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Formulation, Evaluation, and Antimicrobial Efficacy of a Polyherbal Hand Sanitizer Gel Utilizing Aloe barbadensis Miller, Azadirachta indica, and Ocimum sanctum

Prashant Pathak, Sandeep Soni, Narendra Kumawat, Manish, Yogesh Sharma

Department of Pharmacy, Mahatma Gandhi Engineering College, Shivdaspura, Jaipur, Rajasthan, India

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Corresponding author: Prashant Pathak
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Abstract:

Background: Frequent use of conventional alcohol-based hand sanitizers often triggers adverse dermatological effects such as skin barrier disruption, contact dermatitis, and severe dryness. Integrating medicinal plant extracts offers a viable path to mitigate these side effects while preserving broad-spectrum antimicrobial action.

Objectives: The objective of this study was to formulate a stable polyherbal hand sanitizer gel using Aloe vera (gel), Neem, and Tulsi (leaf extracts) combined with 70% w/w isopropyl alcohol (IPA), and evaluate its physicochemical attributes, skin tolerability, and antimicrobial performance.

Methods: Neem and Tulsi extracts were prepared via cold maceration using 50% aqueous ethanol. A 1% w/w Carbopol 940 gel base was hydrated and neutralized using Triethanolamine. Physicochemical features, viscosity, spreadability, drying time, and accelerated stability (over 30 days at 25°C, 40°C, and 60°C) were comprehensively evaluated. Antimicrobial efficacy was verified against Staphylococcus aureus and Escherichia coli via the agar well diffusion method. Skin safety was monitored using a standard patch test on human volunteers.

Results: The developed formulation was a smooth, homogeneous, clear pale-yellow gel with a pleasant minty aroma, a physiological skin-compatible pH range of 6.8–7.2, and an optimal viscosity of 4800–5200 cP. It demonstrated a rapid skin drying time of 15–20 seconds and produced zero irritation, erythema, or edema during patch testing. Agar diffusion assays revealed significant zones of inhibition (ZOI) against S. aureus (~18 mm) and E. coli (~16 mm), which closely parallel standard 70% IPA controls. The formulation maintained structural integrity across 30 days of ambient and accelerated stability testing.

Conclusion: The polyherbal hand sanitizer gel effectively balances potent broad-spectrum antimicrobial performance with enhanced dermoprotective benefits, establishing a highly acceptable, green pharmacy alternative for healthcare and domestic sanitation.

Introduction

Hand hygiene stands as the definitive operational measure for cutting off pathogen transmission pathways and preventing infectious diseases. The hands act as major mechanical vehicles for cross-contamination. Consequently, the World Health Organization (WHO) identifies hand hygiene as the primary frontline intervention to curb healthcare-associated infections. While traditional mechanical washing with soap and water remains standard, instances lacking reliable plumbing infrastructure demand practical alternatives like waterless antiseptic hand rubs.

The systemic urgency of hand hygiene was brought to light by the COVID-19 pandemic, which generated global shortages and triggered an influx of substandard chemical formulations. Most conventional commercial sanitizers depend heavily on synthetic chemical antimicrobials or high concentrations (60% to 80%) of ethanol or isopropyl alcohol (IPA). Although highly effective at denaturing viral and bacterial proteins, routine application routinely strips natural lipid layers from the stratum corneum, culminating in contact dermatitis, painful skin dehydration, and reduced compliance.

To counter this, pharmaceutical research is turning to green chemistry and the principles of Ayurveda to design herbal-based alternatives. Polyherbal combinations can bridge this gap by joining standard volatile alcohol agents with therapeutic plant extracts. This research develops and tests an optimized hand rub incorporating three foundational medicinal plants:

- **Aloe vera (Aloe barbadensis Miller):** Serves as a direct humectant and emollient, preventing alcohol-induced moisture loss.
- **Neem (Azadirachta indica):** Acts as a strong antibacterial agent rich in tetranortriterpenoids.
- **Tulsi (Ocimum sanctum):** High in volatile phenolic compounds that bolster antimicrobial activity while imparting a natural aroma.

This study aims to evaluate a topical antiseptic gel that meets official antimicrobial baselines while actively improving skin compatibility.

Review of Literature

The clinical precedent for chemical hand antisepsis dates back to Ignaz Semmelweis, who used chlorinated lime solutions to drastically drop puerperal fever mortality rates in 19th-century obstetric wards. This was reinforced by Pasteur's germ theory and Lister's focus on aseptic environments. Modern clinical epidemiology confirms that structured hand hygiene programs significantly lower community and nosocomial respiratory and gastrointestinal outbreaks.

However, conventional alcohol rubs present clear physiological drawbacks. Mechanistically, frequent exposure to high-titer alcohols extracts essential intercellular lipids from the outer skin barrier. This process causes elevated transepidermal water loss (TEWL), deep skin cracking, and regular inflammation. Industrial surveys note that hand dermatitis impacts up to 25–30% of healthcare workers who rely on conventional sanitizers. Furthermore, synthetic additives and chemical fragrances routinely spark secondary contact allergies.

Phytotherapy offers an evidence-based approach to address these compliance barriers. Bioactive plant elements can safely interact with alcohol to widen or preserve antimicrobial scope while concurrently soothing the skin barrier.

Aloe vera leaf parenchyma contains more than 75 distinct active constituents, including the core therapeutic polysaccharide acemannan. Formulations including 5% to 20% Aloe vera gel demonstrate an ability to preserve epidermal barrier function and minimize TEWL. Concurrently, Azadirachta indica extracts demonstrate robust efficacy against major target pathogens such as Staphylococcus aureus and Escherichia coli.

Its mechanism relies on complex triterpenoids like nimbidin, gedunin, and the flavonoid quercetin, which disrupt bacterial cell wall replication and ergosterol biosynthesis.

Ocimum sanctum leaves yield substantial percentages of eugenol, an active phenolic compound that drives cell membrane lysis in target microbes. It also contains rosmarinic and ursolic acids, which offer topical antioxidant benefits. Past studies confirm that merging these three specific herbal ingredients maintains zones of inhibition comparable to commercial synthetic sanitizers while significantly reducing skin dryness and irritation profiles.

Aim and Objectives

Aim

The primary aim of this study is to formulate and visually, physically, and microbiologically evaluate a stable polyherbal hand sanitizer gel utilizing Aloe vera leaf gel, Azadirachta indica extract, and Ocimum sanctum extract in an isopropyl alcohol base.

Objectives

1. To isolate and extract active fractions of Neem leaves, Tulsi leaves, and fresh Aloe vera inner parenchyma using validated pharmacognostic protocols.
2. To prepare a stable, visually clear, and homogeneous gel matrix utilizing Carbopol 940 as a gelling polymer, neutralized by Triethanolamine to a skin-compatible pH.
3. To comprehensively assess the physicochemical metrics of the final formulation, spanning pH, structural viscosity, spreadability index, drying time, and organoleptic properties.
4. To determine comparative antimicrobial efficacy against index Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacterial strains via agar well diffusion assays.
5. To conduct accelerated and environmental stress stability profiles across a 30-day monitoring timeline at 25°C, 40°C, and 60°C.
6. To verify dermatological safety and lack of skin irritation via structured human patch testing.

Materials and Methods

Materials

Fresh, authentic leaves of Aloe barbadensis Miller, Azadirachta indica, and Ocimum sanctum were harvested from the institutional medicinal garden. Pharmaceutical-grade Carbopol 940, Triethanolamine (TEA), Isopropyl Alcohol (99% v/v), Glycerin, Hydrogen Peroxide (3% v/v), and pure Peppermint oil were sourced at analytical grade purity.

Formulation Composition

The quantitative multi-component formulation design for a standardized 60-gram batch is outlined in Table 1 below.

Table 1: Complete quantitative formula for the polyherbal sanitizer gel.

Sr. No.	Ingredient	Concentration (% w/w)	Quantity (g)	Function in Formulation
1	Isopropyl Alcohol (IPA)	70.0%	42.00 g	Primary active antimicrobial agent
2	Glycerin	3.0%	1.80 g	Humectant, skin moisturizer
3	Aloe Vera Gel	5.0%	3.00 g	Soothing agent, anti-inflammatory emollient
4	Neem Extract	2.0%	1.20 g	Antimicrobial and antifungal active
5	Tulsi Extract	1.5%	0.90 g	Antibacterial and antioxidant active
6	Carbopol 940	1.0%	0.60 g	Gelling / structural viscosity agent
7	Triethanolamine (TEA)	q.s.	0.30 g	pH adjuster; neutralizes Carbopol matrix
8	Hydrogen Peroxide 3%	0.125%	0.075 g	Auxiliary antiseptic / spore inactivator
9	Peppermint Oil	0.3%	0.18 g	Natural fragrance, mild antimicrobial
10	Purified Water	q.s.	to make 60.0 g	Primary vehicle / continuous phase solvent

Methodology

Extraction of Active Herbal Ingredients

Aloe vera Gel Isolation: Mature leaves were washed and surface sterilized with 70% IPA. The outer green rind was sliced away using a sterile knife. The clear internal parenchymal gel was carefully collected, homogenized in a mechanical blender, and passed through a double-layer muslin cloth matrix to remove extraneous structural fiber. The filtered gel was stored in a dark amber container at 4°C.

Cold Maceration of Neem and Tulsi: Leaves from both plants were washed thoroughly, separated, and shade-dried at room temperature for 5–7 days to safely lower baseline water content. Dried tissues were reduced to a coarse powder using a mechanical grinder and sized uniformly through a mesh No. 40 sieve. Exactly 50 grams of each dried powder was transferred to independent, airtight vessels and submerged in 500 mL of a 50% aqueous ethanol menstruum. The systems were sealed and allowed to stand for 7 days at 25 ± 2°C with scheduled daily mechanical agitation. The solutions were filtered through Whatman No. 1 filter paper. The resulting filtrates were concentrated using a water bath set at 60°C to yield stable, semi-solid herbal extracts.

Development of the Polyherbal Gel Matrix

Carbopol 940 (1% w/w) was slowly sifted into 15–20 mL of sterile purified water and left to hydrate completely for 30–45 minutes to encourage uniform particle swelling. Once fully hydrated, measured quantities of Aloe vera gel (5% w/w), concentrated Neem extract (2% w/w), and Tulsi extract (1.5% w/w) were added sequentially with continuous stirring. Glycerin (3% w/w) was then integrated into the mixture.

Subsequently, 42.0 grams of Isopropyl Alcohol was slowly added to prevent polymer shock. This step was performed in a closed, low-velocity air environment to minimize volatile evaporation losses. Hydrogen peroxide (3%) and peppermint oil were then added to the mixture. Finally, Triethanolamine was added dropwise under digital pH control to neutralize the acidic Carbopol solution, inducing cross-linking to form a smooth, transparent gel structure with a targeted physiological pH. Purified water was added to achieve the final target weight of 60 grams.

Observations and Results

Physicochemical and Organoleptic Profile

The generated polyherbal sanitizer gel was subjected to rigorous physical characterization. The definitive test outcomes are compiled in Table 2.

Table 2: Physicochemical properties of the formulated polyherbal hand sanitizer gel.

Sr. No.	Evaluation Parameter	Applied Experimental Method	Documented Analytical Observation
1	Physical Appearance / Color	Visual inspection under direct light	Clear, pale yellow-green translucent gel
2	Odour	Sensory panel assessment	Pleasant, refreshing minty aroma
3	Structural pH	Calibrated digital pH meter (1% w/v)	6.8 – 7.2 (Highly skin-compatible)
4	Rheological Viscosity	Brookfield Viscometer (Spindle 5, 20 rpm)	4800 – 5200 cP
5	Spreadability Index	Parallel glass slide method	Spreads uniformly under standard load
6	Structural Homogeneity	Manual texture and tactile touch feel	Extremely smooth, total absence of lumps
7	Total Alcohol Content	Gas Chromatography / Specific Gravity	~70% v/v baseline compliance
8	Epidermal Drying Time	In-vivo stopwatch topical evaluation	15 – 20 seconds total drying time
9	Human Skin Irritation	Structured Draize forearm patch test	Zero irritation, erythema, or edema
10	30-Day Storage Stability	Incubation at 25°C, 40°C, and 60°C	Physically stable; zero phase separation

Rheological and Aesthetic Parameters

The formulation exhibited excellent structural attributes. It maintained a viscosity of 4800–5200 cP, ensuring optimal retention on the hands without excessive dripping or unappealing tackiness. The addition of peppermint oil effectively masked the strong, sharp scent of isopropyl alcohol, resulting in a highly acceptable sensory profile. The pH remained stable between 6.8 and 7.2, which is exceptionally safe for human skin and protects the skin's natural protective barrier.

In-Vitro Antimicrobial Efficacy

Antimicrobial performance was evaluated using agar well diffusion assays. The measured zones of inhibition (ZOI) for the polyherbal sanitizer were compared against plain 70% Isopropyl Alcohol (positive control) and pure distilled water (negative control).

Against Staphylococcus aureus, the polyherbal formulation achieved a mean zone of inhibition of approximately 18 mm, which closely approaches the 20 mm zone produced by pure 70% IPA. Against Escherichia coli, the formulation generated a mean zone of 16 mm, compared to 18 mm for the positive control. The negative water control produced 0 mm of active inhibition.

Comparative Zones of Inhibition (ZOI) Profile:
Polyherbal Sanitizer Gel (S. aureus):
[redacted] 18 mm
70% IPA Standard Control (S. aureus):
[redacted] 20 mm
Polyherbal Sanitizer Gel (E. coli):
[redacted] 16 mm
70% IPA Standard Control (E. coli):
[redacted] 18 mm

Discussion

The successful formulation of this polyherbal hand sanitizer gel demonstrates that natural plant extracts can be integrated into alcohol-based vehicles without reducing antimicrobial efficacy. The core structure relies on a neutralized Carbopol 940 polymer matrix. Unneutralized Carbopol remains highly acidic and low in viscosity. Adding Triethanolamine triggers chain ionization and cross-linking, transforming the solution into a thick, clear, three-dimensional gel network. This matrix ensures a uniform distribution of both the volatile alcohol and the dense herbal molecules.

The antimicrobial data indicates that combining Isopropyl Alcohol with plant-derived secondary metabolites provides robust broad-spectrum performance. The active compounds in Neem (such as nimbin, nimbidin, and azadirachtin) degrade bacterial cell membranes and inhibit replication enzymes. Concurrently, eugenol from Tulsi denatures structural membrane proteins, increasing bacterial cell permeability. When paired with 70% IPA, these mechanisms work together to deliver reliable antimicrobial action against both Gram-positive and Gram-negative targets.

Dermatologically, the severe drying effect characteristic of conventional alcohol rubs was successfully neutralized by incorporating Aloe vera gel. The long-chain acemannan polysaccharides in Aloe vera create a protective hydrophilic layer on the skin, sealing in moisture and reducing trerespirational water loss. This effect was confirmed by human patch testing, which resulted in zero irritation, redness, or skin sensitization across all participants.

Stability testing over 30 days showed excellent physical consistency under both room temperature (25°C) and accelerated (40°C) conditions. At an elevated stress temperature of 60°C, the gel color deepened slightly over time. This color change is caused by the mild thermal breakdown of natural plant chlorophyll molecules. However, the gel maintained stable viscosity, pH, and structural integrity, confirming its suitability for commercial storage and transport.

Conclusion

This study successfully developed an optimized polyherbal hand sanitizer gel utilizing Aloe vera, Neem, and Tulsi extracts in a 70% isopropyl alcohol base. The formulation meets strict physicochemical standards for topical cosmetics, including a skin-safe pH, uniform spreadability, and stable viscosity. It provides reliable antimicrobial action against Staphylococcus aureus and Escherichia coli while delivering exceptional skin safety with zero irritation. By replacing synthetic conditioning agents with natural Aloe vera gel, this formulation offers a highly acceptable, eco-friendly option that supports public health and aligns with green pharmacy initiatives.

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