GREEN SYNTHESIS OF COPPER NANOPARTICLES USING HIBISCUS ROSA-SINENSIS STEM: EVALUATION ON ANTI-OXIDANT, ANTI-INFLAMMATORY, ANTI-BACTERIAL & ANTI-CANCER ACTIVITIES:

Dissertation Submitted to Vels University in Partial Fulfilment of the Award of the Degree of

MASTERS OF SCIENCE

IN

APPLIED MEDICAL BIOTECHNOLOGY & CLINICAL RESEARCH

Submitted by

DEEPTHIMAHANTHI YAMINIPRIYA

(Reg No: 23223101)

Under the Guidance of

Dr. M. JAYANTHI M. Sc; M. Phil; Ph. D

Assistant Professor

INSTITUTE OF SCIENCE, TECHNOLOGY & ADVANCED STUDIES (VISTAS)
(Deemed to be University Estd. u/s 3 of the UGC Act, 1956)
PALLAVARAM, THALAMBUR, PERIYAPALAYAM-CHENNAI
ACCREDITED BY NAAC WITH 'A++' GRADE

APRIL 2025

VELS

INSTITUTE OF SCIENCE, TECHNOLOGY & ADVANCED STUDIES (VISTAS)
(Deemed to be University Estd. u/s 3 of the UGC Act, 1956)
PALLAVARAM, THALAMBUR, PERIYAPALAYAM-CHENNAI
ACCREDITED BY NAAC WITH 'A++' GRADE

CERTIFICATE

This is to certify that project entitled "**GREEN SYNTHESIS OF COPPER NANOPARTICLES USING HIBISCUS ROSA-SINENSIS STEM: EVALUATION ON ANTI-OXIDANT, ANTI-INFLAMMATORY, ANTI-BACTERIAL & ANTI-CANCER ACTIVITIES",** submitted for the degree of Masters of Science in Applied Medical Biotechnology & Clinical Research, **Deepthimahanthi Yaminipriya, Reg. No: 23223101,** is the record of research work carried out under the guidance of **Dr. M. Jayanthi,** Assistant Professor, Department of Biotechnology, Vels Institute of Science and Advanced Studies, during the year 2024-2025.

Signature of the Guide **Head of the Department**

Internal Examiner **External Examiner**

BioEdge SOLUTIONS

Dr. Shruthi SD

+91 94827 74014
+91 80733 69271
services@bioedgesolutions.com
sdshruthi@gmail.com
1st floor, M-400, 8th cross, 1st Stage
Peenya Industrial Area, Bangalore 560058

No: BES/CER/550/25-26 Apr 17th, 2025

CERTIFICATE

This is to certify that **Ms. Deepthimahanthi YaminiPriya, Reg no:23223101,** MSc Applied Medical Biotechnology and Clinical Research from Vels Institute of Science, Technology and Advanced Studies(VISTAS), Chennai, Tamilnadu, has successfully completed 4 months final year dissertation entitled "**Green Synthesis of Copper Nanoparticles Using *Hibiscus Rosa-sinensis* Stem : Evaluation of Anti-Oxidant, Anti-Bacterial, Anti-Inflammatory and Anti-Cancer Activities**" at BioEdge Solutions, Bangalore under the guidance of Dr. SHRUTHI SD during 30-12-2024 to 15-04-2025. She has successfully learnt and handled Phytochemistry, Pharmacology, Biochemistry and Animal cell culture techniques. I am pleased to state that she has completed the term with punctuality, hard work and as an inquisitive student.

Place: Bangalore
Date: 17-04-2025

Yours sincerely,

For BioEdge Solutions

Proprietress

DECLARATION

I declare that the research work entitled **"GREEN SYNTHESIS OF COPPER NANOPARTICLES USING HIBISCUS ROSA-SINENSIS STEM: EVALUATION ON ANTI-OXIDANT, ANTI-INFLAMMATORY, ANTI-BACTERIAL & ANTI-CANCER ACTIVITIES:"** submitted by me for the Degree of Masters of Science in Applied Medical Biotechnology & Clinical Research is the Original Work carried out by me under the guidance of **Dr. M. Jayanthi,** Department of Biotechnology, Vels Institute of Science Technology and Advanced Studies, during the year 2024-2025.

Signature of the Candidate

Place: Chennai

Date:

ACKNOWLEDGEMENT

I thank GOD for the blessing shown to carry out this project without any obstacles in the work and health. Inadequate thought in terms of words and expression, this project owes a lot to my beloved parents for their support, inspiration, encouragement, and good wishes for the success of my project.

With immense pleasure, I express my sincere thanks and indebtedness to **Dr.Ishari K. Ganesh,** Chancellor, **Dr.A.Jothi Murugan,** Pro Chancellor, **and Dr.Arthi Ganesh,** Pro-Chancellor. **Dr.Preethaa Ganesh,** Vice President, **Dr.S.Sriman Narayanan,** Vice Chancellor, **Dr.M. Bhaskaran,** Pro- Vice Chancellor, **Dr.P.Saravanan,** Registrar and **Dr.A.Udaya Kumar,** Controller of Examinations for their valuable support and also for providing laboratory facilities to complete my project work.

I take pleasure in expressing my sincere gratitude to **Dr.K.Ashok Kumar,** Head of the Department of Biotechnology for his encouragement and support to complete this project successfully.

In pursuit of academic endeavor, I feel that I have been especially fortunate, as inspiration, guidance, direction, cooperation, love and care all came my way in abundance and it seems almost an impossible task for me to acknowledge the same in adequate terms. I express my sincere thanks to my admired guide **Dr.M.Jayanthi**, Assistant Professor, Department of Biotechnology, Vels Institute of Science, Technology, and Advanced Studies (VISTAS), Chennai for her esteemed supervision, incessant support, inspiration, and constructive criticism throughout my research work.

I want to thank all the faculties **Dr.B.Sampath Kumar, Dr.S.Vijayalakshmi, Dr.N.K.UdayaPrakash,Dr.M.Thenmozhi, Dr.G.Abirami, Dr.M.Suganthi, Dr.B.Prakash, Dr.J.Vardhana, and Ms.N.Preethi** and last but not the least my gratitude towards non-teaching staffs of our department Dharshini and Vedhavalli for their upliftment towards our project. I too want to thank our beloved friends who made us uphold our project in a great manner. I thank all my batch mates for their kind support and suggestions.

INDEX

ABBREVIATIONS

Abbreviations	Expansions
Amp	Ampicillin
Abs	Absorbance
AE	Aqueous Extract
CuNPs	Copper Nanoparticles
Cuso4.5H2o	Copper (II) Sulfate Pentahydrate
CFU	Colony Forming-Unit
Caco-2	Human Colorectal Adenocarcinoma Cell Line
DMSO	Dimethylsulfoxide
DMEM	Dulbecco's Modified Eagle Medium
E.coli	Escherichia coli
EDTA	Ethylenediaminetetraacetic acid
FBS	Fetal Bovine Serum
GS-CuNPs	Green synthesized copper Nanoparticle
Hrs	Hibiscus rosa-sinensis
H2O2	Hydrogen Peroxide
IC50	Half Maximal Inhibitory Concentration
LB Agar	Luria-Bertani Agar
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
MTT	3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide Assay
Ml	Milliliter

MM	Mill molar
Min	Minute
NC	Negative Control
Nm	Nanometer
NP	Nanoparticle
PC	Positive Control
PMo Assay	Phosphomolybdate Assay
PDI	Protein Denaturation Inhibitory
PIA	Protein Inhibitory Activity
PBS	Phosphate Buffer-Saline
Rpm	Revolutions per minute
S.aureus	Staphylococcus aureus
Stdev	Standard deviation
TAC	Total Antioxidant Capacity
TE	Tris EDTA
UV-vis	Ultraviolet-visible
ZOI	Zone of Inhibition
°C	Degree Celsius
µl	Micro molar
µg	Microgram

ABSTRACT

The green synthesis of copper nanoparticles (CuNPs) represents a robust and environmentally sustainable approach to nanotechnology, harnessing the bio-active molecules present in plants as reducing and stabilizing agents. In this investigation, the stem extract of *Hibiscus rose-sinensis* was employed for its traditional medicinal applications. The synthesis of CuNPs offers a cost-effective and non-invasive alternative to conventional chemical methodologies. The bio-synthesized copper nanoparticles were characterized using UV-Vis spectroscopy, which confirmed their size, morphology, stability, concentration, and refractive index. The biological efficacy of these nanoparticles was extensively evaluated through antioxidant assays, including hydrogen peroxide (H_2O_2) scavenging and the total antioxidant capacity (TAC) assessed by the phosphomolybdenum method, which validated the radical-neutralizing capability of CuNPs. The TAC was determined based on the reduction of Mo (VI) to Mo (V) by the extract, leading to the formation of a green phosphate (Mo (V)) complex under acidic conditions. The anti-inflammatory potential was assessed via protein denaturation inhibition and proteinase inhibitory assays, demonstrating significant inhibition rates, with diclofenac serving as a reference drug. One of the underlying causes of inflammation is believed to be protein denaturation. Non-steroidal anti-inflammatory drugs (NSAIDs) mitigate protein denaturation while concurrently inhibiting the COX enzyme. The antibacterial activity was evaluated using the agar well diffusion method against intestinal pathogens such as *Escherichia coli* (*E.coli*) and *Staphylococcus aureus*, indicating pronounced microbial suppression. Furthermore, the anticancer efficacy of copper nanoparticles was analyzed using the MTT assay on CaCO-2 cells, revealing dose-dependent cytotoxicity and a promising IC_{50} value. Our findings indicate that *Hibiscus rose-sinensis* stem-mediated CuNPs have great potential for biomedical applications like managing oxidative stress, microbial infections, inflammation, and cancer treatment.This study underscores the significance of green nanotechnology in advancing bio-compatible therapeutic solutions. Consequently, CuNPs from Hibiscus rose-sinensis stem extract show strong antioxidant, anti-inflammatory, antibacterial, and anticancer properties. The results accentuate the capacity of these bio-fabricated nanoparticles to contribute to biomedical innovations. Future research should prioritize mechanistic studies and *in vivo* evaluations to further explore their therapeutic efficacy.

Keywords: *Hibiscus rose-sinensis; Copper nanoparticles; Green synthesis; Antioxidant; Bio-compatibility.*

INTRODUCTION

Copper nanoparticles (CuNPs) have significant attention due to their diverse applications in biomedical, agricultural, and environmental fields (Pourmadadi ***et al.,*** 2024). The synthesis of CuNPs using plant-primarily based and experienced chemistry methods has come to be a sustainable and eco-friendly alternative to standard chemical techniques (SALAMI ***et al.,*** 2023). Plants act as herbal decreasing and stabilizing agents, removing unwanted harmful chemical substances. *Hibiscus rosa-sinensis*, commonly known as the hibiscus plant, is widely recognized for its medicinal purposes. The stem of this plant carries various bioactive compounds, including flavonoids, polyphenols, and tannins, which could facilitate the reduction of copper ions to copper nanoparticles (Al-Hakkani ***et al.,*** 2020)**.** The synthesized CuNPs exhibit remarkable biological activities like antibacterial, antioxidant, anti-inflammatory, and anticancer properties. These features lead them to potential applications in cancer therapy, oxidative stress management, and preventing bacterial infections (Dharma raja ***et al.,*** 2017).

1.1 Nanotechnology and Green synthesis

1.1.1 Introduction to Nanotechnology:

Nanotechnology is an interdisciplinary area that deals with a vast area of synthesis and alertness of materials at the nanoscale, typically within the variety of 1-100 nm (Khan ***et al.,*** 2022). At this scale, substances show particular physicochemical properties due to the increased surface area, altered digital systems, and quantum confinement effects. Nanomaterials have received significant interest in diverse fields, together with medicine, drugs, agriculture, environmental technological know-how, and cloth engineering. Among those, metal nanoparticles (MNPs) were drastically explored for their catalytic, antimicrobial, antioxidant, and biomedical applications (Ahmad ***et al.,*** 2019). Copper nanoparticles (CuNPs) are of particular interest due to their extraordinary conductivity, catalytic efficiency, optical properties, and wide spectrum antimicrobial activity (Usman ***et al.,*** 2013). However, traditional methods of synthesizing CuNPs often involve chemical and physical methods, which depend upon hazardous chemicals, high electricity intake, and high-priced instrumentation. These drawbacks necessitate the development of green and sustainable synthesis strategies, leading to the emergence of green nanotechnology (Ying ***et al.,*** 2022).

1.1.2 Importance of Green Synthesis:

Green synthesis refers to the biosynthesis of nanoparticles using herbal, renewable assets inclusive of plant extracts, bacteria, fungi, and algae (Salem ***et al.,*** 2021). Among these,

phyto- assisted synthesis (plant-mediated nanoparticle synthesis) is favored because of its value, effectiveness, non-toxicity, scalability, and environmental sustainability (Chopra ***et al.,*** 2022). The fundamental aspects of green synthesis over chemical and physical strategies consist of:

- Bio compatibility: No poisonous decreasing agents or stabilizers are required.
- Eco-friendliness: Avoids dangerous waste generation.
- Cost-effectiveness: Uses with no trouble with available plant materials.
- Simplicity: Requires minimal energy and mild reactions.

Plants comprise a numerous array of phytochemicals, including polyphenols, flavonoids, tannins, alkaloids, terpenoids, and proteins, which act as natural reducing and stabilizing agents(Doughari ***et al.,*** 2012). These biomolecules not only lessen metallic ions but also additionally enhance the stability and organic nature of the synthesized nanoparticles (Roy ***et al.,*** 2019).

1.2 Copper Nanoparticles(CuNPs) and Their Significance

1.2.1 Properties of Copper Nanoparticles:

Copper nanoparticles (CuNPs) exhibit distinct physiochemical properties, making them fairly valuable in diverse medical and industrial applications (Crisan ***et al.,*** 2021). Some key characters consist of:

- High floor- to-extent ratio: It enhances reactivity and interaction with biomolecules.
- Excellent electrical and thermal conductivity: It is useful in electronics and sensors.
- Broad-spectrum antimicrobial activity: It is effective against bacteria, fungi, and viruses.
- Catalytic efficiency: It is applied in industrial reactions, wastewater treatment, and degradation of pollutants.

1.2.2 Applications of Copper Nanoparticles:

A. Biomedical Applications:

- **Antimicrobial Agents:** Copper nanoparticles have been shown to have strong antibacterial and antifungal activity officials against pathogens such as Escherichia coli, staphylococcus aeurgonisa and Candida albicans (Jafari ***et al.,*** 2015).
- **Antioxidant Capacity:** Copper nanoparticles free radical scavenging activity, causing them valuable in preventing oxidative- stress related diseases (Liu ***et al.,*** 2020).

- **Anti-Inflammatory Application:** Copper nanoparticles can modify inflammatory routes by preventing protein denaturation and enzyme activity (Thiruvengadam ***et al.,*** 2019).
- **Anti Cancer Therapy:** Copper nanoparticle induces cytotoxicity in cancer cells through oxidative stress and apoptosis mechanisms (Fahmy ***et al.,*** 2009).
- **Drug Delivery Systems:** Functionalized copper nanoparticles can be used as a carrier for targeted drug distribution in cancer therapy(Denoyer ***et al.,*** 2015)

B. Environment Applications:

- **Water Purification:** Copper nanoparticles are used to remove microbial disinfection and heavy metals.
- **Catalytic degradation of pollutants:** Copper nanoparticles serve as catalysts in breaking organic pollutants in wastewater.
- **Agricultural Use:** Copper nanoparticles have been discovered to increase insect control and plant growth.

Despite these benefits, chemical synthesis of copper nanoparticles leads to aggregation and oxidative stress, which reduces their stability and efficacy (Naz ***et al.,*** 2020). Therefore, green synthesis is a preferred option for the production of stable, biofuctured copper nanoparticles with enhanced biocompatibility.

1.3 Why Hibiscus rosa - sinensis stem?

1.3.1 Taxonomical Classification of Hibiscus rosa – sinensis:

- **Kingdom :** Plantae
- **Phylum :** Magnoliophyta
- **Class :** Magnoliopsida
- **Order :** Malvales
- **Family :** Malvaceae
- **Genus :** Hibiscus
- **Species :** *Hibiscus rosa sinensis.*L
- **Varieties:** Gator Magic, Donna Lynn
- **Medicinal Properties:** Heart, Anti - Oxidant, Wound Healing, Inflammation
- **Traditional Properties:** Anti- Inflammatory, Anti- Microbial
- **Ayurvedic Properties:** Anti Diabetic, Anti – Inflammatory

1.3.2 Phytochemical of Hibiscus rosa sinensis stem:

The stem of Hibiscus rosa-sinensis is rich in bioactive a compound that plays a crucial role in nanoparticle synthesis (Nayak ***et al.,*** 2015). Some of the most important phytochemicals identified:

- Flavonoids (quercetin, kaempferol, myrecitin) act as stabilizing and lowering agents.
- Tannins act as for contributing antioxidant and antibacterial activites.
- Alkaloids are recognized for their anti – inflammatory and cytotoxic consequences.
- Saponins act as establishing the biocompatibility of synthesized nanoparticle.
- Phenolic compounds are responsible for free radical scavenging and metallic ion reduction.

The presence of hydroxyl (-OH) and carboxyl (-COH) functional groups in these phytochemicals provide cu2+ ions deficiency to CuNPs (Al-senani ***et al.,*** 2020). Additionally, biomolecules coats the nanoparticles, preventing aggregation and oxidation which increases their stability. While various parts of *Hibiscus rosa- sinensis* (leaves, flowers, roots) are used for medicinal properties whereas, stem is inferior to nanoparticle synthesis (Missoum ***et al.,*** 2018). Thus, the purpose of this study is to strengthen this research gap by evaluating the capacity of *Hibiscus rosa- sinensis* stem extracts of green synthesis of CuNPs.

AIM & OBJECTIVE

AIM

To synthesize copper nanoparticle from Hibiscus rosa sinensis stem and it's Anti-cancer, Anti-inflammation, Anti-oxidant and Anti- bacterial.

OBJECTIVE

- ✓ Collect the plant sample of Hibiscus stem and from it prepare aqueous extract.
- ✓ Synthesize copper nanoparticles and characterize.
- ✓ To perform in-vitro Anti-oxidant studies and compare between extract & nanoparticles.
- ✓ Then check Anti-bacterial effect against intestinal pathogen.
- ✓ To evaluate the in-vitro Anti-inflammatory activity by assessing their ability to inhibit protein denaturation and proteinase inhibitory activity.
- ✓ To perform Anti-cancer studies against intestinal cancer cells ***Caco-2*** cell line.

REVIEW OF LITERATURE

This study evaluates the green synthesis of gold and silver nanoparticles have been extensively explored in recent years due to its eco-friendly, cost- effective and sustainable approach. Nanoparticles are synthesized at room temperatures in an aqueous medium forming a self assembly of gold and silver nanoparticles. The modulation of the size and shape of the gold nanoparticles has been achieved by varying the ratio of the metal salt and plant extract in the reaction medium. Similarly silver nanoparticles of different shapes have been obtained through adjustments in the pH of the reaction medium. This variation highlights the versatility of the synthesis method. Characterization of the synthesized nanoparticles has been performed using UV- Vis spectroscopy, transmission electron microscopy (TEM), X- RAY diffraction (XRD) and Fourier transfer infrared spectroscopy (FTIR). The crystalline nature of the nanoparticles has been confirmed by XRD, with peaks corresponding to (111), (200), and (220) and (311) planes, indicating an FCC structure. Further evidence of crystalline has been provided by bright circular spots in selected area diffraction (SAED) patterns and clear lattice fringes observed in high resolution TEM images. FTIR spectroscopy has revealed that amine groups are involved in binding gold nanoparticles while carboxylate ion groups are associated with silver nanoparticles. These findings underscore the role of functional groups in the reduction and stabilization of nanoparticles during synthesis.

Here they conclude that the synthesis of gold nanoparticles of varying size and shapes has been carried out using *Hibiscus rosa sinensis* leaf extract, with the ratio of metal salt to extract being altered to achieve the desired variations. Silver nanoparticles of different shapes have been produced by adjusting the pH of the reaction medium, which comprised silver nitrate and hibiscus leaf extract. The nanoparticles have been characterized using UV-vis spectroscopy, transmission electron microscopy (TEM), X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FTIR). The crystalline nature of the nanoparticles has been demonstrated by the presence of bright circular spots in the selected area electron diffraction (SAED) patterns, clear lattice fringes visible in high resolution TEM images and characteristic peaks in the XRD pattern(Philip,Diazy., 2010).

This study evaluates the Nanocrystalline ZnFe2O4 samples have been synthesized using both conventional combustion methods (CCM) and microwave assisted combustion methods (MCM) for comparative purposes utilizing *Hibiscus rosa sinensis* plant extract. The synthesized ZnFe2O4 samples have been characterized using several advanced techniques including X-Ray diffraction(XRD), riveted analysis, Fourier transform infrared spectroscopy(FTIR),high resolution scanning electron microscopy(HRSM),energy dispersive

X-Ray analysis(EDX),diffuse reflectance spectroscopy(DRS). The formation of single phase ZnFe2O4 has been confirmed through XRD & FTIR analysis. Lattice parameters have been calculated using riveted analysis. HRSEM image has revealed particle size variations with sizes ranging from 372.0 to 541.7nm for the CCM samples and 23.4 to 48.5 nm for the MCM samples. The smaller particle and higher surface area of the MCM samples have been highlighted as key advantages of the microwave assisted method.

UV visible diffuse reflectance spectroscopy has been used to determine the band gap of ZnFe2O4 which is approximately 2.1 eV. Photoluminescence analysis has shown emission bands at 486,530,542 & 566 nm for single phase ZnFe2O4. Magnetic properties have been studied using VSM with hysteresis loops analyzed at room temperature. The saturation magnetization (MS) has been found to be significantly higher for ZnFe2O4 synthesized via the MCM method (255.7 emu/g) compared to the CCM method (63.61 emu/g).

The results indicate that the microwave assisted combustion method yields ZnFe2O4 nanoparticles with superior properties including higher surface area, lower crystallite size, and enhanced magnetic performance compared to the conventional combustion method.ZnFe2O4 nanoparticles have been successfully synthesized using both conventional combustion methods(CCM) and microwave assisted combustion method(MCM) by employing metal nitrates and *Hibiscus rosa-sinensis* plant extract solutions. The use of *H.rosa sinensis* leaf extract has been allowed the synthesis to proceed without need for harmful& toxic reducing or stabilizing agents making this an environmentally friendly, simple, efficient approach (Kombaiah *et al*.**,** 2016).

This study evaluates the Antibacterial activity using Iron oxide nanoparticles (alpha-Fe2O3) have been synthesized using an unconventional and eco- friendly technique, where *H.rosa sinensis* flower extract served as both a reducing and stabilizing agent. The antibacterial activity of the synthesized iron oxide nanoparticles has been investigated against bacteria such as *staphylococcus aureus, pseudomonas aeruginosa, klebisella pneumonia* and *Escherichia coli*. The results revealed that the nanoparticles exhibited inhibitory effects on all tested bacteria, demonstrating their potential as an antibacterial activity. The antibacterial activity trend has suggested a relationship between nanoparticle size and the number of particles present in the solution.

The alpha FeO_3 nanoparticles synthesized have been found to possess high stability, low toxicity and biocompatibility, making them suitable for antibacterial applications. These nanoparticles are proposed as potential replacements for antibiotics in bacterial disease

treatment and environmental applications. Ongoing investigations aim to explore and compare the effects of iron nanoparticles with drugs commonly used for the treatment of these pathogens (Buarki *et al.*, 2022)

Copper oxide (CuO) nanoparticles were synthesized using a green chemistry approach with leaf extracts from *Azadirachta indica*, *Hibiscus rosa-sinensis*, *Murray koenigii*, *Moringa oleifera*, and *Tamarindus indica*. To compare their efficiency, the same copper oxide nanoparticles were also synthesized through a chemical method. Phytochemical screening of the leaf extracts revealed the presence of carbohydrates, flavonoids, glycosides, phenolic compounds, saponins, tannins, proteins, and amino acids. FTIR spectra confirmed the bimolecular potentially responsible for nanoparticle formation. The surface Plasmon resonance absorption band observed at 220–235 nm in the UV–Vis spectra supported the successful synthesis of copper oxide nanoparticles.The monoclinic phase of the synthesized nanoparticles was confirmed through XRD patterns, while SEM, TEM, and SAED analyses determined the average size, shape, and crystalline nature.EDX analysis verified the elemental composition of the nanoparticles.Antioxidant activity was assessed using three distinct free radical scavenging assays. Cytotoxicity was evaluated against four cancer cell lines, including human breast (MCF-7), cervical (HeLa), epithelioma (Hep-2), and lung (A549), as well as a normal human dermal fibroblast (NHDF) cell line. Morphological changes in treated cells were observed through Hoechst 33258 staining assay. Copper oxide nanoparticles synthesized via the green method exhibited superior antioxidant activity and cytotoxicity compared to those synthesized chemically.

Copper oxide (CuO) nanoparticles were synthesized using various plant extracts, alongside a chemical synthesis method for comparison. Phytochemical studies confirmed the presence of flavonoids and phenolic compounds, which facilitated the formation of CuO nanoparticles. FTIR spectra validated the presence of Cu–O bonding, while XRD patterns indicated the monoclinic phase of the nanoparticles.The UV–Vis spectra of CuO nanoparticles synthesized by both green and chemical methods exhibited a characteristic absorption peak at 220–235 nm. The morphology, size, and crystalline nature of the nanoparticles were confirmed through SEM, TEM, and SAED analyses, with EDX analysis verifying their elemental composition. In vitro antioxidant assays conducted using various methods demonstrated that CuO nanoparticles synthesized via the green method exhibited higher activity compared to those synthesized chemically. Cytotoxicity assays revealed that all synthesized nanoparticles displayed significant activity against tested cancer cell lines while sparing normal cells, relative to the standard drug cisplatin. Among them, CuO-S6

showed enhanced cytotoxicity against all tested cancer cell lines, with apoptosis identified as the likely mechanism of cell death. The superior efficacy of CuO-S6 was attributed to the influence of additional phytochemicals present in the plant extract used during synthesis. These findings suggest that copper oxide nanoparticles synthesized using plant extracts hold significant promise as chemotherapeutic agents for future biomedical applications (Rehana *et al.*, 2017).

This study outlines an environmentally friendly approach to synthesize copper oxide nanoparticles (CuONPs) using *Hibiscus rosa-sinensis* flower extracts at room temperature. The green synthesis method avoids the use of toxic solvents, ensuring a sustainable process. The flower extract of Hibiscus rosa-sinensis was prepared in deionized water and added to a copper acetate solution. A visible color change from blue to sea green confirmed the formation of CuONPs, attributed to the bio-reduction of copper ions by the plant extract. The biosynthesized CuONPs were characterized using various techniques. The UV-visible spectrum showed an absorbance peak at 505 nm, indicative of surface Plasmon resonance vibrations. FTIR analysis revealed functional groups associated with the reduction and stabilization of CuONPs. X-ray diffraction (XRD) patterns confirmed the formation of CuO with a monoclinic crystal structure. Further characterization using SEM revealed the morphological features, while EDX confirmed the elemental composition. The synthesized CuONPs were also evaluated for antibacterial activity, showcasing their potential applications in antimicrobial treatments. This green synthesis approach provides an effective and sustainable route for producing CuONPs with promising functional properties.

The rapid biological synthesis of copper oxide nanoparticles (CuONPs) using *Hibiscus rosa-sinensis* flower broth offers an eco-friendly, simple, and efficient method. The UV-Vis absorption spectrum confirmed the presence of CuO nanoparticles with a characteristic peak at 505 nm. FT-IR analysis further validated the formation of CuO by showing an absorption peak at 676 cm^{-1}, attributed to Cu-O bonds. The product was confirmed to be copper oxide, with its crystal structure and d-spacing analyzed using XRD, indicating the monoclinic phase of CuO nanoparticles. SEM images revealed that the CuO nanoparticles exhibited a spherical morphology, while EDX analysis demonstrated that the synthesized nanoparticles primarily consisted of copper and oxygen, confirming their purity and composition (Rajendran *et al.*, 2018).

Examples of plant – Based CuNPs Synthesis

Several plant species have been explored for CuNP synthesis due to their high phytochemical content. Some examples include:

Plant Species	Part Used	Size of CuNP
Azadirachta indica(Neem)	Leaves	20-50nm
Ocimum Sanctum(Tulsi)	Leaves	10-30nm
Moringa Oleifera	Seeds	15-40nm
Aloe vera	Gel	30-60nm
Hibiscus rosa- sinensis	Stem (Current study)	100-300nm

The stem of *Hibiscus rosa-sinensis* has not been extensively studied for CuNP synthesis, making it a novel and valuable research focus.

Research Gap and Need for this Study

Identified Research Gaps:

- Limited studies on *Hibiscus rosa-sinensis* stem extract for CuNPs.
- Most researches focused on flowers, leaves, calyx and less research focused on stem.
- No systematic comparison of Antioxidant, Anti- inflammatory, Anticancer, Antibacterial results of CuNPs synthesized from *Hibiscus rosa – sinensis.*
- Further mechanistic studies on how CuNPs exert biological effects are needed.

Significance of this study:

- Eco-friendly and sustainable method to CuNPs synthesis.
- Novel source (stem extract) of *hibiscus rosa – sinensis.*
- Potential biomedical applications in drug development and nanomedicine.

MATERIALS AND METHODS

Materials and Reagents Used

Chemicals and Reagents:

The following chemicals and reagents were used for the synthesis and biological evaluation of CuNPs:

- Copper sulfate pent hydrate(CuSO4.5H2O) : Source of cu^{+2} ions (Merck, India)
- Distilled water : Used for the preparation of all solutions
- Hibiscus rosa - sinensis stem extract : Natural reducing and stabilizing agent
- Hydrogen Peroxide(H2O2, 30%) : Used for the Antioxidant assay(Sigma-Aldrich,USA)
- Ammonium Molybdate : Used for the phosphomolybdate assay
- Luria-Bertani agar : Medium for Antibacterial studies
- Nutrient Broth : For bacterial culture growth
- Ethanol and Methanol : Used as solvents for Extract preparation
- Dimethyl sulfoxide (DMSO) : Solvent for cell culture experiments
- Dulbecco's Modified Eagle Medium(DMEM) : Culture medium for caco-2 cells
- Fetal Bovine serum(FBS) : Growth supplement for cancer cell culture
- Penicillin-streptomycin solution : Antibiotic to prevent contamination in cell culture
- Diclofenac : Used for the Antibiotic
- MTT reagent(3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide) : Used for cell viability

Microbial and cell culture strains:

- **Bacterial Strains :** ***Escherichia coli***(E.coli), ***staphylococcus aureus***(S.aureus)
- **Human cancer cell line :** ***Caco-2*** (colorectal Adenicarcinoma Cell line)

Green synthesis of Copper Nanoparticles (CuNPs)

Preparation of *Hibiscus rosa-sinensis* stems extract:

1. **Collection of plant sample:** Fresh *Hibiscus rosa-sinensis* stem was collected around in Bangalore, Karnataka India. The stems were collected and thoroughly washed with distilled water to remove dust and impurities. The stems are then cut into small pieces and dried at room temperature (or at 40-50ºc in a hot air oven for faster drying). After that boiled it for some time and again after 10min boiled it again. (For contamination of antibacterial).Cooled it for room temperature.

Fig 1: Representing the different parts of *Hibiscus rosa-sinensis* plant that includes the stem, Leaves, Flowers that have wide range of the Medicinal and the Traditional Uses.

Extract Preparation:

Weigh approximately 5g of plant sample of stem. Boiled it in 20ml of distilled water for 10-30 minutes at 60-80ºc.After 24hrs again boiled and I allowed it for the extract to cool temperature and then filtered it by using whatman filter paper or muslin cloth. The filtrate (aqueous extract) is stored at 4ºc for further use. (For antibacterial contamination we have to boil)

Synthesis of Copper Nanoparticle (CuNPs):

- **Prepare 0.1m CuSO4 solution:** Dissolve CuSo4.5H2O in 15ml of distilled water to make 0.1m solution.
- **Mix plant extract with CuSO4 solution:** 5ml of aqueous extract added drop wise into 15ml of 0.1m CuSO4 solution under continuous stirring at 60°C for 2 hours. Then the solution gradually changes from blue to green which is indicating CuNP formation. Then the solution was centrifuged at 10,000 rpm for 15 minutes, and the pellet was washed with distilled water and ethanol to remove unreacted compounds.

Fig 2: Sample preparation using *H.rosa sinensis* stem Aqueous Extract and synthesis of Nanoparticle

Characterization of Copper Nanoparticle (CuNPs)

UV –Visible Spectroscopy: It is used for measuring how they absorb & scatter light. It works for the measuring the magnitude, peak wavelength, and spectral band width of a nanoparticles absorption. The data is usually plotted as extinction vs. wavelength. It can be used to identify, characterize & study nanomaterials. Information about NP's size, shape, stability, concentration refractive index. It is useful for the simple, inexpensive & non-invasive technique that can provide real-time evaluation of Nanoparticles.

After that Record the absorption spectrum at 200-800nm to confirm the formation of CuNps. Every 24hrs observe the absorption spectrum and took the OD. After taking OD take petriplates and poured the extract and nanoparticle were dried at 60°C in a hot air oven and stored for further analysis. After drying prepare stock solution from it. Take four 15ml test tubes and label it as aqueous extract and NPs for preparing different standards. Then dissolve dried mixture it with 2ml of DMSO and from it prepare different standards (1000µg, 500µg).Stored at 4ºC in a sterile vial. Diluted as required for antioxidant, anticancer, antibacterial and anti-inflammatory assays. After adding keep nanoparticle in dark room and extract in the freezer.

Scanning Electron Microscopy (SEM): It is a valuable technique for characterizing copper nanoparticles, providing insights into their morphology, size and distribution. SEM images can reveal whether the nanoparticles are spherical, cuboid or cluster-like. Additionally, SEM analysis can be used to determine the size of distribution of the nanoparticles, with sizes often ranging from a few nanometers to hundreds of nanometers.SEM can visually depict the shape and surface of features of copper nanoparticles. By examining SEM images and using appropriate scale bars, the size of individual nanoparticles and the distribution of sizes within a sample can be determined.

Evaluation of Biological Activity

Antioxidant Studies:

Free radicals are either oxygen derived reactive species (ROS) or nitrogen derived reactive species (RNS). Antioxidants are accepted to play vital part within the body defense system against free radicals. The existence of antioxidants in plants is more as numerous plants utilize as a source of dietary antioxidants. High concentrations of phytochemicals, which may protect against free radical damage, accumulate in fruits and vegetables.

Determination of Total Antioxidant capacity (TAC) by the phosphomolybdenum Assay:

The total antioxidant capacity of crude extracts will be evaluated by the phosphomolybdenum assay. This assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate (Mo (V)) complex at acidic pH. One milliliter each of 0.6 M sulfuric acid(H2So4), 28mM sodium phosphate(NaH2Po4) and 4mM ammonium molybdate((NH4)2MoO4) are added in 20 ml of distilled water and made up volume to 50 ml by adding distilled water to prepare Phosphomolybdenum reagent. 0.5 ml of crude extracts in different concentrations was added to different test tubes individually containing 3 ml of reagent solution (make up to 4ml). These tubes were kept incubated at 95°C for 90 min. Then, the absorbance of the solution was measured at 695 nm using a UV–VIS spectrophotometer against blank after cooling to room temperature. Only H2O was used as the blank and Ascorbic acid was used as positive reference standard. The antioxidant capacity was estimated using the following formula:

$$\text{Antioxidant effect\%} = \frac{\text{A control} - \text{A sample} \times 100}{\text{A control}}$$

Where, A sample is the absorbance of the sample and A control is the absorbance of the control.

The concentration of extract at which 50% inhibition is observed (IC50) were calculated in μg/ml.

Determination of Antioxidant Activity by Hydrogen Peroxide Scavenging (H2O2) Assay:

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Al-Amiery et al. with a minor modification. A solution of hydrogen peroxide (2mM) was prepared in 0.2 M phosphate buffer (pH 7.4). Briefly, about 0.2M potassium dihydrogen phosphate and 0.2 M sodium hydroxide solutions were prepared, 50 ml potassium dihydrogen phosphate solution was placed in a 200 ml volumetric flask and 39.1 ml of 0.2 M sodium hydroxide solution was added, and finally, volume was made up to 200 ml with distilled water to prepare phosphate buffer (pH-7.4). 50 ml of phosphate buffer solution was added to equal amount of hydrogen peroxide to generate the free radicals and solution was kept at room temperature for 5 min to complete the reaction. 1ml of sample extract of different concentrations is added to 1ml of hydrogen peroxide solution. After 10 min incubation in the dark, the absorbance at 230nm was recorded using a UV–Vis Spectrophotometer. A different concentrated solution of ascorbic acid was prepared (500,

1000µg/ml). H2O was used as the blank. The percentage inhibition values are calculated.

$$H_2O_2\ \%Scavenging = \frac{A\ blank - A\ sample \times 100}{A\ blank}$$

Where, A blank is the absorbance of the blank and A sample is absorbance of the sample or standard.

Antibacterial Studies

Agar well Diffusion Method:

The agar well diffusion method was employed to determine the antibacterial activity of sample against the selected bacterial strain, *E.coli, S.Aureus*. A subculture of bacterial strains at a volume of 200 µl, equivalent to 10^6 CFU/ml, was uniformly spread onto the surface of a Petri dish containing 20ml of LB agar broth, using a sterile cotton swab and wells were punched using a sterile gel borer. On the agar, five wells with a diameter of 8mm each were created for the bacterial strains. The first well was designated as the negative control and was loaded with 100µl of DMSO, using which sample was dissolved in the concentrations of 500mg/ml, 1000mg/ml. While second well served as the positive control and contained 100µl of Ampicillin (an antibiotic). Rest of the wells contained 100µl of test drug in above mentioned concentrations. Subsequently, the plates were incubated at 37°C for duration of 24 hours for bacterial growth, following which the measurement of the zone of inhibition surrounding the wells was performed after the incubation period. The concentration of positive control used was 0.1 mg/ml.

Anti-Inflammatory Studies

Inhibition of Protein Denaturation :

The reaction mixture consisted of 1 ml of 1% bovine albumin, 20µl of Conc. HCl(to adjust pH 6.3) and 1ml of sample extract, and the mixture was mixed, and was incubated in a water bath at 37 °C for 20 min, and then the reaction mixture was heated at 51 °C for 30 min. After cooling, the turbidity was measured at 660 nm using a UV/VIS spectrometer. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\%\ inhibition\ of\ denaturation = \frac{Absorbance\ of\ control - Absorbance\ of\ sample \times 100}{Absorbance\ of\ control}$$

Where A as control means negative control.

Proteinase Inhibitory Activity:

Briefly, the reaction solution (1 ml) consisted of 0.06 mg trypsin, 1 ml of 20 mM Tris-HCl buffer (pH 7.4), and 1 ml test sample (0.02 ml extract 0.980 ml methanol). The solution was incubated (37 ℃ for 5 min) water bath, and then 1 ml of 0.7% (w/v) casein was added, and the mixture was further incubated for an additional 20 min at 37°c. At the end of incubation, 1 ml of 70% perchloric acid was added to terminate the reaction. The mixture was centrifuged for 10min 4°c at 6000rpm, and the absorbance of the supernatant was measured at 210 nm against buffer as the blank. Phosphate buffer solution was used as the control. The percentage inhibition of proteinase activity was calculated by using the following formula:

$$\% \text{ inhibition of proteinase} = \frac{\text{Absorbance of control- Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

Anticancer Studies

Caco-2 cell Culture and Maintenance:

Caco-2 cells were grown in DMEM with 10% FBS and 1% antibiotics at 37°C in 5% CO_2 incubator. Thawed the Frozen Caco-2 Cells and from cryopreserved Caco-2 vial from liquid nitrogen storage removed .Then quickly thawed the vial in a 37°C water bath for 1–2 minutes (without overheating). Transferred the thawed cells to a 15 ml centrifuge tube containing 5 ml pre-warmed DMEM + 10% FBS. Later Centrifuge at 1000 rpm for 5 minutes to remove cryopreservative (DMSO).Discard the supernatant and resuspend the cell pellet in fresh DMEM.Transfer cells into a petridish with 5 ml of DMEM and incubate at 37°C, 5% CO_2. Daily need to check the cells for growth, confluency and contamination. Healthy cells will be visible like polygonal, monolayer. Every 2-3 days removed old medium and then washed the cells with PBS and added fresh DMEM+10% FBS and return to the incubator. Passage when the cells reached about80-90% confluence then removed spent medium and washed the cells with PBS.Added 1ml of trypsin-EDTA (0.25%) and incubated for 2-5mins at 37°c until cells detach. Then neutralized the trypsin by adding a fresh DMEM(with FBS) I transferred the suspension to a centrifuge tube and spin at 1000rpm for 5min.Discard the supernatant, and resuspend cells in fresh medium in DMEM and seed them into new petridishes at the required density.

Cell Counting and Seeding:

Cells were counted using a hemocytometer and 50-80 cells/well were seeded in a 96-well plate. Removed the spent medium from petriplate containing 80-90% confluent Caco-2 cells. Washed the cell layer gently with 5 ml PBS to remove dead cells and debris and add 1 ml

Trypsin-EDTA (0.25%) to the flask and incubate at 37°C for 2-5 minutes (until cells detach). Gently tap the flask to dislodge the cells and add 5 ml DMEM + 10% FBS to neutralize trypsin and resuspend the cells. Transfer the suspension to a 15 mL centrifuge tube and centrifuge at 1000 rpm for 5 minutes. Discard the supernatant and resuspend the pellet in 1 ml fresh DMEM.Take 10 µL of cell suspension and mix with 10 µL Trypan Blue (1:1 dilution). Load 10 µL of the mixture into a Neubauer hemocytometer chamber. Using an inverted microscope, count live cells in 4 large squares of the grid. Calculate cell concentration using the formula:

$$\text{Cells concentration (cells/ml)} = \frac{\text{total viable cells in 4 squares} \times 100}{\text{No. of squares counted}}$$

Seeding cells in plates:

Calculate the required volume of cells to obtain the desired cell density. Transfer 100 µl (for 96-well) or 2 ml (for 6-well) of the prepared suspension into each well. Gently swirl the plate for even cell distribution. Place the plate in a CO_2 incubator (37°C, 5% CO_2).Allow cells to attach and grow for 24 hours before starting experiments.

Drug Treatment with CuNPs:

Prepare Drug Stock Solution, If using CuNPs, dissolve them in sterile water or DMEM.Prepare serial dilutions (e.g., 10, 50, 100, 200 µg/ml) in DMEM complete medium. Removed the old medium from the wells and gently wash cells with PBS.Add 100 µl (for 96-well) or 2 ml (for 6-well) of drug solution at different concentrations. Include negative control (untreated cells, only DMEM) and positive control (standard anticancer drug, e.g., doxorubicin or 5-FU). Incubate for 24-48 hours at 37°C in 5% CO_2.

MTT Assay and IC50 Calculation:

Add 100 µl of cells to each well and incubate for 2-3 days. Dissolve 5 mg MTT in 1 ml 1X PBS. Sterilize by filtration. Add 10 µl of MTT stock solution to each well. Incubate for 2-5 hours at 37°C. Solubilizing the formazan. Carefully remove media from each well without disturbing cells. Add 100 µl of DMSO to each well and mix by pipetting up and down. Incubate at 37°C for 15 minutes.MTT reagent (0.5 mg/Ml was added, and formazan crystals were dissolved in DMSO.Absorbance was recorded at 570 nm, and IC50 was calculated using non-linear regression analysis.

Data analysis

For cell proliferation assay:

1. Average the duplicate reading for each sample.

2. Subtract the culture medium background from your assay reading. This is the corrected absorbance.

For cell counting, a standard curve can be established with known cell number and fixed incubation times with the assay reagent.

Note: The amount of absorbance is proportional to cell number.

For cell cytotoxicity assay:

1. Average the duplicate reading for each sample.

2. Subtract the culture medium background from your assay readings. This is the

Corrected absorbance.

3. Calculate percentage cytotoxicity with the following equation, using correcte Absorbance.

% cytoxicity = (100 x (control - sample))

RESULTS

Characterization of CuNPs

UV-Visible Spectroscopy Analysis: The synthesis of CuNPs was confirmed by UV-Vis spectroscopy, which exhibited a surface Plasmon resonance (SPR) peak. For copper nanoparticle (CuNPs), SPR typically ranges from **300-600nm**, depending on particle size, shape.

Observations and Peak Intensity Values:

Wavelength(nm)	Nanoparticle(NP)	Aqueous Extract(AE)
200	0.301	0.254
250	0.317	0.255
300	2.324	2.281
350	2.056	2.013
400	1.913	1.826
450	1.632	1.583
500	1.348	1.254
550	0.932	0.827
600	0.741	0.638
650	0.714	0.681
700	0.648	0.532
750	0.509	0.491
800	0.416	0.354

Table 1: It represents the OD values which are taken through UV spectroscopy and the SPR peak at ≈300nm for Aqueous Extract and the Nanoparticle.

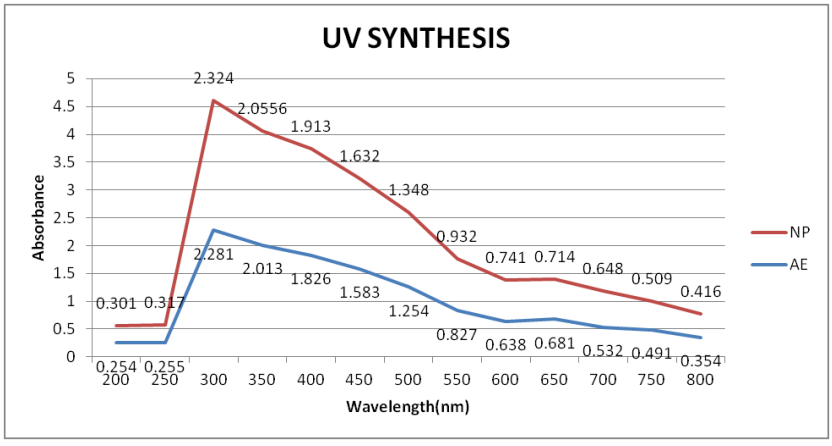
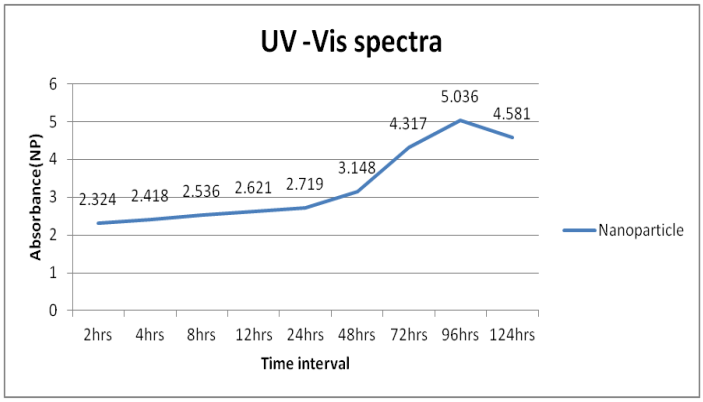


Fig 1: It shows the graphical representation of Aqueous Extract & Nanoparticle through UV spectroscopy, which indicates high absorbance in Nanoparticle i.e. 300nm.

So I conclude that the UV-Visible spectroscopy analysis of synthesized CuNPs using ***Hibiscus rosa-sinensis*** stem extract shows a characteristic SPR peak at ~300 nm, confirming the successful synthesis of copper nanoparticles. The enhanced absorbance in the nanoparticle solution (NP) compared to the aqueous extract (AE) indicates strong reducing and stabilizing capabilities of the plant stem phytochemicals. This optical signature further validates the bio-reduction of Cu^{2+} ions, forming stable and dispersed copper nanoparticles with efficient capping agents from the extract.

Time Interval(300nm)	NP
2hrs	2.324
4hrs	2.418
8hrs	2.536
12hrs	2.621
24hrs	2.719
48hrs	3.148
72hrs	4.317
96hrs	5.036
124hrs	4.581

Table 2: Time-Dependent UV-Visible Spectroscopy Analysis of CuNPs

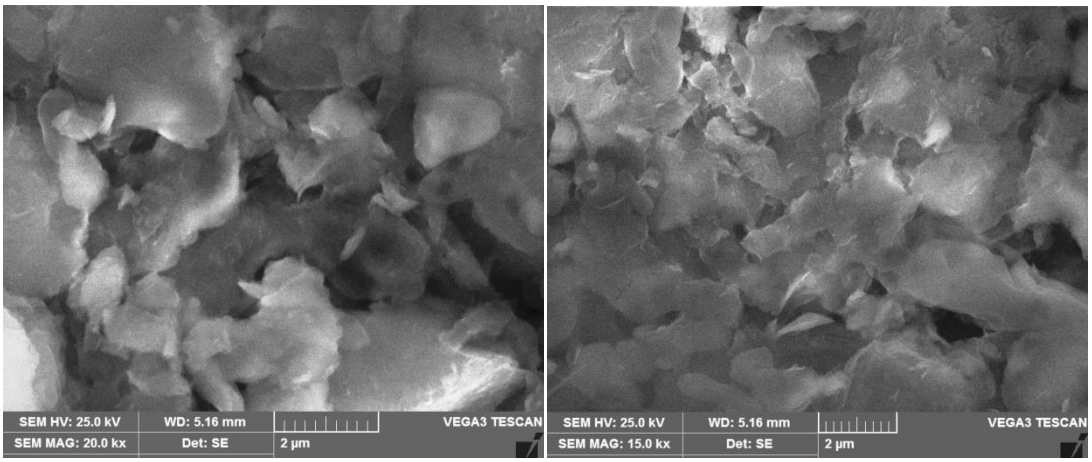


Graph 2: It shows the graphical representation of Nanoparticle which is indicating the highest peak of time interval.

This graph illustrates the growth kinetics and stability of copper nanoparticles synthesized using Hibiscus rosa-sinensis stem extract, by monitoring absorbance at the SPR peak (~300

nm) over time. So conclude that the time-based absorbance confirms that maximum nanoparticle synthesis occurs between 72–96 hours. Beyond this, stability may decline, so 96 hours is suggested as the ideal synthesis duration for optimal CuNP yield using Hibiscus rosa-sinensis stem extract.

Scanning Electron Microscopy (SEM) Analysis:



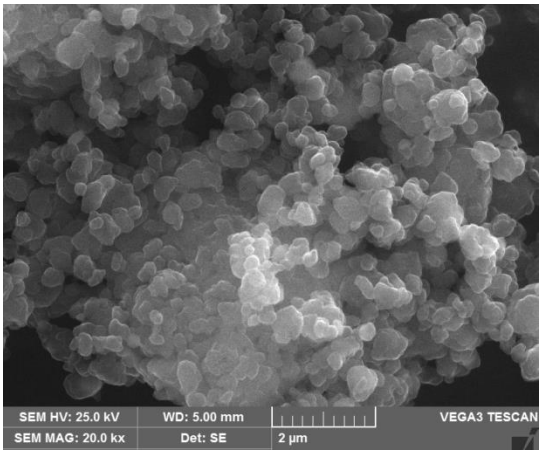


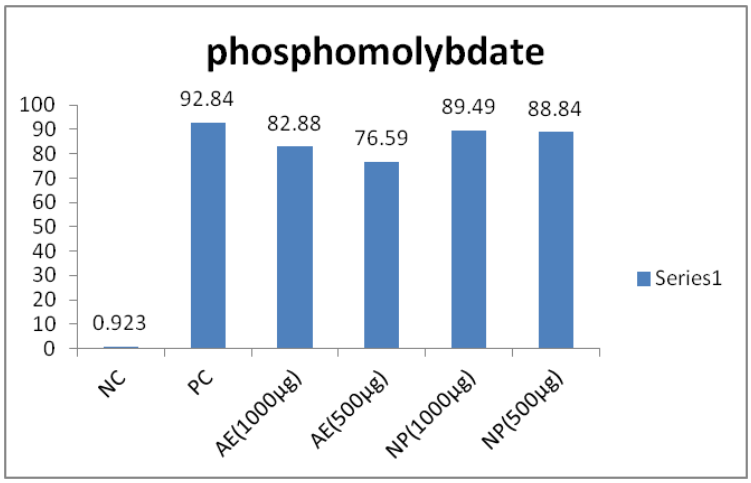
Fig 3: The figure represents the SEM analysis of copper nanoparticles

Antioxidant Activity Results

Determination of total antioxidant capacity (TAC) by the phosphomolybdenum Assay:

Concentration(µg/ml)	%Antioxidant Activity(TAC)
NC(Negative control)	0.923%
PC(Positive control)	92.84%
AE(1000µg/ml)	82.88%
AE(500µg/ml)	76.59%
NP(1000µg/ml)	89.49%
NP(500µg/ml)	88.84%

Table 3: It represents the dose dependent TAC of the Phosphomolybdenum Assay of different concentrations (µg/ml)

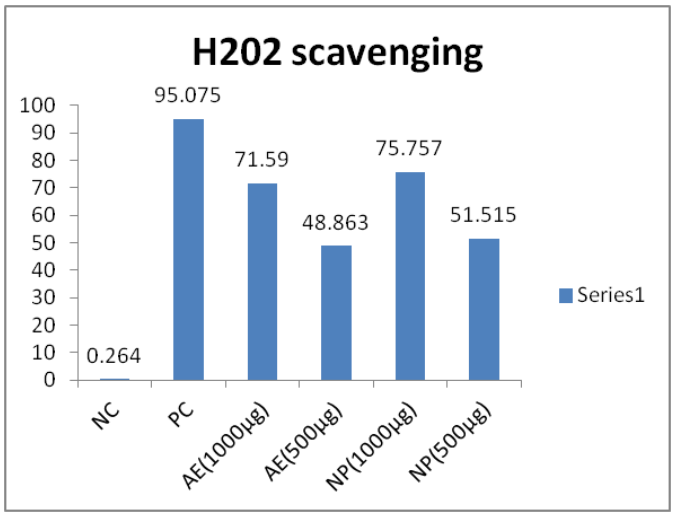


Graph 3: It shows the graphical representation of phosphomolybdenum of TAC activity of bar diagram which are plotted against the AE&NP of different concentrations (µg/ml)

Hydrogen Peroxide (H2O2) Scavenging Assay:

Concentration(µg/ml)	%H2O2 scavenging activity
NC(Negative control)	0.264
PC(Positive control-Standard antioxidant ascorbic acid)	95.07%
AE(1000µg/ml)	71.59%
AE(500µg/ml)	48.86%
NP(1000µg/ml)	75.75%
NP(500µg/ml)	51.51%

Table 4: It represents the dose dependent ofH2O2 scavenging activity of different concentrations (µg/ml)



Graph 4: It shows the graphical representation of Hydrogen peroxide scavenging of bar diagram which are plotted against the AE&NP of different concentrations (µg/ml)

Antibacterial Activity

Agar Well Diffusion Method:

- **Staphylococcus Aureus:**

Treatment	Zone of Inhibition(mm)
Ampicillin(PC)	21mm
Negative control(NC)	7mm
AE	2mm
NP(1000µg/ml)	6mm
NP(500µg/ml)	5mm

Table 5: It represents the zone of inhibition of different concentrations (µg/ml)

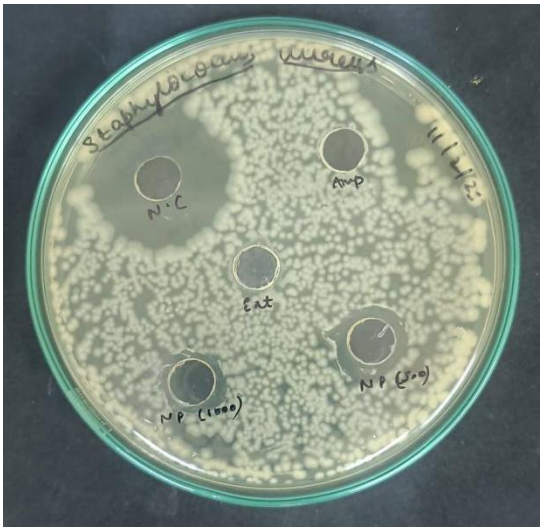
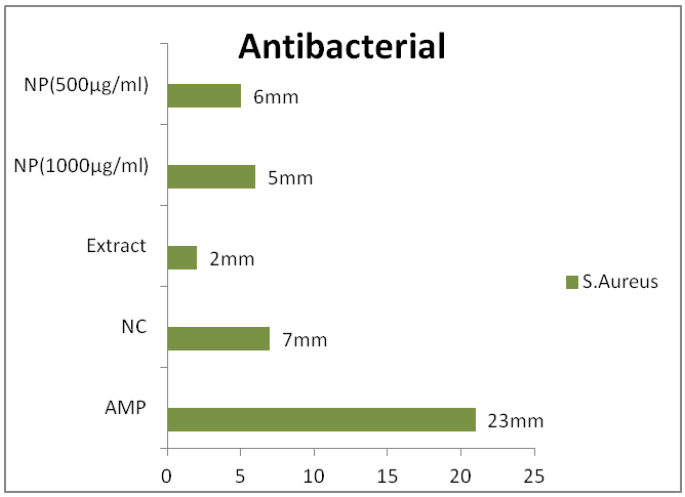


Fig 4: It shows the petriplate containing the staphylococcus aureus bacteria against the intestinal pathogen by the *Hibiscus rosa-sinensis* stem extract and Nanoparticle and the zone of inhibition is observed.



Graph 5: The bar diagram shows the antibacterial of *S.Aureus* against intestinal pathogen.

- **Escherichia Coli:**

Treatment	**Zone of Inhibition(mm)**
Standard Antibiotic(Ampicillin)	23mm
Negative control(NC)	7mm
AE	21mm
NP(1000µg/ml)	18mm
NP(500µg/ml)	16mm

Table 6: It represents the zone of inhibition of different concentrations (µg/ml)

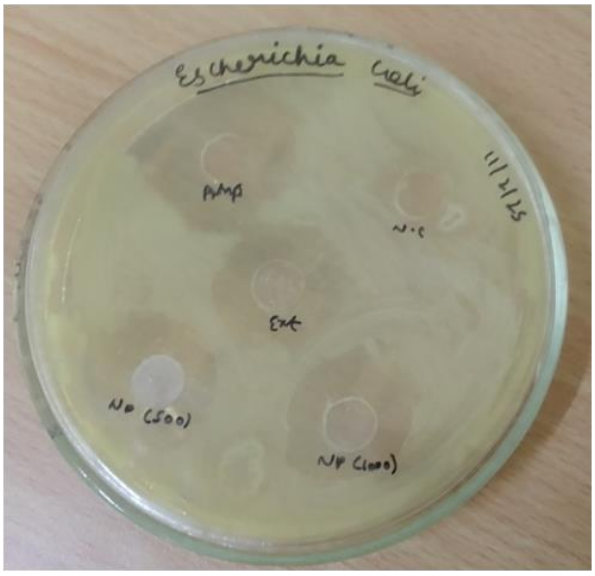
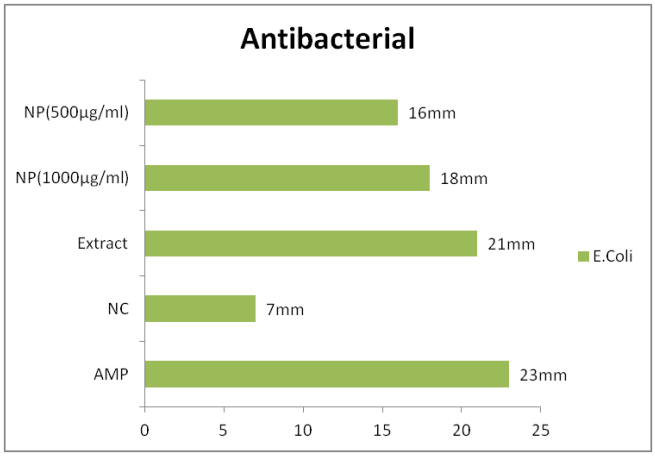


Fig 5: It shows the petriplate containing the Escherichia coli bacteria against the intestinal pathogen by the Hibiscus rosa-sinensis stem extract and Nanoparticle and the zone of inhibition is observed.



Graph 6: The bar diagram shows the antibacterial of *E.Coli* against intestinal pathogen.

Anti-inflammatory Activity Results

Protein Denaturation Inhibitory Assay:

Treatment	**%Inhibition of protein denaturation**
Negative control(NC)	0.305%
Positive control(PC)-Diclofenac	86.22%
AE(1000µg/ml)	51.14%
AE(500µg/ml)	26.88%
NP(1000µg/ml)	54.42%
NP(500µg/ml)	53.11%

Table 7: It represents the anti-inflammatory of protein denaturation inhibition

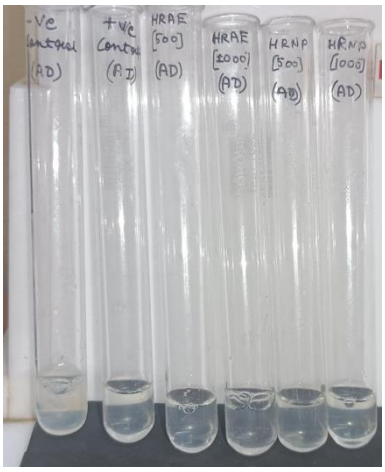
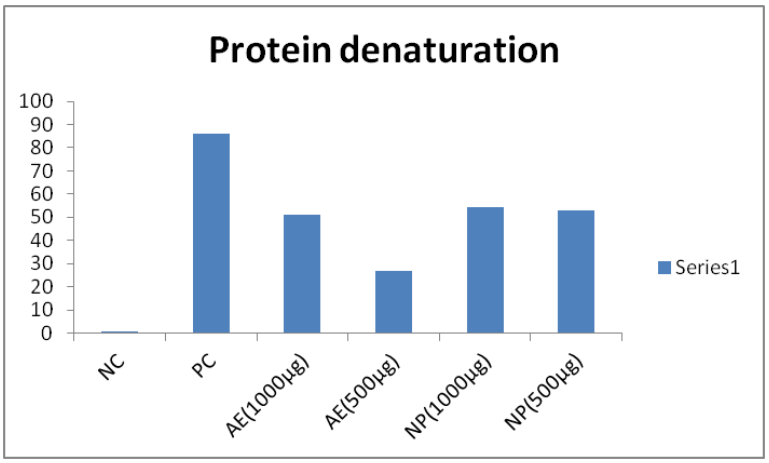


Fig 6: It shows the Albumin Denaturation i.e. protein denaturation of anti-inflammatory



Graph 7: The bar diagram shows the Anti-inflammatory of protein denaturation inhibition based up on different concentrations and also comparison between AE & NP

Proteinase Inhibitory Activity:

Sample	Inhibition (%)
NC	0.911%
PC(Diclofenac)	88.25%
AE(1000µg/ml)	72.88%
AE(500µg/ml)	63.22%
NP(1000µg/ml)	83.64%
NP(500µg/ml)	66.19%

Table 7: It represents the anti-inflammatory of proteinase inhibitory activity

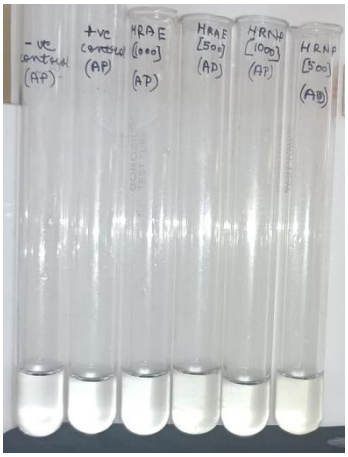
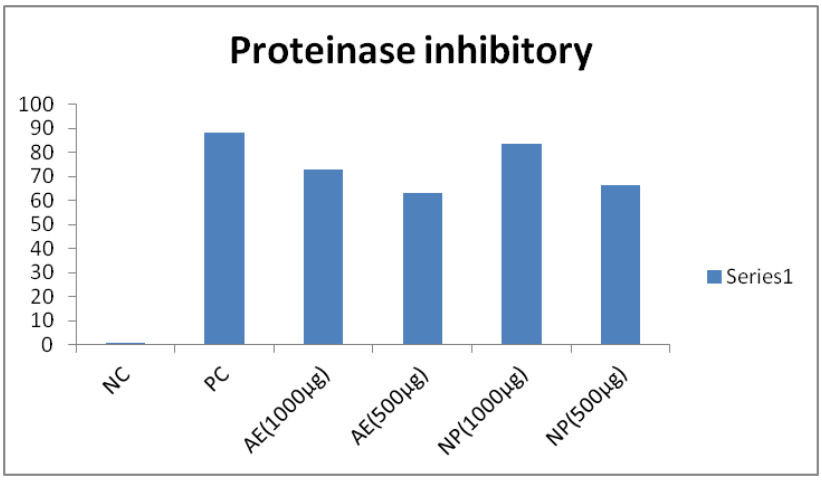


Fig 7: It shows the proteinase inhibitory activity of anti-inflammatory



Graph 7: The bar diagram shows the Anti-inflammatory of proteinase inhibitory activity based up on the concentrations and also comparison between AE & NP

Anticancer Activity

MTT Assay for cytotoxicity Assay Evaluation:

The anticancer potential of the green synthesized copper nanoparticles (CuNPs) and the aqueous extract (AE) of Hibiscus rosa-sinensis stem was assessed using the MTT assay on Caco-2 cell lines. This assay evaluates the metabolic activity of viable cells by measuring the reduction of MTT to formazan, which is directly proportional to the number of live cells.

The Caco-2 cells were treated with increasing concentrations (250 µg/ml, 500 µg/ml, and 1000 µg/ml) of both AE and CuNPs for 2 hours. The untreated cells served as the control, while a standard drug-treated group acted as the positive control. Absorbance was measured at 570 nm.

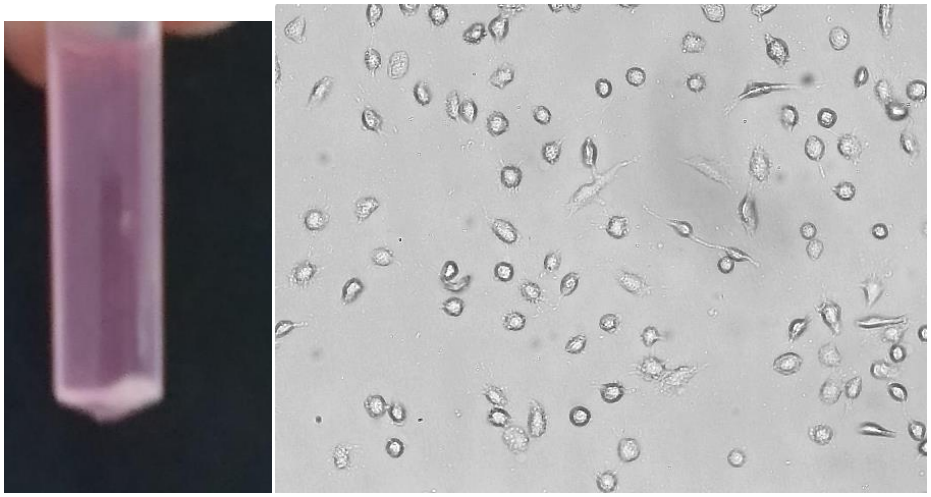


Fig 8: shows the cell pellets of the Caco-2 cell line that are get after the centrifugation process that includes by the seeding of the cells and the next image shows the Caco-2 cells.

Results and Interpretation:

- **Optical Density Values(OD):**
  - ✓ Control group shows high OD (1.993), indicating maximum cell viability.
  - ✓ Treatment with AE and CuNPs results in a dose-dependent decrease in OD, indicating that it has increased cytotoxicity.
  - ✓ The CuNPs demonstrated greater cytotoxicity compared to the AE at each concentration.

Sample	Average OD	%Cell viability	% Inhibition
Control	1.993	100.00%	0.00%
AE(250µg/ml)	0.762	38.23%	61.77%
AE(500µg/ml)	0.381	19.12%	80.88%
AE(1000µg/ml)	0.190	9.56%	90.44%
NP(250µg/ml)	0.521	26.14%	73.86%
NP(500µg/ml)	0.2605	13.07%	86.93%
NP(1000µg/ml)	0.1302	6.53%	93.47%

Statistical Summary:

- The standard deviation and standard error values for each treatment group were minimal, confirming consistency and reproducibility across replicates.
- The calculated % inhibition values showed a progressive increase with higher concentrations for both AE and CuNPs.
- CuNPs exhibited significantly higher cytotoxic effects compared to AE at each respective dose, supporting the hypothesis that nanoparticle formulation enhances therapeutic efficacy.

Discussion:

The MTT assay clearly demonstrates that both the aqueous extract and CuNPs derived from Hibiscus rosa-sinensis stem possess remarkable anticancer properties. The CuNPs displayed superior cytotoxic effects, especially at 1000 µg/ml, where cell viability dropped to 6.53%, indicating a 93.47% inhibition of cancer cell proliferation.

- The increased efficacy of CuNPs can be attributed to:
- Enhanced cellular uptake due to nanoscale size.
- Synergistic effects of copper’s redox properties and plant phytoconstituents.

- Possible disruption of mitochondrial function and induction of oxidative stress in cancer cells.

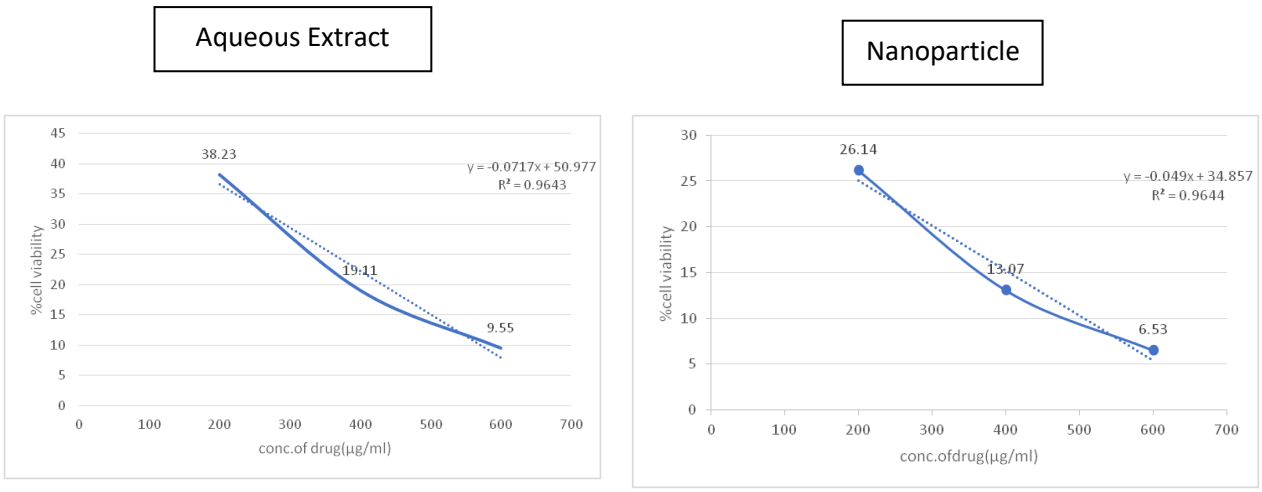
Data Analysis:

Data analysis is done using the Excel software using the data analysis linear graph was done against the concentration of drug vs. % cell viability with comparison of aqueous extract and nanoparticle.IC50 concentration with the linear graph equation MTT assay summary with the% of the cell inhibition with the comparison of aqueous extract and nanoparticle.

Aqueous extract	
Conc. of drug(µg/ml)	**% cell viability**
Control	100%
Std Control	4.13%
250µg/ml	38.23%
500µg/ml	19.11%
1000µg/ml	9.55%

Nanoparticle	
Conc. of drug(µg/ml)	**%cell viability**
Control	100%
Std Control	4.13%
250µg/ml	26.14%
500µg/ml	13.07%
1000µg/ml	6.53%

Table 8: Shows the concentration of the drug and the % cell viability between aqueous extract and nanoparticle. Here the control is taken as the cell lines that are treated with the DMEM media and the standard control as doxorubicin drug and the different concentration of 250µg/ml, 500µg/ml and 1000µg/ml of the stock solution



Graph 8: It represents the linear curve that is plotted against the concentration of drug vs. % cell viability between the aqueous extract and nanoparticle.

IC_{50} Determination for Anticancer Activity:

Introduction: The IC_{50} (half maximal inhibitory concentration) is a key parameter in cytotoxicity assays and represents the concentration of a compound at which 50% of the cancer cells are inhibited (i.e., cell viability is reduced by half). Lower IC_{50} values indicate higher cytotoxic efficacy. Here it is determined by the formula using the linear equation Y=mx+c to determine the IC50 between the aqueous extract and nanoparticle and the results for IC50 shown below:

MTT ASSAY SUMMARY IC50(AE)	
Y=MX+C	
X=(Y-C)/M	
Y	50
M	-0.0717
C	50.977
X	13.62

MTT ASSAY SUMMARY IC50(NP)	
Y=MX+C	
X=(Y-C)/M	
Y	50
M	-0.049
C	34.857
X	-309.04

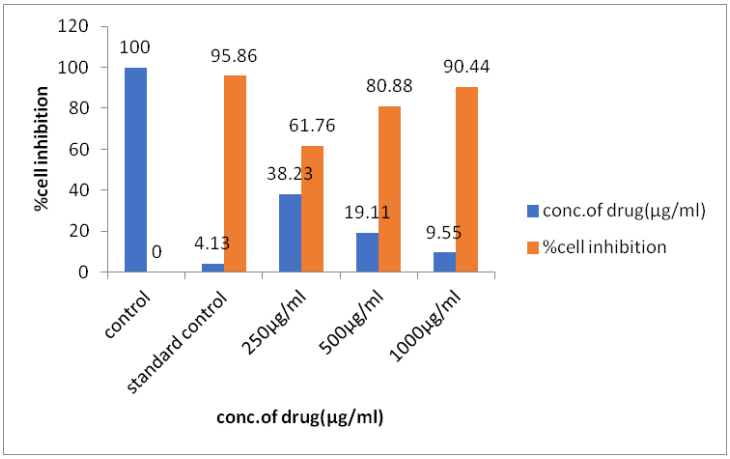
Table 9: It shows the IC50 values that are calculated by the Y=mx+c formula using the excel software.

MTT ASSAY Overall Summary & The % Of Inhibition:

This summary is done in the excel sheet format to show the % of inhibition rate. Here it is plotted against the concentration of drug vs. % of inhibition between aqueous extract and nanoparticle.

Aqueous extract		
Culture Conditions	Conc. of drug(µg/ml)	% cell inhibition
Control	100	0
Standard control	4.13	95.86
250µg/ml	38.23	61.76
500µg/ml	19.11	80.88
1000µg/ml	9.55	90.44

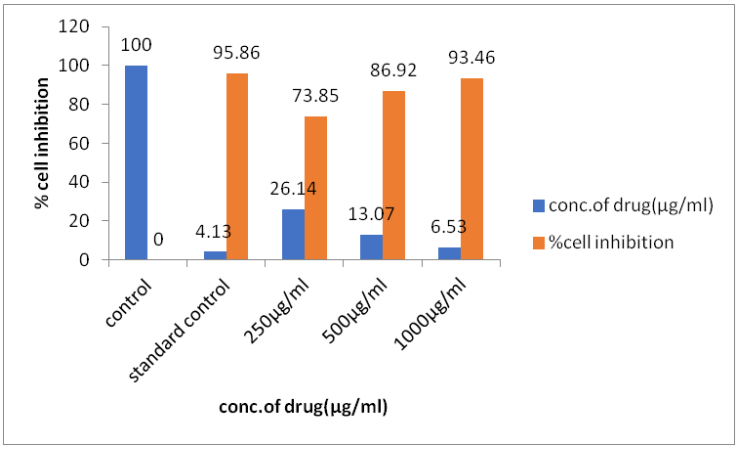
Table 10: It shows the values against the culture conditions vs. the % inhibition rate of aqueous extract.



Graph 9: It represents the Bar diagram shows the different concentrations of the culture conditions against % inhibition rate of aqueous extract.

Nanoparticle		
Culture conditions	**Conc. of drug(µg/ml)**	**% cell inhibition**
Control	**100**	**0**
Standard control	**4.13**	**95.86**
250µg/ml	**26.14**	**73.85**
500µg/ml	**13.07**	**86.92**
1000µg/ml	**6.53**	**93.46**

Table 11 : It shows the values against the culture conditions vs. the % inhibition rate of Nanoparticle.



Graph 10: It represents the Bard diagram shows the different concentrations of the culture conditions against % inhibition rate of Nanoparticle.

Mechanism of Action and Comparison with Previous Studies:

Mechanism of Action of CuNPs

- **Antioxidant Mechanism:** CuNPs neutralize free radicals by electron donation and inhibiting lipid peroxidation.
- **Antibacterial Mechanism:** CuNPs disrupt bacterial membranes, generate ROS, and interfere with DNA replication.
- **Anti-inflammatory Mechanism:** CuNPs inhibit denaturation of proteins involved in inflammation, such as albumin.
- **Anticancer Mechanism:** CuNPs induce mitochondrial dysfunction, ROS-mediated apoptosis, and DNA fragmentation in cancer cells.

Comparison with Previous Studies

CuNPs synthesized using Hibiscus rosa-sinensis showed stronger biological activity compared to CuNPs from Neem, Aloe Vera, and Moringa. The lower IC50 value and higher antibacterial efficiency indicate enhanced activity due to bioactive phytochemical capping agents. Many of the researchers worked on Hibiscus rosa-sinensis Flower, Leaves, Calyx. But less researched on the stem and they didn't even worked on it. Compared to previous studies stem has moderate results which is useful for the traditional and medicinal purposes.

Limitations and Future Directions

- **Limitations of the Study:** While this study successfully demonstrated the green synthesis and biological efficacy of CuNPs.
- **Scalability issues:** The batch synthesis process needs optimization for large-scale production.
- **Stability in biological environments:** Although CuNPs exhibited good stability in aqueous solutions, their long-term stability in physiological conditions needs further evaluation.
- **Mechanistic insights:** The exact molecular pathways of CuNP-induced cytotoxicity and anti-inflammatory mechanisms require more in-depth investigation.

Future Research Directions

- **In Vivo Studies:** Further research should focus on animal model studies to evaluate the pharmacokinetics, biodistribution, and toxicity of CuNPs.
- **Targeted Drug Delivery:** CuNPs can be functionalized with specific ligands for targeted drug delivery in cancer therapy and infectious diseases.
- **Combination Therapy:** Exploring synergistic effects of CuNPs with existing antibiotics and chemotherapeutic agents to enhance therapeutic efficacy.
- **Biodegradability and Safety Studies:** Investigating biodegradability and clearance mechanisms of CuNPs in biological systems to ensure their safety for clinical applications.

DISCUSSION

Green synthesis of copper nanoparticles (CuNPs) using ***Hibiscus rosa-sinensis*** stem offers a sustainable and eco-friendly approach to nanoparticle production. This method leverages the plant's inherent phytochemicals, which act as natural reducing and stabilizing agents, facilitating the formation of CuNPs without the need for hazardous chemicals. These plants contain numerous secondary metabolites with significant anticancer properties. Among them, alkaloids, flavanoids have demonstrated strong anticancer activity. This study focused on the cytotoxicity assay, biomedical applications of extracts and isolated compounds from ***Hibiscus rosa-sinensis*** stem extract as a reducing and stabilizing agent. The cytotoxicity activity of the aqueous extract and fractions of the plant stem on *Caco*-2 cancer cell line was evaluated using IC50 values, as presented in Table. Lower IC50 values indicates higher toxicity whereas, higher IC50 values indicates lower toxicity effects. The results revealed that the aqueous extract of the stem exhibited the higher cytotoxicity activity, with an IC50 value of 13.62µg/ml and also nanoparticle exhibited the lower cytotoxicity activity, with an IC50 value of -309.04µg/ml. These findings are comparable to the IC50 values of standard drug such as doxorubicin. This suggests that the aqueous extract shows higher toxicity compared to nanoparticle. Antioxidant activity results states that the phosphomolybdate assay result demonstrate that both the Hibiscus rosa-sinensis stem of aqueous extract and the green-synthesized of CuNPs possess strong total antioxidant capacity. Notably, CuNPs exhibit higher and more consistent TAC at both concentrations, implying that the nanoformulation significantly boosts antioxidant potential. AE at 1000 µg shows 82.88% activity, this reflects a dose-dependent antioxidant capacity, attributed to phytoconstituents like Polyphenols, Flavonoids&Tannins. NP at 1000 µg/ml shows 89.49% TAC, very close to the positive control. The total antioxidant capacity (TAC) of CuNPs was higher than synthetic antioxidants, confirming their strong electron-donating ability. H2O2 scavenging demonstrates the AE at 1000 µg exhibits 82.88% scavenging, indicating a strong antioxidant potential. The presence of phenolic compounds, flavonoids, and tannins in the Hibiscus rosa-sinensis stem extract are likely responsible for this effect.NP 1000 µg shows 89.49% activity, which is closer to the standard than the AE. So I conclude that the H_2O_2 scavenging activity of CuNPs synthesized from ***Hibiscus rosa-sinensis*** stem demonstrates significant antioxidant potential, especially at lower concentrations, compared to the aqueous extract. The results reveal a dose-dependent scavenging activity for AE, while CuNPs maintain consistently high performance even at reduced doses. This enhancement can be attributed to the synergistic

effect of metal nanoparticles and plant-derived phytochemicals, positioning green-synthesized CuNPs as promising candidates in oxidative stress-related therapeutic applications. Antibacterial activity demonstrates that the CuNPs showed strong antibacterial effects E.coli and S.Aureus with the inhibition zones of 6mm and 18mm, respectively. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) studies confirmed their dose-dependent bactericidal activity. So I conclude that the agar well diffusion results highlight the moderate but significant antibacterial activity of green-synthesized copper nanoparticles from Hibiscus rosa-sinensis stem. While the crude extract alone exhibited minimal inhibition (2 mm), the nanoparticle formulation (5–6 mm) showed improved activity, underscoring the role of nanotechnology in enhancing phytochemical efficacy. These findings suggest potential for CuNPs as a supplementary antimicrobial agent, particularly in resistance-prone environments, though further optimization (e.g., size control, concentration tuning, and combinatorial studies) is necessary for therapeutic applications. So I conclude that both Hibiscus rosa-sinensis stem extract and its synthesized CuNPs exhibit significant inhibitory effects against Escherichia coli. The aqueous extract demonstrated strong natural antibacterial action (21 mm), likely due to its rich phytochemical content. Meanwhile, CuNPs also showed notable activity (16–18 mm), showcasing the effectiveness of the green synthesis method. These results confirm that both extract and nanoparticles hold promising potential for antibacterial applications, with CuNPs offering the added benefit of controlled release and enhanced bioactivity at lower concentrations. Future studies could explore mechanism of action and synergistic combination with antibiotics. The superior activity of CuNPs can be attributed to ROS production and bacterial membrane disruption. CuNPs exhibited strong antibacterial activity; with larger zones of inhibition compared to standard antibiotics.Anti-inflammatory AE effectively inhibited protein denaturation showing 51.14% inhibition at 1000µg/ml, indicating moderate anti-inflammatory activity. The effect is attributed to bioactive phytoconstituents such as flavonoids, saponins, and polyphenols known to prevent protein denaturation—one of the causes of inflammation. CuNPs at 1000µg/mL exhibited 54.42% inhibition, slightly better than AE at the same concentration, suggesting enhancement in bioactivity due to nanoscale delivery.Proteinase inhibitory shows (AE) at 1000 µg/mL, the extract inhibited proteinase by 72.88%, indicating considerable anti-inflammatory potential. The NP (1000 µg/ml) showed higher inhibition (83.64%) than the extract, approaching the efficacy of the standard.

SUMMARY

Research work in this thesis has investigated how copper nanoparticle is used for the stem extract of ***H.rosa-sinensis*** which is tested against Antibacterial, Antioxidant, Anti-Inflammatory and Anti cancer. In previous studies many of the researchers are worked on the Leaves, Calyx, and Flowers of ***Moringa, Azardechta Indica, and Aloe Vera***. but less focused on the stem of ***Hibiscus rosa-sinensis***. H.rosa-sinensis having bioactive compounds like flavonoids, terpenoids etc. It will also useful for medicinal properties against Antibacterial, Anti-inflammatory and also it is useful for traditional purposes.

Our study investigated the synthesis of copper nanoparticle from *H.rosa-sinensis* stem which is compared between AE & NP. The range of SPR peak shows 300nm with the help of UV-Visible spectroscopy confirming the successful synthesis of copper nanoparticles. It indicates the strong reducing and stabilizing capabilities of the plant stem phytochemicals. Also, The nanoparticles were synthesized and allowed to stabilize over 96 hours for optimal formation and maturation.SEM (Scanning Electron Microscopy) Analysis ,the average particle size of the nanoparticles was approximately 2 µm.Morphological analysis indicated that the particles were moderately uniform, suggesting controlled synthesis conditions. Antioxidant Activity of Phosphomolybdenum Assay is compared between Positive Control 92.84% and Nanoparticles (NP) 89.49%. The nanoparticles exhibited high antioxidant activity, nearly comparable to the positive control, suggesting strong radical scavenging capacity.H_2O_2 Scavenging Assay is also compared between Positive Control 95.07% and Nanoparticle 75.75%. Nanoparticles showed moderate to high scavenging activity against hydrogen peroxide, indicating a protective effect against oxidative stress. Antibacterial Activity against Staphylococcus aureus (Gram-positive) comparison between Positive Control 21 mm zone of inhibition and Nanoparticles 6 mm zone of inhibition. Against Escherichia coli (Gram-negative) comparison between Positive Control 23 mm zone of inhibition. And Nanoparticles 18 mm zone of inhibition.Nanoparticles were more effective against E. coli than S. aureus.Antibacterial activity was moderate, suggesting that Hibiscus nanoparticles are better at targeting Gram-negative bacteria compared to Gram-positive.Anti-inflammatory Activity for Protein Denaturation Assay is also compared between Positive Control 86.22% inhibition and Nanoparticles 54.42% inhibition. Proteinase Inhibitory Activity is also compared between Positive Control 88.25% inhibition and Nanoparticles 83.64% inhibition.Nanoparticles demonstrated strong inhibition of proteinase activity, almost equal to the positive control. The inhibition of protein denaturation was moderate, indicating anti-

inflammatory potential. Anticancer Activity of IC_{50} Value (Concentration required to inhibit 50% cell viability) compared between Aqueous Extract 13.62 µg/ml which is highly potent anticancer activity and Nanoparticles -309.04 µg/ml.The aqueous extract of Hibiscus rosa-sinensis showed excellent anticancer activity. A negative IC50 value suggests poor or no significant anticancer effect from the nanoparticles under the tested conditions.

CONCLUSION

This study highlights the potential of Hibiscus rosa-sinensis stem extract as a valuable source of bioactive compounds with Antioxidant, Antibacterial, Anti-inflammatory and Anticancer properties. ***Hibiscus rosa-sinensis*** copper nanoparticles were successfully synthesized and characterixed.The cytotoxicity assay of ***H.rosa-sinensis*** Aqueous extract on *Caco-2* cancer cell line revealed an IC50 value of AE 13.62µg/ml suggesting a higher cytotoxicity effect compared to NP -309.04µg/ml it suggests that nanoparticle didn't show good anticancer activity. Additionally, the extract demonstrated excellent antioxidant properties by reducing oxidative stress. This study, also examined the antibacterial activity of ***H.rosa-sinensis*** stem against intestinal pathogens of bacterial cultures S.aureus and ***E.coli*** using the agar well diffusion method. Results showed ***E.coli*** as a greater zone of inhibition against ***S.aureus***, confirming its moderate antibacterial potential. Also, examined the anti-inflammatory activity of phosphomolybdenum and H2O2 scavenging activity which shows strong anti-inflammatory activity. Anticancer activity was very promising for the aqueous extract, but nanoparticles require further optimization for better anticancer effects. This study suggests that ***Hibiscus rosa-sinensis*** has high therapeutic potential as a natural antioxidant, anti-inflammatory, antibacterial agent, and as an anticancer source (especially in non-nanoparticle form). Moving forward, further research is needed to isolate and characterize its bioactive compounds, explore their mechanisms of action, and conduct clinical trials to determine safety and efficacy. The promising biological properties of ***Hibiscus rosa-sinensis*** suggest its potential for future pharmaceutical development as a natural alternative for cancer treatment, oxidative stress reduction and infection management.

REFERENCES

1. Pourmadadi, Mehrab, Roghaieh Holghoomi, Reza Maleki-baladi, Abbas Rahdar, and Sadanand Pandey. "Copper nanoparticles from chemical, physical, and green synthesis to medicinal application: a review." Plant Nano Biology (2024): 100070.
2. SALAMI, Latifat Olajumoke. "CHEMICAL PROPERTIES OF TREATED NEEM (Azadirachta indica A. Juss) SEED AND PRESERVATIVE POTENTIALS OF ITS OIL ON ONIONS, SELECTED GRAINS AND FERMENTED LOCUST BEANS." PhD diss., 2023.
3. Al-Hakkani, Mostafa F. "Biogenic copper nanoparticles and their applications: A review." SN Applied Sciences 2, no. 3 (2020): 505.
4. Dharmaraja, Allimuthu T. "Role of reactive oxygen species (ROS) in therapeutics and drug resistance in cancer and bacteria." Journal of medicinal chemistry 60, no. 8 (2017): 3221-3240.
5. Khan, Yousaf, Haleema Sadia, Syed Zeeshan Ali Shah, Muhammad Naeem Khan, Amjad Ali Shah, Naimat Ullah, Muhammad Farhat Ullah et al. "Classification, synthetic, and characterization approaches to nanoparticles, and their applications in various fields of nanotechnology: a review." Catalysts 12, no. 11 (2022): 1386.
6. Ahmad, Fiaz, Noreen Ashraf, Tayyba Ashraf, Ren-Bin Zhou, and Da-Chuan Yin. "Biological synthesis of metallic nanoparticles (MNPs) by plants and microbes: Their cellular uptake, biocompatibility, and biomedical applications." Applied microbiology and biotechnology 103 (2019): 2913-2935.
7. Usman, Muhammad Sani, Mohamed Ezzat El Zowalaty, Kamyar Shameli, Norhazlin Zainuddin, Mohamed Salama, and Nor Azowa Ibrahim. "Synthesis, characterization, and antimicrobial properties of copper nanoparticles." International journal of nanomedicine (2013): 4467-4479.
8. Ying, Shuaixuan, Zhenru Guan, Polycarp C. Ofoegbu, Preston Clubb, Cyren Rico, Feng He, and Jie Hong. "Green synthesis of nanoparticles: Current developments and limitations." Environmental Technology & Innovation 26 (2022): 102336.
9. Salem, Salem S., and Amr Fouda. "Green synthesis of metallic nanoparticles and their prospective biotechnological applications: an overview." Biological trace element research 199, no. 1 (2021): 344-370.

10. Chopra, Hitesh, Shabana Bibi, Inderbir Singh, Mohammad Mehedi Hasan, Muhammad Saad Khan, Qudsia Yousafi, Atif Amin Baig et al. "Green metallic nanoparticles: biosynthesis to applications." Frontiers in Bioengineering and Biotechnology 10 (2022): 874742.
11. Doughari, James Hamuel. Phytochemicals: extraction methods, basic structures and mode of action as potential chemotherapeutic agents. Rijeka, Croatia: INTECH Open Access Publisher, 2012.
12. Roy, Anupam, Onur Bulut, Sudip Some, Amit Kumar Mandal, and M. Deniz Yilmaz. "Green synthesis of silver nanoparticles: biomolecule-nanoparticle organizations targeting antimicrobial activity." RSC advances 9, no. 5 (2019): 2673-2702.
13. Crisan, Michaela Corina, Mocan Teodora, and Mocan Lucian. "Copper nanoparticles: Synthesis and characterization, physiology, toxicity and antimicrobial applications." Applied Sciences 12, no. 1 (2021): 141.
14. Jafari, Azam, Latifeh Pourakbar, Khalil Farhadi, Lida MOHAMMAD GHOLIZAD, and Yobert Goosta. "Biological synthesis of silver nanoparticles and evaluation of antibacterial and antifungal properties of silver and copper nanoparticles." Turkish Journal of Biology 39, no. 4 (2015): 556-561.
15. Liu, Tengfei, Bowen Xiao, Fei Xiang, Jianglin Tan, Zhuo Chen, Xiaorong Zhang, Chengzhou Wu et al. "Ultrasmall copper-based nanoparticles for reactive oxygen species scavenging and alleviation of inflammation related diseases." Nature communications 11, no. 1 (2020): 2788.
16. Thiruvengadam, Muthu, Ill-Min Chung, Thandapani Gomathi, Mohammad Azam Ansari, Venkatesan Gopiesh Khanna, Vaishnavi Babu, and Govindasamy Rajakumar. "Synthesis, characterization and pharmacological potential of green synthesized copper nanoparticles." Bioprocess and biosystems engineering 42 (2019): 1769-1777.
17. Fahmy, Baher, and Stephania A. Cormier. "Copper oxide nanoparticles induce oxidative stress and cytotoxicity in airway epithelial cells." Toxicology in Vitro 23, no. 7 (2009): 1365-1371.
18. Denoyer, Delphine, Shashank Masaldan, Sharon La Fontaine, and Michael A. Cater. "Targeting copper in cancer therapy:‘Copper That Cancer’." Metallomics 7, no. 11 (2015): 1459-1476.
19. Naz, Sania, Ayesha Gul, and Muhammad Zia. "Toxicity of copper oxide nanoparticles: a review study." IET nanobiotechnology 14, no. 1 (2020): 1-13.

20. Nayak, Debasis, Sarbani Ashe, Pradipta Ranjan Rauta, and Bismita Nayak. "Biosynthesis, characterisation and antimicrobial activity of silver nanoparticles using Hibiscus rosa-sinensis petals extracts." IET nanobiotechnology 9, no. 5 (2015): 288-293.
21. Al-Senani, Ghadah M., and Nada Al-Kadhi. "The synthesis and effect of silver nanoparticles on the adsorption of Cu2+ from aqueous solutions." Applied Sciences 10, no. 14 (2020): 4840.
22. Missoum, Asmaa. "An update review on Hibiscus rosa sinensis phytochemistry and medicinal uses." Journal of ayurvedic and herbal medicine 4, no. 3 (2018): 135-146.
23. Philip, Daizy. "Green synthesis of gold and silver nanoparticles using Hibiscus rosa sinensis." Physica E: Low-Dimensional Systems and Nanostructures 42, no. 5 (2010): 1417-1424.
24. Kombaiah, K., J. Judith Vijaya, L. John Kennedy, and M. Bououdina. "Studies on the microwave assisted and conventional combustion synthesis of Hibiscus rosa-sinensis plant extract based ZnFe2O4 nanoparticles and their optical and magnetic properties." Ceramics International 42, no. 2 (2016): 2741-2749.
25. Buarki, F., H. AbuHassan, F. Al Hannan, and F. Z. Henari. "Green synthesis of iron oxide nanoparticles using Hibiscus rosa sinensis flowers and their antibacterial activity." Journal of Nanotechnology 2022, no. 1 (2022): 5474645.
26. Rehana, Dilaveez, D. Mahendiran, R. Senthil Kumar, and A. Kalilur Rahiman. "Evaluation of antioxidant and anticancer activity of copper oxide nanoparticles synthesized using medicinally important plant extracts." Biomedicine & Pharmacotherapy 89 (2017): 1067-1077.
27. Rajendran, A., E. Siva, C. Dhanraj, and S. Senthilkumar. "A green and facile approach for the synthesis copper oxide nanoparticles using Hibiscus rosa-sinensis flower extracts and it's antibacterial activities." J Bioprocess Biotech 8, no. 3 (2018): 324.