Developing An Antimicrobial Spray Solution for Smartphones Using Nanoparticles

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***Abstract*—This research aims to address the growing concern of microbial contamination on Smartphones by developing an innovative antimicrobial coating solution. Smartphones are frequently handled devices, often coming into contact with user's hands and faces, making them potential hotspots for the transmission of harmful microorganisms, including Bacteria and Viruses. Traditional cleaning methods are not only labor-intensive but also often fail to provide long-lasting protection. Therefore, the development of a durable antimicrobial coating offers a promising and practical solution to enhance Smartphone hygiene and reduce infection risks. The first step involves identifying biocompatible antimicrobial agents that are effective against a wide range of pathogens, including *E. coli, Staphylococcus aureus,* and *Influenza viruses*. These agents must be safe for human contact and should not cause any adverse reactions upon prolonged exposure. Secondly developing a coating formulation that can be easily applied to smartphone surfaces without affecting their functionality is crucial. The formulation process will involve optimizing the concentration of antimicrobial agents, ensuring strong adhesion to various smartphone materials, and maintaining transparency to preserve the device's aesthetics.**

Rigorous laboratory testing will be conducted to evaluate the antimicrobial efficacy of the developed coating. This will include testing the coating's ability to inhibit microbial growth and kill pathogens on contact. The tests will be performed under various conditions to simulate real-world usage and environmental exposure. To ensure the long-term effectiveness of the antimicrobial coating, durability tests will be conducted

I. INTRODUCTION

In today's digital age, mobile devices have become an indispensable part of our daily lives. These devices, particularly smartphones, are not only communication tools but also gateways to information, entertainment, and social interaction. However, the frequent handling of mobile screens exposes them to a myriad of microbial contaminants, making them potential vectors for a pathogen, highlighting the urgent need for effective hygiene solutions.

The necessity of maintaining hygienic mobile devices has led to the exploration of innovative solutions, one of which is the development of antimicrobial protective sprays. Such sprays aim to provide a barrier that not only protects the screen from physical damage but also inhibits the growth of harmful microorganisms. This research project delves into the formulation of an effective antimicrobial protective spray for mobile screens. The goal is to create a solution that combines durability, transparency, and potent antimicrobial properties without compromising the functionality or user experience of the device.

By investigating various active ingredients and their synergistic effects, this study seeks to identify a formulation that can be easily applied, remains effective over extended periods, and is safe for both the device and the user. Key ingredients being explored include nano-silver particles, copper compounds, and essential oils known for their antimicrobial properties. The findings of this research have the potential to enhance mobile device hygiene, thereby contributing to public health and well-being. Additionally, this project aims to set a benchmark for future innovations in mobile device care, ensuring that users can enjoy their technology with peace of mind.

Adverse Effects of Microorganisms on Screens

For Children:

- Respiratory Issues:

Children often touch their faces and put their fingers in their mouths, which can transfer bacteria and viruses from screens to their respiratory system, causing infections such as colds, flu, and other viral infection

- Skin Infections:

The presence of bacteria on screens can cause skin infections, particularly if children have cuts or abrasions on their hands.

- Allergic Reactions:

Some microorganisms can trigger allergic reactions, leading to symptoms like itching, redness, and swelling on the skin. This can be particularly troublesome for children with sensitive skin or pre-existing allergies.

For Adults:

- Eye Infections:

Adults who frequently touch their faces and eyes can transfer bacteria from their screens, leading to eye infections such as conjunctivitis (pink eye), which can cause redness, swelling, and discharge.

- Immune System Compromise:

Continuous exposure to harmful microorganisms can weaken the immune system over time, making adults more susceptible to illnesses. This is particularly concerning for those with compromised immune systems.

- Gastrointestinal Issues:

They can ingest harmful bacteria, if eat something after handling phone.

Aim and objectives

Aim: The primary aim of this research project is to formulate an effective antimicrobial protective spray for mobile screens utilizing nanoparticles, which will provide long-lasting protection against harmful microorganisms without compromising device functionality or user experience.

Objectives

1. Identify Suitable Nanoparticles:

Research and select nanoparticles (e.g., silver, copper) known for their antimicrobial properties.
Evaluate the effectiveness of these nanoparticles in inhibiting microbial growth on various surfaces.

2. Develop a Formulation:

Formulate a spray that integrates these nanoparticles, ensuring it is safe for use on mobile screens.
Optimize the formulation for durability, transparency, and ease of application.

3. Evaluate Antimicrobial Efficacy:

Conduct laboratory tests to measure the antimicrobial effectiveness of the formulated spray against common pathogens.
Compare the performance of the nanoparticle-based spray to existing antimicrobial solutions.

4. Assess User Safety and Device Compatibility:

Ensure that the spray does not damage mobile screens or interfere with touchscreen functionality.
Conduct safety assessments to confirm that the spray is non-toxic and safe for regular use.

5. Environmental Impact Analysis:

Study the environmental implications of using nanoparticles in antimicrobial sprays.
Develop eco-friendly practices to minimize any potential negative impact on the environment.

6. Market and Commercial Viability:

Analyze the commercial potential and market demand for the antimicrobial spray.
Develop a strategy for product launch and distribution, targeting key consumer demographics.
Optimize the formulation for durability, transparency, and ease of application.

7. Evaluate Antimicrobial Efficacy:

Conduct laboratory tests to measure the antimicrobial effectiveness of the formulated spray against common pathogens.

8. Assess User Safety and Device Compatibility:

Optimize the formulation for durability, transparency, and ease of application.

9. Evaluate Antimicrobial Efficacy:

Conduct laboratory tests to measure the antimicrobial effectiveness of the formulated spray against common pathogens.
Compare the performance of the nanoparticle-based spray to existing antimicrobial solutions.

10. Market And Commercial Viability:

Analyze the commercial potential and market demand for the antimicrobial spray.
Develop a strategy for product launch and distribution, targeting key consumer demographics.

II. NEED OF THE STUDY

2.1. New challenges related to hygiene

The wide use of mobile bias has introduced new challenges related to hygiene and the transmission of microbial pathogens. Mobile screens are high- touch shells that constantly come into contact with hands, faces, and other polluted objects, making them implicit budgets for bacteria, contagions, and fungi. Research has shown that mobile phones can harbor a variety of pathogenic microorganisms, including *Staphylococcus aureus, Escherichia coli,* and indeed *SARS-CoV- 2*, the contagion responsible for COVID-19 (Gohil, 2021). These pathogens can persist on mobile screens for extended ages, adding the threat of cross-contamination and infection. Traditional cleaning styles, similar as using detergent wipes and sprays, can be inconvenient and may damage the delicate shells of mobile defenses. Also, frequent cleaning is necessary to maintain hygiene, which can be impracticable for numerous users. In this environment, the development of antimicrobial defensive sprays using nanoparticles presents a promising result.

Nanoparticles, due to their small size and high surface area, parade unique antimicrobial parcels that can effectively inhibit the growth and survival of microorganisms on treated shells (Rai et al., 2016). These antimicrobial nanoparticles, including tableware, bobby, zinc oxide, and titanium dioxide, have been extensively studied for their efficacity against a broad diapason of pathogens (Yilmaz et al., 2023). Recent advancements in nanotechnology and accoutrements wisdom have led to the development of transparent and durable antimicrobial coatings suitable for operation on mobile defenses. A study by Cho et al. (2021) demonstrated the efficacity of a transparent nanostructured bobby face (TANCS) in reducing bacterial impurity by over 99.9%. The coating was developed by depositing an ultra-thin bobby film onto a glass substrate, followed by rapid-fire thermal annealing to form bobby nanoparticles.

The performing nanostructured face was not only largely effective in inhibiting microbial growth but also retained the optic clarity and touch perceptivity needed for mobile bias. The integration of antimicrobial nanoparticles into defensive sprays for mobile defenses offers several advantages. These include dragged antimicrobial exertion, reduced need for frequent cleaning, and minimum impact on the functionality and aesthetics of the device.

2.2. Overview of Antimicrobial Nanoparticles

Antimicrobial nanoparticles have gained significant attention due to their capability to inhibit the growth of microorganisms. Generally used nanoparticles include tableware, bobby, zinc oxide, and titanium dioxide (Yilmaz et al., 2023). These nanoparticles parade strong antimicrobial parcels by dismembering microbial cell membranes, generating reactive oxygen species, and snooping with microbial metabolism (Yilmaz et al., 2023). Bacterial infections are a major cause of habitual infections and mortality. Antibiotics have been the favored treatment system for bacterial infections because of their cost- effectiveness and important issues. Still, several studies have handed direct substantiation that the wide use of antibiotics has led to the emergence of multidrug- resistant bacterial strains. In fact, super-bacteria, which are resistant to nearly all antibiotics, have lately developed due to abuse of antibiotics. Studies have shown that these bacteria carry a super-resistance gene called NDM- 1. utmost of the antibiotic resistance mechanisms is inapplicable for nanoparticles (NPs) because the mode of action of NPs is direct contact with the bacterial cell wall, without the need to access the cell; this raises the stopgap that NPs would be less prone to promoting resistance in bacteria than antibiotics. thus, attention has been concentrated on new and instigative NP- grounded accoutrements with antibacterial exertion. utmost bacteria live in the form of a biofilm, which frequently contains different species that interact with each other and their terrain. Biofilms are specifically microbial summations that calculate on a solid face and extracellular products, similar as extracellular polymeric substances (EPSs). Bacteria move reversibly onto the face, but the expression of EPSs renders the attachment unrecoverable. Once the bacteria are settled, conflation of the bacterial flagellum is inhibited, and the bacteria multiply fleetly, performing in the development of a mature biofilm. At this stage, the bacteria are stuck together, forming a hedge that can repel antibiotics and give a source of systemic habitual infections. Therefore, biofilms are a serious health trouble. also, the bacteria within biofilms can produce superantigens to shirk the vulnerable system.

thus, despite the cornucopia of antimicrobial medicines and other ultramodern antibacterial agents, bacterial infections remain a major issue. The habitual infections related to planktonic bacteria and biofilms are always delicate to cure because of their essential resistance to both antimicrobial agents and host defenses. Biofilms are less restrained by antibacterial agents than the separate planktonic bacteria are. (Romero, D., Aguilar, C., Losick, R., & Kolter, R. (2010). Nanomaterials are accoutrements that have at least one dimension (1 – 100 nm) in the nanometer scale range or whose introductory unit in the three-dimensional space is in this range. NPs have demonstrated broad- diapason antibacterial parcels against both Gram-positive and Gram-negative bacteria. For illustration, ZnO NPs were set up to inhibit *Staphylococcus aureus*, and Ag NPs parade attention-dependent antimicrobial exertion against *Escherichia coli* and *Pseudomonas aeruginosa*. still, the detailed antibacterial mechanisms of NPs have not been completely explained, and the same types of NPs frequently present differing goods. The antimicrobial medium of action of NPs is generally described as clinging to one of three models oxidative stress induction, essence ion release, or non-oxidative mechanisms.

2.3. Current Landscape

Antimicrobial nanoparticles have emerged as a promising avenue for combating microbial contamination on various surfaces. These nanoparticles exhibit unique properties due to their size, high surface area to volume ratio, and ability to generate reactive oxygen species, which enable them to effectively kill bacteria, viruses, and fungi. Metallic nanoparticles (e.g., silver, copper), polymeric nanoparticles, lipid-based nanoparticles, and carbon-based nanoparticles are among the most studied for their antimicrobial actions. The field of antimicrobial nanoparticles is a dynamic and rapidly evolving area of research, driven by the increasing need for effective solutions to combat microbial contamination.

Here is a more detailed look into the current landscape:
Advancements in Nanotechnology: -
Nanoparticles are particles with dimensions between 1 and 100 nanometers. Due to their small size, they have a high surface area- to-volume ratio, which enhances their interaction with microbial cells. This property makes them highly effective as antimicrobial agents.
Some key findings about nanoparticles are:

Types of Nanoparticles:
Metallic Nanoparticles:
Silver (Ag), copper (Cu), and zinc oxide (ZnO) nanoparticles are widely studied for their antimicrobial properties. Silver nanoparticles are known for their broad-spectrum antimicrobial activity (Rai et al., 2012; Morones et al., 2005).

Polymeric Nanoparticles:
These are composed of polymers and can be engineered to release antimicrobial agents in a controlled manner, providing sustained antimicrobial activity (Dizaj et al., 2014).

Lipid-based Nanoparticles:
These are used to deliver antimicrobial agents directly to the microbial cells, improving the efficacy of the treatment (Ivask et al., 2014).

Carbon-based Nanoparticles:
Graphene and carbon nanotubes have shown promising antimicrobial properties due to their ability to physically damage microbial cell membranes (Klaine et al., 2008).

2.4. Challenges and Considerations

Despite the promising potential of antimicrobial nanoparticles, several challenges remain:
1. Cytotoxicity and Biocompatibility:
Ensuring that nanoparticles are non-toxic to human cells while being effective against microbes is crucial. Research is ongoing to optimize the size, shape, and surface properties of nanoparticles to achieve this balance (Xu et al., 2015).

2. Environmental Impact:
The potential environmental impact of nanoparticles, including their persistence and accumulation in ecosystems, must be carefully assessed. Developing eco-friendly formulations and disposal methods is essential to minimize any negative effects (Klaine et al., 2008).

3. Regulation and Standardization:
Establishing standardized guidelines and regulatory frameworks for the use of nanoparticles is critical to

ensure their safe and effective use. This includes setting limits on nanoparticle concentrations and assessing long-term safety (Bouwmeester et al., 2009).

4. Standardization and Regulation:
There is a need for standardized guidelines and regulations to ensure the safe use of nanoparticles. This includes- Tunable Synthesis: Developing methods to precisely control the size, shape, and surface properties of nanoparticles to optimize their antimicrobial activity and reduce toxicity. (Zhang et al., 2018).

III. MATERIALS AND METHODS

The methodology for developing an antimicrobial protective spray for mobile screens using nanoparticles is divided into several phases:

PHASE 1: ISOLATION AND IDENTIFICATON OF MICROORGANISMS FROM SMARTPHONE SCREENS

The general methodology for isolating and identifying microorganisms from smartphones are as follows:

A. Sample Collection:
Materials:
Sterile cotton swabs, sterile saline solution, sterile containers, smartphones.

Procedure:
Use a sterile cotton swab moistened with sterile saline to swab the surface of the smartphone, focusing on areas that are frequently touched (e.g., screen, buttons, back cover).

B. Culturing and isolation
Materials:
Nutrient agar, Potato dextrose agar (PDA), Sterile Petri plates, incubator, weighing balance machine, autoclave.

Procedure:
Nutrient and PDA agar plates was prepared. then Streaked the swab of the sample onto nutrient agar plates for bacterial growth and onto PDA plates for fungal growth and incubated the plates at 37°C for 24 hours for bacterial and at 25°C for 48 hours for fungal growth respectively. Distinct colonies were selected for their further analysis. Plates were further preserved in refrigerator at 4°C

C. Identification of Bacteria:
Materials:
Microscope, glass slides, nichrome wire loop, St. saline, gram staining kit.

Procedure:
Picked an individual colony using an inoculating loop, preparing a bacterial smear on a clean glass slide, which is then air-dried and heat-fixed to ensure the cells adhere to the surface. Gram Staining technique is performed, coloring the Gram- negative bacteria red or pink, while Gram-positive bacteria remain purple or blue. Once the staining process is complete, the slide is rinsed, dried, and observed under a microscope.

D. Identification of yeast and molds
Materials:
Microscope, glass slides, nichrome wire loop, St. saline, Gram staining kit.

Procedure:
A small portion of the yeast/mold is carefully transferred onto a glass slide using a sterile needle or forceps. Gram staining is done for yeast cells and to enhance visibility, a drop of sterile water or saline may be added to the sample before placing a coverslip over it.

PHASE 2: SYNTHESIS AND CHARACTERIZATION OF COPPER AND SILVER NANOPARTICLES

Objective: To select and synthesize nanoparticles with proven antimicrobial properties.

A. Green Synthesis Using Plant Extracts:
This is an eco-friendly method uses plant extracts as reducing and stabilizing agents.

Materials:
- Metal salt (AgNo3)- 2.0mg and (CuSo4)- 5.0mg
- Neem Leaves- 20gm
- Deionized water – 100ml
- Magnetic Stirrer
- Conical flasks and beakers
- Micropipettes and tips

Steps Involved in Green Synthesis of silver nanoparticles

1. Preparation of Plant Extract:
Fresh 20gm Neem leaves are collected, cleaned, and dried. The dried plant material is then ground into a fine powder and mixed with ethanol to extract the active biomolecules.

2. Synthesis of AgNPs:
The plant extract is added to a solution containing silver nitrate (silver nitrate solution is prepared by adding 2.0 mg AgNo3 in 10 ml of deionized water) under constant stirring. The color change from colorless to dark brown indicates the formation of AgNPs.

Steps Involved in Green Synthesis of copper nanoparticles

1. Preparation of Plant Extract:
Fresh Neem leaves are collected, cleaned, and dried. The dried plant material is then ground into a fine powder and mixed with ethanol to extract the active biomolecules.

2. Synthesis of CuNPs:
The plant extract is added dropwise to a solution containing copper sulfate (copper sulphate solution prepares by dissolving 5mg of cuso4 into 10 ml of deionized water) under constant stirring. The color change from blue solution of CuSo4 to green indicates the formation of copper nanoparticles.

B. Characterization of nanoparticles
Materials:
UV- Visible spectroscopy instrument, prepared nanoparticles solutions

Procedure:
To characterize nanoparticles using UV-Visible spectroscopy, prepared a stable colloidal suspension of the nanoparticles in an appropriate solvent, such as water Ensured the dispersion is uniform and free from large aggregates by using ultrasonication. Next, set up the UV-Vis spectrophotometer to scan within the 200-800 nm range, selecting a suitable reference solvent for baseline correction. Carefully transferred the nanoparticle suspension into a quartz cuvette—since glass absorbs UV light—and placed it into the spectrophotometer while ensuring proper alignment. Started the spectral acquisition process to obtain the absorption spectrum, identifying the surface plasmon resonance (SPR) peak, which provides insights into nanoparticle size, shape, and aggregation state. Typically, silver nanoparticles exhibit an SPR peak around 300-450 nm, while copper nanoparticles may show absorbance in the 400-600 nm range. Analyze the peak position and intensity, as a redshift suggests larger particle size or aggregation, whereas a blueshift indicates smaller or well- dispersed nanoparticles.

PHASE 3: - CHECKING ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF NANOPARTICLES
To evaluate the antimicrobial and antifungal activities of nanoparticles, several methods and assays are commonly used:

A. Antibacterial Activities
1. Agar well method
This method relay on the size of the inhibition zone which is then measured to determine the antimicrobial activity.

Materials:
- Test microorganisms isolated from mobile screens (i.e., Gram-positive rods, Gram negative rods and Gram-positive bacilli)
- Nanoparticle solution of copper and silver (prepared and characterized)
- Agar medium (e.g., Mueller-Hinton Agar for bacteria, Sabouraud Dextrose Agar for fungi)
- Sterile petri dishes
- Sterile micropipettes
- Sterile cork borer or punch
- Incubator (set at optimal growth temperature)
- Ruler or caliper for measuring inhibition zones

Bacterial cultures are first prepared by inoculating isolated strains into nutrient broth and incubating them at 37°C for 18–24 hours. The bacterial suspension is then adjusted to 0.5 McFarland standard, prepared and sterilized Mueller-Hinton Agar and transferred 20-20ml into sterile vitamin tubes, into that added the different microorganism's suspension isolated from mobile screens then poured into sterile petri dishes to allow solidification. Once the agar plates are ready,

using a sterile cork borer or punch, wells of approximately 6–8 mm in diameter are created in the agar, carefully removing excess agar to maintain consistent depth. Four different concentrations of the nanoparticles (25, 12.5, 6.25, and 3.125 µg/mL for silver and 80, 40, 20 and 10ug/ml for copper nanoparticles) are prepared 50µL of each concentration is pipetted into separate wells.

The plates are left undisturbed at room temperature for approximately 30 minutes, allowing the antimicrobial agents to diffuse into the surrounding medium. Following this diffusion period, the plates are incubated at optimal temperatures—37°C for bacterial strains and 25–30°C for fungal strains—for 18–24 hours. After incubation, the zones of inhibition, representing areas where microbial growth has been prevented, are measured using a ruler or caliper. The diameter of these zones is compared across different concentrations to determine the relative antimicrobial effectiveness of the formulated coating spray. This method provides a quantitative assessment of antimicrobial activity, allowing for easy comparison with other disinfectants or antimicrobial treatments

2. Disc diffusion method

Materials:

- Nanoparticle solution of copper and silver (prepared and characterized)
- Bacterial strains isolated from mobile screens (i.e., Gram-positive rods, Gram negative rods, Gram positive bacilli)
- Mueller-Hinton agar plates
- Sterile filter paper discs (6 mm diameter)
- Sterile forceps
- Micropipette and sterile tips
- Sterile Petri dishes
- Sterile cotton swabs

Procedure:

To assess the antibacterial activity of a nanoparticle solution using the disc diffusion method, bacterial cultures are first prepared by inoculating isolated strains into nutrient broth and incubating them at 37°C for 18–24 hours. The bacterial suspension is then adjusted to 0.5 McFarland standard, ensuring a uniform bacterial concentration. Mueller-Hinton agar plates are prepared by pouring sterile medium into Petri dishes and allowing it to solidify. Using a sterile cotton swab, the bacterial suspension is evenly spread across the agar surface to create a lawn culture. Four different concentrations of the nanoparticles (25, 12.5, 6.25, and 3.125 µg/mL for silver and 80, 40, 20 and 10ug/ml for copper nanoparticles) are prepared, Sterile filter paper discs, approximately 6 mm in diameter, are impregnated with 10–50 µL of the nanoparticle solutions of different concentrations are carefully placed on the inoculated agar surface using sterile forceps.

The plates are then incubated at 37°Cfor 24 hours, allowing bacterial growth and potential inhibition to occur. After incubation, the zone of inhibition, the clear area around each disc, is measured using a ruler or caliper to determine the antibacterial activity of the nanoparticles.

B. Antifungal activity for yeast

Materials:

- Nanoparticle solution (prepared at four different concentrations)
- Yeast culture (freshly grown in Sabouraud dextrose broth)
- Sabouraud dextrose agar (for fungal growth)
- Sterile Petri dishes, micropipettes, and tips
- Sterile cotton swabs (for fungal inoculation)
- Spectrophotometer (for adjusting fungal concentration)
- Incubator (set at 25°C for optimal fungal growth)

Procedure:

Firstly, a fresh culture of *yeast (isolated from smartphone screens)* is prepared by inoculating the yeast into Sabouraud dextrose broth and incubating it at 37°C for 24 hours to achieve an optimal growth phase. The fungal suspension is then adjusted to 1×10^6 CFU/mL using a spectrophotometer. Meanwhile, Sabouraud dextrose agar is sterilized and maintained at 45–50°C to prevent premature solidification.

Four different concentrations of the nanoparticle solution are prepared, typically ranging from 25, 12.5, 6.25, and 3.125 µg/mL for silver and 80, 40, 20 and 10µg/mL for copper nanoparticles. The molten agar is mixed with the fungal suspension and poured into sterile Petri dishes, ensuring uniform distribution.

Then 1ml of nanoparticle solutions of varying concentrations are carefully incorporated into the agar medium. The plates are incubated at 37°C for 48 hours, allowing fungal growth and potential inhibition zones to develop.

After incubation, the decrease in growth of yeast colonies with increase in concentration of nanoparticles is the indication of antifungal activity exhibited by nanoparticle at specific concentration.

For molds Materials:

- Nanoparticle solution (prepared at four different concentrations)
- Mold culture (freshly grown in Sabouraud dextrose broth)
- Sabouraud dextrose agar (for fungal growth)
- Sterile Petri dishes, micropipettes, and tips
- Sterile cotton swabs (for fungal inoculation)
- Spectrophotometer (for adjusting fungal concentration)
- Incubator (set at 25°C for optimal fungal growth)

Procedure:

A fresh culture of *mold (isolated from smartphone screens)* is prepared by inoculating the molds into Sabouraud dextrose broth and incubating it at 37°C for 24 hours to achieve an optimal growth phase. The fungal suspension is then adjusted to 1×10^6 CFU/mL using a spectrophotometer. Meanwhile, Sabouraud dextrose agar is sterilized and maintained at 45–50°C to prevent premature solidification. Four different concentrations of the nanoparticle solution are prepared, typically ranging from 25, 12.5, 6.25, and 3.125 µg/mL for silver and 80, 40, 20 and 10µg/mL for copper nanoparticles.

The molten agar is mixed with the fungal suspension and poured into sterile petri dishes, ensuring uniform distribution. Then 1ml of nanoparticle solutions of varying concentrations are carefully incorporated into the agar medium. The plates are incubated at 37°C for 48 hours, allowing fungal growth and potential inhibition zones to develop. After incubation, the decrease in growth of mold colonies with increase in concentration of nanoparticles is the indication of antifungal activity exhibited by nanoparticle at specific concentration.

PHASE 4: CHECKING SYNERGISTIC EFFECT OF TWO DIFFERENT
NANOPARTICLES (EG. Cu AND Ag).

Determined the minimum inhibitory concentration (MIC) of individual copper and silver nanoparticles and minimum bactericidal concentration (MBC) of the individual and combined nanoparticle solutions against mixed bacterial cultures.

Step 1: determination Mic of individual copper and silver nanoparticles Materials:

- Silver nanoparticles (Stock - 200 µg/mL)
- Copper nanoparticles (Stock - 100 µg/mL)
- Mueller-Hinton broth – 100 ml
- Bacterial culture (mixed test organisms- culture isolated form smartphones)

Sterile test tubes – 12, Micropipettes and sterile tips, distilled water

- Incubator (37°C)
- Spectrophotometer (optional, OD at 600)

Procedure:

To determine the Minimum Inhibitory Concentration (MIC) of silver and copper nanoparticles using the tube dilution method, prepared stock solutions of each nanoparticle at their highest MIC concentrations (200 µg/mL for silver and 1000 µg/mL for copper) ensuring sterility with distilled water. In a series of sterile test tubes, added 2 mL of Mueller-Hinton broth, then performed two-fold serial dilutions to achieved a range of concentrations: 0.19 to 200 µg/mL for silver and 0.97 to 1000 µg/mL for copper.

After thorough mixing, prepared a mixed bacterial suspension adjusted to 0.5 McFarland standard, and inoculate each tube with 100 µL of this suspension. Included media control to ensure the quality of growth medium. Once inoculation is completed, incubated the tubes at 37°C for 18-24 hours to allow bacterial growth or inhibition. After incubation, examined the turbidity visually, identified the MIC as the lowest nanoparticle concentration of both copper and silver individually that completely inhibits bacterial growth. Below is a dilution chart for determining the Minimum Inhibitory Concentration (MIC) of silver and copper nanoparticles using the tube dilution method.

Serial Dilution Chart for MIC Determination

Tube no.	Silver Nanoparticles (µg/mL)	Copper Nanoparticles(µg/mL)	Mueller-Hinton Broth (mL)	Bacterial Suspension (µL)
1	200	1000	2	100
2	100	500	2	100
3	50	250	2	100
4	25	125	2	100
5	12.5	62.5	2	100
6	6.25	31.25	2	100
7	3.125	15.62	2	100
8	1.56	7.81	2	100
9	0.78	3.90	2	100
10	0.39	1.95	2	100
11	0.19	0.97	2	100
12	Control	Control	2	100

Each tube contains 2mL of Mueller-Hinton broth and 100 µL of bacterial suspension. The nanoparticle concentrations are halved at each step, following a two-fold serial dilution. Tube 12 serves as a control.

Step 2. Determination of MBC of the individual and combined nanoparticle solutions.

Materials:

- Silver nanoparticles (MIC: 6.25 µg/mL)
- Copper nanoparticles (MIC: 62.5 µg/mL)
- Mueller-Hinton broth (or appropriate bacterial growth medium)
- Bacterial culture (E. coli, S. aureus, or other test organisms)
- Sterile test tubes, Micropipettes, and sterile tips
- Agar plates (Mueller-Hinton or Nutrient Agar)
- Incubator (37°C)
- Sterile distilled water or physiological saline
- Positive control (antibiotic with known MBC)
- Negative control (broth without nanoparticles)

Procedure:

To determine the Minimum Bactericidal Concentration (MBC) of silver and copper nanoparticles individually and in combination, the MIC tubes concentrations exceeding their MIC values. (6.25 µg/mL for silver and 62.5 µg/mL for copper). sub cultured 10 µL from each tube onto Mueller-Hinton agar plates by spread plate technique and incubated them at 37°C for 24 hours. The MBC is determined as the lowest concentration at which no bacterial growth occurs on the agar. For the combined MBC determination, mixed the equal volumes of both copper and silver MIC tubes of various range of concentrations at 1:1 ratio the combined concentrations and incubated at 37°C for 18-24 hours. After incubation, subculture 10 µL onto Mueller-Hinton agar plates and incubated them at 37°C for 24 hours. The lowest concentration of the combined nanoparticles that eliminates bacterial growth is recorded as the synergistic MBC. Significantly enhanced antimicrobial activity of combined nanoparticles compared to individual nanoparticles marks the synergistic effect between the two nanoparticles.

PHASE 5: DEVELOPMENT OF STABLE AND EFFECTIVE FORMULATION OF THE ANTIMICROBIAL SPRAY.

Formulating an antimicrobial coating spray using a combination of copper and silver nanoparticles will involve several steps to ensure effective antimicrobial properties and proper application.

Materials Needed:

- Copper nanoparticles (CuNPs)
- Silver nanoparticles (AgNPs)
- Solvent (e.g., ethanol, water)
- Surfactant (optional, for better dispersion)
- Binder (e.g., polyvinyl alcohol, acrylic polymer)
- Spray bottle
- Mixing equipment (e.g., magnetic stirrer, ultrasonicator)

Procedure:

a. Preparation of Nanoparticle Suspension: Dispersed 25ml (concentration-40ug/ml) of copper nanoparticles solution and 25 ml (concentration-12.5 ug/ml) of silver nanoparticles solution separately in a solvent i.e., 50 ml ethanol using a magnetic stirrer to ensure uniform distribution.
b. Added a surfactant -1ml of tween 20 to improve the dispersion of nanoparticles.
c. Combining Nanoparticles: - Mixed the copper nanoparticle suspension and silver nanoparticle suspension in the 1:1 ratio to achieve the desired antimicrobial properties. Stirred the mixture

thoroughly to ensure homogeneity.

d. Adding Binder: Added polyvinyl alcohol to the nanoparticle mixture to help the coating adhere to surfaces. Adjust the concentration of the binder to achieve the desired viscosity for spraying.
e. Final Mixing and filtration: Mixed the entire formulation thoroughly using ultrasonicator to ensure that the nanoparticles and binder are evenly distributed. The mixture is further filtered through Whatman filter paper no. 1 to ensure the desired size of nanoparticles and to achieve fine liquid sprayable solution.
f. Filling the Spray Bottle: Transferred the final mixture into a black color coated spray bottle to prevent photooxidative damage of the solution. Ensured the spray bottle is clean and free of contaminants.
g. Application: Shake the spray bottle well before use. Spray the antimicrobial coating onto the desired surfaces evenly. Allow the coating to dry completely.

PHASE 6: TESTING ANTIMICROBIAL EFFICACY
Objective:
To evaluate the antimicrobial effectiveness of the formulated spray.

TOTAL REDUCTION ANALYSIS
Materials:

- Smartphones of candidates
- St nutrient agar plates
- St. saline, St. cotton swabs, suspension tubes

Procedure:
A. Testing criteria – Age Group
Objective: to evaluate the total reduction in microbial load on the smartphones among the population across different age groups. Swab samples were collected using St. cotton swab and saline solution from the candidate of different age group i.e., 9-14, 16- 25, 26-35, 36- 45, 46-55 and 56-65. The six samples from individual age group candidate are collected before and after application of formulated spray solution, named the samples S1 to S6 and tested for its antimicrobial activity by spread plate method. 0.1ml of samples from before spray and after spray application are spread onto the nutrient agar surface using sterile spreader. Incubated the plates at 37°C for 24 hours for microbial growth and observed the total no. of colonies on plates before spray and after spray solution. Calculated and estimated the total reduction percentage.

B. Testing Criteria – Higher risk profiles
Objective: To evaluate the total reduction in microbial load on the smartphones of the candidates who are at higher risk profiles. Swab samples are collected using St. cotton swab and saline solution from the candidate which were as follows: 2 housewives with small children, 2 heath care workers, 2 food sellers. The samples from individual candidate are collected before and after application of formulated spray solution named the samples S1 to S6 and tested for its antimicrobial activity by spread plate method. For that Nutrient Agar was prepared. 0.1ml of samples from before spray and after spray application are speeded onto the agar surface using sterile spreader. Incubated the plates at 37°C for 24 hours for microbial growth and observed the total no. of colonies on plates before spray and after spray solution. Calculated and estimated the total reduction percentage.

PHASE 7: COMPARATIVE ANALYSIS
TIME KILL ASSAY.
The time-kill assay is a widely used method to evaluate the antimicrobial effectiveness of a coating spray by measuring the reduction in microbial population over time.

Materials. Samples from smartphone screens

- Formulated coating spray
- Sterile saline or phosphate-buffered saline (PBS)
- Nutrient broth as growth medium, Agar plates
- Pipettes and micropipettes, Sterile glass tubes

Procedure:
Two test groups are set up—one treated with the formulated coating spray and the other with the normal cleaning solution. The antimicrobial agents are applied to the smartphone screens and samples are collected at specific time intervals (0 min, 30min, 60min, 90min, 120 min and 150 min) these samples are plated onto agar plates by spread plate technique.

These plates are incubated for 24 hours at room temperature. allowing viable microorganisms to form colonies. The number of colonies is counted and

compared across different time points to determine microbial reduction. The reduction in CFU is calculated to assess the antimicrobial effectiveness of each treatment. A greater reduction in CFU over time indicates superior antimicrobial activity.

IV. RESULTS, OBSERVATIONS AND ANALYSIS

Phase 1: Isolation and identification of microorganisms from smartphone screens

Isolated bacterial and fungal colonies on Nutrient and Potato dextrose (PDA) agar plates:

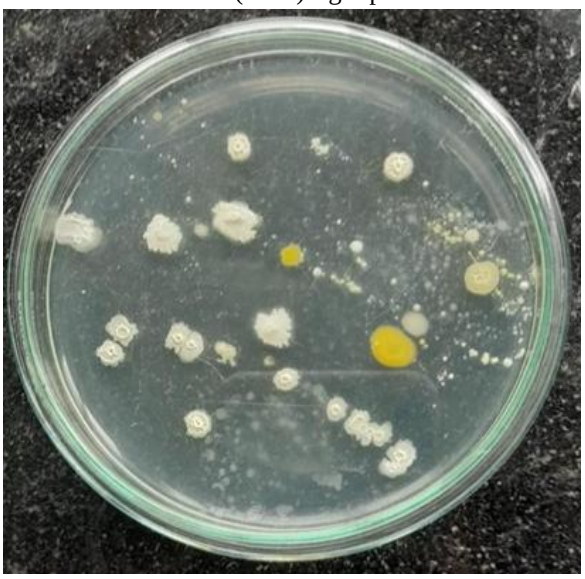


Fig 1.1: Sample 1 (bacterial growth results on nutrient agar plate)

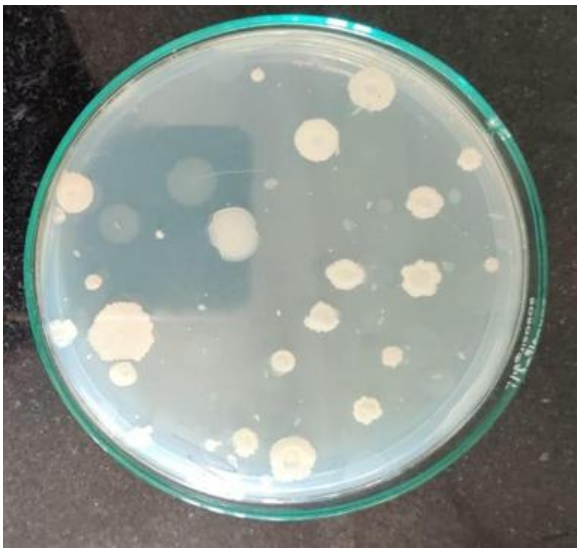


Fig 1.2: Sample 2 (bacterial growth results on Nutrient agar plate)

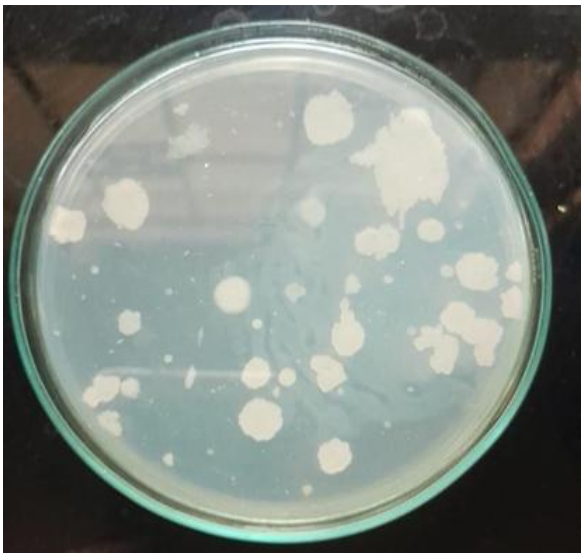


Fig 1.3: Sample 1 (fungal growth results on PDA plate

Fig 1.4: Sample 2 (fungal growth results on PDA plate

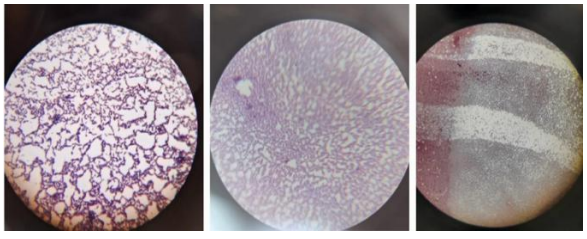


Fig 1.5 Sample 1: Gram staining results of different isolated colonies from nutrient agar plate (from left hand side colony 1, colony 2 and colony 3 respectively.)

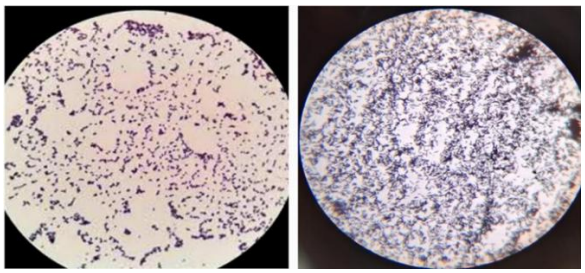


Fig 1.6 Sample 2- Gram staining results of different isolated colonies from nutrient agar plate (from left hand side colony 1 & colony 2 respectively.)

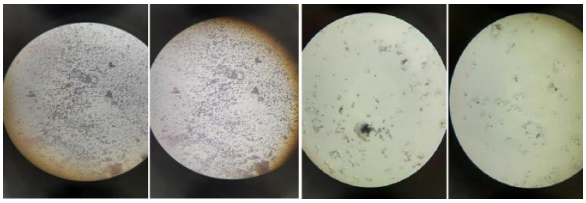


Fig 1.7 sample 1 and 2 (microscopic examination of isolated yeast and mold colonies from PDA Agar plate)

Table 1.1: Observation Table for identification of Bacterial colonies:

Sample	Colony	Observed microscopic characteristics
Sample 1.	Colony 1.	Rod shaped, Gram- positive bacteria in short chains.
	Colony 2.	Spherical shaped, Gram- positive bacteria in clusters.
	Colony 3.	Rod shaped, Gram- negative bacteria in pairs or in short chains.
Sample 2.	Colony 1.	Spherical shaped, Gram- positive bacteria in clusters.
	Colony 2.	Rod shaped, Gram- negative bacteria in pairs or in short chains.

Table 1.2: Observation Table for identification of fungal colonies:

Sample no.	Fungal colony	Observed microscopic characteristics
Sample 1 and 2	yeast	Small, round shaped, budding yeast cells.
Sample 2.	mold	Hyaline hyphae, filamentous and branched.

Results

The microbial isolates displayed varied morphologies across nutrient agar and PDA agar plates as described in above table no.1.1 1nd 1.2. Microscopic examination confirmed the presence of distinct bacterial colonies along with budding yeast cells and branched hyaline hyphae characteristic of mold.

Interpretation

The study successfully differentiated and identified distinct bacterial and fungal species based on colony morphology, agar selectivity, and microscopic analysis. Nutrient agar effectively supported bacterial growth, revealing Gram-positive and Gram-negative species with varying morphologies, while PDA agar selectively facilitated fungal colony formation, distinguishing yeast from mold. The microscopic assessment provided crucial insights into cellular structures, confirming budding yeast morphology and hyaline hyphae indicative of molds. These findings emphasize the importance of agar-based cultivation and microscopic evaluation for accurate microbial identification, aiding in future microbial studies and applications.

Phase 2: Synthesis and Characterization of Copper and Silver Nanoparticles

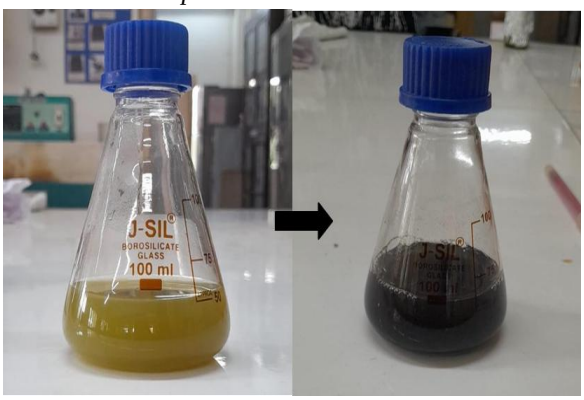


Fig. 2.1 Green synthesis of silver nanoparticles

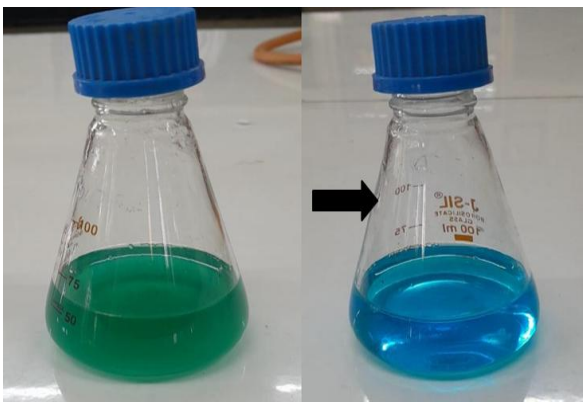


Fig 2.2 Green synthesis of copper nanoparticles

Characterization Of Silver Nanoparticles
Product Name: nanoparticle analysis Batch No.
Examine Item: copper nanoparticles
Operator: admin
Print Person: admin
Save Path: Not Saved
Method: None
Test Mode: Abs.
Test WL:300nm ~ 600nm
Test Slit:2.0nm
Device Type: L6S
Test Time:25/04/11 13:46:32
Report Generate Time:25/04/11 13:48:09
Scan Map:

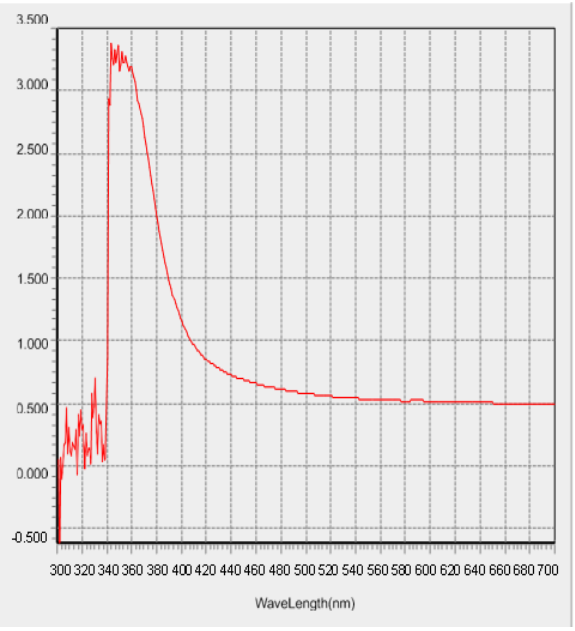


Fig 2.3 absorption spectrum of silver nanoparticles at 300-600 wavelengths

Characterization Of Copper Nanoparticles
Product Name: Yashashree analysis Batch No.:
Examine Item: nanoparticles
Operator: admin
Print Person: admin
Save Path: Not Saved
Method: None
Test Mode: Abs.
Test WL:300nm ~ 700nm
Test Slit:2.0nm
Device Type: L6S
Test Time:25/04/11 13:36:15
Report Generate Time:25/04/11 13:41:30
Scan Map:

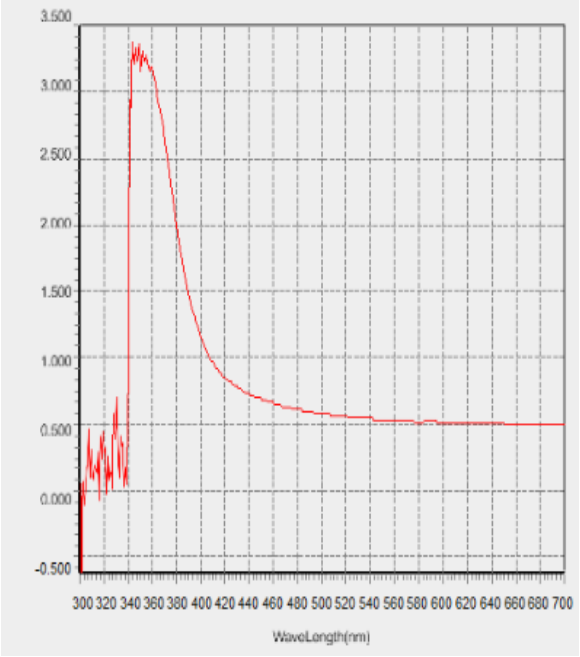


Fig 2.4 absorption spectrum of silver nanoparticles at 300-600 wavelengths

Result
The green synthesis of silver and copper nanoparticles was successfully achieved using environmentally friendly reducing agents. Upon characterization using UV- Visible spectroscopy, distinct surface plasmon resonance (SPR) peaks were observed for both metal nanoparticles. For copper nanoparticles, the SPR peaks appeared within the range of 340 to 460 nm when scanned between 300 to 600 nm, indicating successful formation and stabilization of colloidal copper nanoparticles. Meanwhile, the silver nanoparticles exhibited SPR peaks between 340 to 400 nm when scanned over 300 to 700 nm, confirming their characteristic optical properties. The peak positions suggest uniform dispersion of nanoparticles, with minimal aggregation.

Interpretation
The study successfully demonstrated the green synthesis of silver and copper nanoparticles, with UV-Visible spectroscopy confirming their formation and stability. The SPR peaks observed align with known nanoparticle optical properties, supporting their nanometer-scale size and colloidal stability. The results validate the effectiveness of biogenic reduction in nanoparticle synthesis, offering a sustainable approach to nanoparticle production.

PHASE 3: - CHECKING ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF NANOPARTICLES

Antibacterial activity of silver nanoparticles

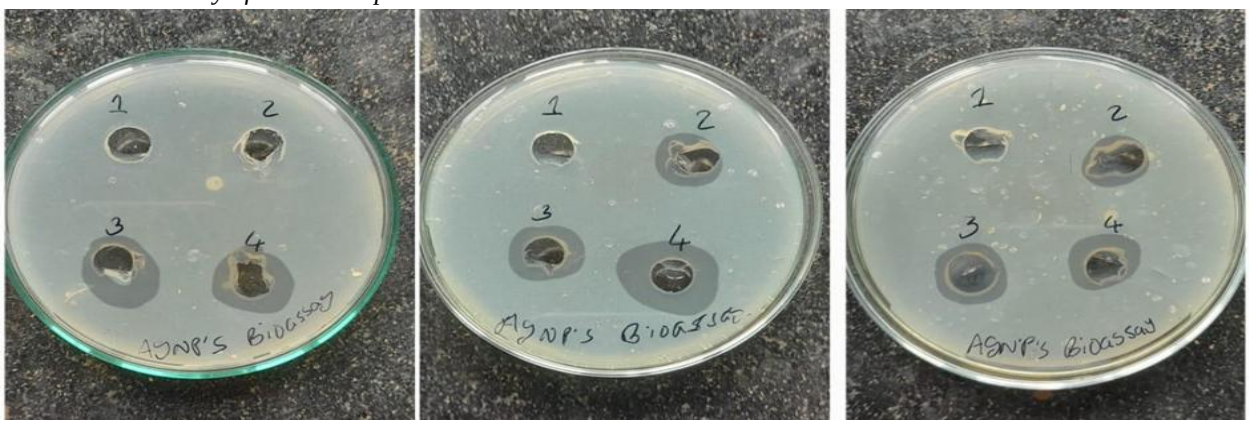


Fig. 3.1 Antibacterial Activity of silver Nanoparticles by agar well assay against isolated bacterial strains i.e., gram-positive rods, gram negative rods and gram-positive bacilli

Fig. 3.2 Antibacterial Activity of silver Nanoparticles by disc diffusion assay against isolated bacterial strains i.e., gram-positive rods, gram negative rods and gram-positive bacilli

Table no. 3.1 (observation Table of antibacterial activity of silver nanoparticles)

Sr.no.	Name of Bacterial colony		Concentration of silver nanoparticles	Dimeter of zone of inhibition (mm) by agar well assay	Dimeter of zone of inhibition (mm) by disc diffusion assay
1.	Gram rods	positive	3.12 µg/mL	No zone	No zone
			6.25 µg/mL	No zone	No zone
			12.5 µg/mL	13mm	1.5mm
			25.00 µg/mL	15mm	1.0mm
2.	Gram rods	negative	3.12 µg/mL	No zone	No zone
			6.25 µg/mL	8mm	No zone
			12.5 µg/mL	9mm	0.8mm
			25.00 µg/mL	13mm	1.0mm
3.	Gram bacilli	positive	3.12 µg/mL	No zone	No zone
			6.25 µg/mL	5mm	0.4mm
			12.5 µg/mL	7mm	0.8mm
			25.00 µg/mL	7mm	1.2mm

Results

The antimicrobial activity of silver nanoparticles was evaluated against three bacterial groups using agar well diffusion and disc diffusion assays. The findings reveal the following trends:

Gram-positive rods:

No inhibition was observed at lower concentrations (3.12 and 6.25 µg/mL). At 12.5 µg/mL, an inhibition zone of 13 mm was observed via the agar well method, but only a minimal 1.5 mm zone was seen in the disc diffusion assay. At 25.0 µg/mL, the zone increased to 15 mm (agar well assay), but decreased slightly to 1.0 mm in the disc diffusion method.

Gram-negative rods:

No inhibition occurred at 3.12 µg/mL. At 6.25 µg/mL, an 8 mm zone was observed only in the agar well assay, while no inhibition was seen in disc diffusion. At 12.5 µg/mL, moderate inhibition was recorded (9 mm in agar well, 0.8 mm in disc diffusion). At 25.0 µg/mL, larger zones were observed (13 mm in agar well, 1.0 mm in disc diffusion).

Gram-positive bacilli:

No inhibition at 3.12 µg/mL. At 6.25 µg/mL, smaller inhibition zones (5 mm and 0.4 mm) were seen. At 12.5 µg/mL, the inhibition zones slightly increased (7 mm in agar well, 0.8 mm in disc diffusion). The highest concentration (25.0 µg/mL) maintained a 7 mm agar well zone but showed a 1.2 mm disc diffusion inhibition.

Interpretation:

Silver nanoparticles exhibit concentration-dependent antibacterial activity, with higher concentrations leading to larger inhibition zones. Gram-positive rods demonstrated the strongest response to silver nanoparticles, followed by Gram-negative rods and Gram- positive bacilli.

The agar well diffusion assay consistently showed larger inhibition zones than the disc diffusion method, suggesting better diffusion and availability of silver nanoparticles in liquid medium compared to solid-embedded discs.

Lower concentrations (3.12 and 6.25 µg/mL) were largely ineffective against all bacterial groups. The variation in inhibition zones across bacterial types may be attributed to differences in cell wall composition, permeability, and nanoparticle interaction.

Antibacterial activity of copper nanoparticles

Fig. 3.3 Antibacterial Activity of copper Nanoparticles by agar well assay against isolated bacterial strains i.e., gram-positive rods, gram negative rods and gram-positive bacilli

Fig. 3.4 Antibacterial Activity of silver Nanoparticles by disc diffusion assay against isolated bacterial strains i.e., gram-positive rods, gram negative rods and gram-positive bacilli

Table no. 3.2 (observation Table of antibacterial activity of copper nanoparticles)

Sr. no.	Name of Bacterial colony	Concentration of copper nanoparticles	Dimeter of zone of inhibition (mm) by agar well assay	Dimeter of zone of inhibition (mm) by disc diffusion assay
1.	Gram positive rods	10 µg/mL	No zone	No zone
		20 µg/mL	No zone	No zone
		40 µg/mL	No zone	0.6mm
		80 µg/mL	8mm	0.8mm
2.	Gram negative rods	10 µg/mL	No zone	No zone
		20 µg/mL	No zone	0.9mm
		40 µg/mL	18mm	1.8mm
		80 µg/mL	19mm	0.8mm
3.	Gram positive bacilli	10 µg/mL	No zone	0.9mm
		20 µg/mL	No zone	1.0mm
		40 µg/mL	No zone	1.0mm
		80 µg/mL	5mm	1.5mm

Results:

Gram-positive rods: At lower concentrations (10–40 µg/mL), copper nanoparticles showed no inhibition in either assay. At 80 µg/mL, the agar well diffusion assay displayed an 8 mm zone of inhibition, while the disc diffusion assay showed only 0.8 mm, indicating minimal sensitivity.

Gram-negative rods:

Copper nanoparticles exhibited significant antibacterial activity at higher concentrations. At 40 µg/mL, the inhibition zones were 18 mm (agar well assay) and 1.8 mm (disc diffusion assay). At 80 µg/mL, the agar well assay maintained an inhibition zone of 19 mm, but the disc diffusion assay showed a smaller inhibition zone (0.8 mm).

Gram-positive bacilli:

There was no inhibition observed in the agar well assay at 10–40 µg/mL, though the disc diffusion assay showed small inhibition zones (0.9–1.0 mm). At 80 µg/mL, the agar well assay demonstrated a 5 mm inhibition zone, and the disc diffusion assay showed 1.5 mm inhibition, indicating moderate antibacterial activity.

Interpretation:

Gram-negative bacteria exhibited the highest susceptibility to copper nanoparticles, showing significant inhibition zones, particularly in the agar well diffusion method. This suggests that Gram-negative bacteria may be more vulnerable due to differences in cell wall composition. Gram-positive

rods showed minimal sensitivity, with an inhibition zone appearing only at the highest concentration (80 µg/mL), suggesting greater resistance. Gram-positive bacilli exhibited slight inhibition at higher concentrations but were generally less affected. The agar well diffusion assay consistently yielded larger inhibition zones compared to the disc diffusion assay, likely due to better nanoparticle diffusion in liquid media. Higher concentrations (≥40 µg/mL) were necessary to exhibit antimicrobial effects, with optimal activity observed against Gram-negative bacteria at 40–80 µg/mL.

These findings suggest copper nanoparticles are more effective against Gram- negative bacteria than Gram-positive bacteria, potentially due to differences in membrane permeability and nanoparticle interactions.

Antifungal Activity of Silver Nanoparticles

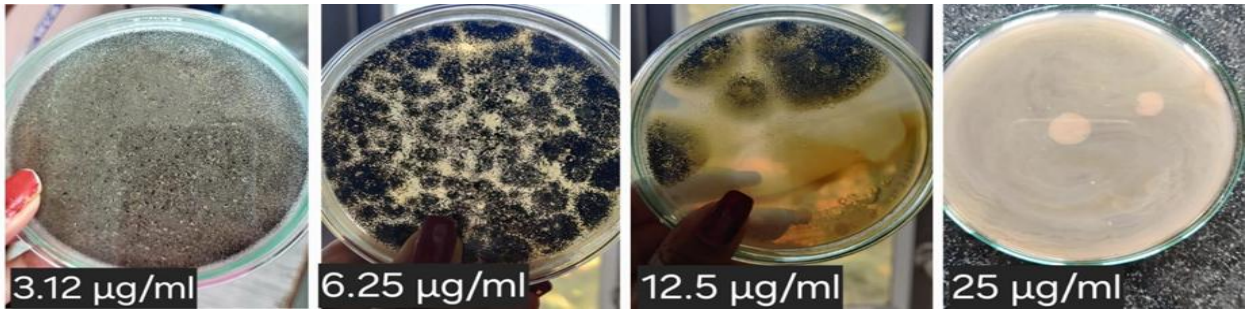


Fig. 3.5 Antifungal activity of silver nanoparticles at different concentrations against molds by pour plate technique.

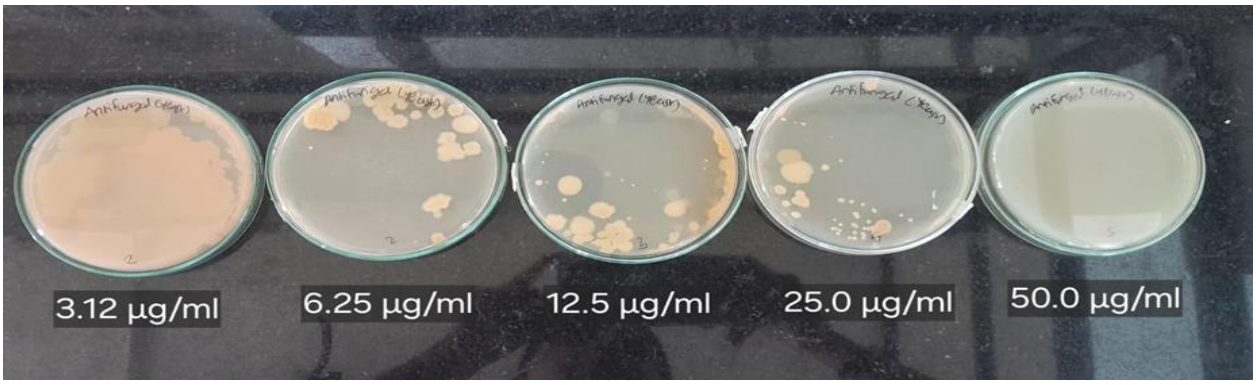


Fig 3.6 Antifungal activity of silver nanoparticles at different concentrations against yeast's by pour plate technique.

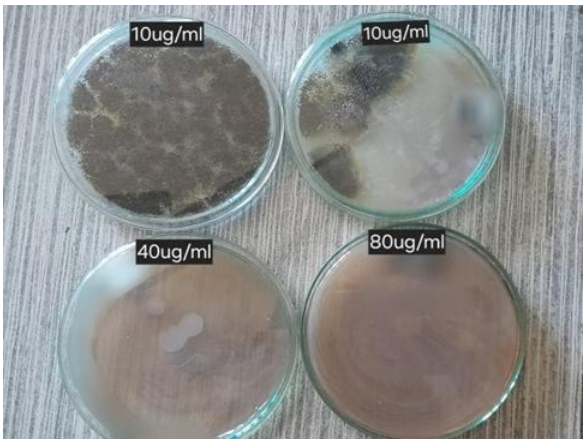


Fig 3.7 Antifungal activity of copper nanoparticles at different concentrations against molds by pour plate technique.

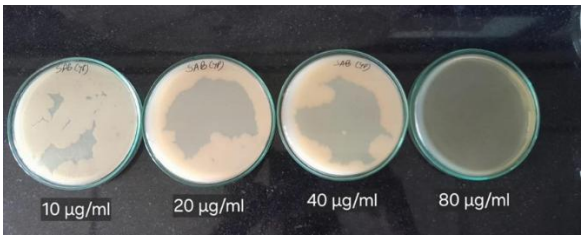


Fig 3.8 Antifungal activity of copper nanoparticles at different concentrations against Yeast's by pour plate technique.

Result:

The antifungal activity of silver and copper nanoparticles was assessed against molds and yeast using the pour plate technique. The results indicate a concentration-dependent inhibitory effect:

Silver Nanoparticles:
As concentration increased, fungal colony counts progressively declined.
Mold growth was entirely inhibited at 25 µg/mL, demonstrating strong antifungal efficacy at this concentration.
Yeast showed complete inhibition at 50 µg/mL, requiring a higher nanoparticle concentration for suppression compared to molds.

Copper Nanoparticles:
Increasing concentrations led to a gradual decline in fungal growth.
Both mold and yeast growth were completely inhibited at 80 µg/mL, indicating copper nanoparticles' potent antifungal properties at higher concentrations.

Interpretation:
Silver and copper nanoparticles exhibit strong antifungal activity, with effectiveness increasing at higher concentrations. Molds were more sensitive to silver nanoparticles, showing complete inhibition at 25 µg/mL, whereas yeast required 50 µg/mL for full suppression.
Copper nanoparticles required higher concentrations compared to silver but ultimately demonstrated complete inhibition of both yeast and molds at 80 µg/mL. The results suggest potential for nanoparticle-based antifungal applications.

PHASE 4: CHECKING SYNERGISTIC EFFECT OF TWO DIFFERENT NANOPARTICLES (EG. CU AND AG).

Mic of silver and copper nanoparticles

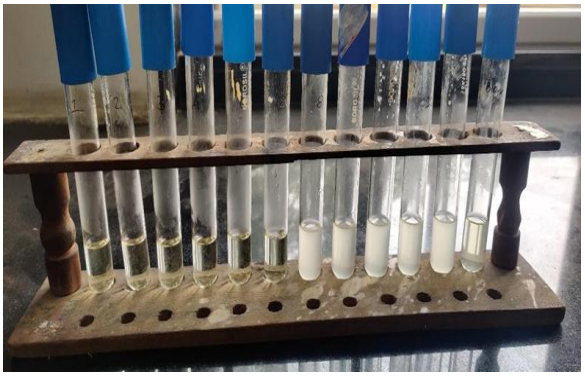
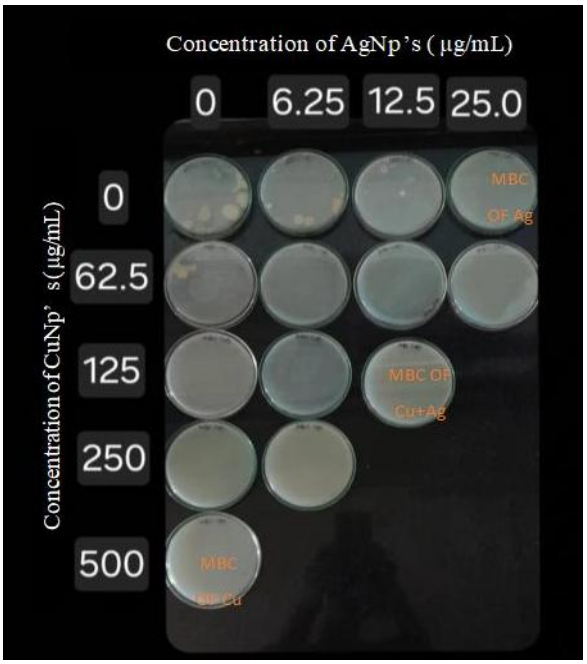


Fig 4.1: MIC of silver nanoparticles against mixed bacterial culture (concentration of AgNp's from left hand side ranging 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19 and 0 µg/mL respectively.)

Fig 4.2: MIC of copper nanoparticles against mixed bacterial culture (concentration of CuNp's from left hand side ranging 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90, 1.95, 0.97 and 0 µg/mL respectively.)

Observation:
The MIC of silver nanoparticles against mixed bacterial culture were found out to be 6.25 µg/mL whereas the mic of copper nanoparticles was found to be at 62.5 µg/mL against mixed bacterial cultures isolated from the screens of smartphone.

MBC of the individual and combined nanoparticle solutions



Observation:
The Minimum Bactericidal Concentration (MBC) results indicate distinct efficacy patterns for individual and combined nanoparticle suspensions. Individual copper nanoparticles exhibited bactericidal activity at

500 µg/mL, while individual silver nanoparticles required a significantly lower concentration of 25 µg/mL to achieve the same effect. However, when combined, the MBC of the mixed nanoparticle suspension was observed at 125 µg/mL for copper and 12.5 µg/mL for silver.

Result:
The results demonstrate distinct antimicrobial efficacy of silver and copper nanoparticles against mixed bacterial cultures isolated from smartphone screens. The Minimum Inhibitory Concentration (MIC) of silver nanoparticles was found to be 6.25 µg/mL, significantly lower than the MIC of copper nanoparticles, which was 62.5 µg/mL.

This indicates that silver nanoparticles exhibit stronger antimicrobial activity at lower concentrations compared to copper nanoparticles. Similarly, the Minimum Bactericidal Concentration (MBC) results align with this trend, showing that individual copper nanoparticles required a concentration of 500 µg/mL for bactericidal activity, whereas silver nanoparticles achieved the same effect at 25 µg/mL.

Notably, when combined, the MBC of the mixed nanoparticle suspension was reduced to 125 µg/mL for copper and 12.5 µg/mL for silver, suggesting a synergistic effect that enhances bactericidal efficacy while lowering the required dosage.

Interpretation:
These findings highlight the potential advantages of using mixed nanoparticle formulations in antimicrobial applications. The observed synergistic interaction suggests that silver and copper nanoparticles, when used together, can achieve optimal antibacterial effects at lower concentrations, potentially reducing material costs and minimizing toxicity concerns associated with higher doses of individual nanoparticles.

This study supports the idea that nanoparticle-based antimicrobial solutions can be fine- tuned to enhance efficacy and sustainability, making them promising candidates for practical applications, such as disinfectants for smartphone screens and other frequently touched surfaces.

PHASE 5: DEVELOPMENT OF STABLE AND EFFECTIVE FORMULATION OF THE ANTIMICROBIAL SPRAY.

Fig 5.1 Formulation of the antimicrobial spray using nanoparticles.

Observation:
The developed antimicrobial spray utilizing nanoparticles exhibits excellent consistency, forming a uniform dispersion without aggregation. The liquid formulation is easily sprayable, ensuring smooth application across the smartphone screen.

Upon application, the spray demonstrates rapid evaporation, preventing excess moisture accumulation while maintaining effective antimicrobial action. The nanoparticles remain well-distributed on the surface, enhancing long-term efficacy. The formulation does not leave visible residue, ensuring optical clarity and maintaining touch responsiveness of the screen.

Result:
The optimized antimicrobial spray successfully achieved better consistency, ensuring homogeneous nanoparticle dispersion. The enhanced evaporation rate allows quick drying, preventing smudges or streaks on the smartphone screen.

The formulation's spray ability ensures effortless application without clogging or excessive dripping. Initial microbial assessments indicate significant reduction in bacterial and fungal presence, demonstrating the effectiveness of the nanoparticle-based antimicrobial mechanism.

The spray maintains screen transparency and smooth touch sensitivity, making it a viable solution for everyday use.

PHASE 6: TESTING ANTIMICROBIAL EFFICACY

A. Testing criterion – Age Group

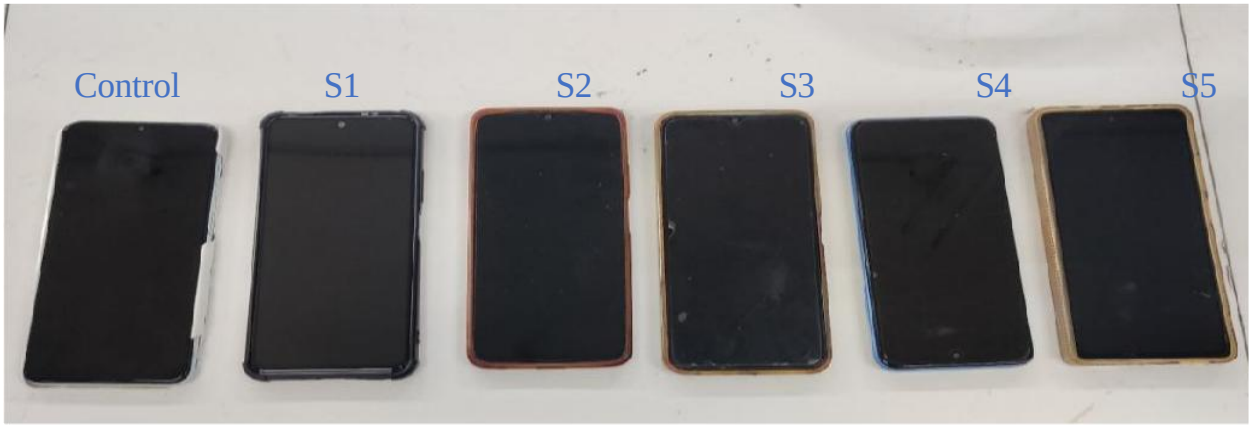


Fig 6.1 Smartphones samples for testing antimicrobial effectiveness (Criterion- A)

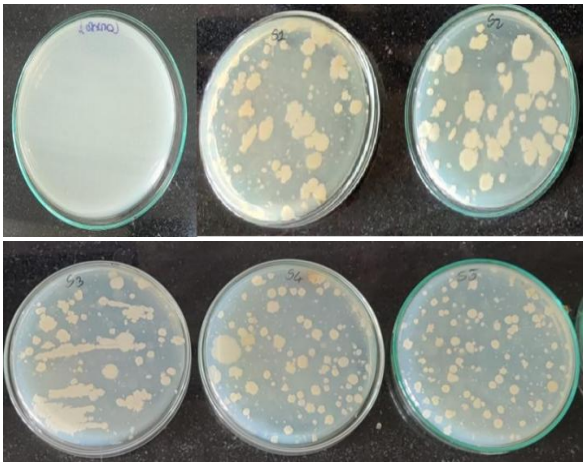


Fig 6.2 Total viable microbial colonies before spray application (criterion-A)

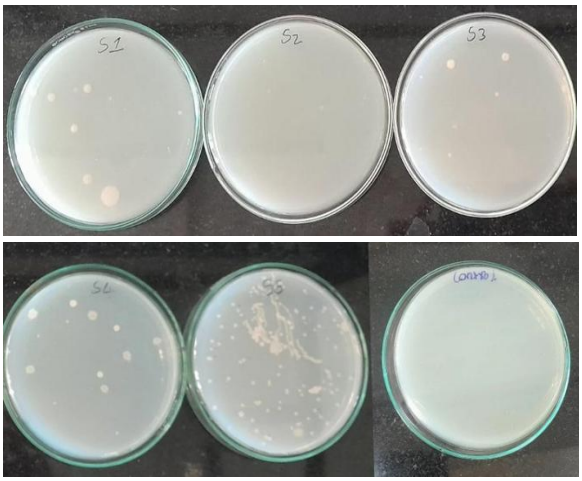


Fig 6.3 Total viable microbial colonies After spray application (criterion A)

Observation Table no. 6.1 (total reduction analysis for criterion A by TVC method)

Sample No.	Age Group of users	Total viable count before spray (cfu/ml)	Total viable count after spray (cfu/ml)
control	9 to 14	0	0
S1	15 to 25	122	10
S2	26 to 35	103	04
S3	36 to 45	119	07
S4	46 to 55	143	13
S5	56 to 65	232	33

Calculation:

Average reduction = Initial average – finale average/ initial average x 100

= 143.5 – 67/ 143.5 x 100

= 96%

Average reduction (age group criteria) = 96%

B. Testing Criteria – Higher risk profiles

Fig 6.4 smartphones samples for testing antimicrobial effectiveness (Criterion - B)

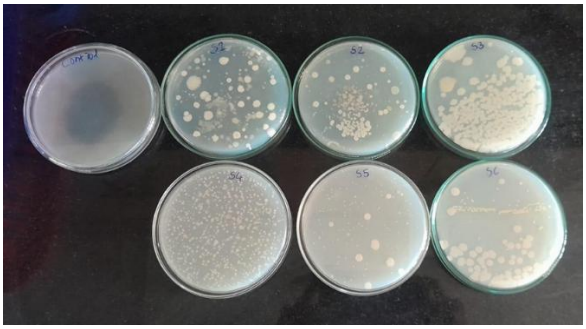


Fig 6.5 total viable microbial colonies before spray application (criterion B)

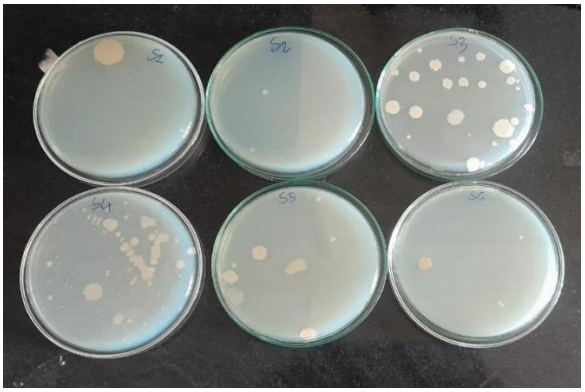


Fig 6.6 total viable microbial colonies After spray application (Criterion B)

Observation Table no. 6.2 (total reduction analysis for high-risk profiles criteria by TVC method)

Sample No.	Profiles of users	Total viable count before spray (cfu/ml)	Total viable count after spray (cfu/ml)
control	NA	0	0
S1	Housewives	218	06
S2	Housewives	117	04
S3	Healthcare workers	311	23
S4	Healthcare workers	522	53
S5	Food sellers	202	13
S6	Food sellers	109	8

Calculation:
Average reduction = Initial average – finale average/ initial average x 100
= 246.1 – 19.5/ 246.1 x 100
= 92%
Average reduction (high risk profiles criteria) = 92%

Result
The antimicrobial spray solution demonstrated remarkable efficacy across both testing criteria.

Age Group Criterion:
The spray effectively reduced microbial contamination on smartphones collected from various age groups, achieving a 96% reduction in viable bacterial count. This indicates consistent effectiveness regardless of user demographics, highlighting its broad applicability.

High-Risk Profiles Criterion:
In samples collected from individuals with potentially higher microbial exposure, including healthcare workers, housewives, and food sellers, the average microbial reduction was 92%. Despite the elevated contamination risk, the spray maintained strong antimicrobial performance, proving its reliability in high-exposure environments.

PHASE 7: COMPARATIVE ANALYSIS

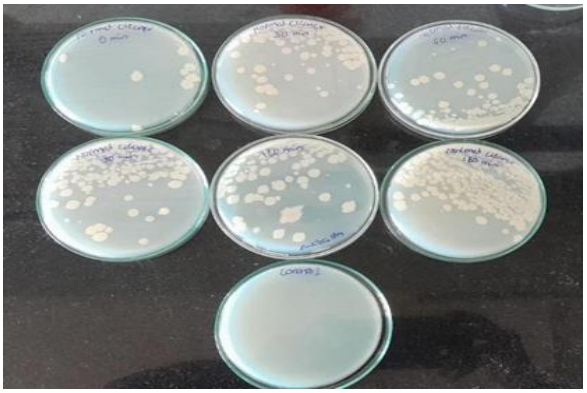


Fig 7.1 Time kill assay of normal cleaning solution for smartphones

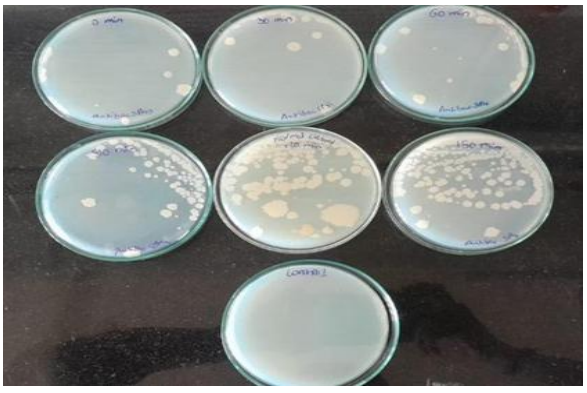


Fig 7.2 Time kill assay of antimicrobial solution using nanoparticles for smartphones

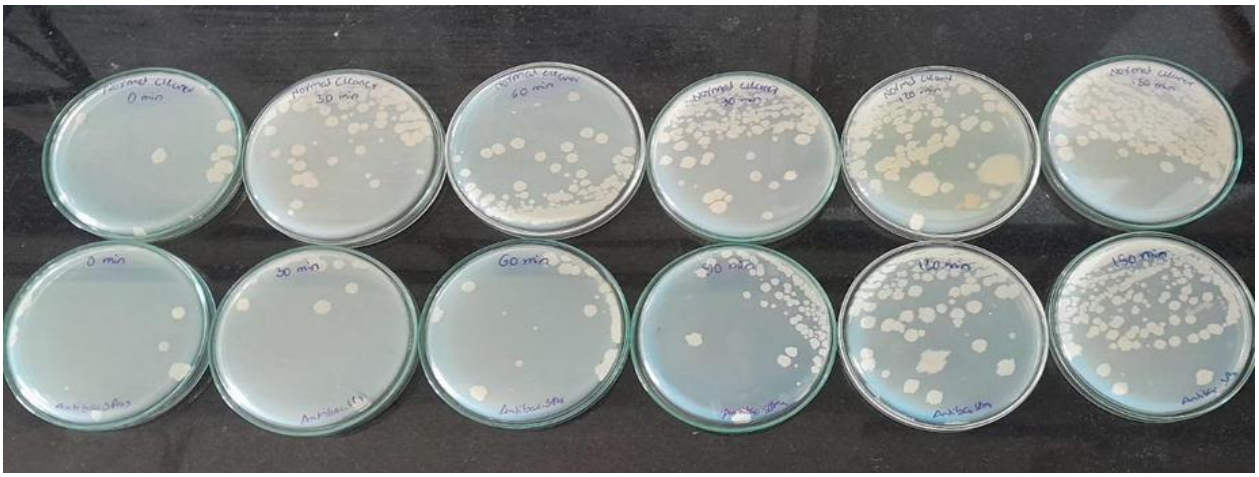


Fig 7.3 Time kill assay of normal cleaning solution Vs antimicrobial solution formulated using nanoparticles for smartphones.

Observation Table 7.1

Sr No.	Time in Minutes	No. of colonies in cfu/ml (normal cleaning solution)	No. of colonies in cfu/ml (formulated cleaning solution)
1.	o	12	5
2.	30	57	14
3.	60	62	23
4.	90	107	58
5.	120	221	98
6.	150	262	113

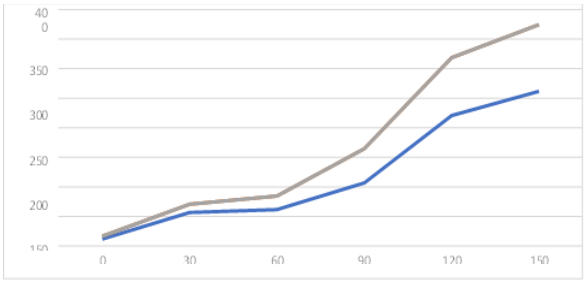


Fig 7.4 Graphical representation for time kill assay

Note: grey line indicates the time kill assay activity of normal cleaning solution and blue line indicates the time kill assay activity exhibited by formulated antimicrobial solution.

Result:

The formulated cleaning solution demonstrates significantly greater antimicrobial effectiveness compared to the normal cleaning solution across all time intervals.

- At 0 minutes, both solutions showed minimal bacterial presence, with the formulated solution maintaining a lower initial colony count (5 cfu/ml vs. 12 cfu/ml).
- By 30 minutes, the normal solution resulted in 57 cfu/ml, while the formulated solution maintained only 14 cfu/ml, showing a strong reduction.

As time progressed, microbial load increased in both cases, but the formulated solution consistently showed lower colony counts, indicating greater and sustained antimicrobial activity.

- At 150 minutes, the normal cleaning solution resulted in 262 cfu/ml, whereas the formulated solution remained lower at 113 cfu/ml, maintaining superior antimicrobial effectiveness over time.

This indicates that the formulated cleaning solution significantly reduces microbial contamination more effectively, proving its efficiency for practical applications.

Interpretation

The formulated cleaning solution demonstrates superior antimicrobial effectiveness, maintaining lower bacterial colony counts across all observed time intervals compared to the normal solution. The initial reduction in microbial load suggests an immediate antimicrobial effect, while the sustained lower counts over time highlight its prolonged activity.

The notable difference in bacterial presence at 30 and 150 minutes indicates that the formulated solution offers enhanced microbial suppression, likely due to

optimized nanoparticle dispersion and prolonged antibacterial interaction. These results validate its practical applicability.

PHASE 8: COMPATIBILITY ASSESSMENT

Table 8.1 User Feedback Chart on Smartphone Spray Solution

User ID	Touch Sensitivity	Transparency of Screen	Evaporation Rate	Other Screen Issues
User 1	No sensitivity change, feels smooth	Perfectly clear screen	Ideal drying time	No complaints
User 2	No noticeable impact	Maintains full transparency	Balanced drying speed	Enhances smoothness of screen
User 3	Responsive, no lag	Completely transparent	Quick drying, easy to apply	No major issues
User ID	Touch Sensitivity	Transparency of Screen	Evaporation Rate	Other Screen Issues
User 4	Excellent touch responsiveness	Crystal-clear visibility	Medium drying time	No residue or streaks
User 5	Feels smoother to the touch	No change in screen clarity	Evaporates evenly	No negative effects
User 6	No sensitivity loss, improved feel	Fully clear display	Quick and uniform evaporation	Minor fingerprint attraction but easy to clean
User 7	Slight improvement in swipe experience	Slightly glossy finish, enhances clarity	Ideal drying time	Reduces glare effectively
User 8	No adverse effects	Maintains full screen transparency	Evaporates seamlessly	Screen remains clean and smooth
User 9	Feels slightly sticky (needs adjustment)	No noticeable transparency loss	Dries slower than expected	Attracts mild dust particles
User 10	No touch delay	Clear display, no residue	Balanced drying speed	Works perfectly with regular use

Result And Interpretation:

The results of the smartphone spray solution feedback indicate an overwhelmingly positive response from users. Nine out of ten users found the spray to enhance touch sensitivity, maintain full screen transparency, and evaporate at an optimal rate without leaving streaks or residue. Many users noted improved smoothness and an overall clean feel on their screens, with some even observing a slight reduction in glare. The only minor concern raised by one user was mild stickiness, which suggests the need for slight formulation adjustments. Overall, the interpretation of this data highlights the effectiveness of the spray in maintaining usability and screen clarity while ensuring a seamless user experience.

V. CONCLUSION AND RECOMMENDATION

The formulated cleaning solution demonstrated significantly greater antimicrobial effectiveness compared to the normal cleaning solution across all measured intervals. The sustained lower bacterial colony counts highlight its prolonged antimicrobial activity, reinforcing its potential for high-touch surface sanitation. Its ability to maintain microbial suppression over time confirms its viability for practical applications, particularly in environments requiring consistent hygiene management. The study validates the optimized nanoparticle formulation, ensuring efficient bacterial reduction while maintaining user-friendly application properties.

To enhance its practical adoption, further refinements could focus on long-term stability testing, biofilm resistance evaluations, and formulation adjustments for extended antimicrobial efficacy. Developing targeted delivery mechanisms, such as spray modifications or enhanced surface adherence properties, could maximize effectiveness in household, healthcare, and commercial settings.

To further enhance the effectiveness of the formulated cleaning solution, future research should explore extended antimicrobial retention, ensuring long-term

protection against microbial contamination. Investigating its performance against resistant bacterial strains and biofilms could provide valuable insights into broader applications, particularly in healthcare and sanitation sectors. Additionally, optimizing surface adherence properties to maintain prolonged efficacy and evaluating environmental stability factors such as temperature and humidity will contribute to its reliability in diverse conditions. These refinements will support its potential commercialization as an advanced hygiene product, ensuring both efficacy and practicality for widespread use.

To support the commercialization and large-scale implementation of the formulated cleaning solution, further investigations into cost-efficiency, production scalability, and regulatory compliance are essential. Optimizing manufacturing protocols to ensure consistent nanoparticle dispersion and stability will enhance both effectiveness and shelf life, making the product more viable for market adoption. Additionally, conducting comprehensive safety assessments, including skin compatibility and toxicity studies, will be critical for regulatory approval, ensuring the formulation meets hygiene and environmental standards. Exploring packaging innovations, such as spray mechanisms that enhance uniform application while minimizing product waste, could further improve consumer experience and practicality. A comparative analysis against existing commercial cleaning solutions will validate its competitive advantages, solidifying its potential as a next-generation antimicrobial cleaning product for household, healthcare, and industrial applications.

To ensure widespread adoption and consumer trust, further studies should examine user experience, storage stability, and application versatility across various environmental conditions. Establishing eco-friendly disposal and sustainability measures will reinforce its appeal in modern hygiene solutions while aligning with global environmental standards. Additionally, conducting large-scale antimicrobial efficacy trials will validate its long-term effectiveness, supporting its transition from laboratory testing to commercial availability. By integrating these refinements, the formulated cleaning solution can be positioned as a high-performance, user-friendly, and sustainable antimicrobial product, ready for broader implementation across healthcare, household, and industrial sectors

Recommendations

To maximize the effectiveness and commercial viability of the formulated cleaning solution, several key areas of improvement and further research are recommended:

1. Long-Term Antimicrobial Stability
- Conduct extended efficacy testing to determine how long the antimicrobial properties remain active on surfaces post-application.
- Investigate formulation adjustments to prolong antimicrobial retention without compromising usability.

2. Biofilm and Resistant Strain Evaluation
- Assess the solution's effectiveness against biofilms, which are more difficult to eradicate compared to planktonic bacteria.
- Test against antibiotic-resistant bacterial strains to explore broader applications in healthcare and sanitation.

3. Surface Compatibility and Material Safety
- Expand testing to various surface types, including plastics, glass, and metals, to ensure no adverse reactions or degradation.
- Evaluate skin safety and toxicity profiles for safe user interaction and regulatory compliance.

4. Optimized Application Methods
- Develop advanced spray mechanisms that enhance coverage while minimizing product wastage.
- Explore nanoparticle encapsulation to improve controlled release and sustained microbial protection.

5. Environmental and Sustainability Considerations
- Investigate biodegradable carrier formulations to align with eco-friendly sanitation solutions.
- Conduct environmental impact studies to assess potential ecological effects from prolonged use.

6. Scalability and Commercialization Strategies
- Optimize production processes for large-scale manufacturing, ensuring cost efficiency and product consistency.
- Develop marketing strategies for targeted industries, including healthcare, food safety, and consumer hygiene.

- Seek regulatory approvals to meet industry standards and certifications, ensuring global compliance.

By addressing these recommendations, the formulated cleaning solution can evolve into a highly efficient, safe, and sustainable antimicrobial product, ready for widespread adoption across healthcare, households, and industrial settings.

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