Formulation of nanoparticles by green synthesis of *psidium guajava* pulp, seed and leaves to check the antimicrobial activity against *Bacillus, E. coli, Salmonella.*

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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($CuSO_4$) nanoparticles using extracts from guava (*Psidium guajava*) pulp, seeds, and 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 *Salmonella* demonstrating significant antibacterial effects due to the disruption of microbial cell membranes and oxidative stress induction. The results suggest that guava-based $CuSO_4$ 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*), Copper sulphate nanoparticles, Green synthesis, Antimicrobial activity

1. INTRODUCTION

Nanotechnology is the science of dealing with materials on a billion scale Counters (i.e.10-9 m=1nm) and is also a study of the manipulation of materials[1].Atomic and molecular scales. Nanoparticles are the most basic components Much less in nanostructure manufacturing in the everyday world Objects that are described by law by Newton's motion, but are more than atoms Or a simple molecule regulated by quantum mechanics[2].Generally, nanoparticle sizes extend on beaches between 1 and 100 nm. Metal nanoparticles have different physical and chemical properties Metals (for example, a melting point,a higher specific magnetizations[3].Nanotechnology is defined as atomic, molecular, or polymer scale research and development[4]. Particles in these dimensions have been used by several industries and humanity for thousands of years, However, recent updates in the ability to synthesize and manipulate such materials[5].Nano-sized materials are used in a variety of fields, including electron, An increase in investment in nanotechnology research and development particularly from the plant Psidium guajava can be used to synthesized metallic nanoparticles due to the presence of phytochemical like flavonoids, terpenoids and phenolics [6]. which act is reducing agent the Psidium guajava [7].

Green synthesis of nanoparticles is an eco-friendly and sustainable approach that utilizes biological [8] such as plant extracts, microorganisms, and biomolecules to produce nanomaterials [9]. This method chemical and physical synthesis techniques which often involve toxic reagents[10] .plant-based green synthesis has gained significant attention due to its cost – effectiveness, biocompatibility[11]. Various plant components, such as leaves,seed,fruit pulp contain natural phytochemicals[12] that as reducing and stabilizing agents foe nanoparticle formation[13].

2. MATERIALS AND METHOD

2.1 Sample collection- Sample collection in Psidium guajava (guava)the market Vasant Vihar Dehradun Uttarakhand.

Fig.1 Guava pulp

Fig.2 Guava Seed

Fig.3 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]

Fig.4 Showing *Psidium guajava* pulp extract, seed extract and 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.

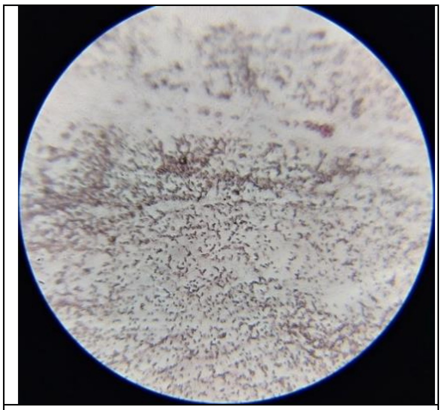

Fig.5 Showing gram staining *Bacillus.*

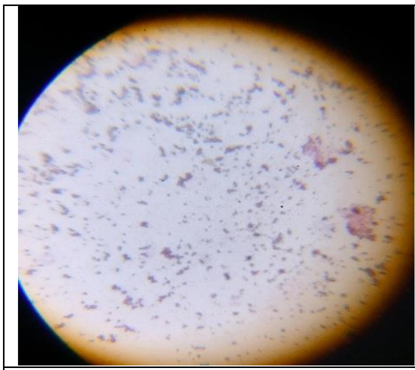

Fig.6 Showing gram staining *E.coli.*

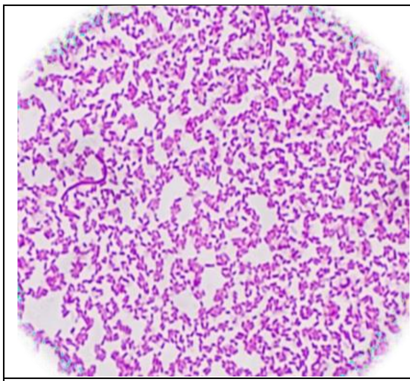

Fig.7 Showing gram staining *Salmonella.*

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.[16-26]

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

S.no	Test name	*Bacillus*	*Salmonella*	*E.coli*
1.	Sugar test • Dextrose • Sucrose • Maltose • D-mannitol	 Positive Positive Positive Positive	 Positive Negative Positive Negative	 Positive Positive Positive Positive
2.	Citrate test	Negative	Negative	Negative
3.	H2s test	Positive	Positive	Positive
4.	Catalase test	Negative	Positive	Negative
5.	Motility test	Non-motile	Positive	Negative
6.	MR- test	Positive	Positive	Positive
7.	MR-VP test	Positive	Negative	Positive
8.	Urease test	Positive	Negative	Negative
9.	Indole test	Positive	Negative	Positive
10.	Nitrate test	Positive	Negative	Positive

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

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

S.no	Phytochemical test	Pulp	Seed	Leaves
1.	Saponin	Negative	Negative	Positive
2.	Tanin	Positive	Positive	Positive
3.	Flavanoid	Positive	Positive	Positive
4.	Alkaloid	Negative	Positive	Positive
5.	Terpenoid	Negative	Positive	Negative
6.	Phenolic	Positive	Positive	Positive
7.	Alkaloids(mayers reagent)	Positive	Positive	Positive
8.	Dragendroffs	Negative	Negative	Negative
9.	Ammonia	Negative	Positive	Positive
10.	Carbohydrate	Negative	Negative	Negative

3. GREEN SYNTHESIS OF COPPER NANOPARTICLES USING COPPER SULPHATE ($CuSO_4$)

Green synthesis of copper nanoparticles (CuNPs) using copper sulphate ($CuSO_4$) 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 [36-39].

3.1 Materials Required

Copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) – 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 $CuSO_4$ Solution – Dissolve 0.01–0.1 M $CuSO_4 \cdot 5H_2O$ in distilled water. Mix with Plant Extract Add the extract dropwise to the $CuSO_4$ 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.

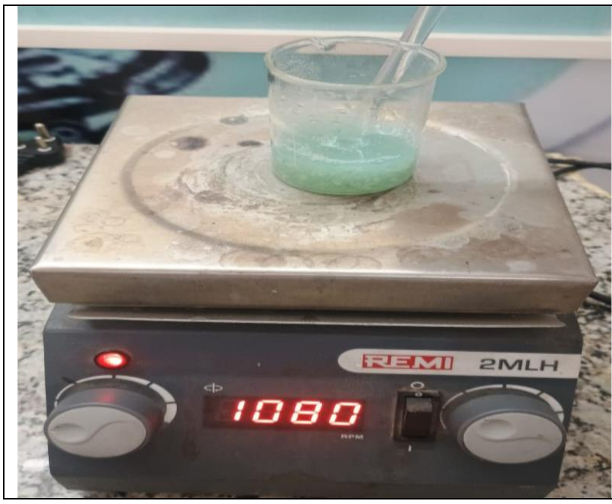

Fig.8 Guava pulp sample in production of copper sulphate nanoparticles.

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.

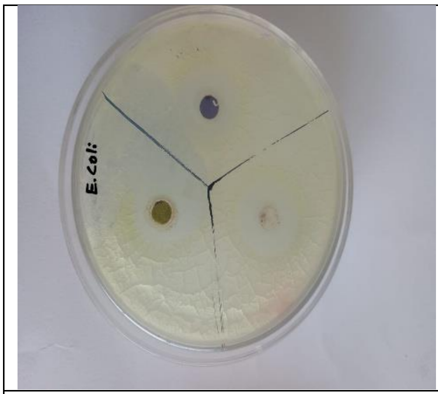

Fig.9 Showing zone of inhibition E.coli.

Fig.10 Showing zone of inhibition Bacillus.

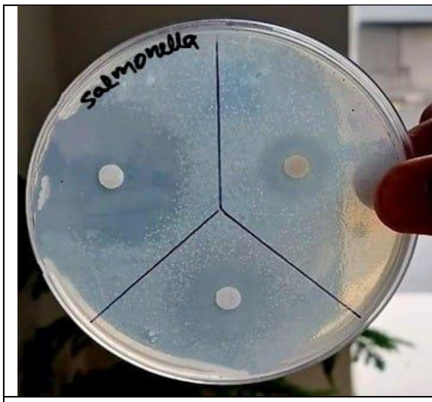

Fig.11 Showing zone of inhibition Salmonella.

Table no.3 MIC Results

Strain	Zone of Inhibition (mm) *Psidium Guajava* Pulp	Zone of Inhibition (mm) *Psidium Guajava* Seed	Zone of Inhibition (mm) *Psidium Guajava* Leaves
Ecoli	3.4 ± .25	2.1 ± .15	2.1 ± .10
Bacillus	3.6 ± .23	3.7 ± .25	3.8 ± .28
Salmonella	2.8 ± .12	3.1 ± .24	3.7 ± .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 *E.coli* 3.4 ± .25mm, 2.1 ± .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 pulp and seed extracts are effective in inhibiting Bacillus and Salmonella growth.
- A larger zone of *Bacillus* 3.6 ± .23mm, 3.7 ± .25mm, 3.7 ± .28mm and *Salmonella* 2.8 ± .12 mm, 3.1 ± .24mm, 3.7 ± .25mm.
- stronger antimicrobial activity guava seed and 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. DISCUSSION

Nanotechnology has emerged as a crucial field in various industries, including food, medicine, and agriculture. Guava (*Psidium guajava*), a tropical fruit rich in bioactive compounds, has been explored for its role in nanoparticle synthesis and applications. Guava extracts (from pulp, seeds, and leaves) contain polyphenols, flavonoids, tannins, and other phytochemicals, which can act as reducing, stabilizing, and capping agents in green nanoparticle synthesis. Additionally, guava-derived nanoparticles exhibit promising antimicrobial, antioxidant, and biomedical properties.

6. CONCLUSION

The of using nanoparticles derived from guava pulp, seeds, and 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*) pulp, seeds, and 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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