

# A Comprehensive Review on the Formulation and Evaluation of Herbal Hand Sanitizer Gel

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Publication Date: 2026/06/10

**Abstract:** Hand hygiene is one of the most essential practices in preventing the transmission of infectious diseases. While alcohol-based sanitizers are widely used due to their rapid antibacterial action, their frequent application may lead to skin dryness, irritation, and flammability concerns. As a safer and more sustainable alternative, herbal hand sanitizer gels formulated with plant extracts and essential oils offer significant antimicrobial potential along with better skin compatibility. This review focuses on the formulation techniques, components, mechanisms, and evaluation parameters involved in the development of herbal and polyherbal hand sanitizer gels. Herbal sanitizers utilize plant-derived bioactive compounds such as tannins, flavonoids, alkaloids, and terpenoids from extracts like Neem, Tulsi, Turmeric, Clove, and essential oils including tea tree, lemon, and lavender. These constituents exhibit antimicrobial action through mechanisms such as disruption of microbial membranes, inhibition of protein synthesis, and interference with metabolic pathways. Different gelling agents (Carbopol, xanthan gum), penetration enhancers (propylene glycol, menthol), and preservatives are incorporated to ensure optimal consistency, stability, and efficacy. Preparation methods include simple mixing, cold processing, essential-oil-based blending, and nanoparticle-assisted formulations using silver nanoparticles for enhanced antimicrobial activity. The evaluation of herbal sanitizer gels involves physical, chemical, and microbiological testing, including pH, viscosity, spreadability, homogeneity, and antimicrobial assays such as agar well diffusion, disc diffusion, pour plate, and spread plate methods. Results from various studies indicate that herbal formulations demonstrate moderate to strong antimicrobial activity, good stability, and favourable sensory attributes. Overall, herbal hand sanitizer gels represent an effective, eco-friendly, non-toxic, and economical alternative to chemical sanitizers, supporting improved hygiene with reduced adverse effects.

**Keywords:** Herbal Hand Sanitizer; Polyherbal Gel; Antimicrobial Activity; Essential Oils; Plant Extracts; Formulation and Evaluation.

**How to Cite:** Dr. Sonali Vijaykumar Magdum; Pranita Santosh Bhavare; Prathamesh Ravindra Patil; Pranali Parasharam Bhendulkar; Prajwal Prashant Deshmukh; Pranoti Anil Pandav (2026) A Comprehensive Review on the Formulation and Evaluation of Herbal Hand Sanitizer Gel. *International Journal of Innovative Science and Research Technology*, 11(5), 3927-3936. <https://doi.org/10.38124/ijisrt/26may2098>

## I. INTRODUCTION

Hand hygiene plays a major role in preventing the transmission of infections and diseases. Hands washing is an essential precaution to shield the skin from dangerous microorganisms and stop the spread of many infectious diseases. Hand washing eliminates visible dirt from hands and lowers the quantity of dangerous microorganisms that humans, animals, and food can carry, such as salmonella and E. coli.

According to a number of studies, hand sanitizers containing at least 70% alcohol may eradicate 99.9% of bacteria. Plants were the primary treatment for a variety of illnesses before to the development of modern medicine[1]. Plant extracts may function as antimicrobial substances against a range of pathogenic microorganisms that cause illness, infection, and medication resistance. When opposed to chemical products, the main advantage of choosing natural sources is that they are inexpensive, easily accessible, and safe [2].

In today's busy life style consumers demand are increased for ready-made alcohol-based hand rubs over traditional soap for hand washing. Plants are contains various secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which shows antimicrobial properties. Hand sanitizers, also referred as hand antiseptics or hand rubs, which are used to remove common pathogens from the hands and come in various forms, including foam, gel, and liquid. To meet this need, we developed a herbal sanitizer using plant extracts from traditionally available plants and other ingredients to meet increasing demand for effective hand hygiene product[3].

➤ *Gels*

A gel is a semi-solid dosage form or substance consisting of a network of interlinked molecules (usually polymers) that trap and immobilize liquid, giving it a solid-like appearance but with liquid-like properties.

➤ *Polyherbal Gels*

Poly herbal → means it includes multiple plant extracts rather than a single herb, based on the Ayurvedic principle of synergism (combined effect is greater than individual effects).

A poly herbal gel is a semisolid formulation prepared by incorporating two or more herbal extracts into a gel base, intended for topical or transdermal use, to achieve synergistic therapeutic benefits.

➤ *Route of Penetration*

- Disrupting lipid structure: Drug interact with lipid polar head of Stratum corneum and increasing lipid matrix

➤ *Properties*

fluidity. This fluidization of lipid creates temporary channel thus facilitating drug passage.

- Enhancing drug solubility: Drug alter the stratum corneum's solvent properties and increase the solubility of drug, thus increase the partitioning.
- Increasing water content: Drug increase free water in lipid bilayers, enhancing polar drug diffusion.
- Acting on corneocytes: Compounds interact with keratin fibrils, alter their intracellular structure which induce the modification and make layer more permeable[4] [17].

➤ *Advantage of Herbal Gel Formulation*

- It is sophisticated and non-greasy in nature
- Gel provide good adhesion property to applied region
- They are eco-friendly and biodegradable
- Potentially Low cost
- Easy to carry
- Broader Efficacy
- Synergistic Effects
- Reduced side effects

➤ *Disadvantages of Herbal Gel Formulation*

- Some Herbal ingredient can cause skin irritation
- Variability in Herb quality
- Lack of standardization
- Temperature Sensitivity[5].

Table 1 Properties of Gel

Sr. No.	Physical	Therapeutic	Safety
1	Smoot Texture	Efficacy	Non toxic
2	Viscosity	Bioavailability	Hypoallergic
3	pH Balance	Anti-inflammatory	Biocompatibility
4	Stability	Antimicrobial	

➤ *Classification of Herbal Gels*

• *Alcohol Containing Sanitizer Gel*

Alcohol containing sanitizers typically contain ethanol / isopropanol as the main ingredient. These gels work by:

- ✓ Denaturing proteins: Alcohol disrupts the proteins on the surface of microorganisms, ultimately killing them.
- ✓ Disrupting cell membranes: Alcohol damages microorganism cell membranes, leading to their death[6].

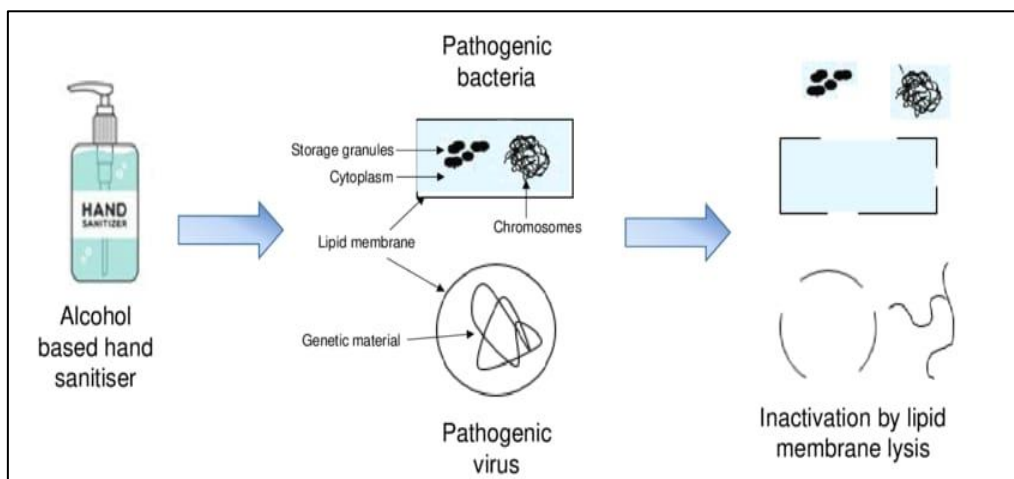


Fig 1 Mechanism of Alcohol Based Sanitizer Gel

➤ *Non-Alcohol Based Sanitizer Gel*

Non-alcohol-based hand sanitizers often contain alternative active ingredients such as:

- Herbal Extract: Certain herbal extract containing Neem extract, Tulsi extract.
- Essential oils: such as tea tree oil, Lemon Oil, Lavender Oils shows antimicrobial properties [7].

➤ *Mechanism of Essential Oil*

- **Disrupting Cell Membranes:** Essential oils can disrupt microorganism cell membranes, ultimately leading to their death.
- **Inhibiting Microbial Growth:** Certain essential oils can inhibit the growth of microorganisms by obstruct their metabolic processes.

➤ *Difference between Alcohol Based and Non -Alcohol Based Hand Sanitizer Gel*

Table 2 Difference between Alcohol Based and Non-Alcohol Based Sanitizer Gel

Alcohol Containing sanitizer Gel	Non-Alcohol based Hand sanitizer Gel
This sanitizers are usually contains 60% and 95% alcohol.	This sanitizers are does not contain alcohol.
Contain ethanol, isopropyl alcohol or n-propanol	Contain essential oil instead of alcohol
Bactericidal effect	Bacteriostatic effect
Flammable	Non Flammable
Can be unsafe for young children	Are usually non toxic

**II. COMPOSITION OF HERBAL HAND SANITIZER GEL**

- Antimicrobial agent
- Gelling agent
- Penetration enhancers
- Neutralizing agent
- Preservatives

• *Antimicrobial Agent*

The substance that kills or inhibits the growth of microorganisms such as bacteria, viruses, fungi, protozoans. These agents can be natural or synthetic which are used in various substance to prevent the spreading of infections and reduce microbial contamination.

Example-Neem, Tulsi, Turmeric, Liquorice, Clove, Thyme, Tridax daisy.

• *Gelling Agent*

Gelling agent is a substance, often a hydrophilic polymer or polysaccharide, that creates a semi-solid gel by forming a weak, cohesive network within a liquid, providing a jelly-like consistency to a formulation. Common examples

include carbomer derivatives used in food, medicine, and other industries.

Example-Carbapol, Hydroxy poly methyl cellulose, Xanthum Gum .Choice of gelling agent depends on factors like desired viscosity, stability, pH and compatibility with other ingredients.

• *Penetration Enhancers*

Penetration enhancers are chemical compounds, or in some cases physical agents like ultrasound, that reversibly reduce the barrier resistance of the stratum corneum to allow active ingredients to more readily penetrate into or through biological membranes .Penetration enhancers are used in herbal hand sanitizers to improve the skin's ability to absorb the sanitizing agents, such as alcohol and other herbal ingredients, by temporarily reducing the barrier function of the stratum corneum.

✓ Example: Propylene Glycol, Clove, Menthol.

• *Neutralizing Agent*

In herbal hand sanitizer gels, Triethanolamine (TEA) is a common neutralizing agent used to adjust the pH and form

a stable gel by neutralizing the acidity of other ingredients like Carbomer. It helps transform the carbomer powder into a hydrated gel, increasing its viscosity and stability, and making the product easier to spread.

- *Preservatives*

Preservatives are substances added to food, medicines, cosmetics, and other products to prevent or delay decomposition, spoilage, and undesirable chemical changes caused by microbial growth or oxidation, thus extending their shelf life. Example: Methyl Paraben, Propyl Paraben[5].

### III. METHOD OF PREPARATIONS

- *Alcohol-Based Hand Sanitizer Gels*

- *Methods of Preparation*

- ✓ Simple Mixing
- ✓ Cold Process
- ✓ Heat Method

- *Simple Mixing*

Mix isopropyl alcohol or ethanol with a gelling agent like Carbopol 940 or hydroxypropyl cellulose, and other ingredients like glycerine and water. The simple method is a variation of the cold process often used for at-home preparation, though it may lack the precision of industrial or laboratory-made products.

- *Procedure*

- ✓ *Step 1: Preparation of Gel Base*

The gel base is prepared by disperse gelling agent in a portion of distilled water until it hydrates and become a gel. Stir slowly using a mechanical stirrer or magnetic stirrer to avoid air Entrapment. Let the dispersion stand for 1-2 hours to fully hydrate the gelling agent.

- ✓ *Step 2: Prepare the Alcoholic Solution*

In a separate container, mix alcohol (Denatured alcohol 60-70% v/v of total formulation), Penetration enhancer, stabilizing agent and other excipient.

Add the herbal extracts mix thoroughly to ensure even distribution.

- ✓ *Step 3: Combine Gel and Alcoholic Herbal Phase*

Slowly add the alcoholic herbal mixture into the hydrated gel base with constant stirring. Stir gently to avoid bubble formation and ensure uniform mixing.

- ✓ *Step 4: Adjust the P<sup>H</sup>*

Check the P<sup>H</sup> of the gel (typically acidic after mixing).

Gradually add Neutralizing agent dropwise with stirring until the pH reaches 6.5-7.

- ✓ *Step 5: Make up Volume*

Add distilled water quantity sufficient to reach the desired final volume. Stir gently to homogenize [8].

- *Cold Process*

Mix the ingredients at room temperature, allowing the gelling agent to hydrate and form a gel. The cold process is the recommended method for making alcohol-based hand sanitizer and is used in the World Health Organization (WHO) formulations. It relies on mixing ingredients at room temperature, without applying any external heat.

- *Procedure*

Disperse the gelling agent into the water using a mixture until it hydrates and becomes a gel. Add the glycerol to the mixture and mix well. In another container mix the alcohol, herbal extract and other excipient. Slowly add the alcohol mixture to the gelling base slurry while stirring continuously. Continue stirring until the gel is uniform. Add the remaining distilled water and mix well, allow the finished product to stand for 72 hrs.

- *Heat Method*

In this mix the ingredients and heat the mixture to dissolve the gelling agent, then cool and fill into containers. Heating alcohol to make hand sanitizer is not a valid method and should never be attempted.

- *Non-Alcohol Based Hand Sanitizer Gels*

- *Methods of Preparation*

- ✓ Herbal Extract Based
- ✓ Essential Oil Based
- ✓ Nanoparticle Based

- *Herbal Extract-Based*

Combine herbal extracts with a gelling agent like Carbopol or xanthan Gum, hydroxyl propyl methyl cellulose, and other ingredients like glycerine and water.

- *Preparation of Gel with Carbopol*

- ✓ Add accurately amount of Carbopol in distilled water and let it stand for 30 minutes to allow swelling.
- ✓ Stir the mixture by using a mechanical or lab stirrer for 30 minutes to form uniform dispersion.
- ✓ In another beaker, mix propylene glycol with propyl paraben and methyl paraben, then add extract stirring until the preservatives are completely dissolved.
- ✓ Add the extract and preservative solution to the beaker containing Carbopol mixture, stirring constantly.
- ✓ Adjust the volume with remaining distilled water and adjust the pH to 6.8-7 by adding Triethanolamine dropwise, achieving the desired gel consistency.

- *Preparation of Gel with Xanthan Gum*

- ✓ Add accurately amount of xanthan gum in distilled water and let it stand for 30 minutes to allow swelling.
- ✓ Stir the mixture by using a mechanical or lab stirrer for 30 minutes to form uniform dispersion.
- ✓ In another beaker, mix propylene glycol with propyl paraben and methyl paraben, then add extract stirring until the preservatives are completely dissolved.

- ✓ Add the extract and preservative solution to the beaker containing xanthan gum mixture, stirring constantly.
- ✓ Adjust the volume with remaining distilled water and adjust the pH to 6.8-7 by adding Triethanolamine dropwise, achieving the desired gel consistency[9].

#### ➤ *Essential Oil-Based*

Blend essential oils with a gelling agent and other ingredients like glycerine and water.

#### • *Procedure*

##### ✓ *Prepare the Gelling Solution*

- Weigh the gelling agent and slowly add it to distilled water while stirring continuously to avoid lumps.
- Continue stirring until the gelling agent is fully dissolved and the solution is clear.

##### ✓ *Mix Aloe Vera Gel and Glycerine*

- In a separate container, mix the aloe Vera gel and glycerine.
- Stir well to ensure uniform mixing.

##### ✓ *Combine the Mixtures*

- Slowly add the aloe Vera-glycerine mixture to the gelling agent solution while stirring continuously.
- Ensure thorough mixing to achieve a uniform gel consistency.

##### ✓ *Add Essential Oils*

- Add essential oils for their antimicrobial properties and fragrance.
- Mix well to ensure even distribution.

##### ✓ *Add Vitamin-E Oil (if Using)*

- Add vitamin E oil for its antioxidant and moisturizing properties.
- Mix well to ensure even distribution.

##### ✓ *Final Mixing and Filling*

- Continue stirring until the gel is homogeneous.
- Fill clean, sterilized containers with the hand sanitizer gel [10].

#### ➤ *Nanoparticle-Based*

Use nanoparticles like silver or copper to create a non-alcohol based hand sanitizer gel. Nanoparticles, typically silver nanoparticles (AgNPs), are used in herbal hand sanitizer gels to enhance their antimicrobial efficacy, providing a broader spectrum of germ, virus, and fungus protection compared to alcohol-based sanitizers. Their small size enables effective interaction and disruption of microbial cell membranes, leading to cell death. Additionally, using green synthesis methods with plant extracts to create nanoparticles makes the sanitizer more skin-friendly, with

potential anti-inflammatory and soothing benefits from the herbal components.

#### • *Procedure*

##### ✓ *Prepare the Plant Extract*

Boil a plant material known for its reducing properties (such as banana peels or neem leaves) in distilled water. Strain and collect the extract.

##### ✓ *Combine with Silver Nitrate*

Mix the plant extract with a silver nitrate (AgNO<sub>3</sub>) solution (approximately 1.75 mM). The bioactive compounds in the extract act as reducing agents for the silver ions.

##### ✓ *Incubate*

Incubate the solution in a dark room. The appearance of a brownish-yellow colour indicates the formation of silver nanoparticles.

##### ✓ *Coat with Chitosan (Optional But Recommended)*

To prevent nanoparticle aggregation and boost antimicrobial effects, coat the AgNPs with water-soluble chitosan. Mix the AgNPs with a chitosan solution and stir with magnetic stirrer. [11]

## IV. EVALUATION

#### ➤ *Physical Evaluation (Organoleptic Properties)*

This is the initial assessment to ensure the product has the correct appearance, consistency and smell.

#### • *Colour and Clarity*

The sanitizer is visually inspected for its colour and transparency. Some herbal sanitizers may have a natural brownish or pale green colour due to the plant extracts used. Clarity is evaluated by checking for any visible particles.

#### • *Odour*

The smell is manually evaluated to confirm the desired fragrance, which often comes from essential oils like clove or mint.

#### • *Texture and Feel*

Researchers assess the product's feel, looking for a smooth, non-sticky gel consistency that is easy to apply.

#### • *Homogeneity*

A homogeneity test ensures all ingredients are uniformly mixed, and no coarse grains or clumps are present by smearing the gel on a glass slide.

#### • *Irritancy Test*

Select a 5 healthy volunteers. The sanitizer was applied on palm and time was recorded. The parameter such as Irritancy, redness, dryness and itching were checked[12].

#### • *Chemical Evaluation*

These tests measure key chemical properties that affect the sanitizer's performance and skin compatibility.

• *pH Measurement*

The pH is measured using a digital pH meter to ensure it falls within the skin-friendly range of 5.5 and 7. Beyond this range can cause skin irritation.

• *Viscosity*

This is measured with a viscometer to ensure the gel has the right thickness for proper application and does not drip.

• *Spreadability*

A spreadability test determines how easily the gel spreads across the skin. Optimal spreadability ensures even application across the hands.

$$S = \frac{m \times l}{t}$$

Where,

m = weight tied to upper slide

l = glass slide length

t = time taken to separate the slides [13].

• *Evaporation Rate*

Researchers test how quickly the sanitizer evaporates after application. An ideal evaporation rate is quick, usually under a minute[12].

• *Alcohol Content*

If the formulation is alcohol-based, the alcohol concentration is measured to confirm it meets recommended levels for antimicrobial action, which is typically 60%–70% [1].

**V. ANTI-MICROBIAL ACTIVITY**

Antimicrobial activity is the capacity of a substance either to kill or suppress the growth of microorganisms like bacteria, fungi, viruses, and protozoa. This characteristics plays an important role in infection control, food preservation, hygiene maintenance and manufacturing of medicines and disinfectant.

➤ *Antimicrobial Agent Work by:*

- Inhibition of cell wall synthesis
- Inhibition of Protein synthesis
- Interruption of Nucleic acid synthesis
- Damage the cell membrane
- Interference in Metabolic pathway

➤ *Agar Well Diffusion Method*

The Agar Well Diffusion Method is a commonly used in vitro technique for evaluating the antimicrobial efficacy of plant extracts, essential oils, or formulated products such as herbal hand sanitizer gels. It evaluates the capacity of a sample to inhibit the growth of specific microorganisms, as determined by measuring the zone of inhibition.

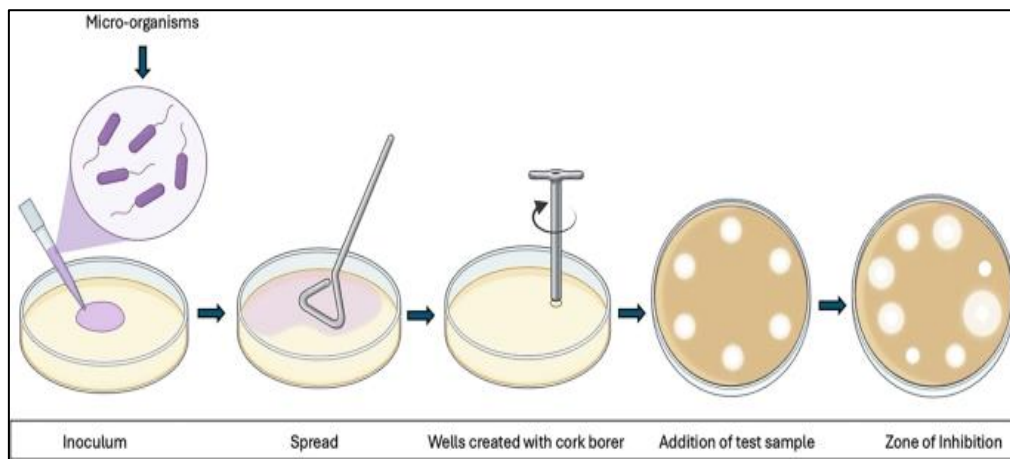


Fig 2 Agar Well Diffusion Method

• *Materials Required*

Microorganisms (Test Organisms)

✓ *Bacteria*

- Staphylococcus aureus
- E.coli, Pseudomonas aeruginosa

✓ *Fungi*

- Candida albicans

✓ *Media*

- Bacteria - Nutrient Agar or Mueller-Hinton Agar.
- Fungi Sabouraud Dextrose Agar (SDA).

• *Procedure*

✓ *Preparation of the Inoculum*

- Prepare fresh cultures of test organism.
- Adjust the turbidity of bacterial suspension.

✓ *Inoculation of Agar Plates*

- In the sterile Petri plates Pour the molten nutrient agar and allow to solidify.
- Inoculate the agar surface evenly with the microbial culture using a sterile Cotton swab.

✓ *Formation of Agar Wells*

- Create wells in the agar by using a sterile cork borer and carefully removing the agar plug.

✓ *Addition of Test Samples*

- Add appropriate volume of the sanitizer gel into each well.

✓ *Incubation*

- Incubate the plates:
- Bacteria: At 37°C (22 hours)
- Fungi: At 25-28°C (48 hours)

✓ *Observation and calculation*

- After incubation observe clear zones of inhibition around the wells
- Measure the diameter in mm of the zones using a ruler.

• *Interpretation of Result*

Table 3 Interpretation of Result

Zone Diameter in mm	Interpretation
No Zone	No Antimicrobial Activity
1 to 10 mm	Less Activity
11 to 15 mm	Intermediate Activity

➤ *Disc Diffusion Method*

It is also referred as the Kirby-Bauer method. It is a standard in vitro assay used to assess the antimicrobial efficacy of various substances, including herbal extracts or formulations. In this method, antimicrobial agents diffuse from paper discs into agar medium inoculated with test microorganisms. The zone of inhibition around the disc shows the antimicrobial activity.

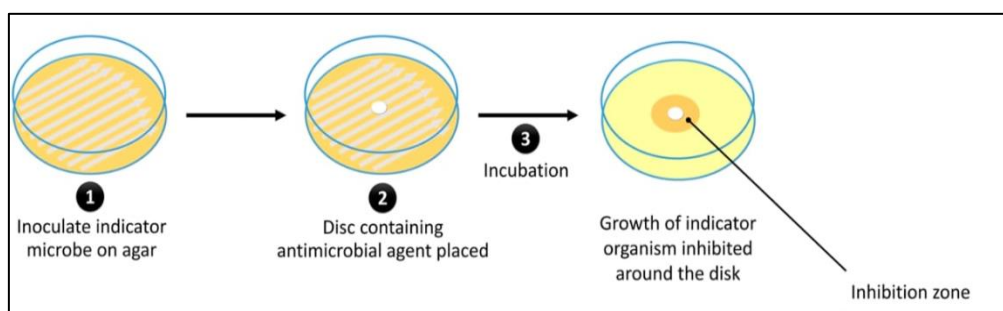


Fig 3 Disc Diffusion Method

➤ *Materials Required*

• *Microorganisms (Test Organisms)*

- ✓ Staphylococcus aureus
- ✓ E. coli, Pseudomonas aeruginosa
- ✓ Candida albicans

• *Culture Media*

- ✓ Bacteria - Mueller-Hinton Agar
- ✓ Fungi - Sabouraud Dextrose Agar

• *Procedure*

✓ *Step 1: Preparation of Inoculum*

- Grow test organisms in nutrient broth for 18-24 hours.
- Adjust the turbidity up to 0.5 McFarland Standard.

✓ *Step 2: Formation of Agar Plates*

- In the sterile Petri plates Pour the Mueller-Hinton Agar and allow to solidify.
- Inoculate the agar surface evenly with the microbial culture using a sterile Cotton swab.

✓ *Step 3: Employment of Discs*

- Sterilized forceps and place sterile discs on the agar surface.
- Impregnate each disc with 10-20 microliter of the herbal hand sanitizer gel.

• *Include*

- ✓ Test discs: Herbal sanitizer gel
- ✓ Positive control: Disc with standard antimicrobial
- ✓ Negative control: Disc with sterile water

✓ *Step 4: Incubation*

- Incubate the plates at
- Bacteria - At 37°C (24 hours)
- Fungi - At 25-28°C (48 hours)

✓ *Step 5: Observation and Result Interpretation*

- After incubation, examine the zone of inhibition around the disc.
- Diameter of each zone is measured using a ruler or Vernier calliper.

• *Interpretation of Result*

Table 4 Interpretation of Result

Sample	Zone Diameter mm	Activity
Herbal Sanitizer Gel	15 mm	Moderate activity
Positive control	20 mm	Strong activity
Negative control	0 mm	No activity

➤ *Pour Plate Method*

It is a quantitative microbiological technique used to determine the number of viable microorganisms (colony-forming units) in a sample. It can be adapted to assess the

antimicrobial efficacy of herbal sanitizer gels by comparing the microbial activity before and after exposure to the product.

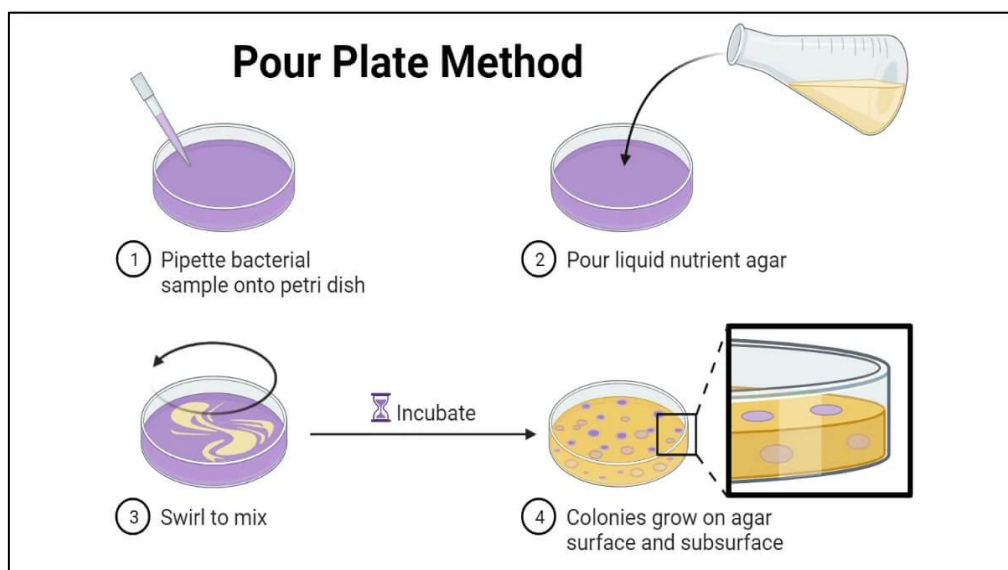


Fig 4 Pour Plate Method

• *Procedure*

✓ *Step 1: Preparation of Microbial Suspension*

- Grow the test organism in nutrient broth for 18-24 hours.
- Adjust the turbidity up to 0.5 McFarland standard.
- Dilute the culture to get ~10<sup>6</sup> Colony Forming Unit / milliliter

✓ *Step 2: Treatment with Hand Sanitizer*

- Mix a fixed volume of herbal hand sanitizer gel with an equal volume of the microbial suspension.
- Allow contact for specific times.
- After each time point, neutralize the sanitizer using a neutralizing agent or dilution in sterile saline.

✓ *Step 3: Serial Dilution*

- Perform serial dilutions to get Countable colony ranges.

✓ *Step 4: Pour Plate Preparation*

- To each sterile Petri dish, add 1 milliliter of the diluted sample.
- In the sterile Petri plates Pour the Mueller-Hinton Agar and allow to solidify.

✓ *Step 5: Incubation*

- Allow incubation of the plates at 37°C for 24 hours.

✓ *Step 6: Colony Counting*

- Count colonies on plates that have 30-300 Colony Forming Units.
- Multiply by the dilution factor to calculate Colony Forming Units/milliliter.
- Compare treated sample Colony Forming Units to control (untreated) Colony Forming Units.

• *Interpretation of Result*

- ✓ Calculate Log Reduction
- ✓ Log Reduction = log<sub>10</sub> (CFU control) - log<sub>10</sub> (CFU treated)

➤ *Spread Plate Method*

It is a quantitative microbiological method used to evaluate how effectively an antimicrobial agent-such as a herbal hand sanitizer gel-reduces the number of viable microorganisms. This method is simple, reliable, and ideal for measuring microbial load reduction after exposure to the sanitizer.

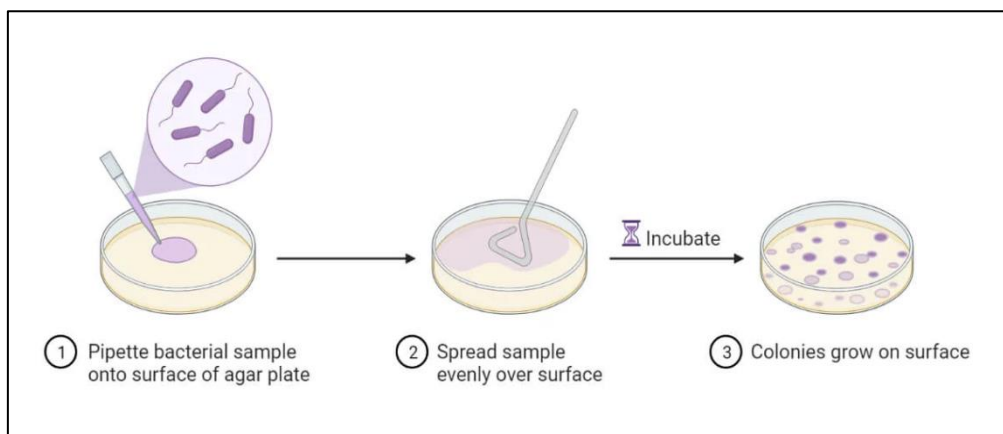


Fig 5 Spread Plate Method

- *Procedure*

- ✓ *Step 1: Preparation of Inoculum*

- The test organism grow in nutrient broth for 18-24 hours.
- Adjust the turbidity to ~10<sup>6</sup> Colony Forming Unit/milliliter (0.5 McFarland standard).

- ✓ *Step 2: Treatment with Herbal Hand Sanitizer*

- Mix equal volumes of Microbial suspension and Herbal hand sanitizer gel
- Allow contact for selected time intervals.
- At the end of each time point, neutralize the sample using a neutralizing agent.

- ✓ *Step 3: Serial Dilution*

- Perform 10-fold serial dilutions of the neutralized mixture in sterile saline solution.

- ✓ *Step 4: Spread Plating*

- Transfer 100 microliter of each dilution onto the surface of sterile agar plates.
- Spread evenly using a sterile L-rod or glass spreader.
- Allow plates to dry for 10-15 minutes.

- ✓ *Step 5: Incubation*

- Incubate plates at
- Bacteria - 37°C (24 hours)
- Fungi - 25-28°C (48 hours)

- *Interpretation of Result*

- ✓ Count colonies on plates with 30-300 Colony Forming Unit.
- ✓ Multiply by the dilution factor to calculate Colony Forming Unit/milliliter.
- ✓ Log Reduction Calculation
- ✓ Log Reduction = log<sub>10</sub> (CFU control) - log<sub>10</sub> (CFU treated) [14 15 16].

## VI. CONCLUSION

This review highlights the growing significance of herbal hand sanitizer gels as safe, effective, and eco-friendly alternatives to conventional alcohol-based sanitizers. Herbal formulations enriched with plant extracts and essential oils—such as Neem, Tulsi, Turmeric, Clove, tea tree oil, and lemon oil—exhibit notable antimicrobial activity through mechanisms that disrupt microbial membranes, inhibit protein synthesis, and interfere with metabolic pathways. The use of suitable gelling agents, penetration enhancers, and preservatives ensures optimal texture, stability, and performance of these formulations. Evaluation through physical, chemical, and microbiological methods, including agar well diffusion, disc diffusion, pour plate, and spread plate techniques, consistently demonstrates moderate to strong antimicrobial efficacy of herbal sanitizer gels. Overall, herbal hand sanitizers offer key advantages such as non-toxicity, skin compatibility, biodegradability, and cost-effectiveness, making them ideal for regular and long-term use. With increasing consumer preference for natural and sustainable products, herbal sanitizer gels hold strong potential as a practical and beneficial alternative in promoting hand hygiene and preventing infectious diseases.

## ACKNOWLEDGMENT

I extend my gratitude to the individuals and organizations who collaborated with me on this college project. I would like to thank Dr.Sonali Magdum for their willingness to collaborate and their valuable inputs throughout the project. Their expertise and contributions have significantly enhanced the quality of this work.

I am also grateful to my friends who worked alongside me, sharing their knowledge and skills. Our collaboration fostered a creative and productive environment, leading to the successful completion of this project.

Lastly, I would like to acknowledge the support received from Dr. Shitalkumar Patil Sir (Honourable principal of Dr.J.J.Magdum Pharmacy College Jaysingpur) for providing the necessary resources and encouragement that enabled this collaborative effort.

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