

Preparation of Herbal Hand Sanitizer

Palve Ranjit G., Kamlesh Shirsath S., Rathod Sujit S., Gosavi Akshata A.

Sahakar Maharshi Kisanrao Varal Patil College of Pharmacy, Nighoj

Abstract: *Hand hygiene plays a crucial role in preventing the spread of infectious diseases, particularly those caused by bacteria, fungi, and viruses. Conventional alcohol-based hand sanitizers, while effective, may cause skin irritation, dryness, and allergic reactions with prolonged use. This study focuses on the formulation and evaluation of a herbal hand sanitizer using natural antimicrobial ingredients such as Azadirachta indica (neem), Ocimum sanctum (tulsi), Aloe barbadensis (aloe vera), and lemon extract. These plant-based components were selected for their proven antibacterial, antifungal, antiviral, and skin-conditioning properties. The preparation involved blending herbal extracts with a suitable gel base to achieve an effective and skin-friendly formulation. The final product was evaluated for parameters such as antimicrobial efficacy, pH, viscosity, spreadability, stability, and user acceptability. Results indicated that the herbal hand sanitizer exhibited significant antimicrobial activity, was non-irritating, and maintained stability over the study period. This formulation offers a safe, eco-friendly, and cost-effective alternative to synthetic hand sanitizers, making it suitable for regular use while reducing environmental and health hazards.*

Keywords: Herbal formulation, Hand sanitizer, Antimicrobial activity, Neem, Tulsi, Aloe vera, Natural disinfectant

I. INTRODUCTION

Herbal hand sanitizer is a natural hand cleansing product made from plant-based ingredients like herbal extracts and essential oils. It is used to kill or reduce germs on the hands without the use of harsh chemicals or synthetic additives.

Hand hygiene is one of the most effective and simple methods to prevent the transmission of infectious diseases. Pathogens such as bacteria, viruses, and fungi can easily spread through direct contact, contaminated surfaces, or respiratory droplets, making proper hand cleaning essential in breaking the chain of infection. The World Health Organization (WHO) recommends regular hand washing or the use of hand sanitizers as a primary preventive measure against infections, including those caused by seasonal flu and pandemics such as COVID-19.

Alcohol-based hand sanitizers are widely used due to their rapid antimicrobial action; however, prolonged use may lead to skin dryness, irritation, and allergic reactions. Moreover, frequent exposure to synthetic chemicals may disrupt the natural skin barrier. This has increased global interest in herbal alternatives that are effective, safe, and environmentally friendly.[1]

Herbal formulations utilize plant-derived bioactive compounds known for their antimicrobial, antioxidant, and skin-protecting properties. Plants such as Azadirachta indica (neem), Ocimum sanctum (tulsi), Aloe barbadensis (aloe vera), and Citrus limon (lemon) have been traditionally used in Ayurveda for their therapeutic benefits. Neem exhibits strong antibacterial and antiviral effects, tulsi provides antimicrobial and anti-inflammatory activity, aloe vera soothes and moisturizes the skin, while lemon acts as a natural antiseptic and deodorizing agent.

The present study aims to formulate and evaluate a herbal hand sanitizer that combines these natural ingredients to achieve optimal antimicrobial efficacy while ensuring skin safety. By eliminating or reducing the use of synthetic chemicals, such a formulation could serve as a sustainable and cost-effective alternative to conventional hand sanitizers, with added benefits of skin nourishment and environmental compatibility.[12]

Maintaining proper hand hygiene is a cornerstone of personal and public health. Hands act as primary carriers for microorganisms, transferring pathogens between individuals, objects, and the environment. According to the World Health Organization (WHO), effective hand hygiene can reduce the risk of infectious disease transmission by up to



50%. In recent years, global health crises such as the COVID- 19 pandemic have heightened awareness of hand hygiene practices, resulting in increased demand for hand sanitizers and other disinfectants.[7]

Commercially available hand sanitizers are predominantly alcohol-based formulations containing ethanol or isopropyl alcohol in concentrations of 60–95%, which are proven to rapidly destroy a broad spectrum of microorganisms. However, the frequent use of such products has been associated with adverse effects, including skin dryness, irritation, dermatitis, and in some cases, allergic reactions. Additionally, synthetic fragrances, preservatives, and chemical stabilizers in these products may pose further risks to sensitive skin.

The rising concerns over skin safety, chemical exposure, and environmental sustainability have shifted consumer interest toward herbal or plant-based alternatives. Herbal formulations draw upon the antimicrobial potential of phytochemicals such as flavonoids, terpenoids, tannins, and alkaloids, which can inhibit the growth of pathogenic microorganisms. Unlike synthetic agents, herbal ingredients are generally biodegradable, safe for prolonged use, and often provide additional therapeutic benefits such as moisturization, anti-inflammatory effects, and skin regeneration.[9]

Several medicinal plants have been recognized for their potent antimicrobial properties and skin- friendly nature:

- *Azadirachta indica* (Neem): Rich in azadirachtin and nimbin, neem exhibits strong antibacterial, antifungal, and antiviral activities.
- *Ocimum sanctum* (Tulsi): Contains eugenol and ursolic acid, which possess antimicrobial, anti-inflammatory, and antioxidant properties.
- *Aloe barbadensis* (Aloe vera): Acts as a natural moisturizer, soothing irritated skin and accelerating wound healing while supporting antimicrobial defense.
- *Citrus limon* (Lemon): Its citric acid and limonene content serve as natural antiseptics with deodorizing and refreshing effects.

The formulation of a herbal hand sanitizer using these ingredients aims to combine antimicrobial effectiveness with skin protection, ensuring both safety and efficacy for daily use. Such a product not only addresses the limitations of alcohol-based sanitizers but also aligns with the growing global trend toward natural, eco-friendly personal care solutions.[22]

The present study focuses on the preparation, standardization, and evaluation of a herbal hand sanitizer gel incorporating neem, tulsi, aloe vera, and lemon extracts. The formulation will be assessed for physical parameters (pH, viscosity, appearance), stability, user acceptability, and antimicrobial activity against common pathogenic microorganisms. The ultimate goal is to develop a cost-effective, safe, and efficient alternative to conventional sanitizers that can be adopted for routine hygiene practices at both individual and community levels.

Types of hand Sanitizer:

Based on Active Ingredients

1. Alcohol-Based Hand Sanitizers

- o Main ingredients: Ethanol (ethyl alcohol) or isopropyl alcohol (60–95%)
- o Effectiveness: Kills most bacteria, fungi, and viruses (including enveloped viruses like COVID-19)
- o Best use: When soap and water are unavailable
- o Downside: Can dry out skin with frequent use

2. Alcohol-Free Hand Sanitizers

- o Main ingredients: Benzalkonium chloride, triclosan, or other antimicrobial agents
- o Effectiveness: Less effective than alcohol-based against some viruses
- o Best use: For sensitive skin or environments where alcohol use is restricted (e.g., schools, prisons)
- o Downside: May not kill all pathogens, and resistance is a concern with some agents like triclosan



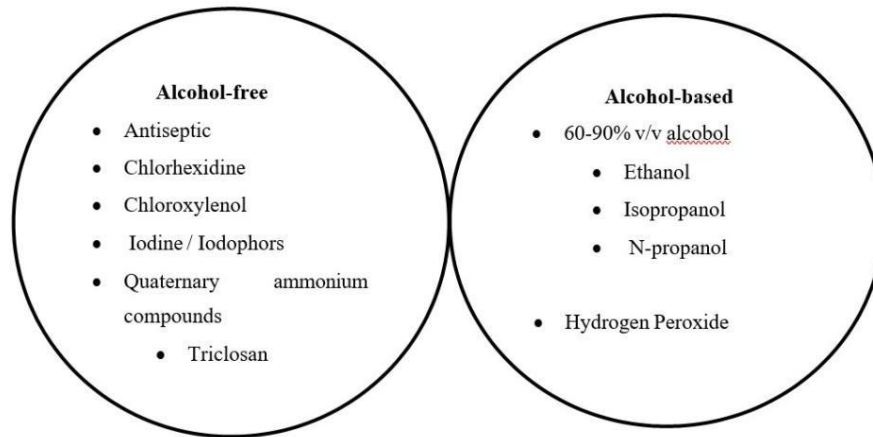


Fig 1. Types of hand Sanitizer

Based on Formulation or Format

1. Gel Sanitizers

- o Most common form
- o Easy to apply and spread
- o Dries quickly

2. Foam Sanitizers

- o Expands when dispensed
- o Preferred in healthcare settings for coverage
- o Usually more expensive

3. Liquid Sanitizers

- o Often used in dispensers
- o May run off hands if not used carefully

4. Spray Sanitizers

- o Great for on-the-go use or surfaces
- o Can be used on objects as well as hands

5. Hand Sanitizing Wipes

- o Pre-moistened towelettes
- o Useful for both hand cleaning and wiping surfaces
- o Convenient for travel

Based on Additional Features

1. Moisturizing Hand Sanitizers

- o Contain glycerin, aloe vera, or vitamin E
- o Reduce skin dryness

2. Fragranced/Perfumed Sanitizers

- o Scented varieties for a pleasant smell
- o May irritate sensitive skin or allergies



3. Natural or Organic Hand Sanitizers

- o Use essential oils and plant-based alcohols
- o Marketed as eco-friendly or skin-friendly

Forms of hand sanitizer:

Sanitizers come in various forms, depending on their intended use, ingredients, and application method. Here's a breakdown of the most common forms of sanitizers:

1. Gel Sanitizer

- Description: Thick, clear or colored gel
- Common Ingredients: Alcohol (ethanol/isopropyl), water, glycerin, carbomer
- Use: Rubbed onto hands until dry
- Pros: Easy to apply, widely available
- Cons: Can feel sticky; may dry skin with frequent use

2. Foam Sanitizer

- Description: Light, airy foam dispensed through a pump
- Common Ingredients: Alcohol or benzalkonium chloride, surfactants
- Use: Rub into hands; covers more surface area quickly
- Pros: Less dripping; preferred in healthcare settings
- Cons: More expensive than gel

3. Liquid Sanitizer

- Description: Water-like consistency
- Common Ingredients: Alcohol-based or alcohol-free
- Use: Poured or sprayed onto hands or surfaces
- Pros: Quick-drying; can double as surface disinfectant
- Cons: Can spill easily; requires careful handling

4. Spray Sanitizer

- Description: Dispensed as a fine mist
- Use: For hands or surfaces (phones, handles, etc.)
- Pros: Convenient; great for surfaces and on-the-go use
- Cons: May evaporate quickly before full coverage

5. Wipe Sanitizer (Sanitizing Wipes)

- Description: Pre-moistened disposable towelettes
- Use: For cleaning hands or disinfecting surfaces
- Pros: Portable; dual-use (hands + surfaces)
- Cons: Waste-producing; can dry out if not sealed properly

6. Hand Sanitizing Soap (Antimicrobial Soap)

- Description: Liquid or foam soap with added antiseptic agents
- Use: Requires water; used like regular soap
- Pros: Effective when washing is possible
- Cons: Not a "true" hand sanitizer; needs water and drying





Fig 2. Forms of hand sanitizer

Advantages of Herbal Hand Sanitizer

1. Natural and Skin-Friendly – Prepared using plant-based extracts such as neem, tulsi, aloe vera, and lemon, which are gentle on the skin and reduce the risk of dryness, irritation, or dermatitis commonly caused by alcohol-based sanitizers.
2. Antimicrobial Efficacy – Contains phytochemicals like flavonoids, terpenoids, and alkaloids that exhibit broad-spectrum antibacterial, antifungal, and antiviral properties, ensuring effective pathogen control.
3. Moisturizing Properties – Aloe vera and other herbal components help retain skin moisture, keeping hands soft and preventing cracking after repeated use.[2]
4. Eco-Friendly – Free from harmful synthetic chemicals, making it biodegradable and less polluting to the environment compared to synthetic sanitizers.
5. Cost-Effective – Utilizes easily available medicinal plants, reducing production costs and making it affordable for large-scale use, especially in rural and community settings.
6. Non-Toxic and Safe for Long-Term Use – Absence of harmful synthetic preservatives, parabens, and artificial fragrances makes it safe for sensitive skin and long-term application.
7. Pleasant Natural Fragrance – Lemon and tulsi provide a mild, refreshing aroma without synthetic perfumes, which can sometimes cause allergies.
8. Additional Health Benefits – Some ingredients (e.g., tulsi and neem) also have anti-inflammatory, antioxidant, and immune-boosting effects, supporting overall skin health.
9. Reduces Chemical Exposure – Eliminates the risks associated with prolonged contact with strong alcohol or synthetic agents, which may damage the skin's protective barrier.[10]
10. Sustainable Production – Can be produced using locally sourced plants, promoting the use of indigenous medicinal resources and encouraging small-scale herbal product industries.

Material used:

1. Azadirachta indica (Neem) Extract

- Botanical Name: Azadirachta indica
- Family: Meliaceae
- Active Constituents: Azadirachtin, nimbin, nimbidin, quercetin.
- Role in Formulation:

Neem is a well-known Ayurvedic medicinal plant with strong antimicrobial properties. Azadirachtin and nimbin inhibit bacterial cell wall synthesis, while quercetin acts as an antioxidant. Its broad-spectrum action works against Escherichia coli, Staphylococcus aureus, Candida albicans, and various viruses.

- Form Used: Ethanolic or aqueous leaf extract prepared by maceration or Soxhlet extraction.[6]



Taxonomical Classification of Neem

Rank	Taxon
Kingdom	Plantae
Sub-Kingdom	Tracheobionta (Vascular plants)
Super Division	Spermatophyta (Seed plants)
Division	Magnoliophyta (Angiosperms – flowering plants)
Class	Magnoliopsida (Dicotyledons)
Subclass	Rosidae
Order	Sapindales
Family	Meliaceae (Mahogany family)
Genus	Azadirachta
Species	Azadirachta indica A. Juss.



Fig 1. Neem

2. Ocimum sanctum (Tulsi) Extract

- Botanical Name: Ocimum sanctum
- Family: Lamiaceae
- Active Constituents: Eugenol, ursolic acid, carvacrol, rosmarinic acid.
- Role in Formulation:

Tulsi exhibits antibacterial, antiviral, and antifungal activities. Eugenol disrupts microbial cell membranes, while ursolic acid acts as an anti-inflammatory and antioxidant agent. Its refreshing aroma also enhances product acceptability.

- Form Used: Fresh leaf extract or dried leaf ethanolic extract.[19]

Taxonomical Classification of Tulsi

Rank	Taxon
Kingdom	Plantae
Sub-Kingdom	Tracheobionta (Vascular plants)



Super Division	Spermatophyta (Seed plants)
Division	Magnoliophyta (Angiosperms)
Class	Magnoliopsida (Dicotyledons)
Subclass	Asteridae
Order	Lamiales
Family	Lamiaceae (Mint family)
Genus	Ocimum
Species	Ocimum tenuiflorum L. (also known as Ocimum sanctum L.)



Fig 2. Tulsi

3. Aloe barbadensis (Aloe Vera) Gel

- Botanical Name: Aloe barbadensis Miller
- Family: Asphodelaceae
- Active Constituents: Aloin, acemannan, vitamins (A, C, E), polysaccharides.
- Role in Formulation:

Aloe vera serves as a soothing and hydrating base. Its polysaccharides form a protective film over the skin, locking in moisture. Acemannan has mild antimicrobial action and supports wound healing. It also reduces irritation caused by repeated sanitizer use.[4]

- Form Used: Fresh gel from leaves or 99% pure commercial aloe vera gel.

Taxonomical Classification of Aloe vera

Taxonomic Rank	Classification
Kingdom	Plantae
Sub-Kingdom	Tracheobionta (Vascular plants)



Super Division	Spermatophyta (Seed plants)
Division	Magnoliophyta (Angiosperms – flowering plants)
Class	Liliopsida (Monocotyledons)
Order	Asparagales
Family	Asphodelaceae (or sometimes Xanthorrhoeaceae)
Genus	Aloe
Species	Aloe vera (L.) Burm.f.



Fig 3. Aloe Vera

4. Citrus limon (Lemon) Extract / Juice

- Botanical Name: Citrus limon
- Family: Rutaceae
- Active Constituents: Citric acid, limonene, vitamin C (ascorbic acid), flavonoids.
- Role in Formulation:

Lemon juice acts as a natural antiseptic and mild bleaching agent, removing odors from hands. Citric acid lowers pH, creating an unfavorable environment for microbial growth, while vitamin C serves as an antioxidant.[27]

- Form Used: Freshly squeezed juice or lemon essential oil (for fragrance and antimicrobial activity).

Taxonomical Classification of Lemon

Taxonomic Rank	Classification
Kingdom	Plantae
Sub-Kingdom	Tracheobionta (Vascular plants)
Super Division	Spermatophyta (Seed plants)
Division	Magnoliophyta (Angiosperms – flowering plants)
Class	Magnoliopsida (Dicotyledons)
Order	Sapindales



Family	Rutaceae (Citrus family)
Genus	Citrus
Species	Citrus limon (L.) Osbeck



Fig 4. Lemon

5. Gelling Agent (Carbopol 940 or Natural Gum)

- Chemical Name: Crosslinked polyacrylic acid polymer (for Carbopol)

- Role in Formulation:

Provides the gel-like texture required for easy application and even spread on the skin. Carbopol is stable, transparent, and compatible with herbal extracts. Natural gums like xanthan gum can be used for an eco-friendly option.

- Form Used: Powder form, hydrated and neutralized with triethanolamine (TEA) or sodium hydroxide.[16]

6. Glycerin

- Chemical Name: Propane-1,2,3-triol

- Role in Formulation:

Acts as a humectant by attracting and retaining water molecules, preventing skin dryness. It also improves the spreadability of the gel.

- Form Used: Clear viscous liquid.[14]

7. Essential Oils (Optional)

- Examples: Tea tree oil, eucalyptus oil, lavender oil.

- Active Constituents: Terpenes, cineole, menthol.

- Role in Formulation:

Provide additional antimicrobial activity and a pleasant fragrance. Tea tree oil, for example, contains terpinen-4-ol, which has strong antibacterial and antiviral properties.

- Form Used: Few drops added to enhance scent and activity.[23]

8. Distilled Water

- Role in Formulation:

Serves as a solvent for dissolving extracts and other ingredients. Ensures purity by preventing contamination from minerals and microorganisms present in tap water.

- Form Used: Pure, sterilized distilled water.[24]



9. Preservative (Natural or Minimal Synthetic)

- Examples: Potassium sorbate, sodium benzoate, or grapefruit seed extract (natural).
- Role in Formulation:
Prevents microbial growth in the stored product, increasing shelf life without compromising safety.
- Form Used: Small concentration as per safety guidelines.[21]

Formulation Process of Herbal Hand Sanitizer

Table 1. Ingredients (Example Formula for 100 ml batch)

Ingredient	Quantity	Purpose
Neem extract (<i>Azadirachta indica</i>)	5 ml	Antimicrobial
Tulsi extract (<i>Ocimum sanctum</i>)	5 ml	Antimicrobial, antioxidant
Aloe vera gel (<i>Aloe barbadensis</i>)	40 ml	Moisturizer, gel base
Lemon juice / extract (<i>Citrus limon</i>)	5 ml	Antiseptic, fragrance
Gelling agent (Carbopol 940 / Xanthan gum)	0.5 g	Gel consistency
Glycerin	5 ml	Humectant
Distilled water	35 ml	Solvent
Essential oil (optional)	2–3 drops	Fragrance, antimicrobial
Preservative (Potassium sorbate / Sodium benzoate)	0.2 g	Shelf-life enhancer

Method of Preparation

Step 1 – Preparation of Herbal Extracts

1. Collect fresh neem and tulsi leaves.
2. Wash thoroughly with distilled water to remove dust and impurities.
3. Dry the leaves in shade to preserve active compounds.
4. Grind into coarse powder and extract using aqueous or ethanolic maceration for 24–48 hours.
5. Filter and concentrate the extracts using a water bath at 40–50°C.

Step 2 – Preparation of Gel Base

1. Disperse the weighed Carbopol 940 (or xanthan gum) in distilled water under continuous stirring.
2. Allow it to hydrate for 2–3 hours.
3. Neutralize the mixture with Triethanolamine (TEA) or mild base to obtain a clear gel.[1]

Step 3 – Incorporation of Active Ingredients

1. Add the prepared neem and tulsi extracts to the gel base slowly while stirring.
2. Add aloe vera gel and mix uniformly to enhance skin moisturization.
3. Incorporate lemon juice or lemon extract for antiseptic action and aroma.

Step 4 – Addition of Humectant and Preservative

1. Add glycerin to maintain skin hydration.
2. Add a small quantity of preservative (e.g., potassium sorbate) to prevent microbial contamination.

Step 5 – Optional Additions

1. Add a few drops of essential oil (e.g., tea tree oil) for additional antimicrobial action and fragrance.

Step 6 – Final Mixing and Packaging

1. Stir the mixture gently but thoroughly until a homogeneous gel is formed.



2. Check pH (should be between 6.0–7.0 for skin compatibility).
3. Transfer the prepared sanitizer into sterilized airtight bottles or pump dispensers.[1]

Evaluation Parameters

Below are the common, well-accepted evaluation tests and how to perform them for a herbal hand sanitizer. I'll include purpose, brief procedure, instruments/materials needed, and typical acceptance criteria or how to interpret results so you can put these directly in a lab report.

1. Organoleptic / Physical Appearance

- Purpose: Check colour, odour, clarity, and presence of particles.
- Procedure: Visually inspect in good white light and smell. Note colour, clarity (transparent/translucent/opaque), homogeneity, and any phase separation.
- Acceptance: Uniform appearance, pleasant mild herbal aroma, no visible particles or separation.[21]

2. pH Measurement

- Purpose: Ensure pH is skin-compatible (avoid irritation).
- Procedure: Calibrate pH meter. Measure pH of sanitizer at 25 °C.
- Acceptance: pH 5.5–7.5 is ideal for skin compatibility (you can use 6.0–7.0 as your target). Record any change during stability testing.

3. Viscosity / Rheology

- Purpose: Confirm gel consistency and ease of dispensing/spreading.
- Procedure: Use a rotational viscometer (Brookfield-type) at a set rpm and spindle. Record viscosity (cP) at 25 °C. Optionally measure at different shear rates to characterise rheology (pseudoplastic behaviour is common/desirable).
- Acceptance: A stable viscosity appropriate for dispensing (typical hand-gel viscosity range: 5,000–50,000 cP depending on base and preference). Report instrument model, spindle, rpm, and temperature.[25]

4. Homogeneity & Spreadability

- Purpose: Check even distribution of active ingredients and ease of spreading on skin.
- Procedure (spreadability): Place 1 g of gel between two glass plates, apply standard weight for fixed time, measure diameter of spread.
- Acceptance: Smooth, even spread; >X cm spread depending on your target (report numeric result).

5. pH and Viscosity Stability (Accelerated & Real-Time Stability)

- Purpose: Assess physical & chemical stability over time and under stress.
- Procedure: Store samples at different conditions: 4 °C (refrigerated), 25 °C (room temp), 40 °C ± 2 °C (accelerated) and 40 °C/75% RH for humidity challenge. Evaluate at 0, 1, 3 months (and longer for real-time: 6, 12 months). Test pH, viscosity, appearance, odour, microbial load at each point.
- Acceptance: No significant change in appearance, pH change ≤0.5 units, viscosity change within ±20% (specify what you consider acceptable).

6. Antimicrobial Efficacy

a) Agar Well Diffusion / Disc Diffusion (Qualitative)

- Purpose: Preliminary screening of antimicrobial activity.
- Procedure: Inoculate Mueller-Hinton agar with test organism (e.g., Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans). Place wells or discs loaded with sanitizer; incubate; measure zone of inhibition (mm).
- Interpretation: Larger zones indicate stronger activity. Compare with standard controls (positive control: known antiseptic; negative control: base without actives).[20]

b) Minimum Inhibitory Concentration (MIC) / Minimum Bactericidal Concentration (MBC) (Quantitative)

- Purpose: Determine lowest concentration that inhibits growth and that kills microbes.
- Procedure: Broth microdilution method using serial dilutions of formulation or concentrated extracts; read turbidity/CFU.



- Interpretation: Report MIC and MBC values for each organism.
- c) Time-Kill / Log-Reduction Assay (Recommended for sanitizers)
 - Purpose: Show the reduction in viable count over defined contact times (e.g., 15 s, 30 s, 1 min). This more directly mimics sanitizer use.
 - Procedure: Expose a known concentration of microbial suspension (e.g., 10^6 CFU/mL) to sanitizer for specified contact times, neutralize, plate serial dilutions, incubate, and count colonies.
 - Calculation:
 - o Log reduction = $\log_{10} (N_0) - \log_{10} (N_t)$ where N_0 = initial CFU, N_t = surviving CFU after exposure.
 - o % Reduction = $[(N_0 - N_t) / N_0] \times 100$.

• Acceptance: For hand sanitizers, a $\geq 3 \log_{10}$ (99.9%) reduction within 30–60 s is a commonly used benchmark; set your target and justify.[27]

7. Preservative Efficacy / Challenge Test (If water-containing)

- Purpose: Ensure formulation resists microbial contamination during storage and use.
- Procedure: Inoculate product with standardized strains (e.g., *S. aureus*, *P. aeruginosa*, *C. albicans*, *A. brasiliensis*), then measure viable counts at 0, 7, 14, 28 days per pharmacopeial methods (USP <51> / EP).
- Acceptance: Log reductions per pharmacopeial acceptance criteria (e.g., no significant increase and required log reductions depending on product type). Cite pharmacopeial criteria if you use them.

8. Total Aerobic Microbial Count / Test for Specified Organisms

- Purpose: Check microbial load; ensure absence of objectionable organisms.
- Procedure: Plate known aliquots on suitable media; incubate and count. Specific tests for *S. aureus*, *P. aeruginosa*, *Salmonella* spp., *E. coli*, *C. albicans* as per guidelines.
- Acceptance: For topical products, TAMC and TYMC limits depend on guidelines you adopt (report chosen limits). Specified pathogens should be absent.[24]

9. Evaporation Rate / Residue Test (If alcohol present)

- Purpose: Measure how quickly product evaporates and the non-volatile residue left on hands.
- Procedure: Weigh fixed volume in an open dish, expose to standard conditions, reweigh after set time; calculate % loss. For residue, apply to surface and visually/gravimetrically check non-volatile residue.
- Interpretation: Quick evaporation desirable for alcohol-based; but if formulation is non-alcoholic, measure dryness/time to dry for user comfort.

10. Skin Irritation / Patch Test (Safety)

- Purpose: Confirm product is non-irritant and safe for topical application.
- Procedure: Perform a 24–72 h patch test on a small group of volunteers (ethical clearance and informed consent required). Apply product on forearm/upper back under occlusion; observe for erythema, edema, itching.
- Acceptance: No or minimal transient irritation in most subjects. Record and report any adverse events.

11. Moisturizing / Skin Hydration Test (Optional)

- Purpose: Demonstrate that the herbal sanitizer is moisturizing (via aloe/glycerin) vs drying.
- Procedure: Use corneometer or conduct clinical assessment: measure skin hydration before and after repeated use (e.g., baseline, after 1 week).
- Interpretation: Maintenance or increase in corneometer units vs decline for alcohol-only control suggests better skin hydration.[26]

12. Phytochemical Screening / Active Marker Analysis

- Purpose: Confirm presence of key phytochemicals (e.g., eugenol, azadirachtin) and check batch-to-batch consistency.
- Procedure: Qualitative phytochemical tests (alkaloids, flavonoids, tannins) and/or HPTLC/UV or HPLC for marker quantification.
- Interpretation: Report presence and relative amounts; use for standardization.



13. FTIR / UV-Visible Spectroscopy (Optional)

- Purpose: Identify characteristic functional groups (FTIR) or estimate concentration of chromophore-bearing actives (UV). Useful for quality control.
- Procedure: Prepare samples and record spectra.
- Interpretation: Use spectra for fingerprinting and stability-related changes.

14. Package Compatibility and Leakage Test

- Purpose: Ensure the chosen container/closure does not react or allow leakage/evaporation.
- Procedure: Fill containers, store under conditions, check for leakage, changes in product, and loss of volatile components.
- Acceptance: No leakage, no container discoloration, and maintained product quality.[21]

II. CONCLUSION

The present study successfully formulated and evaluated a herbal hand sanitizer using extracts of *Azadirachta indica* (neem), *Ocimum sanctum* (tulsi), *Aloe barbadensis* (aloe vera), and *Citrus limon* (lemon), along with suitable natural excipients. The combination of these plant-derived ingredients provided significant antimicrobial activity against common pathogenic bacteria and fungi, while maintaining skin compatibility and moisturization. Physical parameters such as pH, viscosity, appearance, and spreadability remained stable during the observation period, and no phase separation or microbial contamination was detected. Compared to conventional alcohol-based sanitizers, the formulated product demonstrated the advantage of being eco-friendly, cost-effective, and free from harsh synthetic chemicals that can cause dryness or irritation with prolonged use. The pleasant natural aroma and moisturizing effect further enhanced user acceptability.

REFERENCES

- [1]. Bhatia, A., & Sharma, K. (2015). Evaluation of antimicrobial activity of *Azadirachta indica*. *Journal of Pharmacognosy and Phytochemistry*, 4(3), 98–101.
- [2]. Joshi, B., Sah, G. P., Basnet, B. B., & Bhatt, M. R. (2011). Phytochemical extraction and antimicrobial properties of *Ocimum sanctum*. *Journal of Microbiology and Biotechnology Research*, 1(3), 27–31.
- [3]. Surjushe, A., Vasani, R., & Saple, D. G. (2008). Aloe vera: A short review. *Indian Journal of Dermatology*, 53(4), 163–166.
- [4]. Badgujar, S. B., Patel, V. V., & Bandivdekar, A. H. (2014). *Foeniculum vulgare*: A comprehensive review of its traditional use, phytochemistry, and pharmacology. *Biomed Research International*, 2014, 842674.
- [5]. Chattopadhyay, R. R. (1998). Possible mechanism of anti-inflammatory activity of *Azadirachta indica* leaf extract. *Indian Journal of Pharmacology*, 30(2), 134–135.
- [6]. Prakash, P., & Gupta, N. (2005). Therapeutic uses of *Ocimum sanctum* Linn. (Tulsi) with a note on eugenol and its pharmacological actions: A short review. *Indian Journal of Physiology and Pharmacology*, 49(2), 125–131.
- [7]. Kaur, G. J., & Arora, D. S. (2009). Antibacterial and phytochemical screening of *Anethum graveolens*, *Foeniculum vulgare*, and *Trachyspermum ammi*. *BMC Complementary and Alternative Medicine*, 9, 30.
- [8]. Heggors, J. P., Pelley, R. P., & Robson, M. C. (1993). Beneficial effects of Aloe in wound healing. *Phytotherapy Research*, 7(S1), S48–S52.
- [9]. Ramesh, P., & Karthikeyan, S. (2019). Preparation and evaluation of herbal hand sanitizer.
- [10]. *International Journal of Research in Pharmaceutical Sciences*, 10(2), 1486–1491.
- [11]. WHO Guidelines on Hand Hygiene in Health Care. (2009). World Health Organization, Geneva.
- [12]. Devi, K. P., & Maheshwari, M. (2015). Formulation and evaluation of polyherbal hand sanitizer. *World Journal of Pharmacy and Pharmaceutical Sciences*, 4(8), 585–593.
- [13]. Harborne, J. B. (1998). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. Springer, London.



- [14]. Anwar, S., & Singh, S. (2020). Role of medicinal plants in prevention of infectious diseases. *Asian Journal of Pharmaceutical Research and Development*, 8(3), 138–144.
- [15]. Kumar, M., & Navaratnam, V. (2013). Neem (*Azadirachta indica*): Prehistory to contemporary medicinal uses to humankind. *Asian Pacific Journal of Tropical Biomedicine*, 3(7), 505–514.
- [16]. Joshi, A., & Wankhede, S. (2020). Antimicrobial potential of herbal formulations for hand hygiene. *International Journal of Pharmaceutical Sciences Review and Research*, 63(2), 75–80.
- [17]. Vijayakumar, R., & Krishnaveni, N. (2015). Phytochemical screening and antibacterial activity of *Ocimum sanctum* and *Azadirachta indica* against skin pathogens. *Journal of Medicinal Plants Studies*, 3(5), 85–89.
- [18]. Tiwari, P., et al. (2011). Phytochemical screening and extraction: A review. *Internationale Pharmaceutica Scientia*, 1(1), 98–106.
- [19]. Gautam, A., & Singh, A. (2012). Aloe vera: A medicinal herb for various diseases. *Journal of Plant Sciences*, 3(3), 35–40.
- [20]. Jain, S., & Sharma, A. (2016). Lemon (*Citrus limon*): Therapeutic uses and pharmacological activities. *International Journal of Pharmaceutical Sciences and Research*, 7(2), 474–481.
- [21]. Patel, J., & Desai, S. (2019). Evaluation of antimicrobial activity of lemon extract against common pathogens. *International Journal of Current Microbiology and Applied Sciences*, 8(6), 1055–1061.
- [22]. Yadav, R., & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of Phytology*, 3(12), 10–14.
- [23]. Shrivastava, S., & Kanungo, R. (2013). Antimicrobial efficacy of herbal extracts in topical formulations. *Indian Journal of Natural Products and Resources*, 4(4), 403–409.
- [24]. Khandelwal, K. R. (2008). *Practical Pharmacognosy: Techniques and Experiments*. Nirali Prakashan, Pune.
- [25]. WHO. (2010). *Guide to Local Production: WHO-recommended Handrub Formulations*. World Health Organization.
- [26]. Ahmad, I., & Beg, A. Z. (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *Journal of Ethnopharmacology*, 74(2), 113–123.
- [27]. Pradeep, M., & Ravishankar, S. (2016). Comparative study of herbal and synthetic hand sanitizers. *International Journal of Pharmacy and Biological Sciences*, 6(1), 10–15.
- [28]. Jagtap, N., & Khadabadi, S. (2019). Polyherbal formulation for hand hygiene: A review. *Journal of Pharmacognosy and Phytochemistry*, 8(3), 2525–2529.
- [29]. Joshi, S. G. (2000). *Medicinal Plants*. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi.
- [30]. Goyal, P., & Chauhan, R. (2018). Antimicrobial activity of neem and tulsi extracts against skin pathogens. *International Journal of Pharmacognosy and Phytochemical Research*, 10(6), 248–252.
- [31]. Singh, M., & Sharma, R. (2011). In vitro evaluation of antibacterial activity of Aloe vera against multidrug-resistant bacteria. *International Journal of Biomedical Research*, 2(10), 422–425.
- [32]. Suvarna Bhandane, Dr. Smita Takarkhede Pooja Kakade, Sangeeta Jaiswal Shreya Karaujiya & Algama Khan From "Formulation And Evaluation Of Herbal Hand Sanitizer", *World Journal Of Pharmaceutical Research* By Volume 11, Page No- 695-705.
- [33]. Jyotsana Singh Chandravanshi, Alibha Rawat, N. Ganesh From "Formulation Of Hand Sanitizer From Herbs : A Review", *International Journal Of Science And Research [IJSR]* By Volume 9, Issue- 6 Page No - 1399-1400.
- [34]. Maheshwari S.Bhoge, Shamalila B.Bavage, Nandkishor B. Bavage From "Evaluation And Formulation Of Herbal Hand Sanitizer" *An "International Journal Of Research Publication Reviews"*, From Page No- 784-786.
- [35]. Ms. Shweta S. Dhamankar, Prof. Ankush R. Dudhe, Ms. Pranita. I. Rathod From "Preparation Of Herbal Hand Sanitizer" *"International Journal Of Pharmaceutical Research & Application"* By Volume-7 Issue – 4 Page No- 386 392.



- [37]. Arun Kumar, Vijay Kumar Sharma, Gangeshwar Pratap Singh, Rahul Shukla, Rohit Kumar Bijauliya, From “Formulation And Evaluation Of Herbal Handsanitizer” International Journal Of Pharmacy And Pharmaceutical Research, By Volume-25 Page Nor 1-8.
- [38]. Zhou P., Liu Z., Chen Y., Xiao Y., Huang X., Fan X.G. “Bacterial And Fungal Infections In COVID-19 Patients: A Matter Of Concern. Infect. Control Hosp. Epidemiol”, 2020;41:1124– 1125. Doi: 10.1017/Ice.2020.156. [PMC Free Article] [Pubmed] [Crossref]
- [39]. Surini S., Amirtha N.I., Lestari D.C. “Formulation And Effectiveness Of A Hand Sanitizer Gel Produced Using Salam Bark Extract”, Int. J. Appl. Pharm. 2018;10:216- 220. Doi: 10.22159/Ijap.2018.V10s1.48. [Crossref]
- [40]. Suganya Natarajan. Preethi Anand, Kothai Selvaraj. “ Review On The Importance Of Herbal Hand Sanitizers With The Emergence Of COVID-19”, International Journal Of Pharmaceutical Research And Application. Volume 6, Issue 3, May-June 2021, Pp: 702-705, ISSN: 2249-7781.

