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# Formulation of Green Synthesis nanoparticles from psidium guava, Hibiscus and Azadirachta indica and evaluate its antimicrobial property against Klebsiella, E. coli and Bacillus.

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## ABSTRACT

The synthesis of nanoparticles using plant-based extracts has gained significant attention due to its eco-friendly and cost-effective nature. This study focuses on the green synthesis of copper sulphate ( $\text{CuSO}_4$ ) nanoparticles using extracts from guava (*Psidium guajava*), Neem (*Azadirachta indica*), Hibiscus leaves. The bioactive compounds present in guava serve as natural reducing and stabilizing agents, facilitating the formation of nanoparticles without the need for toxic chemicals. The synthesized nanoparticles were characterized using UV-Vis spectroscopy to determine their structural, morphological, and chemical properties. Antimicrobial activity was evaluated against common bacterial pathogens, including *Escherichia coli*, *Bacillus*, and *Klebsiella* demonstrating significant antibacterial effects due to the disruption of microbial cell membranes and oxidative stress induction. The results suggest that guava-based  $\text{CuSO}_4$  nanoparticles can serve as potential antimicrobial agents for applications in biomedicine, food preservation, and environmental protection. This study highlights the potential of plant-mediated nanoparticle synthesis as a sustainable and efficient method for developing antimicrobial nanomaterials.

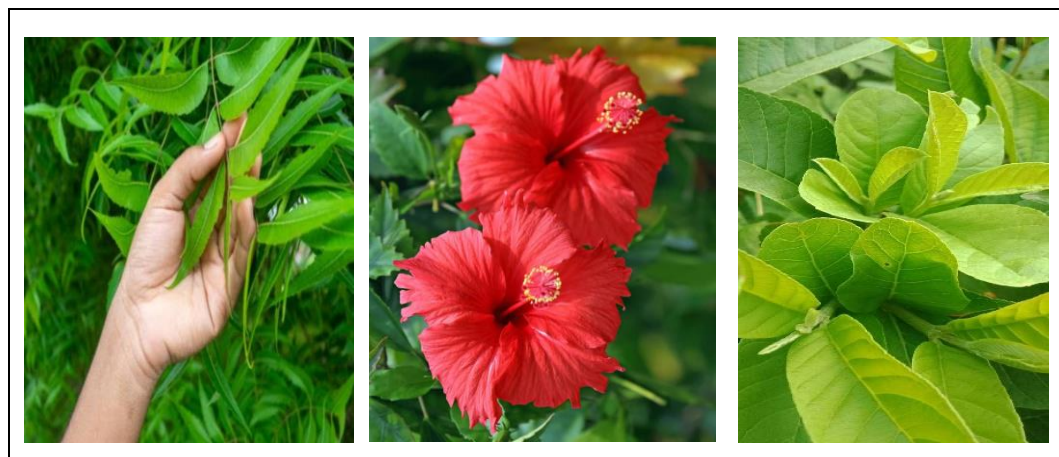
**Keywords-** Guava (*Psidium guajava*), Neem (*Azadirachta indica*), Hibiscus, Copper sulphate nanoparticles, Green synthesis, Antimicrobial activity.

## 1. INTRODUCTION

The field of nanotechnology pertains to manipulating matter at the nanoscale which is as astonishing as it sounds, one billionth of a unit. It encompasses applications in a wide range of disciplines, such as materials science, electronics, medical, and environmental science. The development and fabrication of all innovative materials and tools with distinct qualities that are unachievable at bigger scales falls within the purview of nanotechnology. High-performance materials such as carbon nanotubes (CNT) for energy source, semiconductor nanoparticles such as quantum dots (QD) for electronics, sensors and imputing tools etc. are all boons of nanotechnology. It additionally has found use in biomedicine and environmental science, where it has the potential to bring about a revolution in discovery of novel antibiotic compounds, in disease prognosis, AI nonsurgical robots, bioremediation, water treatment, and waste monument.

## 2. MATERIAL AND METHOD

**2.1 Sample collection-** Sample collection in Guava (*Psidium guajava*), Neem (*Azadirachta indica*), Hibiscus, the market Vasant Vihar Dehradun Uttarakhand.



Neem Leaves

Hibiscus

Guava Leaves

**2.2 Extract preparation-** Stirring-assisted extraction Ten grams of powder mixed with 100 ml of solvent (water) was subjected to continuous shaking at room temperature in a shaking incubator for 24 h at 120 rpm [14]

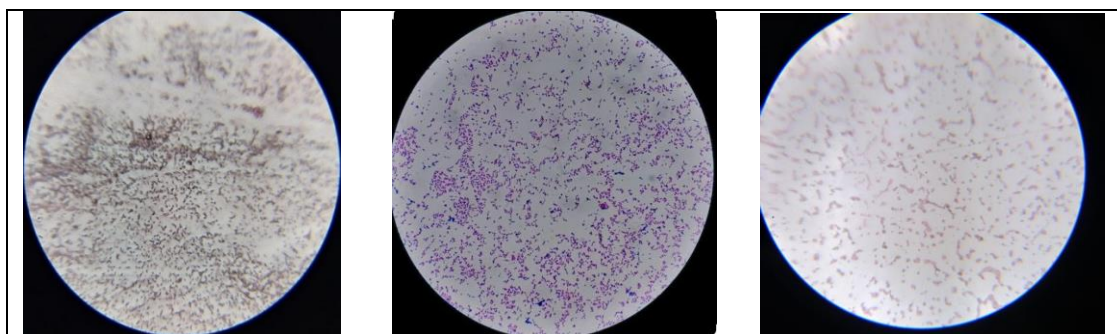


Showing *Psidium guajava* extract, *Azadirachta indica* extract and Hibiscus leaves extract

**2.3 Isolation of Microorganisms-** The microorganisms were isolated and cultured on NAM (Nutrient Agar Medium) plates using the streaking method. The cultures were then incubated at 37°C for 24 hours [15].

**2.4 Colony Selection-** After incubation, different bacterial colonies with distinct shapes were selected for further analysis [16].

**2.5 Gram Staining-**The selected colonies underwent Gram staining to differentiate between Gram-positive and Gram-negative bacteria.



**2.6 Microscopic Examination** -The stained samples were observed under a microscope to characterize the bacteria based on their morphology.

**2.7 Biochemical Testing**- Standard biochemical tests were performed on the isolated bacteria to identify their metabolic and enzymatic properties.[ 16 to 26]

**Table no. 1 Biochemical test describe the characterization isolated bacteria.**

S.N	Test name	Bacillus result	Klebsiella result	E.coli result
1	Sugar test <ul style="list-style-type: none"> <li>• Dextrose</li> <li>• Sucrose</li> <li>• Maltose</li> <li>• D-Mannitol</li> </ul>	Positive Positive Positive Positive	Positive Negative Positive Positive	Positive Positive Positive Positive
2	Citrate	Negative	Negative	Negative
3	H <sub>2</sub> S	Positive	Positive	Positive
4	Catalase test	Negative	Negative	Negative
5	Motility test	Non-motile	Non-motile	Negative
6	MR test	Positive	Positive	Positive
7	MR-VP test	Positive	Negative	Positive
8	Urease test	Positive	Negative	Positive
9	Indole test	Positive	Positive	Positive
10	Nitrate	Positive	Negative	Positive
11	Protease	Negative	Positive	Negative
12	Amylase	Negative	Negative	Negative

**2.8 Phytochemical Testing:** All the phytochemical done by standard method.[27 to 35]

**Table no.2 Phytochemical test describe the characterization isolated bacteria.**

S.N.	Test Name	Azadirachta indica	Hibiscus	Psidium guajava
1.	Saponin	Positive	Negative	Positive
2.	Tanin	Positive	Negative	Positive
3.	Flavanoid	Negative	Negative	Positive
4.	Alkaloid	Negative	Positive	Positive
5.	Terpenoid	Negative	Negative	Negative
6.	Phenolic	Negative	Negative	Positive
7.	Alkaloids(mayers reagent)	Positive	Negative	Positive
8.	Dragendroffs	Negative	Positive	Negative
9.	Ammonia	Positive	Positive	Positive
10.	Carbohydrate	Negative	Negative	Negative

### 1. GREEN SYNTHESIS OF COPPER NANOPARTICLES USING COPPER SULPHATE (CuSO<sub>4</sub>)

Green synthesis of copper nanoparticles (CuNPs) using copper sulphate (CuSO<sub>4</sub>) as a precursor involves plant extracts or natural biomolecules as reducing and stabilizing agents. This method is eco-friendly, cost-effective, and biocompatible, avoiding toxic chemical reducers like sodium borohydride [36 to 39].

#### 3.1 Materials Required

Copper sulphate pentahydrate (CuSO<sub>4</sub>·5H<sub>2</sub>O) – Metal precursor. Plant extract (e.g., Psidium guajava, Aloe Vera, Azadirachta indica, etc.) – Reducing/stabilizing agent. Distilled water – Solvent. Magnetic stirrer & heater.

#### 3.2 Preparation of Plant Extract

**Collection & Cleaning:** Fresh leaves, bark, or fruit of the chosen plant (e.g., Psidium guajava) are collected and washed with distilled water to remove dust and impurities. **Drying & Grinding:** The plant material is shade-dried and ground into a fine powder. **Extraction:** The powder is boiled in distilled water (or ethanol) at 60–80°C for 15–30 minutes. **Filtration:** The extract is filtered using Whatman filter paper or centrifugation to remove solid residues.

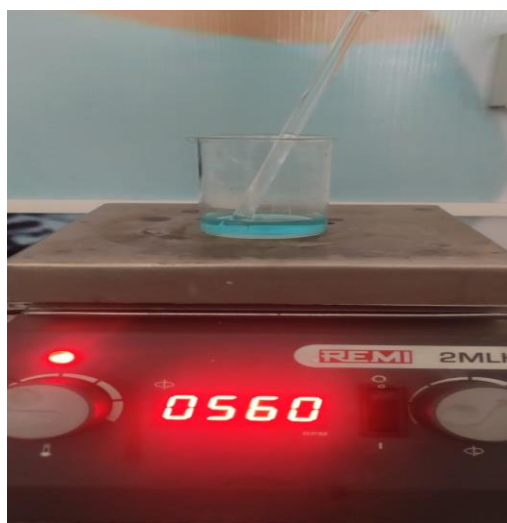
#### 3.3 Synthesis of Copper Nanoparticles

**Prepare CuSO<sub>4</sub> Solution** – Dissolve 0.01–0.1 M CuSO<sub>4</sub>·5H<sub>2</sub>O in distilled water. Mix with Plant Extract. Add the extract dropwise to the CuSO<sub>4</sub> solution under stirring. **Reaction Conditions:** Stir continuously at room temperature or slightly heated (50–80°C). Observe color change from blue to green, yellow, or

reddish-brown, indicating CuNP formation. Adjust pH (6–8) using NaOH or plant extract if needed to optimize reaction. Incubation & Precipitation – The reaction is left for 24 hours to allow complete reduction.

### 3.4 GREEN SYNTHESIS OF COPPER SULPHATE NANOPARTICLES PROCEDURE

0.1M copper sulphate was prepared by 15 ml distilled water. Polyethylene glycol 2.5 ml added containing stire it. 20 ml guava extract was added to the copper sulphate solution. Then add NaOH few drops. PH strip dip the copper sulphate check the Ph range 6. Put the beaker on the oven. Next day centrifuge the eppendroff tube in 10 min 1000 rpm. Supernatant discard gently manner. Then pellet add the ammonia solution. Then preserved it freeze.



## 4. ANTIMICROBIAL ACTIVITY

The Minimum Inhibitory Concentration (MIC) is a critical parameter in microbiology used to determine the lowest concentration of an antimicrobial agent required to inhibit the visible growth of a microorganism.



**Table no.3 MIC Results**

Strain	Zone of Inhibition (mm) <i>Psidium Guajava</i> leaves	Zone of Inhibition (mm) Hibiscus	Zone of Inhibition (mm) Azadirachta indica
<i>Ecoli</i>	2.45 ± .25	2.30 ± .15	2.15 ± .10
<i>Bacillus</i>	3.95 ± .23	3.75 ± .25	3.85 ± .28
<i>Klebsiella</i>	2.85 ± .12	1.15 ± .24	1.5 ± .25

#### 4.1 INTERPRETATION

MIC values help categorize microorganisms as susceptible, intermediate, or resistant to specific antimicrobial agents. These categories are based on extensive research correlating MIC with achievable serum levels, resistance mechanisms, and therapeutic outcomes.

- A Larger Zone of *Bacillus* 3.95 ± .25mm, 2.15 ± .15mm, 2.10 ± .10mm means Inhibition Indicates strong antimicrobial activity. Suggests that E. coli is highly susceptible to the tested antibiotic. A very good Minimum Inhibitory Concentration (MIC) result corresponds to a low MIC value, meaning a small amount of the antibiotic effectively inhibits bacterial growth. MIC and Zone of Inhibition Correlation. MIC (Minimum Inhibitory Concentration) The lowest concentration of an antibiotic that prevents visible bacterial growth. A low MIC means the antibiotic is effective at lower doses, which is good. A high MIC means the bacteria require more antibiotic to be inhibited. Suggests that guava leaf extracts are effective in inhibiting *Bacillus* and *E.coli* growth.
- A larger zone of *Bacillus* 2.45 ± .23mm, 3.75 ± .25mm, 3.85 ± .28mm and *E.coli* 2.85 ± .12mm, 3.15 ± .24mm, 3.75 ± .25mm.
- stronger antimicrobial activity guava leaves a very good result are show. A good MIC result that even at a low concentration, guava extracts successfully inhibit bacterial growth.

#### 4.2 RELATIONSHIP WITH ZONE OF INHIBITION

There is an inverse correlation between MIC and the zone of inhibition; lower MIC values indicate higher susceptibility and larger zones of inhibition.

### 5. CONCLUSION

The of using nanoparticles derived from guava leaves. Guava-based nanoparticles exhibit strong antioxidant, antimicrobial, and anti-inflammatory properties due to the presence of bioactive compounds such as polyphenols, flavonoids, and tannins. These nanoparticles have promising applications in the food industry for preservation, in medicine for drug delivery and wound healing, and in environmental applications for water purification.

The study on nanoparticles derived from guava (*Psidium guajava*), Neem and Hibiscus leaves for the green synthesis of copper sulphate nanoparticles highlights their effectiveness as natural reducing and stabilizing agents. Guava extracts contain bioactive compounds such as flavonoids, tannins, and polyphenols, which facilitate the eco-friendly synthesis of copper nanoparticles.

The results indicate that guava-based nanoparticles exhibit promising antibacterial, antioxidant, and catalytic properties, making them suitable for various biomedical and industrial applications. Additionally, this green synthesis method is cost-effective, sustainable, and environmentally friendly compared to traditional chemical synthesis methods.

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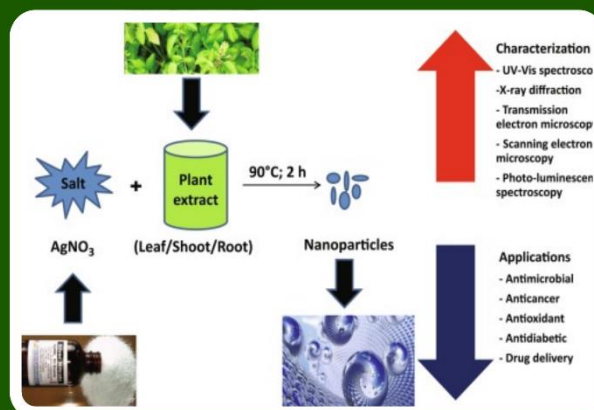
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