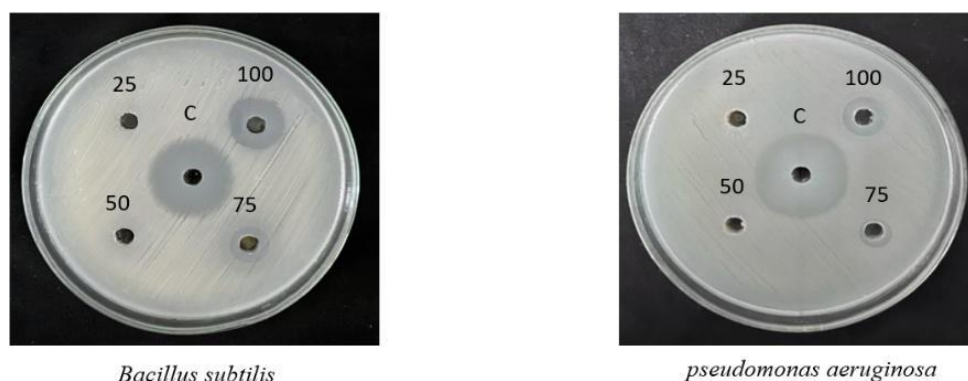


Fig 5.3.1 ZONE OF INCUBATION

Table 5.3.2 Antibacterial activity

Microorganism	25	50	75	100	+ve
BS	0	0	10	16	23
PA	0	0	8	11	24

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.

The ethanolic leaf extracts of *Parkinsonia aculeata* against *S. typhi* shows antimicrobial activity. The antibacterial activity of *Parkinsonia aculeata* ethanolic extracts was tested against *E. coli*, *S. typhi*, *K. pneumonia* bacteria. The results indicated that the three concentrations were effective against tested bacteria. Besides, the effects of pH caused a significant variation in the zone of inhibition.

The maximum antibacterial activity has been checked for ethanolic extract against three bacterial strains. The optimization or maximum antibacterial activity has been checked for ethanolic extracts against bacterial species. After the analysis, the final parameters were determined, and it was concluded that temperature, concentration and pH were significant among the three factors studied against *E. coli*, *S. typhi*, and *K. pneumonia*. By using Response Surface Methodology (RSM). Hence, it is suggested from this review work that *Parkinsonia aculeata* when used in proper PH, Temperature, and Concentration, can work to treat profuse bacterial inflammations in the biological systems.

5.4 ANTICANCER ACTIVITY:

Cancer is the uncontrollable programmed growth of abnormal cells in the body. It can occur in any part of the body which affects the normal mechanism of a working cell. There are different types of cancers which are the leading cause of death. Tobacco is the major cause of cancer, which is responsible for patient's cancer deaths. The cancer treatment includes many treatments such as surgery, chemotherapy, and radiation therapy.

These all treatments have painful side effects. Such as chemical changes in the body, weight loss, difficulty in breathing, fatigue, and hair loss. The plant of

Parkinsonia aculeata having source of providing the latest chemoprotective potential against cancer. The ethanolic leaves extract using the Soxhlet extraction apparatus of *Parkinsonia aculeata* contains parkinsonin-A, parkinsonin-B, and parkintin, have shown that some flavonoids modulate the metabolism and can contribute to cancer prevention. In the In-vitro antimutagenic and DNA protecting potential of methanol, hexane, n-butanol and aqueous extract of *Parkinsonia aculeata*. It was evaluated by two different assays, the Ames assay and DNA nicking assay.

The study found that all the extracts inhibited DNA damage by the hydroxyl radicals. The study found that *Parkinsonia aculeata* effectively act as antimutagenic against 4- Nitro-o-phenylenediamine sodium azide and 2-Aminofluorene in the strains of *Salmonella typhimurium*.

Cell Culture Maintenance

MDA-MB-231 (triple-negative Human breast adenocarcinoma epithelial cell line) was obtained from the National Centre for Cell Sciences (NCCS), Pune, India.

Cells were maintained in the logarithmic phase of growth in Dulbecco's modified eagle medium (DMEM) supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS), 100 U/mL penicillin, 100 µg/mL streptomycin.

They were maintained at 37°C with 5% CO₂ in 95% air humidified incubator. The anticancer activity of the sample was tested against MDA-MB-231 cell line by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (Mossman, 1983).

The cells were seeded in 96-well microplates (1 x 10⁶ cells/well) and incubated at 37°C for 48 h in 5% CO₂ incubator and allowed to grow 70-80% confluence. Then the medium was replaced and the cells were treated with different concentrations of sample and incubated for 24 h.

The morphological changes of untreated (control) and the treated cells were observed under digital inverted microscope (20X magnification) after 24 h and photographed.

The cells were then washed with phosphate-buffer saline (PBS, pH-7.4) and 20 μ L of (MTT) solution (5 mg/mL in PBS) was added to each well.

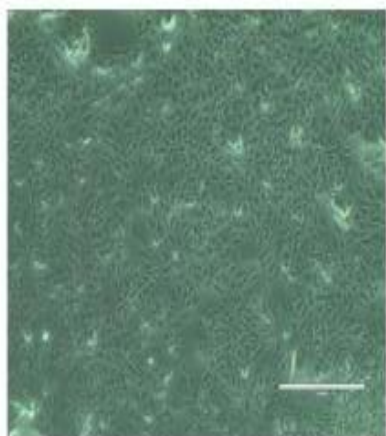
The plates were then stand at 37°C in the dark for 2 h. The formazan crystals were dissolved in 100 μ L DMSO and the absorbance was read spectrometrically at 570 nm.

Percentage of cell viability was calculated using the formula,

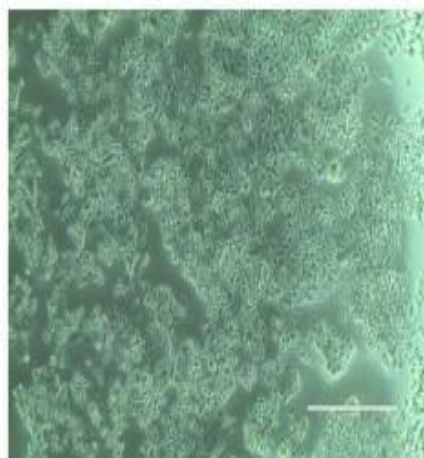
$$\text{Cell viability (\%)} = (\text{Absorbance of sample} / \text{Absorbance of control}) \times 100$$

ANTICANCER TEST

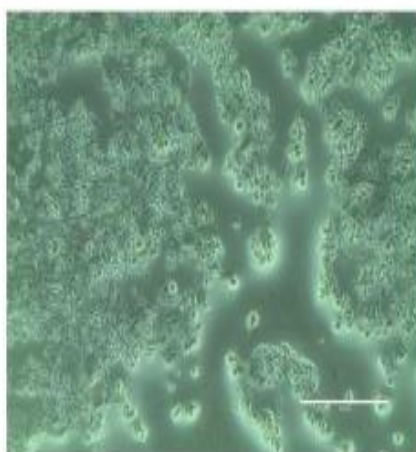
CONTROL20



CONTROL 40



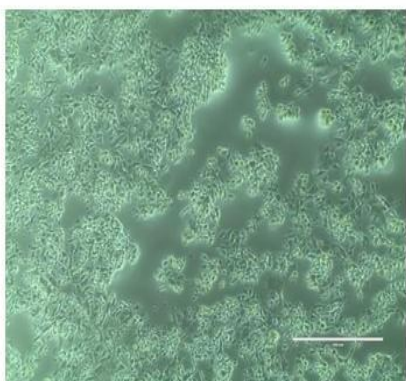
CONTROL 60



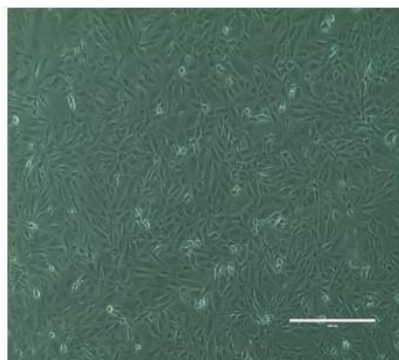
RESULTS: ANTICANCER

Fig 5.4.1 BACTERIAL GROWTH

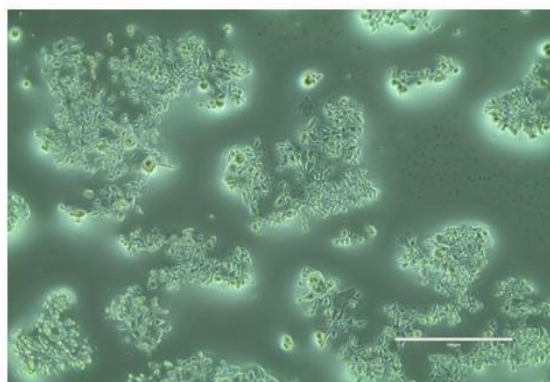
CONTROL 80



NORMAL CONTROL



CONTROL 100



(Fig 5.4.2) BCTERIAL GROWTH

Table 5.4.3 For Anticancer test

MTT READINGS

MDA-MB-231				
Concentrations ($\mu\text{g/mL}$)	Absorbance		Average	Cell Viability (%)
	I	II		
Control	0.613	0.605	0.609	100
20	0.508	0.497	0.5025	82.51231527
40	0.345	0.338	0.3415	56.07553366
60	0.213	0.221	0.217	35.63218391
80	0.156	0.146	0.151	24.79474548
100	0.091	0.098	0.0945	15.51724138

5.6 STAINING CELLS :

DAPI (pronounced ‘DAPPY’), or 4',6-diamidino-2-phenylindole, is a fluorescent stain that binds strongly to adenine–thymine-rich regions in DNA

Fluorescence Microscopy: DAPI is extensively used in fluorescence microscopy. It binds to DNA, allowing researchers to visualize and study cellular nuclei and chromatin structures.

Nuclear Counterstain: In fluorescence microscopy, DAPI serves as a nuclear counterstain. Its blue fluorescence contrasts well with other fluorescent probes used to label different cellular structures.

Fixed Cells Staining: DAPI is typically used to stain fixed cells since it is cell impermeant. However, it can also enter live cells when used at higher concentrations.

Flow Cytometry: DAPI is employed as a DNA stain in flow cytometry, aiding in the analysis of cell populations based on their DNA content.

Chromosome Staining: Researchers use DAPI to stain chromosomes during cytogenetic studies.

Mitochondrial DNA Identification: DAPI was initially used to identify mitochondrial DNA in ultracentrifugation, marking its first recorded application as a fluorescent DNA stain.

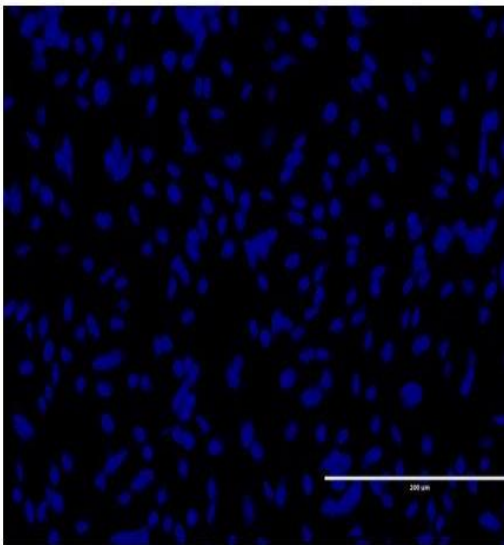
Cell Viability and Membrane Integrity:

Although DAPI is generally used to stain fixed cells (since it is cell impermeant), it can also enter live cells when used at higher concentrations. Thus, it provides a marker for membrane viability.

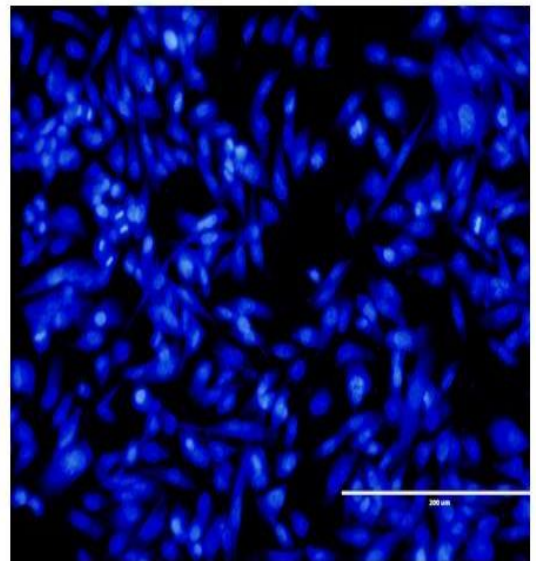
For live-cell staining, Hoechst 33342 dye is a popular alternative as a cell-permeant nuclear counterstain.

Its strong fluorescence when bound to DNA led to its rapid adoption for staining DNA in various organisms, including plants, metazoan, bacteria cells, and virus particles.

CONTROL CELLS



TREATED CELLS



5.6.1.Results for staining cells:

CHAPTER 6.

SUMMARY AND CONCLUSION:

Copper oxides and nanoparticles were synthesized by the chemical reduction method and were characterized by SEM AND EDX, FTIR, XRD, Antibacterial Test, Anticancer Test and Staining Test.

The different concentrations (10,15,20,25,30,35,40,45,50) for antimicrobial and anticancer test. The different peak ranges of each nanoparticle are present in the tests. In the Antibacterial test the microorganisms of *Bacillus subtilis* is present in 10 zone of incubation is formed in 75, and 16 zone of incubation in 100 and *Pseudomonas aeruginosa* is present in 8 zone of incubation in 75 and 11 zone of incubation in 100, the positive concentration of BS is 23 and PA was 24. The nanoparticle size and shape were observed by SEM EDX, XRD and internal compositions are observed by FTIR. finally the copper oxide nanoparticles are observed by the extraction of (*Parkinsonia aculeata*). It is used as medicine like pils, oils, antiseptic creams, ointment, some peoples are taking it as a food.

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