“*FORTUNELLA MARGARITA* LEAF EXTRACT: SUSTAINABLE PRODUCTION AND CHARACTERISATION OF COPPER AND ZINC OXIDE NANOPARTICLES”

A DISSERTATION

Submitted in Partial Fulfilment of the Requirements

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BIOTECHNOLOGY

Submitted by

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CANDIDATE'S DECLARATION

I **Suman,** Roll Number: **2K22/MSCBIO/49** student of M.Sc. Biotechnology hereby declares that the project dissertation titled **"*Fortunella margarita* Leaf Extract: Sustainable Production and Characterisation of Copper and Zinc Oxide Nanoparticles"** submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science is an authentic record of my own work carried out during the period from January 2024 to May 2024, under the supervision of Prof. Jai Gopal Sharma. This work has not been previously formed as the basis for the award of any degree, Diploma Associateship, Fellowship, or other similar title or recognition.

My review paper has been accepted in a Scopus-indexed journal with the following details:

Title of the paper: Phytochemistry of Aloe Vera: A Catalyst for Environment-friendly Diverse Nanoparticles with Sustained Biomedical Benefits.
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Date:

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CERTIFICATE

This is to certify that the project Dissertation titled **"*Fortunella margarita* Leaf Extract: Sustainable Production and Characterisation of Copper and Zinc Oxide Nanoparticles"** which is submitted by **Suman**, roll no. **2K22/MSCBIO/49** from Department of Biotechnology, Delhi Technological University, Delhi, is a record of the original project work carried out by the student herself under my supervision. To the best of my knowledge, this work has not been submitted in part or full for any Degree or Diploma to this university or elsewhere.

Prof. Yasha Hasija	**Prof. Jai Gopal Sharma**
Head of Department	**Supervisor**
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Delhi Technological University	**Delhi Technological University**

Date:

Fortunella margarita Leaf Extract: Sustainable Production and Characterisation of Copper and Zinc oxide Nanoparticles

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ABSTRACT

This research focuses on the environmentally sustainable and economical process of producing copper and zinc oxide nanoparticles by using an extract from *Fortunella margarita* (kumquat) leaves. Absorption peaks at 560 nm for CuNPs and 380 nm for ZnONPs were found during the characterization of the nanoparticles using UV-Vis spectroscopy. ZnO NPs were shown to have less antibacterial activity than Cu NPs when the produced nanoparticles were tested against E. coli by Disc Diffusion approach. The study evaluated the nanoparticle's photocatalytic breakdown efficiency in the presence of sunlight using methylene blue dye. Compared to 47% for Cu NPs, ZnO NPs had higher photocatalytic activity, at 64%. It's clear from this how useful ZnO NPs are for cleaning up the environment, especially when it comes to breaking down organic contaminants. With Cu NPs excelling in antibacterial applications and ZnO NPs displaying great efficiency as dye degradants, the work shows the dual functionality of kumquat-produced nanoparticles and lights the way for sustainable and multifunctional nanomaterials.

LIST OF PUBLICATIONS

1. A paper entitled “Phytochemistry of Aloe Vera: A Catalyst for Environment-friendly Diverse Nanoparticles with Sustained Biomedical Benefits” has been accepted in Nature Environment and Pollution Technology (NEPT).

2. A paper entitled “Bio Synergize: Microbial Synergy Driving Simultaneous Bioremediation and Nanoparticle Synthesis” has been accepted in African Journal of Biological Sciences (AJBS).

3. A paper entitled “Insights into the Inhibition Mechanism of Lipoteichoic Acid Synthase (LtaS) Enzyme by Endophytic Fungal Metabolites: A Molecular Docking and Dynamics Simulation Study for Combatting Drug Resistance” has been accepted in International Conference on Emerging Technologies in Science and Engineering (ICETSE) – 2024.

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NPs – Nanoparticles

FM – *Fortunella margarita*

E. coli – Escherichia coli

B. subtilis – Bacillus subtitlis

ZOI – Zone of inhibition

XRD – X-ray diffraction

XPS - X-ray photoelectron spectroscopy

SEM – Scanning electron microscopy

TEM – Transmission electron microscopy

FTIR – Fourier transform infrared spectroscopy

HRTS - High- resolution transmission electron microscopy

Cu NPs– Copper nanoparticles

ZnO NPs – Zinc oxide nanoparticles

FM NPs- *Fortunella margarita*-mediated nanoparticles

MB – Methylene Blue

SPR – Surface plasmon resonance

TPP – Tripolyphophate

ICP-OES - Inductively coupled plasma optical emission spectrometry

EDAX - Energy dispersive absorption x-ray spectroscopy

GC/MS – Gas chromatography/Mass spectrometry

CHAPTER 1

INTRODUCTION

1.1 General Introduction

With advances in science & technology, Nanotechnology has become 1 of the most active fields in every research area. It is highly used in electronic devices and seeking attention for nanoparticle synthesis. Nanoparticles are nano or minuscule dimensions within a range of 1-100 nanometers with a large surface-volume ratio. They possess unique properties like magnetic, optical, electrical, chemical, and other various biological properties(Zharov et al., 2005). Two approaches for synthesizing nanoparticles (NPs) involve Top-down & Bottom-up with the involvement of physical method & chemical methods. Physical methods involve crushing, grinding, and milling, etc whereas chemical methods such as co-precipitation, sol-gel, hydrothermal, etc methods require excessive energy with the release of toxic carcinogenic by-products(Ying et al., 2022). With the increment in alkaline conditions, metal ions will get reduced to attain a petite size of the nanoparticle, whereas, with the increment in temperature, there is an increment in the absorption spectra (profound effect), which results in the rapid formation of nanoparticles(Song et al., 2009). Synthesis of nanomaterial comprises three phases: (a) activation phase; metal ion gets reduced, (b) growth phase; aggregation of small particles to form large nanoparticles via Ostwald ripening, (c) termination phase; concluded the shape of a nanoparticle. Synthesized nanoparticles have various antimicrobial, anti-inflammatory, drug-delivery, anticancer, and antioxidant properties(Muthu et al., 2021). Using biological methods like plant extracts, bacteria, fungi, and other pathogens, green synthesis has emerged as an ideal option over conventional methods for NP synthesis(Behravan et al., 2019).

"Green synthesis" is the most eco-friendly, non-toxic, and cost-effective as a natural source(Behravan et al., 2019). Plant extracts which contain components like alkaloids, polyphenols, vitamins, and steroids, have great medicinal value and act as reducing and stabilizing agents(Akbar et al., 2020) and result in the formation of stable metal nanoparticles (Sargazi et al., 2022) . Phenolic groups in plant extracts have a higher capacity to reduce the metal ions for nanoparticle synthesis than fungi and bacteria. The extent of metal ions reduction to synthesize specific metal NPs directly depends on the type and concentration of phytoconstituent present in plant extracts(Iravani, 2011). Various nanoparticles such as Cu, Au, Ag, Zn, CO, etc are synthesized by this green route.

1.2 Why Green Synthesis Route Utilized over Conventional Methods?

Different methods like physical, chemical, and biosynthesis methods are used for the synthesis of nanoparticles. Physical methods for synthesis require high energy and release harmful chemicals and waste products. Chemical methods face many problems like using toxic and expensive solvents(Kundu et al., 2014) and heating the chemicals at high temp, with low pressure, which leads to increased risk to human health and surroundings(Li et al., 2011). These methods decline the biological applications and desired mass production.

To overcome this problem, the global market transitioned towards environment-friendly methods. Biosynthesis methods are quite safe, Harmless, and novel approaches including plants and microorganisms for medicinal applications(Rabiee et al., 2020). Microbial systems are cost-effective and nontoxic for the environment including bacteria, fungi, algae, yeast, etc for nano-size particles. These work to reduce and stabilize the particular metal nanoparticle(Gericke Mariekie & Pinches Anthony, 2006). Among these biological systems fungi have better quality in production due to low expenses for downstream processing, highly efficient for extracellular enzymes used for large mass production, good metal reduction, and utilization of biomass(Sarkar et al., 2014). It is quite challenging for researchers because of the complexity of toxic metals for the synthesis of nanoscale materials. Microbes-mediated nanoparticles are not completely viable to form the desired product, they require a proper sterile condition and high maintenance. No control for nanoparticle size, structure, and time for microbial screening are some major drawbacks of microbial systems for nanoparticle synthesis(Kundu et al., 2014). Researchers investigated to reveal that plant parts and their extracts are the most ideal and cost-effective, nontoxic method of nanoparticle synthesis with deficient maintenance of requirements in the system. Plant extracts contain numerous active phytocomponents like flavonoids, amino acids, minerals, enzymes, polysaccharides(Gao et al., 2019), polyols, and polyphenols. Phytochemicals in plant extracts act as stabilizing agents as compared to microbial systems for nanoparticle synthesis to reduce toxic heavy metals. Plant-mediated nanoparticle synthesis is cheap, accelerated, and doesn't require specific conditions and screening of microbial cultures as microbial systems do, and was easily synthesized in a controlled condition at room temperature(Rajakumar et al., 2012) with quite stirring and heating(Acharyulu et al., 2014). This is done by simply mixing the reducing agents present in plant extracts in an aqueous solution of the respective metal of the nanoparticle to be synthesized. A visual change in the color of the solution or by UV-Vis Spectroscopy characterizes the formation of nanoparticles. Finally, nanoparticles are collected by washing before drying at low temperatures. Plant-mediated synthesis of nanoparticles is stable for getting higher yield which makes it a highly preferred method over other biosynthesis methods. Several nanomaterials synthesized by this method have unique properties. Ex- Gold and silver nanoparticles synthesized by plant extracts have anti-microbial, anticancer, and antiviral properties. whereas Copper and Zinc oxide nanoparticles are efficient for the production of cosmetic products and coating purposes(Mittal et al., 2013).

Various plants such as *Medicago sativa, A. indica, aloe vera, Tamarind, Alfa Alfa, Hydrilla sp., Mangifera indica, cassia fistula, Piper betle,* and many more medicinal plants have been used for NP's synthesis.

1.3 Medicinal Plant - *Fortunella margarita*

Fortunella margarita (kumquats) is a citrus fruit bearing plant species belonging to the Rutaceae family, native to South Asia and the Asia-Pacific region. Leaves for this plant are dark green colored with a smooth texture and they can withstand temperatures below 14°F for a very short -period. It is highly rich in active phytoconstituents, including flavonoids, vitamins, essential oils, and phenolic acids(Alkhalifawi et al., n.d.). Phytochemicals in kumquat fruit have high vitamin C and vitamin B2 content(Polatoğlu & Bozkurt, 2021), a vital nutrient that works as an antioxidant & strengthens immunity. It has anticancer, antiviral, and anti-inflammatory health benefits. Additionally, it is used as an ornamental plant and in various food industries such as wine, jams, candies, etc. Essential oils (limonene-87%) isolated from peel of kumquat are used in pharmacy, cosmetics, and aromatherapy. For the current study, copper and zinc oxide nanoparticles are synthesized by *Fortunella margarita* (FM) because Cu and ZnO have high thermal, electrical, and antimicrobial efficiency among various nanoparticles. Both NPs are extensively involved in the remediation of heavy metals, medical treatments, and other industrial processes.

1.4 Objectives

The main objective of this thesis work is to synthesize Cu and ZnO nanoparticles from *Fortunella margarita* plant extract with analysis and characterization using UV-vis spectrophotometry. Additionally, the research will examine the antibacterial effects of NPs on *E. coli* and determine their dye degradation efficiency in breaking harmful dye. The outcome of this study is expected to show practical and environmental benefits to synthesizing Cu and ZnO nanoparticles using the kumquat plant with notable dye degrading and antibacterial effects.

1.5 Organization of the Thesis

This thesis entitled "*Fortunella Margarita* Leaf Extract: Sustainable Production and Characterisation of Copper and Zinc Oxide Nanoparticles" structures several chapters focusing on the different prospects of the study.

Chapter 1. Introduction

This chapter introduces nanoparticles with their different applications in various fields such as medicine, industries, and environmental applications**.** It compares the advantages of using green synthesis methods over conventional methods. This chapter also provides an overview of the plant *Fortunella margarita* with its phytochemical compositions along with their various applications. Objectives are outlined with the aim of the research.

Chapter 2. Review Literature

This chapter covers existing literature of research that focuses on the synthesis of Cu and ZnO nanoparticles and reviews phytochemical properties using kumquat to determine its environmental impacts.

Chapter 3. Methodology of Experiment

It involves a detailed methodology starting from *Fortunella margarita* leaf extract preparation, synthesis of nanoparticles, and characterization using UV-vis spectroscopy technique and also evaluating the antibacterial and dye degradation ability of the synthesized nanoparticles using the plant.

Chapter 4. Results and Discussion

It concludes the resulting data from characterization and other application tests.

Lastly, the Conclusion summarises research findings with stable synthesis of nanoparticles using *Fortunella margarita*, a medicinal plant to optimize and explore their practical implications.

CHAPTER 2

REVIEW LITERATURE

Classification and Description of the *Fortunella margarita* Plant:

Kingdom: Plantae
Clade: Angiosperms, Eudicots, Rosids Order: Sapindales
Family: Rutaceae
Genus: Fortunella
Species: *Fortunella margarita*

Fortunella margarita commonly called kumquat, is a green shrub tree belonging to the Rutaceae family. It’s an ornamental plant with white fragrant flowers and edible fruits that look like small oranges with a sweet-sour taste. This plant has valuable medicinal properties to treat diseases(Swingle, 1915). Diverse form of Fortunella species is present worldwide such as *Fortunella japonica* (Phung Anh et al., 2018a), *Fortunella crassifolia, Fortunella margarita* (Amjad et al., 2021)*, Fortunella venosa, and Fortunella hindsii* (Wang et al., 2022). These species are distinct from each other in characteristic features of leaf, growth habitat, etc.
Some specific phytochemicals present in kumquat with their certain benefits are mentioned in the following:
Quercetin: it is generally found in the peel of the plant species.
It’s a kind of flavonoid having anti-inflammatory and anti-oxidant properties that helps cells from inflammatory damage(Lasota et al., 2024; Tan et al., 2014).

Citric Acid: found in pulp. It's an organic acid that helps in digestion, increases nutrient absorption, and is used in the cosmetics industry to enhance skin health benefits.

Hesperidin: it is also found in pulp(Lasota et al., 2024). A flavonoid helps to balance oxidative stress (O.S) and effectively contributes to cardiovascular health by acting as an anti-oxidant (Tan et al., 2014).

Luteolinidin: found in the peel of plant species, a flavonoid helps neutralize free radicals to act as an anti-oxidant.

Naringenin: found in the pulp of species of Fortunella plant. It acts as an anti-inflammatory agent and protects cells from damage due to inflammation(Tan et al., 2014).

Fortunella margarita phytoconstituents help to provide antioxidant, anti-inflammatory, and possible health promoting effects. These molecules can be used to promote wellness in general in several applications, including dietary supplements, cosmetics, and organic preservatives.

Leaves, bark, fruits, stems, and flowers are common plant parts that can be used for copper and zinc oxide nanoparticle synthesis. Plant extract acts as a capping and reducing agent to reduce metal ions to form eco-friendly and stable metal nanoparticles. Cu and ZnO nanoparticles have strong antibacterial properties against various strains of bacteria such as *E. coli, Staphylococcus aureus, Bacillus subtilis,* etc(Naseer et al., 2020). Now we see some characteristics below of kumquat along with other plant species that are utilized to generate copper & zinc oxide NPs.

Fortunella japonica plant extracts together with a solution of AgNO3 were used in this research to produce silver nanoparticles, which were subsequently investigated using XRD, SEM, TEM, and UV-vis spectrum. This resulted in an increased rate of formation of Ag NPs with an average size of 15 nm under sunlight while no formation occurs in dark conditions. The antibacterial activity of *E. coli* is higher with a diameter of 23mm as compared to *B. subtilis* with 16.3 mm(Phung Anh et al., 2018b).

The current study analyzed the possible antimicrobial effects of extracted essential oils of *Fortunella margarita* and *Pistacia lentiscus* in food products like fruit juice and ice creams. They extract essential oils by using a distillation method and mix them into prepared food products by continuously stirring. Finally, analytical techniques such as GC-MS analyze that alpha-pinene is an abundant compound in *P. lentiscus* essential oil and limonene in F. margarita essential oils. With the disc diffusion method analysis been made that *Pistacia lentiscus* essential oils exhibit strong antimicrobial activity in food supplements as compared to *F. margarita* towards foodborne pathogens such as *Salmonella Typhimurium, S. cerevisiae, Escherichia coli, and Aspergillus flavus,* etc (Mitropoulou et al., 2022).

This study (Oraibi et al., 2023) delves into the synthesis of copper nanoparticles from the *Carum carvi* plant and examines their positive effects on the germination of *Solanum lycopersicum* seeds. Firstly, the production of Cu NPs by mixing *Carum carvi* extract in copper sulphate soln. and observed the color shift from dark brown to a green color. Optical properties of Cu NP detected by UV-vis, XRD, SEM, and AFM. It showed an absorption spectrum with a peak at 320-340 nm while no peak was observed between this wavelength with an aqueous extract of *Carum carvi.* AFM detects NP size with an average of 12.4 nm. To examine the effects of green synthesized NPs on seedlings researchers first sterilized or washed the seeds with distilled water, soaked in ethanol for 4-5 sec, and then again sterilized for 7-10 min with double distilled water on a magnetic stirrer. Lastly, they noted the results of seed germination in soil containing Cu NPs and found enhancement in the chlorophyll content and the activity of peroxidase enzymes on the 21st day after its treatment for germination. By using the poly-electrolyte complexation method this study gives an insight into the environmentally friendly method used to synthesize nano fertilizers. This novel achievement was made by encapsulating copper oxide nanopowder inside the polymeric complex of chitosan & Na alginate to successfully release copper nutrients from its shell that help in the germination of swingle seeds of *Fortunella margarita.* These nanocomposites are characterized by X-ray crystallography, DLS (dynamic light scattering), ICP-OES (inductively coupled plasma optical emission spectrometry), and thermogravimetric analysis. The results evaluated the synergic effects shown by nanocomposites in swingle seed germination by slowly releasing copper out of the shell and it works as a potent nano fertilizer (Leonardi et al., 2021).

This paper addresses the therapeutic ability of synthesized nanocomposites with kumquat against a disease named Toxoplasmosis in mice. This disease is caused by a parasite i.e. *Toxoplasma gondii* in hosts. To make kumquat-mediated chitosan nanocomposites, 200g of kumquat-dried peel powder was mixed in 800 ml of 90% ethanol on a magnetic stirrer at 60 degrees Celsius temp. for 10-15 min and then left in a dark closed-glass bottle for extraction for one day. After 24 hrs extract was filtered. Nanocomposites were prepared by mixing 1 ml of chitosan mixture in kumquat filtrate followed by the addition of 100 microlitres of TPP sol. (tripolyphosphate) In kumquat-chitosan mixture drop by drop with stirring and then centrifuged for 5 -7 minutes, the supernatant was characterized via SEM, TEM, and zeta potential assay. After assessments, these nanocomposites were injected into infected mice resulting in effective changes in oxidative stress and acting as a significant therapy for Toxoplasmosis(El-Hamed et al., 2022).

In a comparative study, researchers assessed the high potency of essential oils in fruits and leaves of the *Fortunella margarita* plant to know which plant organ exhibits better efficiency as an antiviral against avian Influenza Virus i.e. (H5N1) and antimicrobial agent against pathogens such as *B. subtilis, S. aureus, Sarcina luta*, etc. isolation and analysis of essential oils from fresh leaves and fruits were done by hydro-distillation and GC/MS technique. Agar plates were prepared and test samples for antiviral and antimicrobial were applied on pates by agar diffusion assay which was then kept incubated at 28-

37 °C for 1-2 days. The diameter zone revealed that essential oil present in leaves showed more antimicrobial activity than fruit oil. While essential oils in fruits showed high antifungal activity and also antiviral against avian Influenza Virus i.e. (H5N1) (Ibrahim et al., 2015).

This study examines the impacts of extract's concentration *Citrus microcarpa* on the optical properties of ZnO nanoparticles. Peel of *Citrus microcarpa* dried, grounded, and different conc. of powder such as 1, 2, and 4 % dissolved in distilled water with stirring and filtered out after centrifugation. 2 g of zinc nitrate was mixed in diff. extract of solution and stir for one hour. The prepared solution was then kept for a half day in a water bath at 60°C and finally, the sample was dried, and collected after calcination for 45 min at 400°C. ZnO nanoparticles of diff conc. was characterized using FTIR, XPS (x-ray photoelectron spectroscopy), XRD, and PL (photoluminescence). 4% extract-ZnO NP achieved a higher 90% degradation of methylene blue under sunlight and UV light (Villegas-Fuentes et al., 2023).

In this work, researchers investigated the green synthesis of ZnS NPs with combined actions of *Ulva fasciata*, algae, and *Citrus japonica* (kumquat fruit). This combined type of ZnS NP is created to address both antimicrobial effects as well as dye degradation effects. Spherical ZnS NP with a size of 31-nm was assessed via UV-vis, SEM, EDAX (energy dispersive absorption x-ray spectroscopy), TEM, and FTIR. This investigation recommended ZnS NP as a safe and cheaper bio-photocatalyst for impure industrial wastewater owing to its ideal antibacterial and photodegradation performance (El Nady et al., 2022).

Nanoparticles' antibacterial qualities have been proven to be profitable against urinary tract infections (Santhoshkumar et al., 2017). This report describes the chemical and biological processes used to synthesize nanostructured ZnO nanoparticles. Steady and round nanoparticles are produced with zinc nitrate and the aloe vera, with a conversion rate of more than 95% at 25% conc. characterization of ZnONPs was done by SEM & TEM to study the optical, morphology, and structural properties, and resulted in NPs in a range between 25-40nm, content of leaf broth plays a vital way in controlling the size of nanoparticles(Sangeetha et al., 2011).

The researchers generated Cu_2O/CuO NPs, ZnO NPs, and Cu_2O/CuO–ZnO nanocomposites utilizing leaf extracts of *Alchornea cordifolia* plant. By utilizing a simple hydrothermal process NPs were generated and dried in an oven at a temperature of 80-90. UV-vis revealed the production of nanoparticles with amide on their surfaces, indicating their involvement as capping agents. X-ray crystallography studies revealed combined phases of Cu_2O/CuO NPs and hexagonal wurtzite phase for both nanocomposites and ZnO NPs. NPs had an average size of 3.54 nm and had an impact on cervical cancer will be evaluated via the MTT assay technique (Elemike et al., 2020).

The present article suggests a cost-effective and ecologically stable production of CuO and ZnO NPs that utilizes *Ferulago angulate* (schlecht) boiss extract as a nontoxic reducing agent and stabilizer. NPs are studied with techniques, XRD confirmed that crystalline CuO & ZnO NPs had greater quality and a particle size of around 44nm. The photocatalytic activity showed against Rhodamine B dye and discovered that ZnO nanoparticles have more dye degradation activity than copper oxide nanoparticles at room temp. under visible light irradiation (Shayegan Mehr et al., 2018). *Melia azedarach* leaf extract and zinc nitrate were applied as an initial material in the synthesis of ZnO NPs. Alkyl halides, aliphatic amines, and carboxylic acids were involved in the production of NP, which displays an absorption peak at 372nm. SEM and TEM revealed a hexagonal spherical shape, and EDAX verified that NPs included zinc, demonstrating that the biosynthetic method completed the outcomes. Green synthesized ZnO NPs have significant anti-oxidant & antibacterial characteristics which makes them interesting in a broad-spectrum way for biological applications. The anti-oxidant property in this research revealed robust scavenging action in it (Dhandapani et al., 2020).

CHAPTER 3

EXPERIMENTAL

The objective of this project work "*Fortunella Margarita* Leaf Extract: Sustainable Production and Characterisation of Copper and Zinc Oxide Nanoparticles"

3.1 Materials and Pieces of Equipment

During experimental work, chemicals such as copper sulphate pentahydrate, zinc sulphate heptahydrate, and antibiotics i.e. Drotvine-M are used.
Sterile Equipment such as falcon tubes, measuring cylinders, beakers, Eppendorf tubes, tweezers, swab sticks, culture media plates, centrifuge machine, magnetic stirrer, incubator, UV-spectrophotometer, and many more are used.

3.2 Collection of Plant Samples

Leaves of plant i.e. *Fortunella margarita* (FM) were gathered from Delhi Technological University (DTU) campus, Delhi. To get all of the dirt off the surface of the leaves, they were cleaned using tap water and continued with distilled water thoroughly.

Fig. 3.1 Collection of *Fortunella margarita* leaves.

3.3 Leaf Extract Preparation

1. 20 grams (total 40 g) of fresh leaves of *Fortunella margarita* (kumquat) were chopped and ground with pestle mortar to make a thin paste.
2. The prepared paste was diluted in 100 milliliters of distilled water and was stored for one hour at ambient temperature.

Fig. 3.2 Leaf extract dissolved in 100 ml of distilled water.

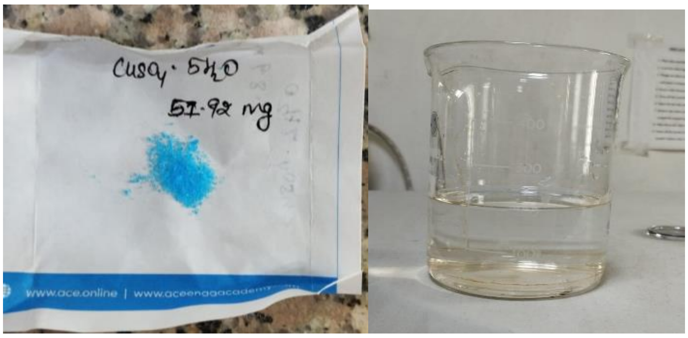
3. After one hour, the residue was removed and the leaf extract solution or mixture was filtered in a beaker to get phytoconstituents using Whatmann's filter paper.

Fig. 3.3 filtrate of *Fortunella margarita* plant

4. The collected filtrate was covered with aluminium foil and is refrigerated for later processing.

3.4 Preparation of Copper and Zinc Sulphate Stock Solutions

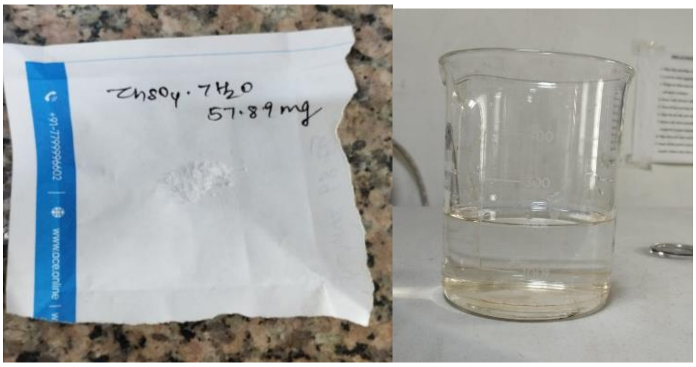
1. To prepare 1 mM copper sulphate solution weighing 51.92 mg of copper sulphate pentahydrate ($CuSO_4.5H_2O$) as a metal precursor by weighing balance and mixing it in 200 milliliters of distilled water using a stirrer with magnetic fields.



$CuSO_4.5H_2O$ compound $CuSO_4.5H_2O$ solution

Fig. 3.4 Preparation of copper sulphate pentahydrate solution by dissolving $CuSO_4.5H_2O$ compound in distilled water.

2. On the other hand, weighed 57.89 mg of zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) and was combined in 200 milliliters of distilled water properly to make a 1 mM zinc sulphate solution.



ZnSO4.7H2O compound ZnSO4.7H2 solution

Fig. 3.5 Preparation of zinc sulphate heptahydrate solution by dissolving ZnSO4.7H2O compound in distilled water.

3.5 Collection of Synthesized Cu & ZnO Nanoparticles

1. The filtrate of the leaf extract mixture was then mixed with 1mM copper & zinc sulphate solution in two separate beakers.
2. Keep the beakers in a water bath @ 70 °C for half an hour.

Fig. 3.6 Copper sulphate pentahydrate solution with plant extract after water bath @70°C.

Fig. 3.7 Zinc sulphate heptahydrate solution with plant extract after water bath @70°C.

3. After 1 hour, two solutions- one containing Cu leaf extract and the rest being Zn leaf extract- were centrifuged at 12,000 rpm @ 30 °C for 10-15 min in 50 ml falcon tubes.
4. Discard the supernatant and the particles in pellet were rinsed thrice with distilled water using a centrifuge machine with the same conditions as earlier to collect synthesized copper & zinc oxide NPs.

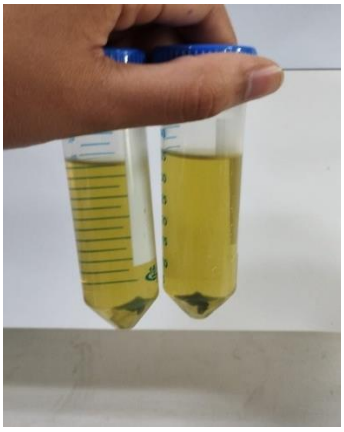
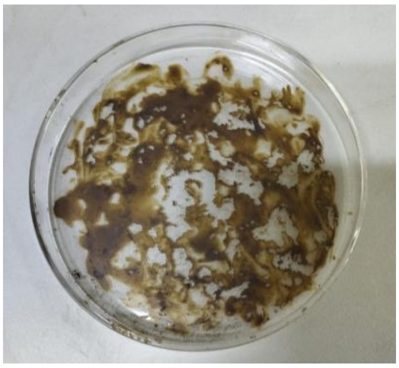
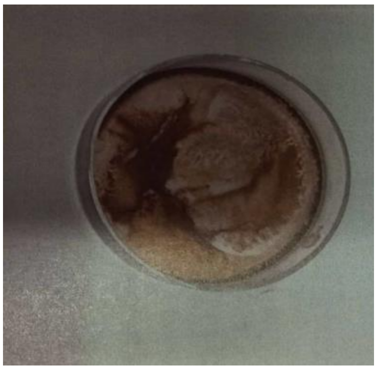


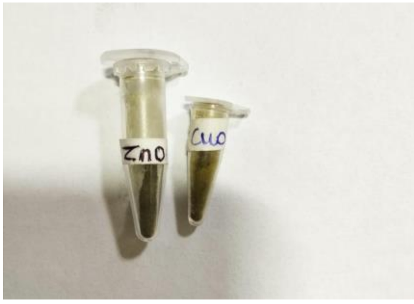
Fig. 3.8 Sedimentation of synthesized and centrifuged nanoparticles in pellets in falcon tubes.

5. Collect pellets in dried glass petri plates and dry them in a hot air oven @ 37 °C overnight.





(A) (B)



(C)

Fig. 3.9 Copper (A), Zinc (B) nanoparticles in a Petri plate, (C) collected in a plastic vial.

6. The dried pellet was scratched away the Petri dish weighed, and gathered in an Eppendorf tube by labeling it as Cu NPs and ZnO NPs.

3.6 Characterization of Copper and Zinc Oxide NPs

Reduction of metal salts in nanoparticles shown by a shift in the color of the solution throughout the reaction

3.6.1 Optical Characterization via UV-vis Spectroscopy:

The absorbance spectrum of synthesized nanoparticles was analyzed by UV-vis spectroscopy. It works on a phenomenon called 'surface plasmon resonance' which indicates that when light with a specific wavelength strikes the nanoparticle surface, it results in vibration & excitation of surface electrons of each NP that gives a solution a vibrant color(Rathi Sre et al., 2015).
Dissolve 5 mg of synthesized Cu and ZnO NPs in two different test tubes and add distilled water (5 ml) to both test tubes. then it was subjected to a vortex and followed by sonication to separate each particle. Load 2 ml of this mixture in the glass cuvette one after the other to measure the absorbance by using a UV-vis spectrophotometer. Copper nanoparticles show absorbance in a range of 540-600nm while zinc oxide NPs show a range between 280-400nm, respectively.

3.6.2 Antibacterial Effects of Cu & ZnO Nanoparticles:

Antibacterial activity can be tested by the AST (Antibiotic Susceptibility Testing) method to examine the antibacterial effects of *Fortunella margarita* (FM) synthesized nanoparticles (Cu & ZnO) against *E. coli* alongside antibiotics (Drotvine-M) towards bacterial pathogens. Here, the disc diffusion technique is employed to measure the zone of inhibition of respective culture plates.

1. Select gram-negative bacteria, *Escherichia coli* for testing.
2. Inoculate E. coli bacterial strain in sterile saline solution (2 ml) in a test tube to make a suspension of E. coli.
3. Utilising a clean swab stick, spread prepared suspension of bacteria on the surface of nutrient agar plates, uniformly in laminar airflow.

4. Prepare a test sample of copper by mixing 25 mg of copper nanoparticle in 500 μL of sterile distilled water in one test tube. Prepare the zinc test sample in the second test tube by adding 12.5 mg of zinc oxide nanoparticles in 250 μL of distilled water, lastly, prepare the Drotvine-M antibiotic test sample by mixing 20 mg of antibiotic in 50 ml of distilled water.
5. Place a sterile filter paper disc in all (three) agar plates with the help of a tweezer and pour 0.5 ml of copper, zinc & antibiotic test samples onto the sterile discs.
6. Seal culture plates with paraffin and place them in an incubator @34-37°C for 24-48 hrs for bacterial growth.
7. Examine the plates of agar to measure the clear area free of inhibition surrounding the discs using a ruler.
8. Compare the effectiveness of copper and zinc oxide nanoparticles with that of Drotvine-M towards bacterial growth.

3.6.3 Photocatalytic Dye Degradation Studies:

In this work, the effects of photocatalysis of synthesized nanoparticles, copper & zinc oxide were analyzed by degradation of Methylene Bue (MB) dye. This efficient degradation of dye can be depicted by recording the intensity of the solution in time intervals. Parameters such as time, dye concentration, pH, and catalyst quantity play a vital role in optimizing dye degradation efficiency(Sreelekshmi et al., 2022).

The degradation percentage of the tested sample can be calculated with this given Eqn. (3.1) (Elbadawy et al., 2023):

$$\% \text{ Degradation} = (C_0 - C_e)/C0 \times 100 \quad (3.1)$$

Here,

C_0 - initial conc. of MB dye solution in mg/L,
C_e - final conc. of MB dye solution in mg/L (Elbadawy et al., 2023).

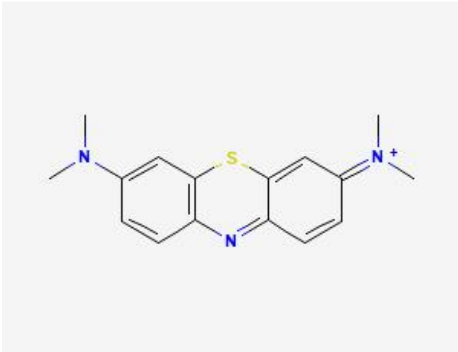


Fig. 3.10 Two-dimensional Methylene Blue (MB) dye structure.

1. In two beakers, 10 mg of Cu -NPs and ZnO -NPs were combined with 100 ml of MB dye solution (10mg of dye/1000mL of distilled water).
2. A control test is conducted using methylene blue dye solution without nanoparticles.
3. This mixture stirs under the dark for 25-30 minutes to reach the level of balance or equilibrium stage before being exposed it to direct sunlight for irradiance.
4. Examine discoloration in the mixture every 15 minutes at a wavelength of 650-670 nm.
5. Compare the dye's rate of degradation utilizing each catalyst (Cu-NP & ZnO-NP).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Colorimetric Indication to NP Formation.

Synthesis of copper and zinc oxide nanoparticles is confirmed by a change in color due to the phenomenon- of surface plasmon resonance (SPR) at room temperature(Alkhalifawi et al., 2015). It occurs when leaf extract solution is added to a 1mM stock solution of copper and zinc sulphate, respectively. The reaction mixture changes color from dark green to bluish-yellow color due to the reduction of copper metal ions to form Cu NPs, while a change from dark green to yellowish-brown confirms the formation of ZnO NPs. So, this depicts the stable formation of NPs, which is further characterized by techniques and other application-based tests.

4.2 UV-visible Spectrophotometric Detection

The SPR property of synthesized metal nanoparticles is successfully characterized by UV-vis spectrum. SPR is responsible for forming a specific absorption spectrum peak at one particular wavelength of light (Zambare et al., 2022). The UV spectrum of copper nanoparticles detected in a range of 400 to 800nm, which gives a strong absorption peak at 560nm as shown in Fig. 4.1 indicating the formation of stable Cu NPs. The blue band in the graph indicates the presence of Cu NPs.

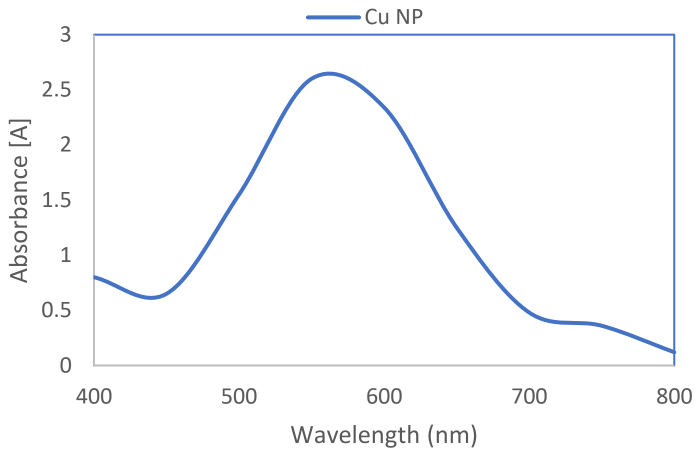


Fig. 4.1 UV-vis spectrum of FM -Cu NPs

Moreover, the Ultraviolet-visible spectrum of zinc oxide's NP is analyzed within a range of 200 to 650 nm, ZnO NPs show an absorption peak at 380nm as shown in Fig. 4.2. Orange band in the below graph indicates the presence of ZnO NPs.

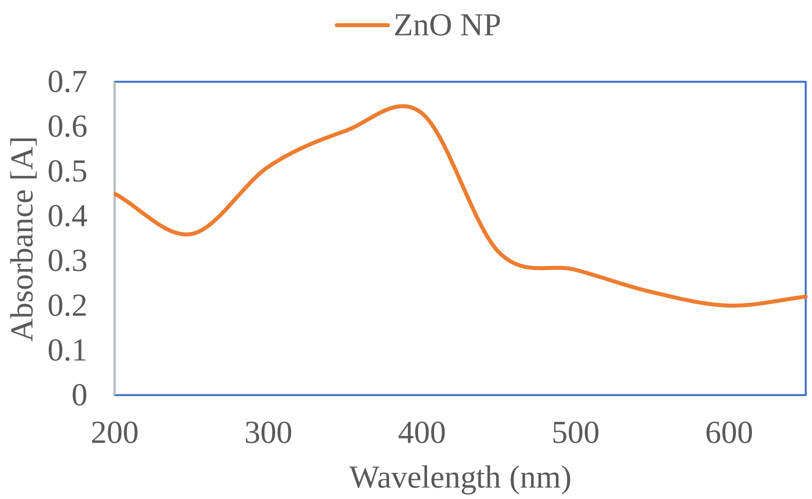
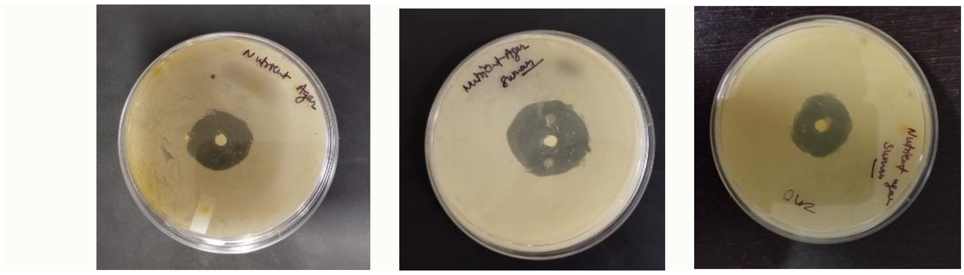


Fig. 4.2 UV-vis spectrum of FM -ZnONPs

4.3 Comparative Analysis of Antibacterial Activities of Test Samples Against *E. coli*

The zone of inhibition of Antibiotics i.e. Drotvine-M, Cu, and ZnO NPs against E. coli is measured after 24 hours of incubation at 37°C Table 4.1. The inhibition zones of each sample are relatively similar. The zone of inhibition of Antibiotic, Cu, and ZnO nanoparticles shown in Fig. 4.3 is 28 mm, 20 mm, and 15 mm, respectively.



(A) (B) (C)

Fig. 4.3 The zone diameter of inhibition showed against *Escherichia coli* of (A) copper NP (20mm), (B) antibiotic (28mm), and (C) zinc oxide NP (15mm).

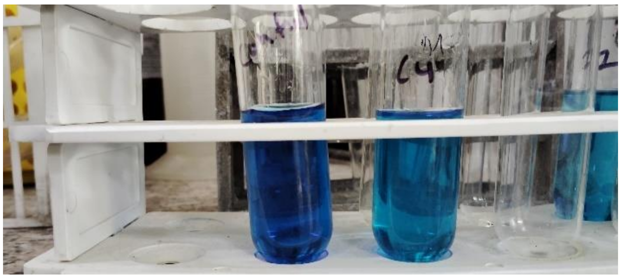
Table 4.1 Diameter of zone inhibition of *Fortunella margarita* -mediated NPs against *E. coli* isolates (Agar Disc Diffusion Method)

Antibacterial test agents or samples	Dose concentration in mg/ml	Diameter of inhibition zones
Drotvine-M	0.4	28 mm
Copper NPs	50	20 mm
Zinc NPs	50	15 mm

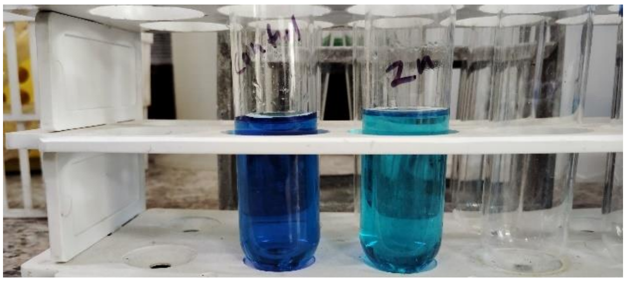
From these disc diffusion tests, we concluded that *Fortunella margarita*-mediated Cu nanoparticles have high antibacterial effects against *E. coli* as compared to ZnO NPs and can efficiently be used as a drug just like an antibiotic with antibacterial activity. This effectiveness is caused by the attachment of metal ions of Cu NP on the surface of the membrane to penetrate inside the cell of bacteria(Morones et al., 2005). Inside the cell, it can interact with the genome of bacteria resulting in damage to it.

4.4 Photocatalytic Action of Cu & ZnO Nanoparticles

The Photocatalytic dye-breaking actions of Cu and ZnO NPs were studied for the disintegration of the dye methylene blue molecules in sun radiations. The absorbance index of both NP solutions was determined with an absorption wavelength near 660 for MB dye that was identified by a shift in shade of the solution between dark blue to a light blue in the case of Cu and a somewhat translucent light blue that reflects electron transfer. The figures below demonstrate the shift in color at 60 min.



(A) Photocatalytic degradation action of copper NP on MB dye after 60 min under sunlight.



(B) Photocatalytic degradation action of zinc oxide NP on MB dye after 60 min under sunlight.

Fig. 4.4 Photocatalytic dye degradation efficiency detection of (A) Cu NP, and (B) ZnO NP synthesized using *Fortunella margarita* on Methylene Blue (MB).

This resulted in zinc oxide nanoparticles with 64% photocatalytic activity for methylene blue degradation compared to copper nanoparticles with 47%.

CHAPTER 5

CONCLUSION AND PROSPECTIVE DIRECTION

The work demonstrated the sustainable production of copper and zinc oxide NPs by utilizing plant extracts. Cu NPs demonstrated noteworthy antimicrobial efficiency against *E. coli*, while ZnONPs showed exceptional photocatalytic performance, making them appropriate for environmental restoration. Bioactive components of the *Fortunella margarita* may have medicinal & health benefits. Gaining insights into its genetic composition can help with conservation efforts and advance our understanding of the evolution of citrus plants. The potential applications of flora-mediated nanoparticles in environment protection and in healthcare that is highlighted in this work. Improving functionality and adaptability for practical uses should be the main aim of future research.

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Final Acceptance Letter

Paper No. B-4182

To,

S. Yadav, A. Khan and J. G. Sharma
Department of Biotechnology, Delhi Technological University,
Delhi-110042, India

Dear Sir/Madam

We are glad to inform you that your paper entitled **"Phytochemistry of Aloe Vera: A Catalyst for Environmentfriendly Diverse Nanoparticles with Sustained Biomedical Benefits"** has been accepted for publication in the scientific research journal *Nature Environment and Pollution Technology* after thorough reviewing, and revision. The paper is likely to come in Vol. 24, No. 1 (March), Year 2025.

Thanking you,

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Date: 27, April,2024

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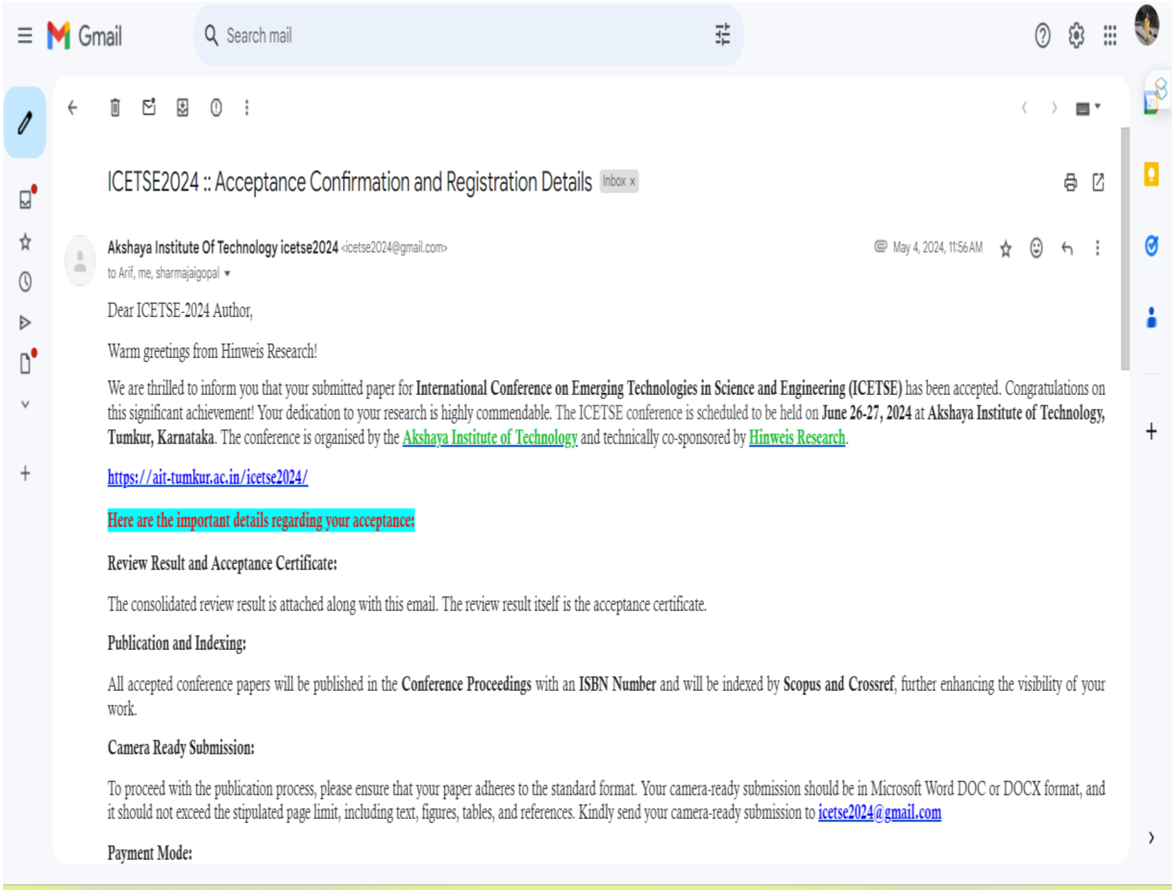

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We are thrilled to inform you that your submitted paper for **International Conference on Emerging Technologies in Science and Engineering (ICETSE)** has been accepted. Congratulations on this significant achievement! Your dedication to your research is highly commendable. The ICETSE conference is scheduled to be held on **June 26-27, 2024** at **Akshaya Institute of Technology, Tumkur, Karnataka**. The conference is organised by the Akshaya Institute of Technology and technically co-sponsored by Hinweis Research.

https://ait-tumkur.ac.in/icetse2024/

Here are the important details regarding your acceptance:

Review Result and Acceptance Certificate:

The consolidated review result is attached along with this email. The review result itself is the acceptance certificate.

Publication and Indexing:

All accepted conference papers will be published in the **Conference Proceedings** with an **ISBN Number** and will be indexed by **Scopus and Crossref**, further enhancing the visibility of your work.

Camera Ready Submission:

To proceed with the publication process, please ensure that your paper adheres to the standard format. Your camera-ready submission should be in Microsoft Word DOC or DOCX format, and it should not exceed the stipulated page limit, including text, figures, tables, and references. Kindly send your camera-ready submission to icetse2024@gmail.com

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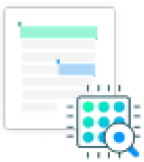
Caution: Percentage may not indicate academic misconduct. Review required.

It is essential to understand the limitations of AI detection before making decisions about a student's work. We encourage you to learn more about Turnitin's AI detection capabilities before using the tool.

Frequently Asked Questions

What does the percentage mean?
The percentage shown in the AI writing detection indicator and in the AI writing report is the amount of qualifying text within the submission that Turnitin's AI writing detection model determines was generated by AI.



Our testing has found that there is a higher incidence of false positives when the percentage is less than 20. In order to reduce the likelihood of misinterpretation, the AI indicator will display an asterisk for percentages less than 20 to call attention to the fact that the score is less reliable.

However, the final decision on whether any misconduct has occurred rests with the reviewer/instructor. They should use the percentage as a means to start a formative conversation with their student and/or use it to examine the submitted assignment in greater detail according to their school's policies.

How does Turnitin's indicator address false positives?
Our model only processes qualifying text in the form of long-form writing. Long-form writing means individual sentences contained in paragraphs that make up a longer piece of written work, such as an essay, a dissertation, or an article, etc. Qualifying text that has been determined to be AI-generated will be highlighted blue on the submission text.

Non-qualifying text, such as bullet points, annotated bibliographies, etc., will not be processed and can create disparity between the submission highlights and the percentage shown.

What does 'qualifying text' mean?

SUMAN YADAV

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SUMMARY

I'm suman and I graduated from Deen Dayal Upadhyaya College (Bsc Lifescience). Now, I'm pursuing my Master's in Biotechnology at Delhi Technological University, Delhi. I love to take up new challenges and explore different domains. I always seeking new opportunities to build up my skills and knowledge. I am looking for related experience and am willing to listen and learn and excited to contribute in a real-world environment to help find practical, actionable solutions for current needs.

SKILLS

- MS Office
- MS DOC
- Outlook
- Basic Excel
- Teamwork
- Team interaction
- Problem-Solving
- Teaching

EXPERIENCE

RESEARCH INTERN, 06/2023 - 07/2023
ESIC Model Hospital, New Delhi, India
- Work experience in labs of Biochemistry, Pathology, and Microbiology.

WEBSITES, PORTFOLIOS, PROFILES

https://www.linkedin.com/in/suman-yadav-386120220

EDUCATION AND TRAINING

Delhi Technological University, *New Delhi, Expected in 05/2024*
M.SC (Biotechnology)
GPA: 9.09

Deen Dayal Upadhyaya College, *05/2022*
B.Sc. (Lifesciences)
GPA: 9.09

Shri Krishan Sr. Sec. School, *03/2019*
HBSE (Class XII)
GPA: 9.09

Shri Krishan Sr. Sec. School, *03/2017*
HBSE (Class X)
GPA: 9.09

CERTIFICATIONS

- BRIDGE PROGRAM (Bring Science Home), Department of Zoology -Deen Dayal Upadhyaya College, 2021
- Indian Academy of Sciences, Ashoka University, Sonepat, India, Sonepat, 2019

COURSEWORK

- Industrial bioprocess development
- Drug discovery
- Science of stem cells

PROJECTS

volunteer, Helping Hut NGO, 06/2020, 07/2021