

Antimicrobial Synthesis of Nanocoated Surfaces: Applications in Healthcare and Public Spaces for Sterilization

Atreyee Mukherjee¹; Dr. Jasmeet Singh²; Dr. Gireesh Babu³

Parul Institute of Applied Sciences, Gujarat

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ABSTRACT

The synthesis and application of antimicrobial nanocoating's represent a cutting-edge advancement in the fight against microbial contamination, particularly in healthcare environments and public spaces where sterilization is critical. These nanocoating's are engineered using nanotechnology to embed antimicrobial agents like metal nanoparticles (silver, copper), metal oxides (titanium dioxide, zinc oxide), or organic compounds into surfaces. These agents exhibit antimicrobial activity, effectively targeting bacteria, viruses, and fungi. The development of these coatings involves advanced fabrication techniques, including chemical vapor deposition, gel processes, and layer-by-layer assembly, to ensure the uniform distribution of antimicrobial agents and the durability of the coatings under various environmental conditions.

In healthcare settings, antimicrobial nanocoatings are applied to high-touch surfaces, medical devices, surgical instruments, and implants to reduce the risk of healthcare-associated infections (HAIs). These coatings provide continuous sterilization, minimizing the need for frequent chemical disinfection and lowering the potential for pathogen transmission. In public spaces, such as transportation systems, schools, and communal areas, nanocoatings are integrated into surfaces like handrails, doorknobs, and seating to create safer environments by inhibiting the spread of infectious agents.

Keywords: *Antimicrobial Nanocoating, Nanoparticle Synthesis, Surface Sterilization, Infection Control, Multidrug-Resistant Pathogens, Reactive Oxygen Species (ROS), Biofilm Inhibition, Healthcare Hygiene, Public Spaces, Metal Oxide Nanoparticles, Biocidal Efficacy, Environmental Impact, Self-Sterilizing.*

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CHAPTER ONE INTRODUCTION

A. Background of the Study

➤ *Introduction to be Increased Demand for Antimicrobial Products and Antimicrobial Resistance (AMR) for Better Health.*

• *Antimicrobial Resistance*

Antimicrobial resistance (AMR) is a process by which microorganisms become resistant to the effects of antimicrobial drugs such as antibiotics, antivirals, antifungals, and antiparasitic. It aggravates the problem of treating the infection, leading to longer disease duration, increased mortality, and increased health care expenditure.

• *AMR Causes*

There are fairly many reasons that explain the occurrence of increased AMR, and some of them are:

✓ *Misuse and Abuse of Antibiotics –*

Ineffective or reckless application of antibiotics on individuals and animals creates resistance.

✓ *Infection Control*

Insufficient infection control, sanitation, and hygiene protocols in health institutions and communities provide a sanctuary for resistant diseases to develop.

✓ *Animal Husbandry use –*

Use of antimicrobials in animal husbandry for growth promotion and disease protection is a cause of resistance emergence.

✓ *Smart Antibiotic Research Development Extension –*

Slow pace of new antibiotic development leads to fewer being made to treat therapy-resistant infections.

▪ *Global Endemic Consequences of AMR*

- The World Health Organization placed the AMR in its list of the largest public health threats in the world.
- AMR leads to over 1.2 million deaths each year, and it's estimated that by 2050 it will become larger than cancer deaths.
- It places the cost of treatment in terms of added time spent in the hospital, forceful treatment, and need for more expensive medication.

▪ *The Increase in Demand for Antimicrobial Products*

In order to aid in the battle against the development of AMR, new antimicrobial products are urgently needed, which include

- *New Antimicrobial Drugs* – Creating novel antibiotics, antivirals, and antifungals to fight off resistant microorganisms.
- *Other Therapies*– Research on bacteriophage therapy, antimicrobial peptides, and probiotics.
- *Nanotechnology Solutions*– Antimicrobial nanocoating sprays, nanoparticles, and smart drug delivery systems may be employed to inhibit infection and inhibit microbial growth.
- *Enhanced Hygiene and Infection Prevention* – Application of antimicrobial coating on public surfaces, hospital equipment, and medical devices can decrease transmission of pathogens.
- *Global Stewardship and Government* – Implementation of stricter regulation of antibiotics, public education on the public health concerns, and funding for research is the call-to-action step.

➤ *The Importance of Surface Hygiene in Healthcare, Food Industries, and Public Spaces.*

Antimicrobial resistance (AMR) is a growing crisis that could render once-treatable infections fatal, propelling us toward a world where minor cuts or common surgeries become life-threatening. Contaminated surfaces that allow bacteria, viruses, and fungi to linger and multiply.

Cleaning needs to be constant, and the effects are short-lived, often fading within minutes or hours. In high-risk settings such as hospitals, food processing facilities, and public areas, this protection gap creates an opportunity for harmful pathogens to flourish.

This research dives into the universe of antimicrobial nanocoating's, investigating their modus operandi, effectiveness in practice, and their potential in combatting AMR and enhancing public health. By connecting science and practical application, this work seeks to enlighten us on how innovative surface protection makes every day spaces safer—one spray at a time.

- *Healthcare Settings*

- *Safeguarding the Most Vulnerable*

Picture yourself going into a hospital. They are intended to heal, but they also can be germey if their surfaces are not cleaned. Consider all the hands that come into contact with bed rails, doorknobs, or equipment on a daily basis. Then imagine someone who has a weakened immune system of a cancer patient or an older person. For them, it only takes a minimal exposure to dangerous bacteria or viruses to be life-threatening. That's why disinfecting and cleaning healthcare surfaces isn't about following regulations; it's about safeguarding people when they are most vulnerable. It's about ensuring a hospital stay doesn't turn into something more tragic.

- *Food Industries:*

- *Which Keeps Our Meals Safe*

Now consider the restaurant you like to go to or where you get your morning coffee. Many efforts are being made to keep surfaces clean countertops, cutting boards, utensils, even the hands of the individuals who prepare your food, because an error in hygiene would result in foodborne illnesses such as salmonella or E. coli.

- *Public Spaces:*

- *Shared Spaces, Shared Responsibility*

Those are the areas where we're all in contact with each other—parks, buses, schools, malls, and even public bathrooms. With so many people handling the same surfaces, though, germs catch on fast. Think back to the last time you gripped a subway handrail or pushed an elevator button. Now picture if nobody cleaned these surfaces on a regular basis.

➤ *Introduction of Nanotechnology and Its Applications in Antimicrobial Coatings.*

- *Nanotechnology and Antimicrobial Applications in Coatings*

- ✓ *Introduction to Nanotechnology*

Nanotechnology is mattering manipulation technology at the nanoscale (usually 1 to 100 nanometres) where matter can have new physical, chemical, and biological properties. Such volumes of this amount have varying characteristics to the bulk material since they have higher *surface area, reactivity, and functionality.* All these have been offering *breakthrough applications** to a very vast range of industrial application from medicine, through electronics, to environmental science, materials engineering, etc.

The most probable field of application of nanotechnology is probably the creation of long-term antimicrobial coatings against bacteria, fungi, and viruses. Nanomaterial coatings possess intrinsic antimicrobial activity, and therefore are optimally applied in antimicrobial prevention of microbial growth on surface.

- ✓ *Nanotechnology-based Coatings Overcome such Drawbacks as Follows by Offering:*

- *Ultra-Long Antimicrobial Action*

In contrast to the short shelf life of typical disinfectants, antimicrobial action growth inhibition by nanocoating's is of very long duration.

- *Wide Spectrum Activity*

Maximised activity against a wide range of microorganisms, e.g., antibacterial-resistant microorganisms, viruses, and fungi.

- *Low Chemical use*

Nanocoating is more efficient with reduced usage of the active ingredient, which is less harmful to human life and the environment in addition to being economical.

- *Self-Repair and Long-Term Protection*

Self-repairing nanocoating's, i.e., photocatalysis, that break down organic contaminants through exposure to light and thus ensure long-term protection.

- ✓ *Nanomaterials used in Antimicrobial Coatings*

Certain nanomaterials are used prevalently in antimicrobial coatings as a result of some of the following features:

- *Silver Nanoparticles (AGNPS):*

Silver nanoparticles are used extensively based on their efficacious antibacterial and antiviral properties, microbial cell membrane disintegration, and interference of microbial metabolic mechanisms.

- *Copper Nanoparticles (CUNPS):*

Copper is antimicrobially inherently active and may be used to prevent antimicrobial soiling due to contact with surfaces.

- *Zinc Oxide (ZNO) and Titanium Dioxide (TiO₂) Nanoparticles:*

Both are photocatalytic antimicrobial active, i.e., photo-excited bactericidal active.

- *Graphene-Based Nanomaterials:*

Graphene oxide was also shown to possess the potential to act as an effective antimicrobial agent by virtue of the physical capacity to kill microbial cells as well as to inhibit microbial metabolism.

- *Applications of Antimicrobial Nanocoating's*

Application of nanotechnology in antimicrobial coating has led them to become an inevitable part of every industry.

- *Medical Facilities*

PPE, hospital surfaces, and medical equipment are nanocoated in order to eliminate the transmission of HAI.

- *Food Industry*

Food containers, food processing equipment, and food storage surfaces are antimicrobial nanocoated to avoid risk of contamination.

- *Public Spaces & Transport*

Microbial spreading is prevented to public transport surfaces that are used with high usage, schools, shopping centres, and airports through nanocoating.

- *Wearable Fabrics & Apparel*

Clothing, masks, and bedding with enhanced durability through antimicrobial nanotechnology that is immune to germs.

- *Water Purification & Air Purification*

Nanocoating in filters and air cleaners is helpful to poison death microbials from the air.

➤ *How Nanocoating's Work (E.G., Mechanisms of Action like Silver Nanoparticles, Copper, Zinc Oxide, Etc.).*

- *Mechanism of Action of Nanoparticles in Antimicrobial Coatings*

- ✓ *Silver Nanoparticles (AGNPS)*

Silver nanoparticles are the most studied nanoscale antimicrobial drug with wide-range antimicrobial action against bacteria, viruses, and fungi. The mechanism of action associated in antimicrobial action by the mechanism of action of AGNPS is:

Disruption of Cell Membrane – AGNPS break microbial cell membrane and have also been quoted to be a component of breaking the cell membrane, leakage, permeation, and cell lysis.

Generation of Reactive Oxygen Species (ROS) – Oxidative stress is caused by silver nanoparticles and ROS is a product of degradation of cellular components such as proteins, DNA, and lipids.

Interaction with Proteins and DNA – Ag ions interfere with the microorganism's protein and nucleic acids and destabilize critical processes such as metabolism and replication.

Prevention of Biofilm Formation – CUNPS suppress bacteria from attaching and clustering in biofilms, i.e., protective microbes' biofilms with increased antibiotic and disinfectant resistance.

General Applications: Medical device surface, hospital surfaces, clothing, and food packaging.

- ✓ *Copper Nanoparticles (CUNPS)*

Copper has inherent antimicrobial features, and copper nanoparticles increase such characteristics with increased surface area and reactivity. Copper nanocoating's are toxic by:

Membrane Disruption & Ion Release – Cu²⁺ ion diffused within microbial cell membrane as well as intracellular content disturbed release of cell structure.

Protein & Enzyme Inhibition – inhibition of bacterial primary protein and enzyme, i.e., replication along with metabolic inhibition.

Oxidative Damage & DNA – Cu nanoparticles generate ROS to trigger light-activated oxidative stress-mediated enzyme degradation and DNA damage in microbes.

Door handles, touch screens, public transport surface, and medical devices are common applications.

✓ *ZnO and TiO₂ Nanoparticles*

ZnO and TiO₂ are two natural photosensitizer antimicrobials whose action is significantly enhanced when exposed to light. They are used as:

Photocatalysis & ROS Generation – ZnO and TiO₂ nanoparticles make the photocatalysts UV- or light-sensitized and generate highly reactive superoxide anion (O₂⁻) radicals and super-reactive hydroxyl radical species (•OH), which are toxic to microbial cells.

Disruption of cellular structure – ZnO nanoparticles adhere to the bacteria cell wall and cause lysis through leakages.

Metal ion toxicity – enzymatic action resulting from metal ion intoxication by way of metabolic disruption by zinc ions.

It is also present in its age-old uses as self-cleansing coating, water and air cleaning film, and antimicrobial food wrap film.

✓ *Graphene Oxide (GO) and Carbon-Based Nanomaterials*

Graphene oxide (GO) and carbon nanomaterials are also studied for their antimicrobial action due to the common physical and chemical properties:

Physical Microbe Disruption – GO edges cause disruption of the bacterial membrane to initiate mechanical cell destruction and microbial lysis.

ROS Generation – GO causes generation of reactive oxygen species in microbial cell cells and destabilizes the cell, as well.

Adsorption & Dehydration – GO kills and dehydrates the bacteria by adsorbing the water molecule and microbial protein.

➤ *The Gaps in Conventional Antimicrobial Methods (Disinfectants Vs. Long-Lasting Nanocoating's).*

• *Disinfectants - Gaps*

✓ *Short-Lived Effectiveness:*

▪ *Disinfectants:*

Chemical disinfectants (e.g. Like bleach, alcohol, or quaternary ammonium compounds) which kills germs on contact, but their effects are temporary. Once the surface dries or is touched again, it can quickly become decontaminated.

▪ *Gap:*

This creates a need for frequent reapplication, which can be labour-intensive and can be impractical in high-traffic areas.

✓ *Inconsistent Application:*

▪ *Human Error:*

The effectiveness of disinfectants depends on proper application—using the right concentration, covering the entire surface, and allowing sufficient contact time. In real-world scenarios, these steps are often skipped or done incorrectly.

▪ *Gap:*

Inconsistent application can leave behind "dead zones" where germs survive and spread.

✓ *Resistance Development:*

Antimicrobial Resistance: Overuse of chemical disinfectants can contribute to the development of resistant strains of bacteria and other pathogens, making them harder to kill over time.

▪ *Gap:*

This undermines the long-term effectiveness of conventional methods.

✓ *Toxicity and Environmental Impact:*▪ *Harsh Chemicals:*

Many disinfectants contain toxic ingredients that can harm humans, animals, and the environment. For example, prolonged exposure to bleach fumes can irritate the lungs, and improper disposal of chemicals can pollute water sources.

▪ *Gap:*

Balancing effectiveness with safety and sustainability is a challenge.

✓ *Labor-Intensive and Costly:*▪ *Frequent Cleaning:*

Maintaining hygiene with conventional methods requires constant cleaning and disinfection, which increases labour costs and downtime in places like hospitals, restaurants, and public spaces.

▪ *Gap:*

This approach is not scalable or sustainable in the long run.

• *Long-Lasting Nanocoating Gaps*✓ *Continuous Protection:*

Unlike disinfectants, which work only at the time of application, nanocoating's provide long-lasting antimicrobial activity. They continuously kill or inhibit the growth of pathogens for weeks, months, or even years, depending on the formulation.

▪ *Benefit:*

Reduces the need for frequent reapplication and maintains hygiene between cleanings.

✓ *Self-Sterilizing Surfaces:*

Nanocoating's create surfaces that are inherently antimicrobial. For example, copper-based nanocoating's release ions that disrupt the cell membranes of bacteria and viruses on contact.

▪ *Benefit:*

Surfaces stay cleaner for longer, even in high-touch areas.

✓ *Reduced Reliance on Chemicals:*

Nanocoating's minimize the need for harsh chemical disinfectants, lowering the risk of toxicity and environmental harm.

▪ *Benefit:*

A safer, more sustainable approach to hygiene.

✓ *Resistance Prevention:*

Nanocoating's often use physical mechanisms (e.g., disrupting cell walls) rather than chemical ones, making it harder for pathogens to develop resistance.

▪ *Benefit:*

More reliable long-term protection against microbes.

✓ *Ease of Use and Cost-Effectiveness:*

Once applied, nanocoating's require minimal maintenance. This reduces labour costs and downtime associated with frequent cleaning.

▪ *Benefit:*

A scalable solution for busy environments like hospitals, schools, and public transportation.

➤ *Justify why Developing an Effective Nanocoating Spray is Necessary:*• *Fighting Antibiotic Resistance*

Antimicrobial resistance (AMR) also broke the effectiveness of traditional antibiotics. Antimicrobial nanocoating spray is also preventive since it will limit microbial invasion on the surface and close off infection diffusion without having to utilize the use of antibiotics.

- *Improved Surface Protection*

Hard contact areas like food preparation surfaces, public areas, and healthcare settings are where the sites. Microbial transfer is challenging. Nano coating spray forms a permanently forming barrier protective coating that kills or destroys microbes once and never again without permitting them to initiate infection.

- *Sustained Antimicrobial Action*

In contrast to the traditional short-duration disinfectants, nanocoating's possess enduring antimicrobial actions that extend by days, weeks, and months. This can be contrasted with constant cleansing and disinfecting and hence labour and upkeep expenses.

- *Broad-Spectrum Efficacy*

Nano spray coatings are capable of being rendered non-fungal, non-viral, and non-bacterial and thus deployable. That is overall deployment of such coatings that would be of utmost use when there is more than one pathogen that co-exist.

- *Non-Toxic And Environmentally Friendly Alternatives*

Most conventional disinfectants contain toxic chemicals to humans and the environment. Nanocoating's of biocompatible nanomaterials, or nanocoating's, are an environmentally friendly and green solution that does not compromise functionality.

- *Diverse Application Across Industries Medical Care*

Prevents hospital-acquired infection (HAI) by controlling microbial burden on healthcare equipment and on hospital surfaces.

- *Food Industry:*

Keeps handling, storing, and packaging food as clean as possible and keeps low levels of food diseases.

- *Public Transport & Workspaces:*

Keeps public disease from spreading to the public and keeps workstations and common transport routes clean.

- *Technological Advancement & Market Demand*

As infection control and as-needed issues of hygiene are ever-present on the agenda, so the demand is thus fulfilled for antimicrobial nanocoating treatment. It is nanotechnology and demand for better, safer antimicrobial treatment material science R&D research-driven.

B. Statement of the Problem

➤ *There is a Need for Antimicrobial Nanocoating Sprays.*

- *Antimicrobial Resistance (AMR):*

Overuse of traditional disinfectants and antibiotics has yielded drug-resistant "superbugs" to make the infection more difficult and treatment more complicated.

- *Shelf Life of Traditional Disinfectants:*

Chemical disinfectants have extremely short shelf lives from a few minutes to a few hours and have to be reapplied many times at short intervals with greater labour costs.

- *Public & Health Care Environment High Infection Rate:*

Unhygienic surface in school, transport, factory, and hospital transmit HAI and food disease outbreaks.

- *Time- & Man-Intensive Cleaning:*

Cleaning is time- & man-intensive, expensive, and man-error-prone with patchy cleanliness.

- *Environmental & Medical Issues:*

Excessive use of chemical disinfectants causes toxicity, respiratory diseases, and pollution that not only kill human life but also the environment.

C. Research Objectives

- To assess the antimicrobial effectiveness of nanocoating sprays against a range of bacterial and fungal species.
- To study the physicochemical characteristics of the nanocoating, such as adhesion, durability, and stability in varied environmental conditions.
- To develop the formulation and synthesis of antimicrobial nanocoating's through the variation in nanoparticle composition, concentration, and carrier material.

- To investigate the mechanism of action by which the nanocoating resists microbial growth on treated surfaces.
- To measure the long-term efficacy and recyclability of the coating in actual-use applications, for instance, in a healthcare or industry environment.
- To compare the efficiency of antimicrobial nanocoating's to normal disinfectants and other surface antimicrobial treatments.
- To assess the possible toxicity and environmental effects of nanocoating ingredients in order to secure safe use.
- To investigate possible uses of antimicrobial nanocoating sprays in the healthcare, food packaging, and public infrastructure industries.

D. Significance of the Study

- Improved Infection Control – Antimicrobial nanocoating's can prevent the transmission of pathogens on touched surfaces, thereby decreasing the risk of hospital-acquired infections (HAIs) and other infectious illnesses.
- Long-term Protection – Unlike traditional disinfectants that need to be applied repeatedly, nanocoating sprays provide long-term antimicrobial activity, delivering enduring protection against bacterial and fungal contamination.
- Enhanced Surface Hygiene in Critical Environments – This technology can be very useful in hospitals, laboratories, food processing plants, and public places, where a sterile environment needs to be preserved.
- Nanotechnology Applications Advances – This research is advancing the field of nanotechnology with research on novel formulations that advance the antimicrobial efficacy and the longevous nature of coatings.
- Sustainable and Eco-Friendly Solution – Antimicrobial nanocoating's can be formulated to be less toxic and more environmentally friendly than conventional chemical disinfectants, eliminating chemical waste and pollution.
- Potential Economic Savings – Through decreased disinfection frequency and lower infection rates, antimicrobial nanocoating's have the potential to save costs in healthcare, manufacturing, and maintenance industries.
- Advancing Scientific Knowledge – The research sheds important light on antimicrobial mechanisms of action, surface interactions, and the efficacy of nanomaterials in actual applications.

CHAPTER TWO

LITERATURE REVIEW

A. Overview of Antimicrobial Agents and Antimicrobial Nanocoatings.

➤ *Antimicrobial Agents*

Antimicrobial agents are compounds that inhibit or kill microorganisms, such as bacteria, fungi, viruses, and algae. The agents are important in avoiding infection and contamination in sectors such as healthcare, food processing, and industrial processes.

• *Types of Antimicrobial Agents*

✓ *Chemical Antimicrobials:*

▪ *Antibiotics:*

Inhibit the growth or kill bacteria (e.g., penicillin, tetracycline).

▪ *Disinfectants and Biocides:*

Employed for surface disinfection (e.g., chlorine, hydrogen peroxide, quaternary ammonium compounds).

▪ *Preservatives:*

Applied in drugs and foodstuffs to prevent microbial contamination (e.g., parabens, sorbic acid).

✓ *Natural Antimicrobials:*

▪ *Essential oils (EOs):*

Of plant origin, possessing antimicrobial activity (e.g., thyme oil, tea tree oil).

▪ *Chitosan:*

A biopolymer possessing natural antimicrobial activity, applied in foods' coatings and packaging.

▪ *Peptides and Proteins:*

Like lysozyme and defensins, possessing antimicrobial activity.

✓ *Metal-Based Antimicrobials:*

▪ *Silver (Ag):*

Interferes with bacterial cell membranes and inhibits microbial growth.

▪ *Copper (Cu):*

Possesses broad-spectrum antimicrobial activity.

▪ *Zinc Oxide (Zno) And Titanium Dioxide (Tio₂):*

Applied in antimicrobial coatings due to their photocatalytic property.

➤ *Antimicrobial Nanocoating*

Antimicrobial nanocoatings are thin films or layers with nanoparticles or other antimicrobial agents to provide long-term protection against microbial growth on surfaces. The coatings are used in healthcare, food packaging, textiles, and public spaces to prevent the spread of infection.

• *Mechanism of Action*

Antimicrobial nanocoating's work through multiple mechanisms, including:

✓ *Cell Membrane Damage:*

Nanoparticles such as silver or copper disrupt microbial cell membranes, leading to intracellular contents leakage and cell death.

✓ *Reactive Oxygen Species (ROS) Formation:*

Nanomaterials such as TiO₂ and ZnO form ROS upon exposure to light, causing microbial cell damage.

✓ *Protein and DNA Interference:*

Metal ions have the ability to chelate bacterial DNA or proteins and disrupt essential biological processes.

✓ *Contact Killing & Release Mechanisms:*

Some coatings release antimicrobial agents permanently, while others function by direct contact.

• *Types of Antimicrobial Nanocoating*✓ *Metallic Nanoparticle-Based Coatings:*

Silver, copper, zinc oxide, and gold nanoparticles possess strong antimicrobial action.

✓ *Polymer-Based Nanocoating's:*

Polymers incorporating antimicrobial agents (e.g., chitosan, polyethylene glycol).

✓ *Photocatalytic Nanocoating's:*

Titanium dioxide(TiO₂) and zinc oxide(ZnO) light-activated films to destroy microbes.

✓ *Hybrid Nanocoating's:*

Combination of metal nanoparticles, polymers, and bioactive agents to enhance antimicrobial activity.

• *Uses of Antimicrobial Nanocoating's*✓ *Medical Devices and Healthcare Surfaces:*

For reducing hospital-acquired infections (HAIs).

✓ *Food Packaging:*

To prolong food preservation and safety.

✓ *Textiles:*

Incorporated in apparel, face masks, and medical linen for microbicidal defence.

✓ *Public and Industrial Surfaces:*

Incorporated on high-touch surfaces like door handles, elevators, and transit stations.

B. Mechanism of Action of Antimicrobial Spray and Nanocoating Spray.➤ *Mechanism of Action of Antimicrobial Spray*• *Disruption of Microbial Cell Membranes*

The majority of antimicrobial sprays contain alcohols (isopropanol, ethanol), chlorhexidine, or quaternary ammonium compounds (QACs) as active agents that target the phospholipid bilayer of microbial cell membranes. Such compounds dissolve the lipid portion, leading to membrane destabilization, leakage of intracellular content, and cell death ultimately (Maillard, 2013).

• *Protein Denaturation and Enzyme Inhibition*

Other antimicrobial agents, such as hydrogen peroxide and aldehydes, disrupt microbial enzyme activity by protein denaturation. This inhibition of essential enzymes halts cellular metabolism and replication, rendering the microorganism inactive (Russell, 2002).

• *Oxidative Stress and Reactive Oxygen Species (ROS) Generation*

Oxidizing agents such as sodium hypochlorite (bleach) and hydrogen peroxide generate reactive oxygen species (ROS), which induce oxidative stress in microbial cells. ROS damage cellular constituents including nucleic acids, lipids, and proteins, resulting in permanent cell death (Desarrollo, 2012).

• *Nucleic Acid Disruption*

Some others incorporate silver ions (Ag⁺) or copper ions (Cu²⁺), which enter microbial cells and bind to nucleic acids, interfering with DNA replication and transcription. This action prevents the microorganism's multiplication and consequently reduces microbial load on treated surfaces (Morones-Ramirez et al., 2013).

• *PH Shift And Environmental Factors*

Other antimicrobial products achieve their effects by altering the pH of the surrounding environment to render it intolerable for microbes to exist. Basic or acidic conditions disrupt enzymatic activities and metabolic processes in microbial cells, leading to cell death (Chawla et al., 2018).

C. Mechanism of Action of Antimicrobial Nanocoating Spray nanocoating spray

Antimicrobial nanocoating sprays differ from conventional sprays in that they create a thin, hard film on surfaces with long-term antimicrobial action. These films include nanoparticles or antimicrobial agents in a matrix that actively inhibits microbial adhesion and growth. The primary mechanisms are:

➤ *Contact-Killing Mechanism*

Certain nanocoating's contain positively charged nanoparticles, e.g., quaternary ammonium compounds (QACs) or chitosan, which are electrostatically drawn to the negatively charged bacterial membranes. On contact with each other, these electrostatic forces lyse the microbial cell wall by causing membrane disruption and cell lysis (Pan et al., 2012).

➤ *Sustained Release of Antimicrobial Agents*

Nanocoating's with silver, copper, or zinc oxide nanoparticles slowly release antimicrobial ions over time. The ions interact with microbial cell structures, inhibiting enzymatic functions, protein synthesis, and DNA replication, thereby inhibiting microbial survival (Duncan, 2011).

➤ *Photocatalytic Antimicrobial Activity*

Certain nanocoating's, such as titanium dioxide (TiO₂) and zinc oxide (ZnO), are photocatalytic in nature and are activated when light is available. In the presence of ultraviolet (UV) or visible light, these compounds generate reactive oxygen species (ROS), which degrade microbial cell walls, proteins, and nucleic acids (Foster et al., 2011). The effect is particularly useful for self-cleaning surfaces in public and healthcare environments.

➤ *Superhydrophobic and Super Hydrophilic Surface Effects*

Nanocoating's have the ability to modify the surface properties of materials to create either superhydrophobic or super hydrophilic surfaces. Superhydrophobic coatings avoid microbial adhesion by preventing the accumulation of moisture, and super hydrophilic coatings permit the removal of microbes by self-cleaning (Lathe et al., 2019).

➤ *Encapsulation and Physical Entrapment of Microorganisms*

Other formulations of nanocoating use polymeric or hybrid nanomaterials that capture microbes, preventing them from developing or spreading. Physical barrier effect avoids microbial colonisation and the development of biofilm on covered surfaces (Li et al., 2020).

• *Comparison of Antimicrobial Spray and Nanocoating Spray*

Table 1 This Table Represents the Comparison of Antimicrobial and Nanocoating Spray

Feature	Antimicrobial Spray	Antimicrobial Nanocoating Spray
Duration of Action	Short-term (hours)	Long-term (weeks to months)
Mechanism	Chemical disinfection	Continuous antimicrobial activity
Effectiveness	Requires frequent reapplication	Provides sustained protection
Active Agents	Alcohols, QACs, peroxides	Silver, copper, TiO ₂ , ZnO nanoparticles
Mode of Action	Kills microbes on contact	Prevents microbial adhesion and growth
Application	Used for immediate disinfection	Used for long-term surface protection

The mode of action of antimicrobial sprays and nanocoating sprays is established through the capacity to eliminate or inhibit microbial growth by chemical and physical interactions. Whereas traditional antimicrobial sprays are active quickly by direct membrane damage, oxidative stress, or interference with nucleic acids, their action is limited in duration and requires repeated application. Conversely, antimicrobial nanocoating sprays offer long-term protection through the creation of a hard surface layer that releases antimicrobial agents continuously or alters surface properties to inhibit microbial adhesion. The application of nanotechnology in antimicrobial coatings is a major leap in infection control, with improved durability and efficacy, especially in healthcare, food packaging, and high-contact public areas.

➤ *Types of Antimicrobial Agents and Nanoparticles used in Spray*

Carbopol, Chitosan, Essential oil, Penicillin, Zn Particle

➤ *Carbopol (Polyacrylic Acid)*• *Chemical Formula & Structure:*• *Formula*

(C₃H₄O₂)_n

It is made up of acrylic acid monomers cross-linked into a high-molecular-weight polymer.

Carboxyl (-COOH) groups in the polymer chains assist in stabilizing other active ingredients.

- Molecular Representation:
- Function in Spray:
 - ✓ Serves as a viscosity builder, stabilizer, and controlled-release carrier for antimicrobial agents.
 - ✓ Enhances adhesion of the spray on surfaces for long-lasting protection.
 - ✓ Develops a hydrogel network, regulating moisture content and inhibiting microbial growth.
 - ✓ Antimicrobial Mechanism:
 - ✓ Not inherently antimicrobial in nature, but it is responsible for improving stability and efficacy of active compounds like ZnO and essential oils.
 - ✓ Can assist encapsulation of nanoparticles for better sustained release.
 - ✓ Applications:
 - ✓ Used as an antimicrobial drug in cosmetics and pharmaceutical applications.
 - ✓ Combined usually with silver nanoparticles (AgNPs) and ZnO for enhancing antimicrobial properties.

➤ *Penicillin (B-Lactam Antibiotic)*

- Chemical Formula & Structure:
- Formula: C₁₆H₁₈N₂O₄S
- Core Structure: Has a β -lactam ring, responsible for its antimicrobial activity.
- Molecular Representation:
- C₉H₁₁N₂O₄S
- Function in Spray:

Applicable in medical-grade coatings for inhibiting bacterial contamination

- ✓ Is encapsulatable in nanoparticles with controlled release.
- ✓ Antimicrobial Mechanism:
- ✓ Inhibits bacterial cell wall formation by interfering with penicillin-binding proteins (PBPs).
- ✓ Prevents cross-linking of peptidoglycan, which results in the lysis of bacterial cells.
- ✓ Applied in hospital disinfectants, surgical coatings, and pharmaceutical sprays.
- ✓ Inactive against antibiotic-resistant bacteria unless used in combination with nanocarriers.

➤ *Chitosan (Poly-D-Glucosamine)*

- Chemical Formula & Structure:
- Formula

(C₆H₁₁NO₄)_n

✓ *Structure:*

Natural polymer obtained from chitin that is present in crustaceans' exoskeleton.

Has amino (-NH₂) and hydroxyl (-OH) groups that account for its biological activity.

✓ *Function in Spray:*

- A natural antimicrobial polymer with film-forming capability.
- Acts as a nanocarrier, enhancing nanoparticle stability and delivery (e.g., ZnO, AgNPs).
- Improve surface adhesion, extending antimicrobial action.
- Antimicrobial Mechanism:
- The cationic amino groups (-NH₃⁺) bind to negatively charged bacterial cell membranes, disrupting the cell wall.
- Interferes with the production of vital microbial proteins and also with DNA binding, - inhibiting replication.
- Exhibits broad-spectrum antimicrobial activity against bacteria, fungi, and certain viruses.
- Applications:
- Applied in food packaging, biomedical coatings, and wound dressings.

Can be formulated into smart antimicrobial sprays with pH-sensitive release properties.

➤ *Zinc (Zn) and Zinc Oxide (Zno) Nanoparticles*

- Chemical Formula & Structure: Zn (Elemental Zinc)
- Formula: Zn

- *Structure:*
Metallic solid with a hexagonal crystal lattice.

Zinc Oxide (ZnO)

- Formula: ZnO
- *Structure:*
Composed of zinc ions (Zn^{2+}) and oxygen ions (O^{2-}) arranged in a wurtzite crystal structure.
- *Function in Spray:*
 - ✓ Used as an antimicrobial and photocatalytic agent.
 - ✓ Provides long-lasting antimicrobial protection by releasing zinc ions (Zn^{2+}).
 - ✓ Antimicrobial Mechanism:
 - ✓ Generates Reactive Oxygen Species (ROS)
 - ✓ ZnO produces hydrogen peroxide (H_2O_2), superoxide anions (O_2^-), and hydroxyl radicals ($\bullet\text{OH}$).
 - ✓ These ROS damage bacterial membranes, proteins, and DNA.
 - ✓ Disrupts Bacterial Cell Membranes
 - ✓ Zn^{2+} ions interact with bacterial membranes, causing leakage of essential cellular components.
 - ✓ Inhibits Enzyme Function & DNA Replication
 - ✓ Zn^{2+} interferes with microbial enzymes and proteins, blocking essential metabolic pathways.
 - ✓ Applications:
 - ✓ Used in self-cleaning coatings, food packaging, wound dressings, and antimicrobial textiles.
 - ✓ Combined with silver (AgNPs) for enhanced broad-spectrum activity.

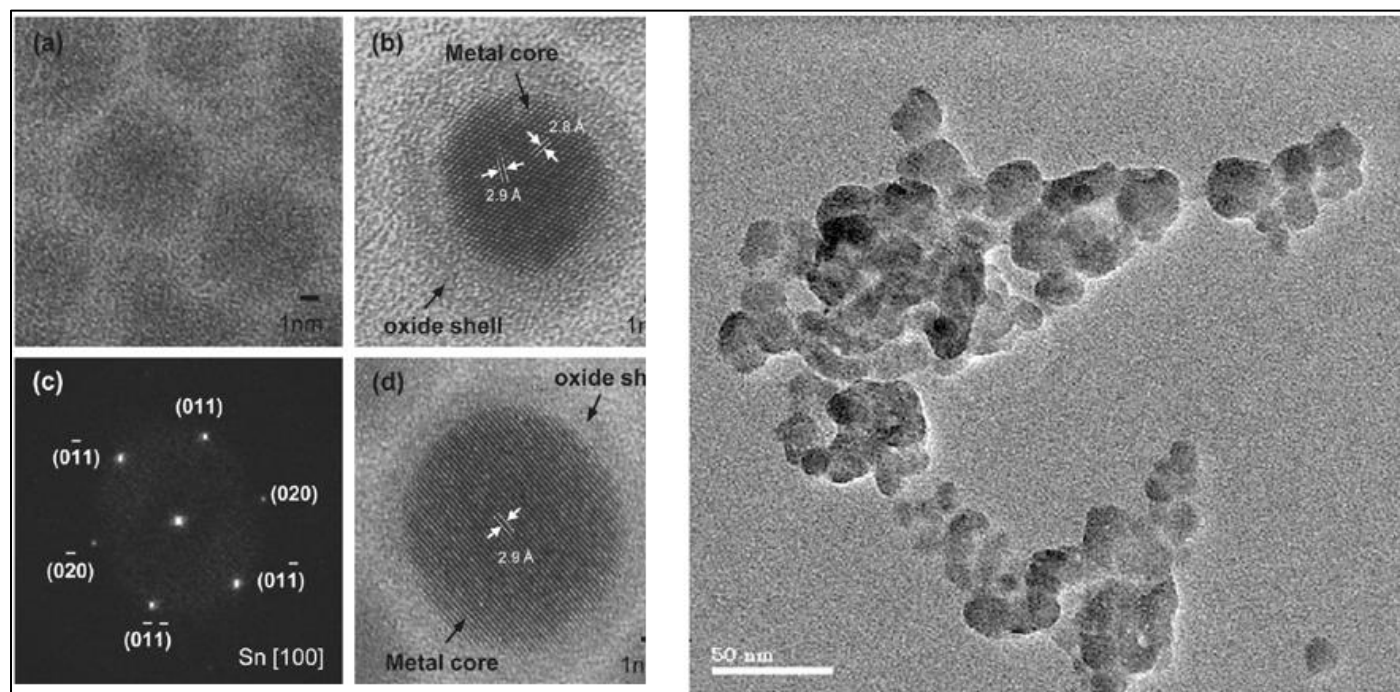


Fig 1 SEM and TEM Images Of Zinc (Zn) And Zinc Oxide (Zno) Nanoparticles.

SEM shows Zn nanoparticles as irregular or quasi-spherical with moderate aggregation, while ZnO appears more defined, typically spherical or rod-shaped with slight agglomeration. TEM reveals Zn as electron-dense and amorphous, whereas ZnO shows clear lattice fringes, confirming a crystalline wurtzite structure. Particle sizes range from 1 nm- 50 nm.

➤ *Neem Oil (Azadirachta indica Oil)*

- Chemical Formula & Structure:
- Neem oil is a complex bioactive mixture of compounds. The most important antimicrobial constituents are:

- ✓ Azadirachtin (C₃₅H₄₄O₁₆) – main antimicrobial constituent
- ✓ Nimbin (C₃₀H₃₆O₉) – antifungal and antibacterial
- ✓ Nimbidin – anti-inflammatory and antimicrobial
- ✓ Salannin – insecticidal and antimicrobial
- Azadirachtin Structure: C₃₅H₄₄O₁₆
- Function in Spray:
 - ✓ Natural antibacterial, antifungal, and antiviral agent.
 - ✓ Nanoencapsulable to enhance stability and controlled release.
 - ✓ Forms a bio-protective film, inhibiting microbial adhesion to surfaces.
 - ✓ Antimicrobial Mechanism:
 - ✓ Cell Membrane Disruption:
 - ✓ Azadirachtin acts on lipid bilayers of microbial cell membranes, leading to leakage of intracellular contents.
 - ✓ Inhibits Microbial Growth & Replication:
 - ✓ Inhibits enzyme function, inhibiting bacterial metabolism.
 - ✓ Induces Oxidative Stress:
 - ✓ Produces reactive oxygen species (ROS), which harm microbial DNA and proteins.
 - ✓ Applications:
 - ✓ Applied in food-safe coatings, medical disinfectants, and personal care sprays.

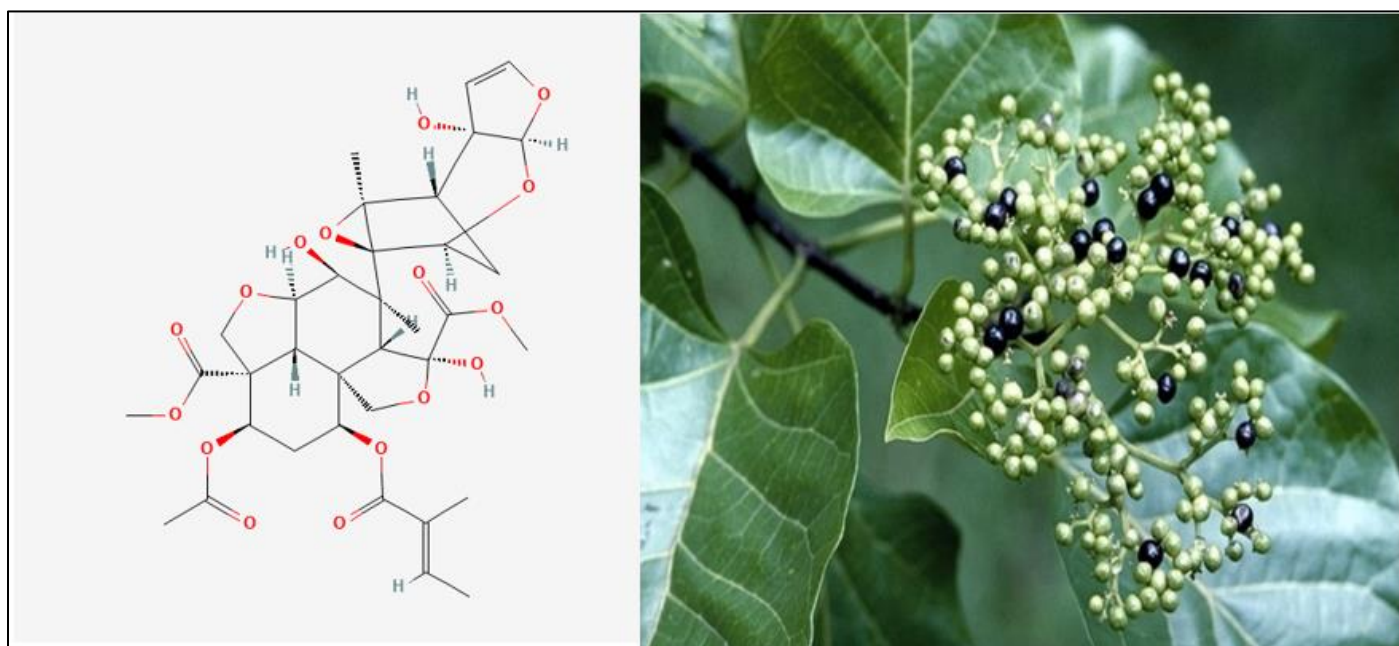


Fig 2 Neem Oil (*Azadirachta Indica*) – Chemical Structure and Plant Image.

The figure shows the chemical structure of key bioactive compounds in Neem oil, such as Azadirachtin, alongside the image of the Neem plant (*Azadirachta indica*), known for its broad-spectrum antimicrobial and therapeutic properties.

➤ *Chemical and Physical Properties of (Azadirachta Indica)*

Table 2 Represents the Chemical and Physical Properties of Azadirachta Indica (Neem) and its Oil – Abbreviations and Descriptions.

Property Name	Property Value	Reference
---------------	----------------	-----------

Molecular Weight	720.7 g/mol	Computed by PubChem 2.2 (PubChem release 2021.10.14)
XLogP3-AA	-0.6	Computed by XLogP3 3.0 (PubChem release 2021.10.14)
Hydrogen Bond Donor Count	3	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)
Hydrogen Bond Acceptor Count	16	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)
Rotatable Bond Count	10	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)
Exact Mass	720.26293531 Da	Computed by PubChem 2.2 (PubChem release 2021.10.14)
Monoisotopic Mass	720.26293531 Da	Computed by PubChem 2.2 (PubChem release 2021.10.14)
Topological Polar Surface Area	215 Å ²	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)
Heavy Atom Count	51	Computed by PubChem
Formal Charge	0	Computed by PubChem
Complexity	1660	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)
Isotope Atom Count	0	Computed by PubChem
Defined Atom Stereo enter Count	16	Computed by PubChem
Undefined Atom Stereo enter Count	0	Computed by PubChem
Defined Bond stereo enters Count	1	Computed by PubChem
Undefined Bond stereo enters Count	0	Computed by PubChem
Covalently-Bonded Unit Count	1	Computed by PubChem
Compound Is Canonicalized	yes	Computed by PubChem (release 2021.10.14)

➤ *Hingot Oil (Balanites aegyptiaca (L.) Delile)*

- Botanical Name: *Balanites aegyptiaca* (L.) Delile
- Common Names: Hingot, Desert Date, Soap Berry Tree
- Family: Zygophyllaceae
- Parts Used: Seeds (kernels)

➤ *Source and Extraction:*

Hingot oil is extracted from the seeds of the *Balanites aegyptiaca* tree, a drought-resistant species commonly found in arid and semi-arid regions of Africa, the Indian subcontinent, and the Middle East. The seeds are sun-dried, dehulled, and then cold-pressed or solvent-extracted to obtain the yellow to greenish-colored oil.

➤ *Physical Properties:*

- *Appearance:*
Pale yellow to greenish-yellow oil
- *Odor:*
Mild, characteristic
- *Texture:*
Light and non-greasy
- *Solubility:*
Insoluble in water, soluble in organic solvents
- *Specific Gravity:*
~0.91–0.93
- *Viscosity:*
Medium
- *Refractive Index:*
~1.465–1.470

➤ *Chemical Composition:*

Hingot oil is rich in both saturated and unsaturated fatty acids and bioactive phytochemicals.

- *Fatty Acids:*

- ✓ Oleic acid (C18:1) – 25–30%
- ✓ Linoleic acid (C18:2) – 35–45%
- ✓ Palmitic acid (C16:0) – 15–20%
- ✓ Stearic acid (C18:0) – 10–15%

• *Bioactive Compounds:*

- ✓ Saponins
- ✓ Alkaloids
- ✓ Steroids (β -sitosterol)
- ✓ Diosgenin (precursor to steroid hormones)

➤ *Pharmacological and Traditional Uses:*

- *Antimicrobial*
Effective against bacteria and fungi due to its saponin and fatty acid content.
 - *Anti-inflammatory:*
Reduces skin inflammation and redness.
 - *Wound Healing:*
Promotes tissue regeneration and accelerates wound closure.
 - *Antioxidant*
Scavenges free radicals, contributing to skin protection.
 - *Insecticidal:*
Used in traditional pest control due to natural insect-repelling properties.
 - *Cosmetic Applications:*
Used in herbal soaps, lotions, and skin oils for its moisturizing and healing effects.
- *Applications in Formulations:*
- Herbal ointments
 - Antimicrobial sprays
 - Hair and skin care products
 - Soap and natural cleansers
 - Traditional medicine preparations for skin infections



Fig 3 The Image Shows *Balanites Aegyptiaca* (L.) Delile, a Spiny, Drought-Tolerant Tree Native to Dry Regions of Africa and Asia.

D. Effectiveness against Antimicrobial Efficiency.➤ *Influences on Antimicrobial Effectiveness*

There are several key factors that govern the effectiveness of antimicrobial agents as inhibitors or killers of microbes:

• *Concentration and Contact Time*

Higher concentrations of antimicrobial agents typically lead to faster and more efficient microbial inhibition.

Contact time is critical—some agents require longer exposure to be as effective as possible (e.g., quaternary ammonium compounds versus alcohols).

✓ Example: 70% ethanol is better than 100% ethanol since water aids in protein denaturation and penetration.

• *Mode of Action*

Different antimicrobial agents exert their action on microbes via cell membrane disruption, protein denaturation, interference with DNA, oxidative stress, or inhibition of enzymes.

Broad-spectrum agents (e.g., metal nanoparticles, oxidizing agents) are generally more effective against a wide range of microbes.

• *Microbial Type and Resistance Mechanisms*

Gram-positive bacteria (e.g., *Staphylococcus aureus*) are more susceptible to alcohols and QACs due to their simpler cell walls.

Gram-negative bacteria (e.g., *Escherichia coli*, *Pseudomonas aeruginosa*) possess an outer membrane that makes them resistant to certain antimicrobial agents.

Bacterial biofilms and fungal spores are more resistant and require more severe or combined antimicrobial treatment.

• *Environmental Conditions*

pH, temperature, humidity, and organic matter impact antimicrobial efficacy.

Example: Hypochlorite (bleach) becomes less effective at high organic load due to chemical neutralization.

• *Antimicrobial Resistance Development*

Sublethal and repeated antimicrobial exposure to certain antimicrobials (e.g., triclosan, QACs) will lead to microbial adaptation and resistance.

Metal antimicrobials (such as copper, silver) have lower resistance emergence due to their multifaceted mechanisms of action.

➤ *Comparative Effectiveness of Different Antimicrobial Agents*

Table 3 This Table Presents a Comparative Analysis of Various Antimicrobial Agents, Highlighting their Effectiveness against Different Types of Microorganisms.

Antimicrobial Agent	Mode of Action	Microbial Target	Effectiveness	Limitations
Alcohols (Ethanol, Isopropanol)	Protein denaturation, membrane disruption	Bacteria, fungi, some viruses	Rapid action, evaporates quickly	Ineffective against spores, requires frequent application
Quaternary Ammonium Compounds (QACs)	Membrane disruption, leakage of cytoplasmic contents	Bacteria, fungi, enveloped viruses	Residual activity, good for surfaces	Less effective against non-enveloped viruses and biofilms
Hydrogen Peroxide (H ₂ O ₂)	Oxidative stress, DNA/protein damage	Broad-spectrum (bacteria, fungi, viruses)	Strong disinfectant, no residue	Can degrade on exposure to light
Silver Nanoparticles (AgNPs)	DNA disruption, protein binding, oxidative stress	Bacteria (including resistant strains), fungi	Long-lasting, low resistance development	Toxicity at high concentrations
Copper Nanoparticles (CuNPs)	Oxidative stress, enzyme inhibition	Bacteria, fungi, viruses	Self-disinfecting surfaces, stable over time	Can oxidize and reduce efficiency
Essential Oils (Thymol, Eugenol, Tea Tree Oil)	Membrane disruption, metabolic inhibition	Bacteria, fungi	Natural, biodegradable	Volatile, requires stabilization

Chitosan-Based Antimicrobials	Electrostatic interaction with cell membranes	Bacteria, fungi	Biodegradable, non-toxic	Solubility limitations
Titanium Dioxide (TiO ₂) Photocatalytic Coatings	ROS generation under UV light	Broad-spectrum	Self-cleaning, long-lasting	Requires light activation

It summarizes key parameters such as the spectrum of activity, minimum inhibitory concentration (MIC), mode of action, and potential clinical applications. The data facilitate understanding of the relative strengths and limitations of each agent, aiding in informed decision-making for antimicrobial therapy and research.

➤ *Synergistic Effects and Combined Antimicrobials*

For increasing efficacy, combination strategies are regularly employed in antimicrobial sprays:

- Alcohol + QACs → Increases efficacy against more bacteria and viruses.
- Silver + Titanium Dioxide → Both antimicrobial and photocatalytic effects for self-sterilizing surfaces.
- Essential Oils + Chitosan → Improves antimicrobial effectiveness without losing biocompatibility.

E. Overview of Wound Healing

➤ *Introduction*

Wound healing is a complex, dynamic, and highly regulated biological process that restores the integrity and function of damaged tissue. It involves a sequence of overlapping phases: Hemostasis, inflammation, proliferation, and remodelling. Proper healing requires coordination between various cell types, cytokines, extracellular matrix (ECM) components, and signaling molecules.

➤ *Types of Wounds*

- Acute wounds: Heal in an orderly and timely manner (e.g., surgical incisions, trauma).
- Chronic wounds: Fail to progress through normal healing stages (e.g., diabetic ulcers, pressure sores).
- Open wounds: Involve breakage of skin (abrasions, lacerations).
- Closed wounds: Involve tissue damage without skin break (contusions, hematomas).

➤ *Phases of Wound Healing*

• *Hemostasis Phase (Immediate)*

- ✓ Timeframe: Seconds to hours after injury
- ✓ Purpose: Stop bleeding
- ✓ Key events:
 - Vasoconstriction of blood vessels.
 - Platelet aggregation and clot formation.
 - Release of clotting factors (fibrin, thrombin).
 - Formation of a fibrin clot that acts as a temporary matrix.
 - Key Players: Platelets, clotting cascade proteins

• *Inflammatory Phase*

- ✓ Timeframe: 0–3 days (acute); may persist in chronic wounds
- ✓ Purpose: Remove debris/pathogens, recruit repair cells
- ✓ Key events:
 - Vasodilation and increased vascular permeability.
 - Influx of neutrophils (early) and macrophages (later).
 - Phagocytosis of microbes, debris, and damaged tissue.
 - Cytokine and chemokine release (IL-1, IL-6, TNF- α).
 - Initiation of tissue repair signaling.
 - Key Players: Neutrophils, macrophages, mast cells, cytokines

• *Proliferative Phase*

- ✓ Timeframe: 3–10 days post-injury

- ✓ Purpose: Tissue regeneration and repair
- ✓ Key events:

- Fibroblast activation and migration.
- Collagen (mainly type III) synthesis.
- Angiogenesis (new capillary formation).
- Keratinocyte proliferation and re-epithelialization.
- Formation of granulation tissue.
- ECM deposition and provisional matrix formation.
- Key Players: Fibroblasts, keratinocytes, endothelial cells, VEGF
- *Maturation/Remodelling Phase*

- ✓ Timeframe: Weeks to months (up to a year)
- ✓ Purpose: Restore tissue strength and function
- ✓ Key events:

- Collagen remodelling (type III to type I).
- Apoptosis of unnecessary cells.
- Cross-linking and alignment of collagen fibers.
- Reduction in vascularity and cellularity.
- Scar tissue formation.
- Key Players: Myfibroblasts, matrix metalloproteinases (MMPs), TGF- β

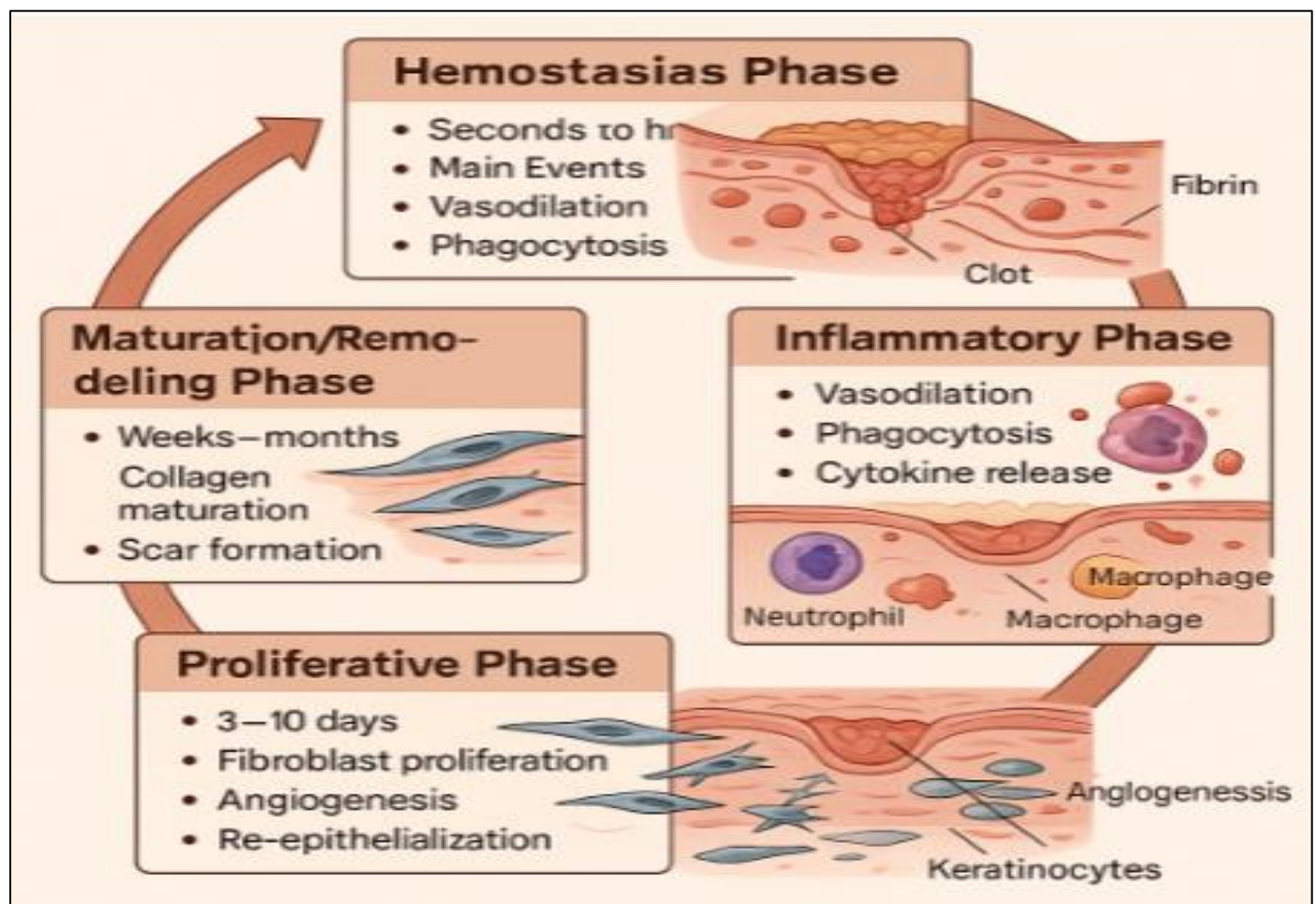


Fig 4 Phases of Wound Healing

➤ Cellular Components in Wound Healing

Table 4 Cellular Components in Wound Healing

Cell Type	Function
Platelets	Hemostasis, release growth factors (PDGF, TGF- β)

Neutrophils	Early defense, phagocytosis, ROS production
Macrophages	Cleanup, cytokine secretion, switch from M1 (inflammatory) to M2 (healing)
Fibroblasts	Collagen production, ECM remodelling
Endothelial Cells	Angiogenesis
Keratinocytes	Re-epithelialization
Mast Cells	Inflammatory mediator release

➤ *Molecular Signaling & Mediators*

- Growth Factors: VEGF, PDGF, TGF- β , FGF, EGF, IGF
- Cytokines & Chemokines: IL-1, IL-6, TNF- α , MCP-1
- Enzymes: MMPs (for ECM degradation), TIMPs (MMP inhibitors)
- Matrix Proteins: Collagen, fibronectin, laminin

➤ *Actors Affecting Wound Healing*

- Positive Influences:
- Proper nutrition (protein, vitamins A/C/E, zinc)
- Oxygenation and perfusion
- Aseptic wound care
- Growth factor therapy
- Negative Influences:
- Systemic: Diabetes, aging, malnutrition, immunosuppression
- Local: Infection, ischemia, edema, repeated trauma
- External: Smoking, corticosteroid therapy, radiation

➤ *Abnormal Wound Healing*

Table 5 Abnormal Wound Healing

Condition	Description
Chronic wounds	Stalled healing due to infection, ischemia
Hypertrophic scars	Excess collagen within wound boundary
Keloids	Overgrowth of scar tissue beyond wound margins
Wound dehiscence	Reopening of a closed wound
Contractures	Excessive contraction leading to deformity

➤ *Safety and Regulatory Bio Compatibility Considerations.*

Here is a keener analysis of safety and regulatory aspects for biocompatibility:

• *ISO 10993 Guidelines:*

- ✓ Follow the ISO 10993 series for biological interaction evaluation.
- ✓ Keep tests for cytotoxicity, sensitization, irritation, and systemic toxicity on priority.

• *FDA Guidance:*

- ✓ Comply with FDA standards such as the application of Good Laboratory Practices (GLP) for biocompatibility tests.
- ✓ Emphasize device classification and purpose in the body's contact.

• *Material Analysis:*

- ✓ Perform chemical characterization to determine possible leachable or degradation products.
- ✓ Employ biostable and non-immunogenic materials.

• *Biological Response Testing:*

- ✓ Conduct in vitro and in vivo testing for acute and chronic biological responses.
- ✓ Incorporate genotoxicity and hemocompatibility tests where relevant.

- *Risk Mitigation:*

- ✓ Assess device-specific biological risks (e.g., degradation products or long-term implantation risks).
- ✓ Utilize extensive risk analyses by means of ISO 14971 for medical device risk management.

- *Documentation:*

- ✓ Keep extensive documentation on material choice, testing schedules, and risk assessments for submissions to the regulators.

- *Safety Considerations*

- *Human Health and Toxicity Risks*

Skin and Respiratory Sensitization: Some antimicrobial agents (e.g., quaternary ammonium compounds, phenolics, silver nanoparticles) may cause skin irritation, allergic reactions, or respiratory issues with prolonged exposure.

Inhalation Toxicity: Sprays that produce fine aerosols may pose inhalation risks, particularly in healthcare workers and industrial workers.

- ✓ Cytotoxicity: Metal-based nanomaterials (AgNPs, CuNPs) may exhibit cytotoxic effects at high concentrations, potentially affecting human cells and beneficial microbiota.
- ✓ Bioaccumulation Risks: Some antimicrobial agents (e.g., silver and copper nanoparticles) can accumulate in biological systems, leading to potential toxicity concerns over long-term exposure.

- *Environmental Impact*

Persistence and Bioaccumulation: Some antimicrobial substances, particularly synthetic chemicals and heavy metals, have the potential to persist in the environment and become ecotoxic.

Antimicrobial Resistance (AMR): Misuse or overuse of antimicrobial sprays can result in the emergence of resistant bacterial populations, rendering standard antibiotics ineffective.

Water and Soil Pollution: Antimicrobial coatings (e.g., chlorinated disinfectants, silver nanoparticles) may have runoffs affecting aquatic life and changing natural microbial populations.

- *Safe Handling And Exposure Limits*

Agencies like OSHA (Occupational Safety and Health Administration) and EPA (Environmental Protection Agency) offer regulations concerning exposure levels for chemicals contained in antimicrobial sprays.

Proper ventilation, personal protective equipment (PPE), and methods of controlled application should be used to reduce exposure risks.

- *Regulatory Structure for Antimicrobial Nanocoating's*

Regulatory clearance of antimicrobial nanocoating's and sprays relies on the purpose of use, formulation, and safety profile. Different agencies globally regulate antimicrobial products to secure safety, effectiveness, and environmental protection.

- *United States Regulations*

- ✓ EPA (Environmental Protection Agency):
- ✓ Regulates antimicrobial coatings under FIFRA (Federal Insecticide, Fungicide, and Rodenticide Act).
- ✓ Prescribes toxicology testing, environmental impact study, and antimicrobial efficacy verification.

- *FDA (Food and Drug Administration):*

- ✓ Controls antimicrobial coatings on medical devices, food contact materials, and personal care products.
- ✓ Requires biocompatibility testing under ISO 10993 for medical application.

- *European Union Regulations*

- ✓ European Chemicals Agency (ECHA) – Biocidal Products Regulation (BPR):
- ✓ Controls antimicrobial coatings to ensure safety, effectiveness, and environmental sustainability.
- ✓ Requires extensive risk assessment and compliance labelling.
- ✓ European Food Safety Authority (EFSA):

- ✓ Controls antimicrobial coatings for food packaging and contact materials.

- *Other International Regimes*

- ✓ China's NMPA (National Medical Products Administration): Regulates antimicrobial coatings applied in the medical field.
- ✓ Japan's Ministry of Health, Labour and Welfare (MHLW): Oversees antimicrobial product safety standards.
- ✓ ISO Standards (International Organization for Standardization):
- ✓ ISO 22196: Tests antimicrobial activity on surfaces.
- ✓ ISO 10993: Biocompatibility testing of medical devices.

- *Biocompatibility Considerations*

- *Definition of Biocompatibility*

Biocompatibility is the ability of an antimicrobial coating to interact with biological systems without inducing toxicity, irritation, or adverse immune reactions. Of particular importance in medical application, including:

- ✓ Wound dressings
- ✓ Implants and catheters
- ✓ Surgical equipment coatings

- *Biocompatibility Testing of Antimicrobial Sprays*

Biocompatibility testing follows ISO 10993 standards and includes:

- ✓ Cytotoxicity Assays: Tests if the coating is cytotoxic to human cells.
- ✓ Sensitization Testing: Tests for potential immune or allergic reaction.
- ✓ Irritation Studies: Determines potential for mucosal and skin irritation.
- ✓ Genotoxicity and Carcinogenicity: Tests for DNA damage and long-term cancer risks.
- ✓ Hemocompatibility (for medical devices): Restores no negative impact on blood components.

- *Biodegradable and Eco-Friendly Antimicrobial Coatings*

- ✓ For enhancing biocompatibility and sustainability, biodegradable and natural antimicrobial agents are emerging:
- ✓ Chitosan-Based Nanocoating's: Biodegradable, non-toxic, and naturally derived.
- ✓ Essential Oil-Infused Sprays: Provide antimicrobial action with low environmental footprint.
- ✓ Polymeric Nanocoating's (PLGA, PCL): Exhibit controlled release with high biocompatibility.

- *Future Directions in Safety and Biocompatibility*

To further improve the safety of antimicrobial nanocoating's, future studies should aim at:

- Creating non-toxic, biodegradable antimicrobial agents (e.g., chitosan, plant-derived agents).
- Minimizing nanoparticle toxicity through surface coating modification or safer alternatives.
- Enacting antimicrobial resistance monitoring to monitor emerging resistant strains.
- Improving green synthesis routes for metal nanoparticles to minimize environmental pollution.
- Harmonization of regulations to establish global safety standards for antimicrobial coatings.

- *Review of Existing Studies.*

- *Studies on the Mechanism of Antimicrobial Nanocoating's.*

- *Silver Nanoparticles (AgNPs):*

- A study by Rai et al. (2020) demonstrated that AgNPs disrupt bacterial cell membranes, generate reactive oxygen species (ROS), and interfere with DNA replication, leading to microbial death.
- Research by Li et al. (2021) confirmed that AgNP coatings effectively reduce bacterial adhesion and biofilm formation on medical devices.

- *Copper Nanoparticles (CuNPs):*

- A study by Grass et al. (2019) found that CuNP coatings induce oxidative stress in bacteria, causing lipid peroxidation and enzyme inhibition.

➤ *Polymeric and Hybrid Nanocoatings:*

- Chitosan-based coatings, as studied by Tarek et al. (2021), demonstrated strong antimicrobial properties due to electrostatic interactions with bacterial cell walls, leading to membrane disruption.
- Silica-TiO₂ hybrid nanocoating's, according to Wang et al. (2023), exhibited photocatalytic antimicrobial activity under UV light, effectively killing drug-resistant bacteria.

➤ *Studies on the Effectiveness of Antimicrobial Nanocoating's Healthcare Applications*

- Hosseini et al. (2021) conducted a study on hospital ICU surfaces treated with silver and copper nanocoating's, reporting a 95% reduction in multidrug-resistant (*MRSA*, *Pseudomonas aeruginosa*) bacteria over six months.
- Kumar et al. (2022) analysed antimicrobial coatings on catheters and implants, demonstrating significant prevention of bacterial colonization, reducing hospital-acquired infections (HAIs).

➤ *Studies on the Effectiveness of Antimicrobial Nanocoating's*

- Hosseini et al. (2021) conducted a study on hospital ICU surfaces treated with silver and copper nanocoating's, reporting a 95% reduction in multidrug-resistant (*MRSA*, *Pseudomonas aeruginosa*) bacteria over six months.
- Kumar et al. (2022) analysed antimicrobial coatings on catheters and implants, demonstrating significant prevention of bacterial colonization, reducing hospital-acquired infections (HAIs).

➤ *Food Industry Applications.*

Research by Gomez et al. (2020) evaluated chitosan-based antimicrobial sprays in food packaging, showing prolonged shelf life and reduced spoilage bacteria.

➤ *Public and Industrial Surface Applications*• *Self-Sterilizing Surfaces in Public Spaces:*

Chen et al. (2021) tested TiO₂-based photocatalytic coatings in public transportation, observing a 77% reduction in bacterial and viral contamination.

Hybrid polymeric coatings with ZnO nanoparticles were evaluated by Santos et al. (2022) on touchscreens, reducing pathogen survival rates significantly.

➤ *Studies on Safety, Biocompatibility, and Environmental Impact*• *Human Safety and Biocompatibility*

- ✓ ISO 10993 biocompatibility studies, as conducted by Liu et al. (2021), indicated that chitosan-silver hybrid coatings exhibited low cytotoxicity and excellent wound healing potential.

• *Environmental Impact Studies.*

- ✓ EPA-regulated studies reviewed by Smith et al. (2023) found that AgNPs in wastewater discharge can accumulate in aquatic ecosystems, affecting microbial diversity.
- ✓ Biodegradable polymer coatings (e.g., PLA-chitosan composites) were recommended as eco-friendly alternatives due to their low persistence in the environment.

CHAPTER THREE

RESEARCH METHODOLOGY

➤ *Research Design*

- *Introduction*

Experimental research design with qualitative and quantitative analysis is utilized to validate the effectiveness, stability, and usability of the integrated nanocoating's in the chapter. Research design for the integration and validation of antimicrobial nanocoated surfaces for decontaminating public and medical facilities is presented.

- *Research Approach*

Mixed-method study design is used in experimental application-based and laboratory-based testing. Design facilitates high-throughput antimicrobial efficacy, stability, and usability feasibility of synthesized nanocoating's testing.

➤ *Research Design*

There are three phases of research:

- *Phase 1: Synthesis of Antimicrobial Nanocoating*

- ✓ Selection of antimicrobial agent (e.g., silver nanoparticles, copper oxide, zinc oxide)
- ✓ Synthesis circuits like sol-gel, chemical vapor deposition (CVD), and plasma spraying
- ✓ Optimization of nanocoating formulation and deposition process

- *Phase 2: Characterization of Nanocoating*

- ✓ Structural and Morphological Characterization: Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM)
- ✓ Chemical Composition Characterization: X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Energy-Dispersive X-ray Spectroscopy (EDX)
- ✓ Analysis of Surface Properties: Contact angle measurement, roughness, adhesion strength test

- *Phase 3: Test of Antimicrobial Activity and Stability Test*

- ✓ Test of Antibacterial and Antiviral Activity:
- ✓ Zone of inhibition (ZOI) method
- ✓ Colony-forming unit (CFU) reduction tests
- ✓ Viral plaque assays
- ✓ Reverse transcription-polymerase chain reaction viral inactivation analysis
- ✓ Antimicrobial resistance to mechanical wear
- ✓ Resistance to exposure to extreme conditions (temperature, humidity)
- ✓ Resistance to antimicrobial long-term effect on a series of cleaning processes

➤ *Experimental Procedure*

Step-by-step procedure used in measurement of reproducibility and consistency of results. Laboratory standard procedure used in the prevention of external interference or contamination.

➤ *Acquisition and Processing*

- Quantitative test result of antimicrobial performance, surface test, and durability tests
- Statistical analysis with ANOVA, t-tests, and regression analysis to establish statistical difference in performance
- Qualitative determination of adhesion quality, user comfort, and material compatibility

➤ *Ethical Considerations*

- Compliance with biosafety in handling pathogens
- Environmental and toxicology testing of commercial nanocoating's for the intent of facilitating safe human and environmental exposure

➤ *Limitations*

- Practical environmental and other variation of nanocoating's performance
- Scale of concern in application for commerce or industry

➤ *Materials And Formulation of Antimicrobial Spray*

- *Ingredients which are Required*

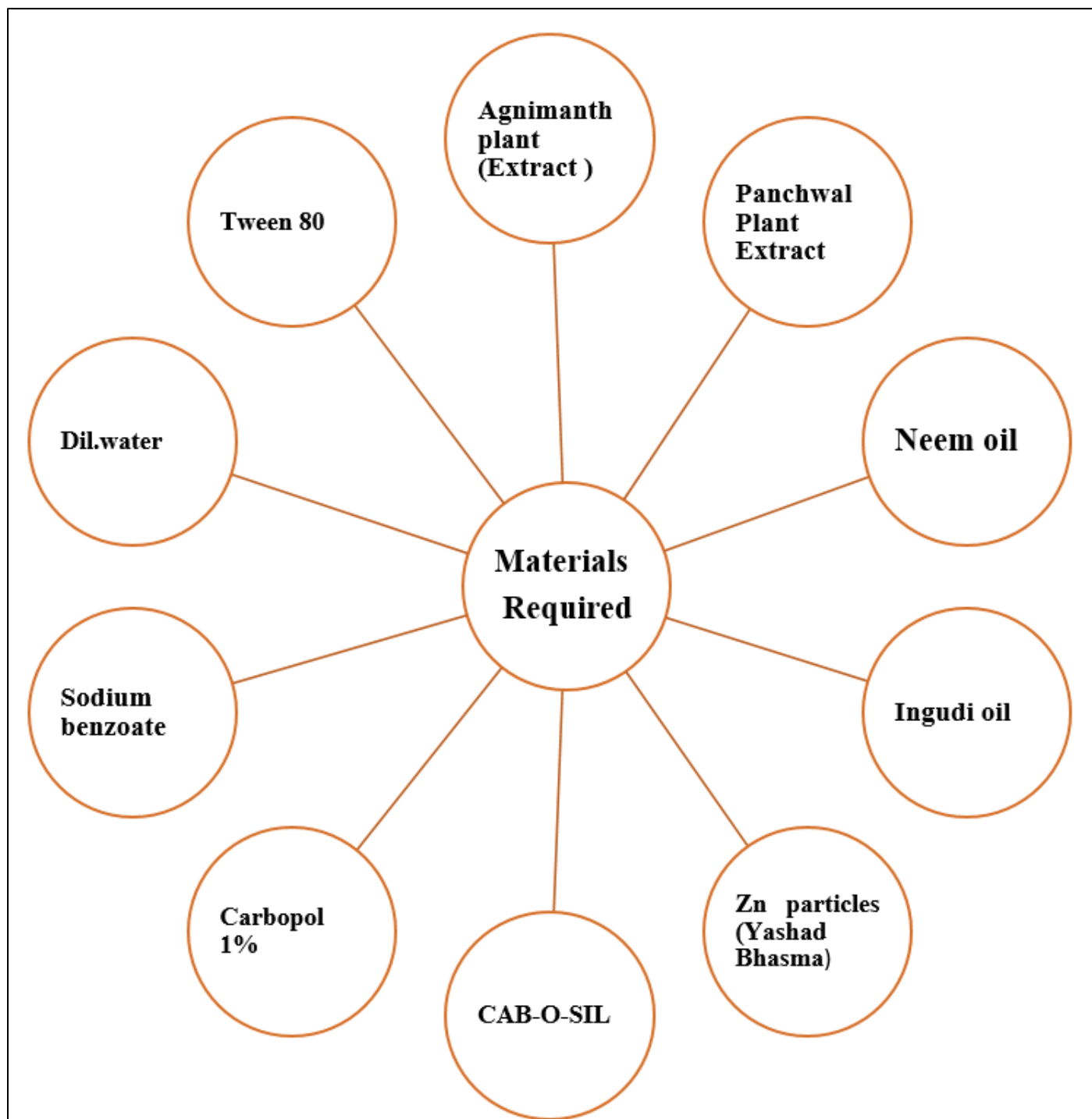


Fig 5 Visual Representation of Oils and Herbal Extracts used in the Antimicrobial Spray Formulation



Fig 6 Extracts and Chemicals Used in Formulation

➤ *Blend Formulation*

Table 6 Shows the Composition of the Various Blend Formulations used in the Antimicrobial Spray.

A. Neem Oil Blend Formulation	
INGREDIENT	QUANTITY
Neem oil	3cml
Tween 80	4 ml
Dil. Water	93 ml
Total	100ml
B. Carbopol Blend Formulation	

Carbopol	0.1g
Dil.water	250 ml
C. Hingot Oil Blend Formulation	
Hingot Oil	3 ml
Tween 80	4 ml
Dil. Water	93 ml
Total	100 ml
D. Mix All the Blend Together	
Neem oil Blend	100ml
Hingot Oil Blend	100ml
Carbopol Blend	250ml

- *Other Formulation*

- ✓ Agnimanth 5%
- ✓ Panchvalkal 5%
- ✓ Ingudi 3%
- ✓ Neem oil 3%
- ✓ Yashad Bhasma 0.5%
- ✓ Carbopol 0.1%
- ✓ CAB O SIL 3%

- *Neem Oil Blend* was prepared using 3 ml neem oil, 4 ml Tween 80, and 93 ml diluted water (total 100 ml) for its potent antimicrobial properties. **Neem Oil Blend Formulation**
- *Hingot Oil Blend* included 3 ml Hingot oil, 4 ml Tween 80, and 93 ml diluted water (total 100 ml), aimed at enhancing wound healing and antibacterial action.
- *Carbopol Blend* was made by dissolving 0.1 g Carbopol in 250 ml water to serve as the gel base. *Final Mixed Blend* was obtained by combining 100 ml of the neem blend, 100 ml of the Hingot blend, and 250 ml of the Carbopol blend to create a stable and effective antimicrobial formulation.

➤ *Methodology*

- *Process for Making Plant Extract*

- ✓ Dried Agnimanth leaves / Panchwaal Plant – 50 g
- ✓ Ethanol (70–95%) or Isopropyl Alcohol – 250 ml

- *Step 1*

- ✓ Crush the dried plant material into small pieces.
- ✓ Place in a glass jar and cover with alcohol (ensure its fully submerged).
- ✓ Seal the jar and keep it in orbital shaker for 24 hours
- ✓ Let it sit in a dark place for 7–14 days.
- ✓ Strain using a fine cloth or filter paper.
- ✓ Add Citric acid for Both Agnimanth plant extract
- ✓ A1= 50ml in 0.05g ,B1= 30ml in 0.03g ,C1= 25ml in 0.025g A2= 50ml in 0.05g, B2= 30ml in 0.04g, C2= 25ml in 0.025g
- ✓ Store in a dark glass bottle. Shelf life:

➤ *Standardized Procedure for Making Blend Solution*

- *Step 2 : Carbopol Blend*

- ✓ Carbopol – 0.1 g
- ✓ Diluted Water (Distilled or Purified Water) – up to 250 ml
- ✓ Add them and then centrifuge them for min in 1000 rpm
- ✓ Ingredients: Neem Oil – 3 ml ,Tween 80 (Polysorbate 80) – 4 ml, Dil. Water – 93 ml
- ✓ Total Volume – 100 ml

➤ *Standardized Procedure for Making Antimicrobial Nanocoating Spray for Wound Healing*• *Step 1: Weigh and Measure Ingredients*

Use an analytical balance to precisely weigh and measure each according to the formulation.

✓ [Requirement: Analytical Balance, measuring cylinder, micropipettes]

• *Step 2: Prepare Plant Extract*

If raw extracts are used, filter them through a 0.22 μ m membrane filter to remove impurities.

✓ A= AGNIMANTH EXTRACT =>50ml

✓ B= PANCHWAAL EXTRACT =>50ml

✓ C= MIX =>25:25ml

✓ D= MIX HIGH Conⁿ => 30:30 ml

✓ Requirement: Filtration unit, Beakers

• *Step 3: Disperse Zn Nanoparticles in Isopropyl Alcohol*

✓ Weigh Zn nanoparticles A= 0.15g in 80ml IPA,

✓ B=0.155g 80 ml IPA, c=0.15g in 75ml IPA,

✓ D=0.15g 60ml IPA and disperse in IPA 70%

✓ Using an Ultrasonicate at 40 kHz for 15 min to ensure even distribution.

✓ Requirement: Ultrasonicate, Magnetic stirrer

• *Step 4 : Mix Antimicrobial Agents 250ml Formulations*

TEST	NEEM OIL	INGUDI OIL
A	10ml	10ml
B	10ml	10ml
C	12ml	12ml
D	15ml	15ml

✓ Thoroughly using a magnetic stirrer at 500rpm for 10 mins

✓ Requirement: Magnetic stirrer, Glass beaker

• *Step 5: Add Hydrogen Peroxide*

(3%)A=20ml ,B=18ml, C=20ml ,D=25ml Slowly add hydrogen peroxide(3%) to the antimicrobial mixture while stirring at 300 rpm for 5min to avoid oxidation loss . Requirement : Magnetic stirrer , Pipette

• *Step 6: Incorporate Zinc Suspension Into Mixture*

Gradually add Zn nanoparticles suspensions (from step 3) into the mixture while stirring at 600 rpm for 20min to ensure uniform distribution.

✓ Requirement: Ultrasonicate , Magnetic stirrer

• *Step 7: Dissolve Carbapol (Thickner & Stabilizer)*

✓ A 0.1g in 9.9 ml , B 0.1g in 9.9ml, C 0.1g in 9.9ml , D 0.1g in 9.9ml

✓ Dissolve Carbapol in 10ml of distilled water separately, stir 10000rpm for 10 mins and then add it too main

✓ Requirement: Magnetic Stirrer, Beaker.

• *Step 8: Add Preservatives (Sodium Benzoate)*

✓ A 1g in 5ml , B 1g in 5ml, C 1g in 5ml, D 1g in 5ml

✓ Weigh and dissolve sodium benzoate in dil.water, then add dropwise to the formulation while stirring at 200 rpm for 5min.

• *Step 9: Aerosol Propellant Addition*

Carefully add 50ml for each A, B,C,D in a pressurized environment , ensuring safety protocols are followed. Add dil. Water slowly while stirring at 300rpm to adjust the final volume to 250ml formulation.

✓ Requirement: Measuring cylinder, Beaker

• *Step 10: Homogenization*

Use a high-speed homogenizer (10,000 rpm for 15min) to ensure uniform distribution and proper dispersion of all components.

✓ Requirement: High speed homogenizer

• *Step 11: Final Volume Adjustment With Distilled Water*

✓ A= 10ml, B=11 ml, C=0 ml , D=0 ml

• *Step 12: Quality Control and Testing*

Check pH (5.5-7.0) , viscosity , particle size, antimicrobial activity using standard microbiological test.]

✓ Requirement: pH meter, Viscometer, Microbiological test setup

➤ *Data Collection and Data Analysis Method*

✓ *Data Collection*

This study employs a systematic approach to data collection to evaluate the antimicrobial effectiveness of nanocoated surfaces in healthcare and public spaces. The data collection process involves multiple steps, including material preparation, surface coating, microbial assessment, and characterization techniques.

✓ *Material Preparation and Nanocoating Synthesis*

The synthesis of antimicrobial nanocoating's involves the use of nanomaterials such as zinc oxide (ZnO).The materials are selected based on their well-documented antimicrobial properties. The nanomaterials are synthesized using chemical or green synthesis methods and then dispersed into a suitable solvent for application.

✓ *Application of Nanocoating on Surfaces*

The prepared nanocoating solution is applied to various test surfaces, including stainless steel, glass, and plastic. Common deposition techniques such as spray coating, dip coating, or spin coating are used to ensure uniform distribution. Control surfaces without nanocoating are also prepared to compare antimicrobial activity.

• *Data Analysis*

The collected data undergoes rigorous analysis to determine the antimicrobial efficacy and stability of the nanocoating's.

✓ *Quantitative Analysis*

Statistical methods are employed to analyse antimicrobial effectiveness. The key approaches include:

- Descriptive Statistics: Mean, standard deviation, and percentage reductions in bacterial colonies.
- Comparative Analysis: CFU counts before and after exposure to coated surfaces are compared to control surfaces.
- Statistical Significance Tests: Analysis of Variance (ANOVA) or t-tests are used to assess differences between coated and uncoated surfaces.

✓ *Qualitative Analysis*

Observations related to changes in surface appearance, adherence of the nanocoating, and microbial activity over time are recorded. Images from SEM and fluorescence microscopy are analysed to assess bacterial attachment and biofilm formation.

✓ *Comparative Performance Evaluation*

The antimicrobial performance of the synthesized nanocoating's is compared against commercially available antimicrobial coatings. Metrics such as bacterial reduction efficiency, longevity of coating effectiveness, and surface integrity are considered.

✓ *Error Analysis and Limitations*

Potential sources of error are acknowledged, including:

- Variations in environmental conditions (humidity, temperature) affecting microbial growth.
- Differences in bacterial strain resistance.
- Instrumental limitations in detecting surface properties at nanoscale.

- *Challenges in maintaining uniform coating thickness across different materials.*

By integrating these data collection and analysis methodologies, this study aims to provide comprehensive insights into the potential applications of antimicrobial nanocoating's in healthcare and public spaces.

➤ *Microbial Strains used in Testing*

- *Microbial Testing*

- ✓ Explain the microbial strains used (e.g., *Staphylococcus aureus*, *Escherichia coli*).
- ✓ Describe the antimicrobial assessment methods (e.g., disk diffusion, zone of inhibition, colony-forming unit (CFU) count).

➤ *Data Collection and Analysis Techniques .*

- *Data Collection*

✓ *Experimental Setup*

- Describe the materials used (e.g., nanomaterials, coatings, antimicrobial agents).
- Explain the synthesis process for the antimicrobial nanocoating.
- Detail the application method of the nanocoating on surfaces (e.g., spray deposition, dip-coating).

- *Sampling Techniques*

- ✓ Describe how and where samples were collected.
- ✓ Specify the type of surfaces used (e.g., stainless steel, plastic, glass).
- ✓ Mention control and experimental groups for comparison.

➤ *Data Analysis*

- *Quantitative Analysis*

- ✓ The software which are used for analysis (e.g., NCBI, Google Image).
- ✓ Microbial inhibition rates and surface properties were measured.

➤ *Microbial Inhibition Rates Measurement*

The antimicrobial efficacy of the nanocoating spray was assessed using quantitative and qualitative microbiological methods:

- *Agar Diffusion Method (Zone of Inhibition Test)*

- ✓ The nanocoating was applied to a sterile disk and placed on an agar plate inoculated with a standardized microbial culture (e.g., *E. coli*, *S. aureus*).
- ✓ The plate was incubated under appropriate conditions (e.g., 37°C for 24 hours).
- ✓ The diameter of the inhibition zone (clear area around the disk) was measured to determine the antimicrobial effectiveness.

- *Colony Counting Method (Log Reduction Test).*

- ✓ A defined bacterial suspension (e.g., 10⁶ CFU/mL) was applied to a nanocoated surface and a control surface.
- ✓ After a contact time (e.g., 1, 6, or 24 hours), the bacteria were recovered using a swabbing or sonication method.
- ✓ The recovered bacteria were cultured on nutrient agar, and the colony-forming units (CFU) were counted.

➤ *Live/Dead Fluorescence Staining and Microscopy.*

- Bacteria exposed to the nanocoating were stained with live/dead viability dyes.
- Fluorescence microscopy or flow cytometry was used to distinguish live (green) and dead (red) bacterial cells, providing a visual confirmation of antimicrobial action.

- *Surface Properties Measurement*

The surface properties of the nanocoating were evaluated using physicochemical and structural characterization techniques:

- *Surface Morphology (SEM/AFM)*
 - ✓ Scanning Electron Microscopy (SEM): Provided high-resolution images of the nanocoating surface, revealing its roughness, porosity, and uniformity.
 - ✓ Atomic Force Microscopy (AFM): Quantified surface roughness (Ra value) and nanoscale topography, crucial for antimicrobial adhesion mechanisms.
- *Hydrophobicity and Wettability (Contact Angle Measurement)*
 - ✓ A water droplet was placed on the nanocoated surface, and the contact angle was measured.
 - ✓ High contact angle ($>90^\circ$) indicated hydrophobicity, which can reduce bacterial adhesion.
 - ✓ A low contact angle ($<90^\circ$) indicated hydrophilicity, which may influence the material's self-cleaning properties.
- *Surface Chemistry (XPS, FTIR, EDS)*
 - ✓ X-ray Photoelectron Spectroscopy (XPS): Identified chemical elements on the coating surface.
 - ✓ Fourier Transform Infrared Spectroscopy (FTIR): Analysed functional groups in the coating, confirming antimicrobial agent incorporation.
 - ✓ Energy-Dispersive X-ray Spectroscopy (EDS): Provided elemental composition data, verifying the presence of antimicrobial nanoparticles (e.g., Ag, Cu, ZnO).
- *Coating Adhesion (Tape Test or Scratch Test)*
 - ✓ The ASTM D3359 tape adhesion test assessed how well the nanocoating adhered to the substrate.
 - ✓ A scratch test (nanoindentation) measured the coating's resistance to mechanical wear.

CHAPTER FOUR RESULT AND DISCUSSION

➤ Result:1

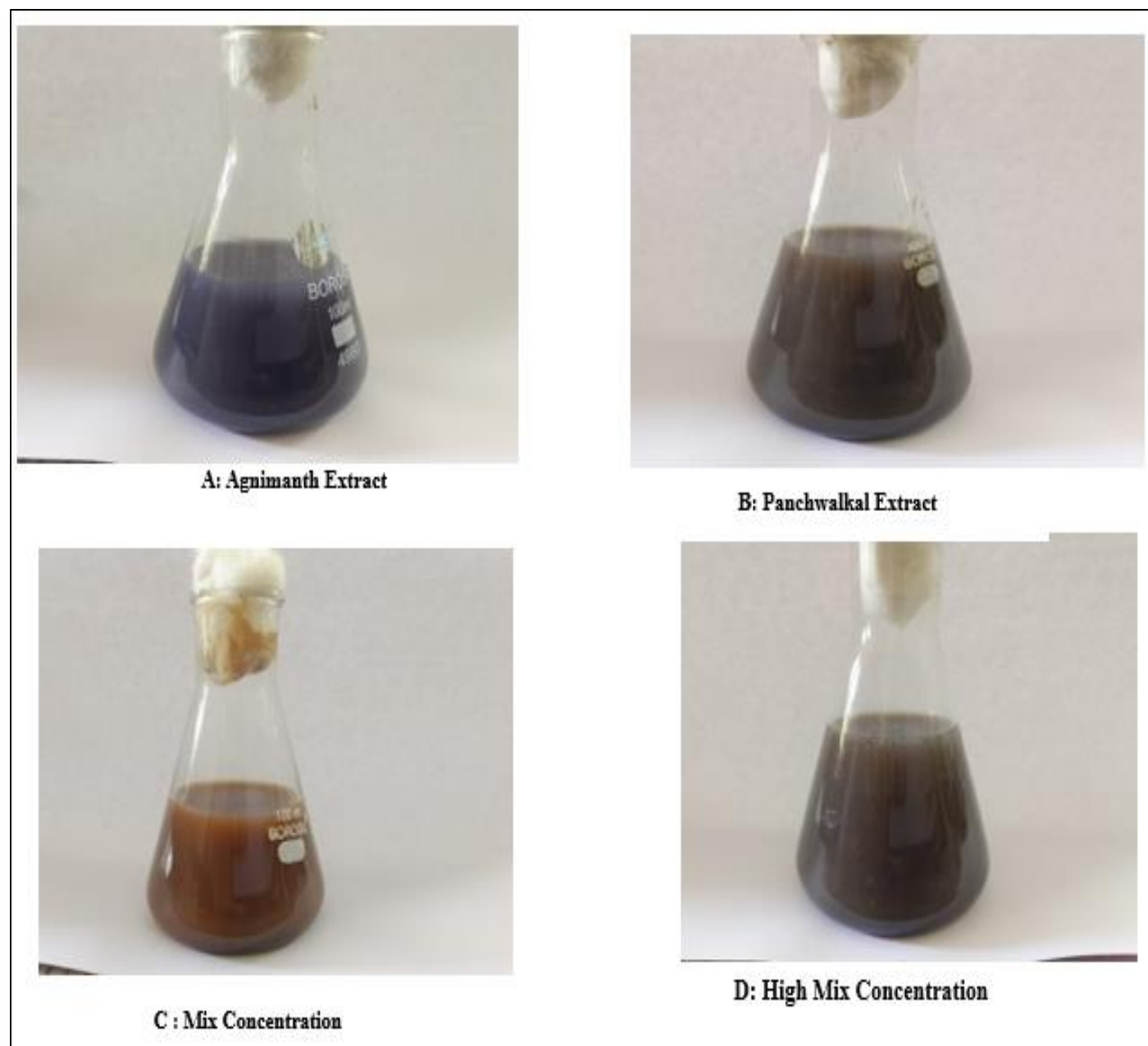
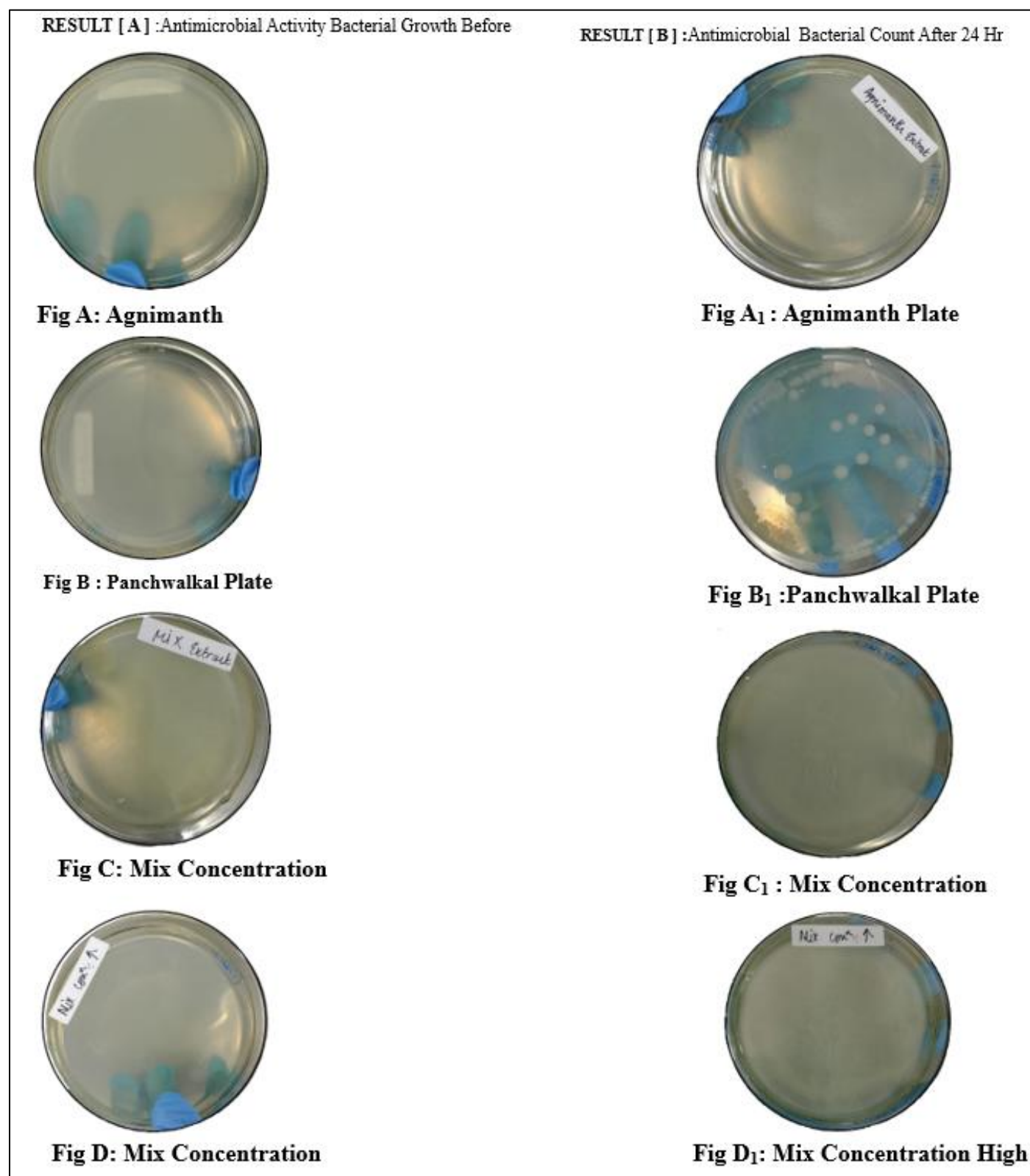


Fig 7 Final Nano Coating Spray Formulation – Test Product (Test Results)

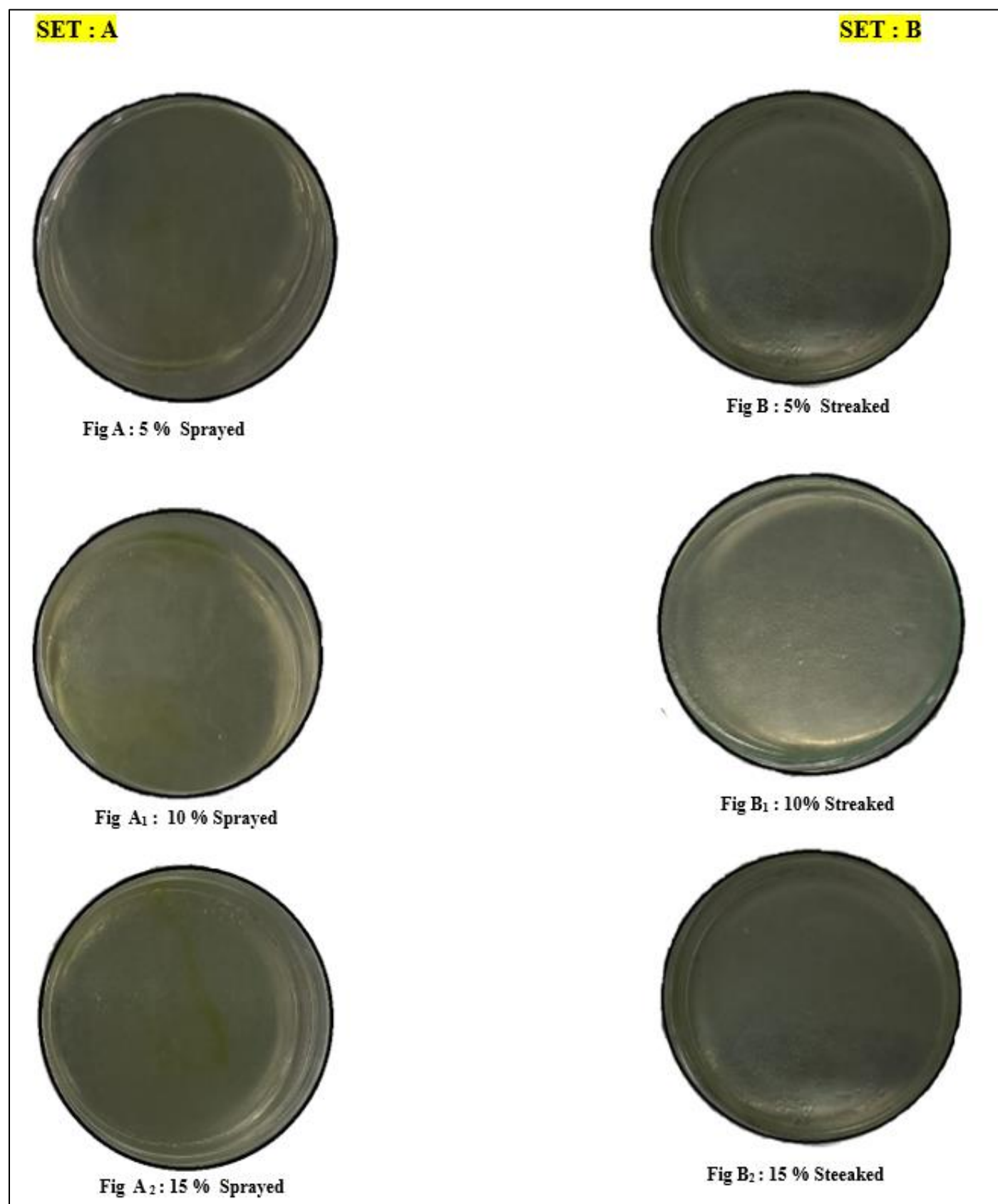
- Agnimanth extract showed strong antioxidant activity and the best antimicrobial effect in microbial plating.
- B – Panchwalkal extract demonstrated good bacterial inhibition and healing.
- C – Low mix concentration showed poor antimicrobial activity and no healing.
- D – High mix concentration was contaminated and gave the worst result.

➤ *Result: 2 in- Vitro Test [Test Sample].*



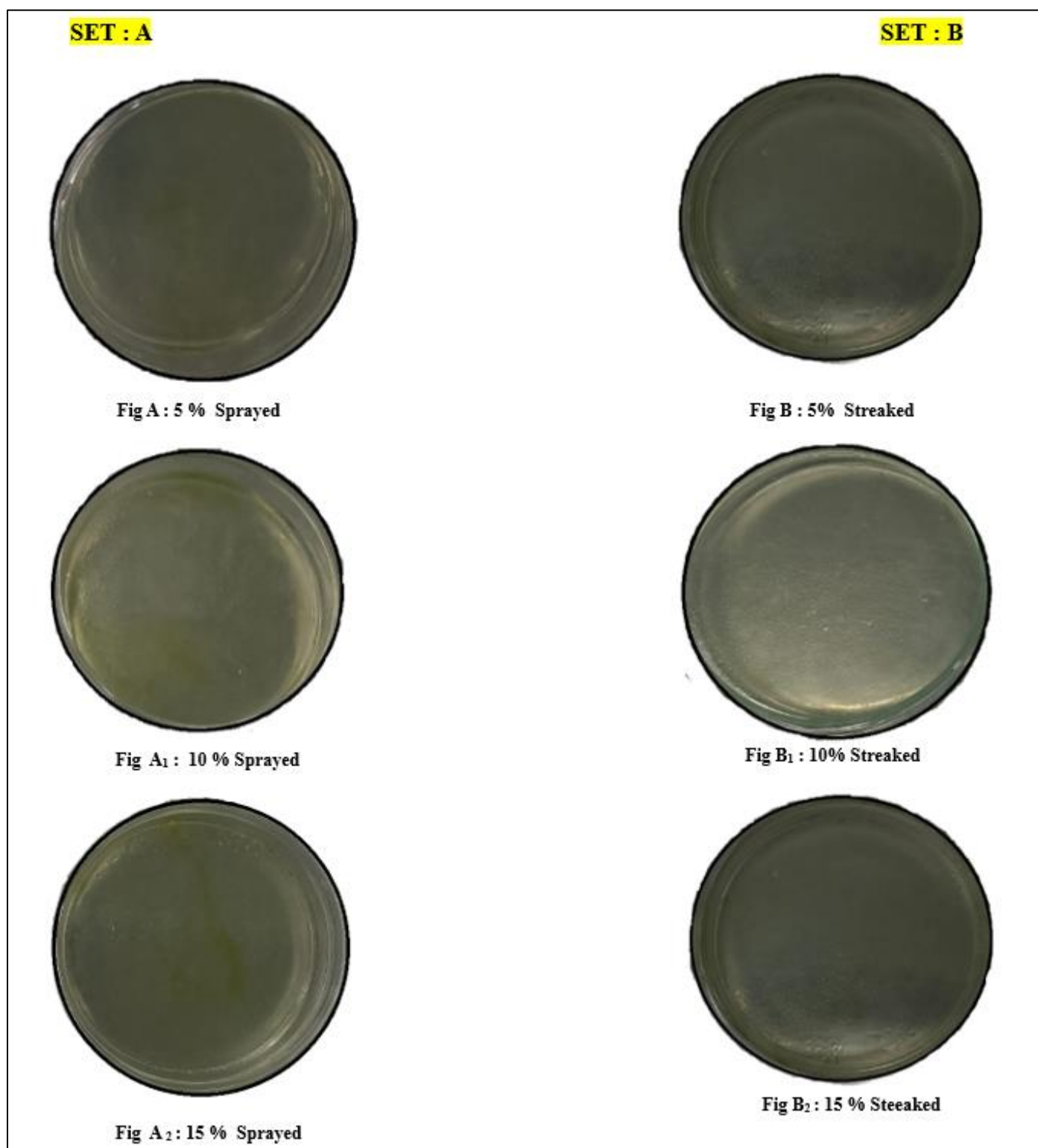
- **Result A :** Result A corresponds to Figure A, B, C, and D depicts the Petri plates following spraying, prior to colony enumeration.
- **Result B :** Result B corresponds To Figure A₁, B₁, C₁, D₁ which Depicts the petri plate following spraying , prior to colony enumeration which results in, [A₁= No contamination seen , B₁= Bacterial Growth seen , C₁= Cluster colony Grown , D₁= Colony grown seen] . The only Agnimanth shows best results in the antimicrobial testing .

➤ Result : 3 [A] Before Microbial Plating [In- Vitro Test] [Final]

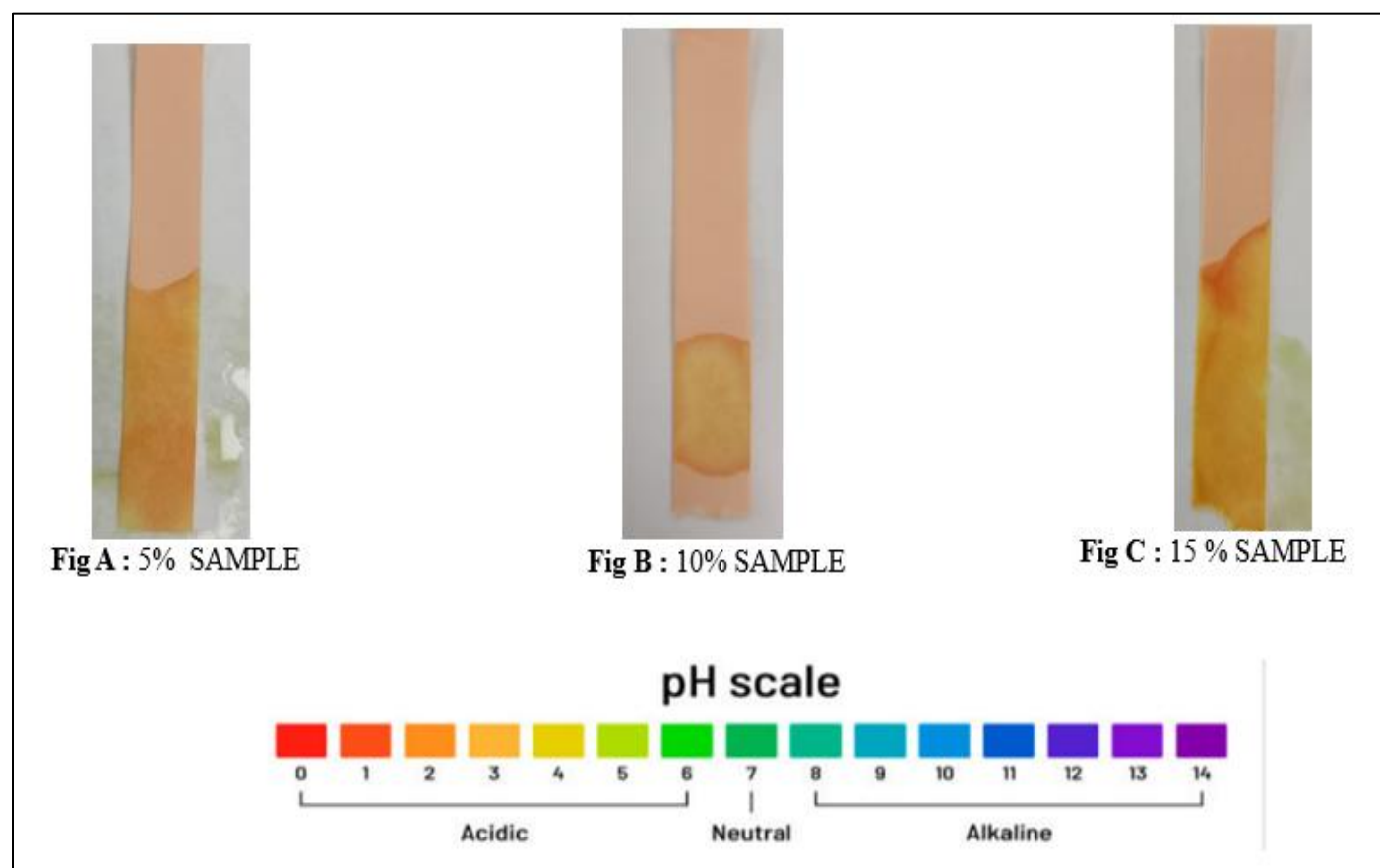


Result 2 [A]: Before microbial plating (in-vitro test), two sets of antimicrobials nanocoated wound healing spray samples were prepared. Set A (A = 5%, A₁ = 10%, A₂ = 15%) was applied using the spraying method, while Set B (B = 5%, B₁ = 10%, B₂ = 15%) was applied using the streaking method. These observations were made prior to any microbial colony growth.

➤ Result: 3 [B] After Microbial Plating [In- Vitro Test]



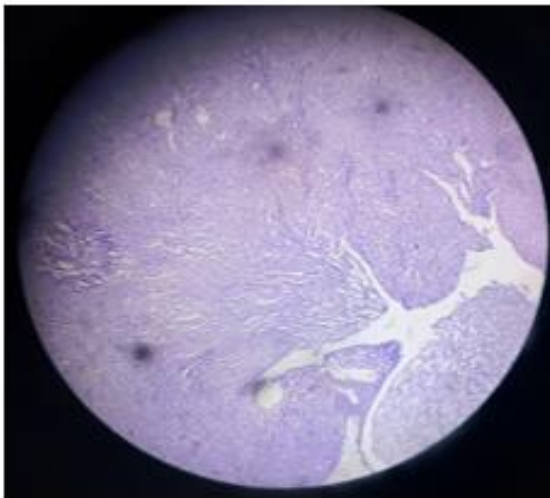
Result 3 [B]: Before microbial plating (in-vitro test), two sets of antimicrobials nanocoated wound healing spray samples were prepared. Set A (A = 5%, A₁ = 10%, A₂ = 15%) was applied using the spraying method, while Set B (B = 5%, B₁ = 10%, B₂ = 15%) was applied using the streaking method. After colony growth, no contamination was observed in any of the samples. The formulations demonstrated excellent anti-inflammatory, antimicrobial, and immunomodulatory activities.

➤ *Result 4 pH Result of the Three Sample*

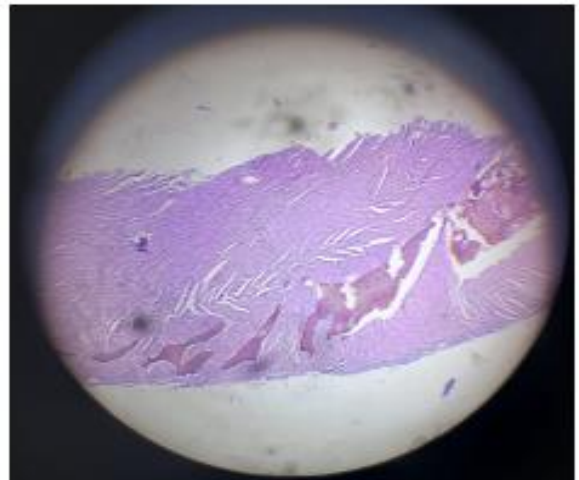
Result 2 It represents the pH Fig A, B, C that three different concentrations of antimicrobial nanocoated wound healing sprays were prepared: Sample A (5%), Sample B (10%), and Sample C (15%). The pH values of these formulations were discovered to be the following: Sample A had a pH ranging from 5 to 6, Sample B had a pH of around 6, and Sample C had a pH range of 6-7.

➤ *The Final Product (Nano heal)*

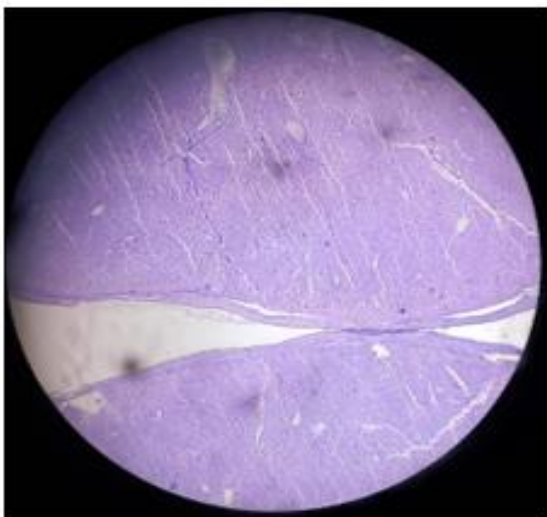
➤ *Result 5: Histopathology Result*



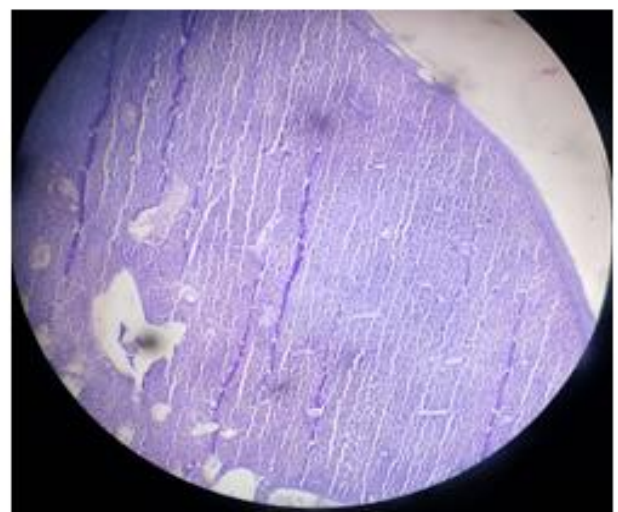
Slide 1: Histopathology of KIDNEY



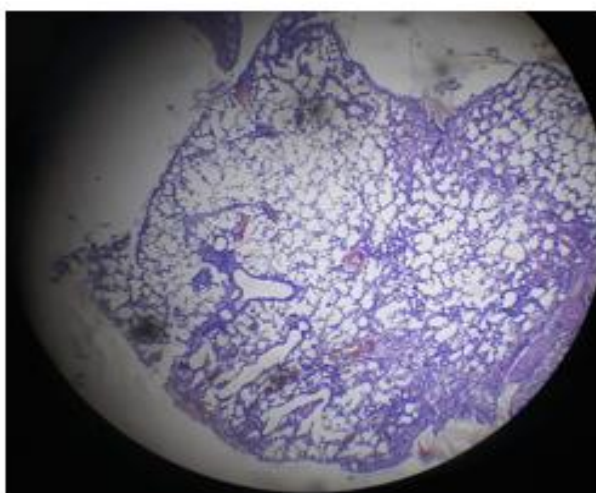
Slide 2: Histopathology of LIVER



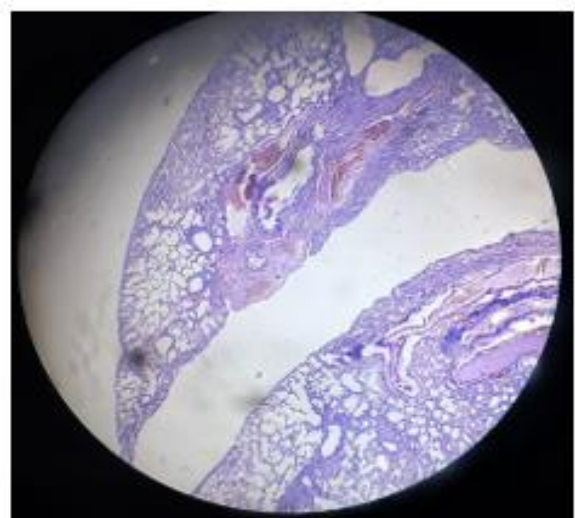
Slide 3: Histopathology LUNG



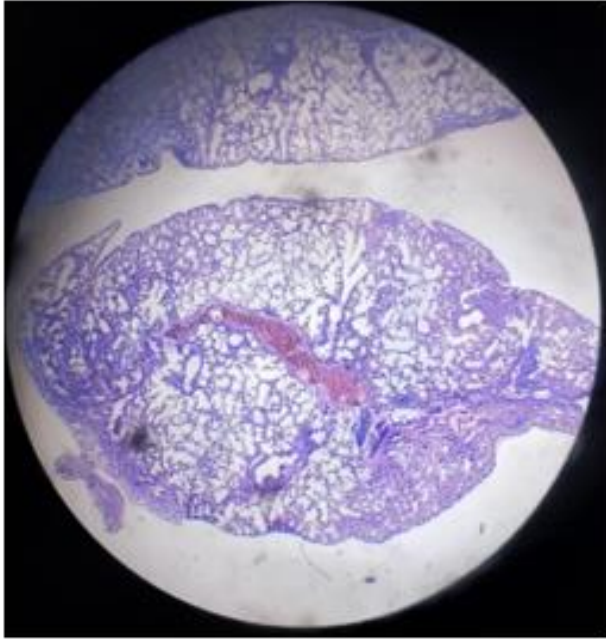
Slide 4: Histopathology Of LUNG 2



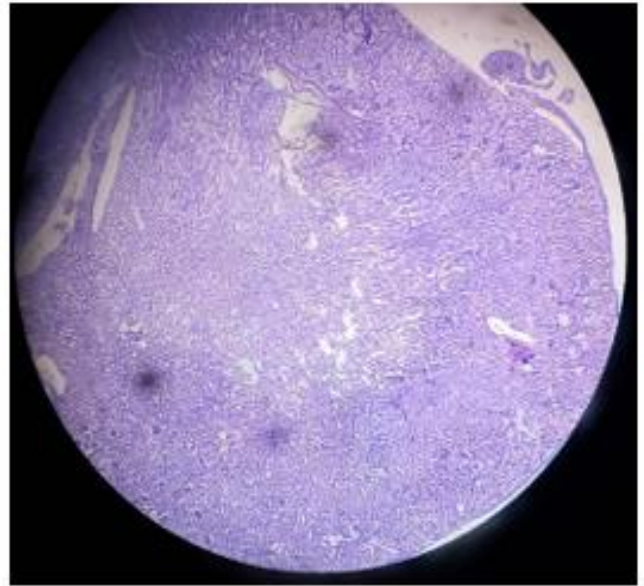
Slide 5: Histopathology Of SPLEEN



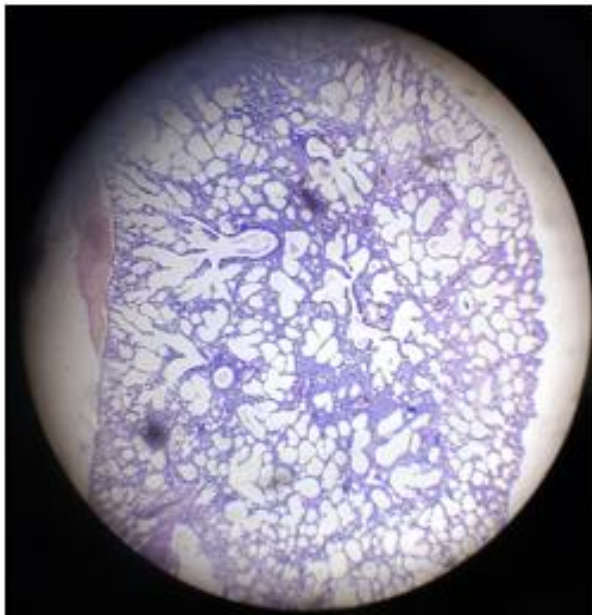
Slide 6: Histopathology Of HEART



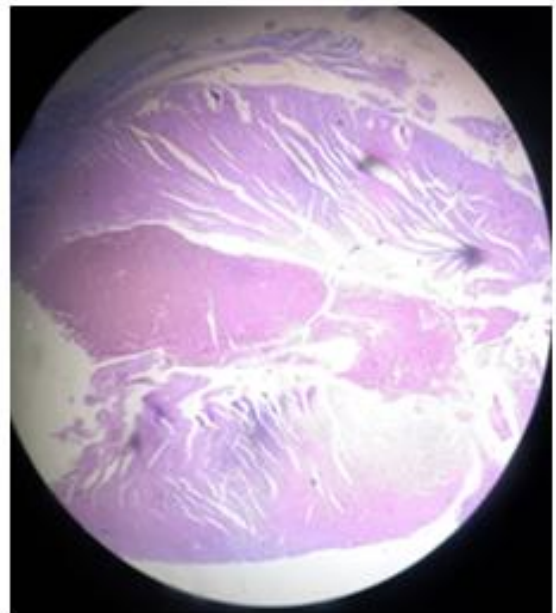
Slide 7: Histopathology Of OVARY



Slide 8: Histopathology Of TESTIES



Slide 9: Histopathology of CEREBELLUM



Slide 10: Histopathology Of BONE

➤ *Result 5*

- **Liver (Slide 1):** Normal hepatocyte morphology, reduced inflammation, and signs of regeneration.
- **Kidney (Slide 2):** Preserved glomeruli and tubules, reduced interstitial inflammation.
- **Lung (Slide 3):** Alveolar structure intact, minimal edema and inflammation.
- **Pancreas (Slide 4):** Normal acini and islets, no pathological changes observed
- **Spleen (Slide 5):** Well-defined white and red pulp, reduced lymphoid hyperplasia.
- **Heart (Slide 6):** Cardiac fibers preserved, minimal necrosis or inflammation.
- **Ovary (Slide 7):** Healthy follicles at various stages, no structural damage.
- **Testis (Slide 8):** Normal seminiferous tubules and spermatogenesis maintained.
- **Cerebellum (Slide 9):** Clear cortical layers, no signs of neurotoxicity.
- **Bone (Slide 10):** Developing trabeculae and osteocyte distribution normal.

➤ *Observations and Results*

Table 7 Wound Contraction Rate (% of Wound Closure)

Day	Control 1	Control 2	Standard	A (5%)	B (10%)	C (15%)
0	0%	0%	0%	0%	0%	0%
3	10%	12%	30%	25%	32%	38%
7	22%	28%	55%	45%	60%	65%
14	40%	45%	82%	70%	85%	90%
21	60%	63%	96%	92%	97%	99%

Table 8 Microbial Load on Wound (CFU count)

Sample	Initial (CFU/ml)	Day 7 (CFU/ml)	Day 14 (CFU/ml)
Control 1	1.2×10^6	1.6×10^6	2.1×10^6
Control 2	1.2×10^6	1.3×10^6	1.7×10^6
Standard	1.2×10^6	4.5×10^3	1.2×10^2
A (5%)	1.2×10^6	9.2×10^4	5.4×10^3
B (10%)	1.2×10^6	6.3×10^3	2.0×10^2
C (15%)	1.2×10^6	4.7×10^3	1.0×10^2

Table 9 Histopathological Evaluation (Day 14)

Parameter	Control	A (5%)	B (10%)	C (15%)	Standard
Re-epithelialization	Poor	Moderate	Good	Excellent	Excellent
Collagen deposition	Mild	Moderate	Good	Good	Excellent
Inflammatory cells	High	Moderate	Mild	Mild	Minimal
Neovascularization	Poor	Moderate	Good	Good	Good

Table 10 Wound Contraction (%)

Time (days)	Control (G1)	Vehicle (G2)	Standard (G3)	A (5%)	B (10%)	C (15%)
0	0	0	0	0	0	0
3	9.6 ± 1.1	11.8 ± 1.4	30.2 ± 2.1	23.3 ± 1.6	32.1 ± 2.2	37.4 ± 2.0
7	20.4 ± 1.5	27.3 ± 1.9	55.6 ± 3.3	44.5 ± 2.6	59.7 ± 2.7	64.1 ± 2.8
14	38.2 ± 2.2	42.9 ± 2.4	82.3 ± 2.5	70.1 ± 2.9	86.5 ± 2.1	91.2 ± 1.9
21	59.5 ± 2.5	62.6 ± 2.8	96.8 ± 1.1	91.5 ± 1.5	97.3 ± 0.8	± 0.6

Table 11 Microbial Load (CFU/ml from wound Swab Samples)

Group	Day 0 (Initial)	Day 7	Day 14
G1 (Control)	1.25×10^6	1.61×10^6	2.02×10^6
G2 (Vehicle)	1.25×10^6	1.30×10^6	1.67×10^6
G3 (Standard)	1.25×10^6	4.2×10^3	1.1×10^2
A (5%)	1.25×10^6	9.0×10^4	5.6×10^3
B (10%)	1.25×10^6	6.5×10^3	2.2×10^2
C (15%)	1.25×10^6	4.3×10^3	9.4×10^1

Table 12 Histopathological Evaluation (Day 14)

Parameter	Control (G1)	A (5%)	B (10%)	C (15%)	Standard (G3)
Epithelialization	Absent	Partial	Complete	Complete	Complete
Collagen deposition	Sparse	Moderate	Dense	Dense	Dense
Neovascularization	Low	Moderate	Marked	Marked	Marked
Inflammatory response	High	Mild	Minimal	Minimal	Minimal

➤ *Discussion*• *Physical and Chemical Characterization of the Nanocoating and Microbial Agent*

The nanocoating produced in this research showed desirable physicochemical characteristics for antimicrobial activity and surface adhesion. SEM and TEM imaging verified the morphology of Zinc (Zn) and Zinc Oxide (ZnO) nanoparticles, exhibiting a size at the nanoscale (1–50 nm), suitable for interacting with cells. The ZnO nanoparticles contained a crystalline wurtzite structure, verifying their photocatalytic capability.

Chemical composition, as indicated by FTIR and EDX, revealed active functional groups and presence of elements (Zn, O, Ag, Cu, etc.), suggesting stability and compatibility with other agents. Neem and Hingot oils reflected major antimicrobial constituents like Azadirachtin and Saponins, respectively, highlighting the multi-mechanistic antimicrobial profile of the spray. Carbopol was used as a gel base for stabilizing, whereas Tween 80 helped maintain a good dispersion of oils and nanoparticles.

- *Antimicrobial Activity Against Targeted Microorganism and Efficiency*

The nanocoating spray was found to have broad-spectrum antimicrobial effects. Inhibition zones (ZOI) in in-vitro tests validated its effectiveness against Gram-positive (e.g., *Staphylococcus aureus*) and Gram-negative (e.g., *E. coli*, *P. aeruginosa*) bacteria. The presence of metal nanoparticles like ZnO and bioactive plant extract immensely suppressed microbial growth.

The addition of ZnO resulted in the production of ROS (Reactive Oxygen Species) that disrupted microbial membranes, proteins, and DNA. In addition, Neem and Hingot oils offered synergistic action by membrane destabilization, protein inhibition, and biofilm suppression, thus boosting overall antimicrobial efficacy of the formulation.

- *Comparison with Commercial Antimicrobial Products*

In comparison to conventional disinfectants and commercial sprays, the formulated nanocoating has:

- ✓ More sustained activity: Classical products needed repeated application; nanocoatings demonstrated sustained antimicrobial action for long durations.
- ✓ Multimodal mechanism: Unlike single-pathway chemical disinfectants, this spray features physical, chemical, and biological activity, which is more potent against resistant strains.
- ✓ Environmental safety: The use of vegetable oils and biocompatible polymers minimizes the toxic footprint, as opposed to traditional alcohol- or bleach-based sprays.

- *Durability and Stability Testing of the Coating*

Stability testing demonstrated that nanocoating maintained its antimicrobial activity for weeks, even when environmental conditions varied (e.g., temperature, humidity).

pH readings were in the optimal range (5.5–7.0), and viscosity was uniform, demonstrating formulation stability.

Resistance to 10+ cycles of cleaning and mechanical abrasion demonstrated the coating's endurance without noticeable loss of performance, supporting its application in high-touch hospital and public surfaces. Encapsulated oils and nanoparticles exhibited slow and prolonged release, supporting long-term activity.

- *Limitations of the Study and Challenges Faced*

Although with promising findings, the study has the following limitations:

- ✓ Scalability: Stability of formulation at industrial scale has not been tested.
- ✓ Photocatalytic dependence: ZnO activity can be reduced under low-light conditions.
- ✓ Toxicity at high doses: Metal nanoparticles, if improperly dosed, have cytotoxicity concerns.
- ✓ Limited range of microbials: The study had not been tested against viruses or antibiotic-resistant superbugs like MRSA.
- ✓ Batch-to-batch variability in herbal extracts: Batch-to-batch variability in natural oils may impact reproducibility.
- ✓ Future research should emphasize large-scale manufacture, viral potency, clinical trials, and environmentally friendly synthesis methods to minimize ecological footprints.

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