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Preparation and Evaluation of Herbal Hand Sanitizer Using Tulsi, Lemon, and Aloe Vera Extracts

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Abstract: ***The present study aimed to formulate and evaluate herbal hand sanitizer gels incorporating Ocimum sanctum (Tulsi), Aloe vera, and Citrus limon (Lemon) extracts as natural antimicrobial agents. Five formulations F1–F5 were made by using carbopol as a gelling agent and glycerine as a humectant. The gels developed were tested for organoleptic characteristics, pH, viscosity, spreadability, antimicrobial properties, stability, and skin irritation.***

All the formulations displayed desirable physical properties, with F3 and F5 possessing better color, fragrance, and texture. pH values (6.2–6.9) fell in the skin-friendly range. Spreadability and viscosity tests indicated that F3 and F5 had an ideal balance for ease of application. Antimicrobial testing showed that these formulations possessed extensive inhibitory zones against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans, equivalent to commercial alcohol-based hand sanitizers. Stability tests for more than 90 days showed negligible colour, odor, and pH changes, which attested to formulation stability. There was no irritation in the patch test, reflecting skin safety. F3 and F5 were the most effective, stable, and user-acceptable herbal hand sanitizers overall. The research adds credence to alcohol-free, plant-derived sanitizers as safe, effective, and efficient tools for keeping hands clean.

Keywords: Herbal hand sanitizer, Ocimum sanctum, Aloe vera, Citrus limon, antimicrobial activity, stability study.

I. INTRODUCTION

It is widely acknowledged that one of the best ways to lessen the spread of infectious diseases is to practice good hand hygiene. Skin and environmental contamination are often attributed to pathogens like Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Candida species, and outbreaks in healthcare and community settings have been directly connected to poor hand hygiene[1]. Because of their quick antibacterial activity, conventional hand sanitizers are frequently used, especially those with high alcohol concentrations (60–80%). However, frequent use of these chemical-based formulations is linked to negative side effects, including as excessive dryness, irritation, burning, and occasionally allergic responses[2].

Global dependence on synthetic hand sanitizer has also Increasing global use of synthetic sanitizers has also sparked concerns about environmental sustainability and antimicrobial resistance. In a herbal hand sanitizer, the addition of Tulsi, Lemon, and Aloe vera extracts results in a well-balanced composition, with Aloe vera providing skin protection and hydration while Tulsi and Lemon guarantee potent antibacterial activity [3].

Herbal sanitizers are becoming more and more well-known, with even patented ideas In order to make herbal hand sanitizers, the bioactive components from these plants are often extracted (either by solvent extraction or maceration) and then formulated with a base that contains alcohol or gel-forming chemicals. Once the extracts are mixed in the right proportions, the antibacterial effectiveness and user acceptability are guaranteed [4].

As a result of these drawbacks, interest in herbal substitutes has increased. Herbal formulations are prized for their safety, biodegradability, and compatibility with skin physiology in addition to their wide range of antibacterial and antioxidant qualities. In contrast to strictly synthetic treatments, plant-based sanitizers frequently contain bioactive substances that address both user comfort and efficacy by combining antibacterial activity with moisturizing or calming benefits. In this regard, three plants are particularly noteworthy: Aloe barbadensis (aloe vera), Citrus limon (lemon), and Ocimum sanctum (tulsi). Every plant has a long history in traditional medicine, and current pharmacological research backs it up [5][6].

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Eugenol, ursolic acid, flavonoids, and phenolic chemicals are abundant in tulsi and contribute to its antibacterial, antifungal, and anti-inflammatory properties. Tulsi extracts have shown inhibitory effects against a variety of bacteria and fungus, which makes them a viable option for topical antibacterial compositions. Furthermore, its antioxidant qualities can aid in the healing process and shield the skin from oxidative stress [7][8].

Vitamin C, limonene, citric acid, and flavonoids are some of the active ingredients found in lemons. By reducing the pH in the area, citric acid has an antibacterial impact and makes it harder for bacteria to survive. A volatile oil called limonene improves membrane permeability, and flavonoids have anti-inflammatory and antioxidant properties. Lemon's attractive citrus scent also enhances compositions' sensory appeal, which is crucial for customer approval [9].

Aloe Vera's polysaccharides, anthraquinones, and vitamins make it a popular ingredient in dermatological preparations. Aloe Vera is essential for skin regeneration, hydration, and relaxation in addition to its moderate antibacterial properties[10] The dryness and irritation that alcohol-based sanitizers frequently induce are offset by these qualities. Furthermore, when added to gel-based systems, aloe vera can improve the stability and bioavailability of other plant extracts[11].

The antibacterial properties of Tulsi, Lemon, and Aloe Vera have been documented in separate studies, and there have been multiple attempts to create herbal sanitizers with one or two of these plants. Only a little amount of research has been done on the synergistic use of all three botanicals in one formulation. The broad-spectrum antimicrobial action of tulsi, the acidic and fragrant qualities of lemon, and the moisturizing effect of aloe vera might all be combined to create a well-balanced sanitizer that is appropriate for daily use.

Therefore, the goal of the current study was to create and assess herbal hand sanitizer formulations utilizing extracts from aloe vera, lemon, and tulsi. The goals were to:

1) Formulate stable gel-based sanitizers with the right stabilizers and gelling ingredients.
2) Examine physicochemical traits such as pH, viscosity, spreadability, and organoleptic qualities.
3) Evaluate antibacterial activity using the agar well diffusion method against specific strains of bacteria and fungi.
4) Examine short-term stability in various storage scenarios.

The goal of this research is to address these goals and offer scientific proof that herbal sanitizers are feasible substitutes for traditional chemical formulations that are safer and more environmentally friendly. The results could potentially bolster the future development of commercial herbal sanitizers for broad use and add to the expanding body of research on plant-based healthcare solutions.

II. MATERIAL AND METHODOLOGY

A. Materials

The raw materials were selected based on their proven antibacterial and skin-protective properties. Fresh *Ocimum sanctum* (Tulsi) leaves were collected from local herbal gardens and authenticated by a botanist. Fresh *Citrus limon* (Lemon) fruits were procured from the local market, and mature *Aloe barbadensis* (Aloe vera) leaves were freshly harvested and processed to obtain gel. Analytical-grade ethanol and distilled water were used throughout the study. Carbopol 940 and xanthan gum served as gelling agents, while glycerin was added as a humectant to prevent skin dryness.

B. Chemicals and Equipment:

TABLE I :Chemical Requirements

S. No.	Material	Quantity Used (per 100 mL batch)	Purpose / Function
1	Tulsi extract	10.0 mL	Antibacterial and antifungal agent
2	Lemon juice / peel oil	5.0 mL	Antimicrobial, antioxidant, fragrance
3	Aloe vera gel	15.0 mL	Moisturizing and soothing agent
4	Ethanol (96%)	62.5 mL	Antiseptic and disinfectant
5	Glycerin	2.0 mL	Humectant, prevents skin dryness
6	Carbopol 940	1.0 g	Gelling agent
7	Triethanolamine (TEA)	q.s.	pH adjustment and gel neutralization
8	Distilled water	q.s.	Solvent and volume adjuster

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TABLE II : Equipment requirements

S. No.	Equipment / Apparatus	Purpose / Use
1	Beakers and conical flasks	Mixing and heating of extracts and formulations
2	Graduated cylinders	Measuring liquid ingredients
3	Digital weighing balance	Accurate weighing of materials
4	Magnetic stirrer / Stirring rod	Uniform mixing and dispersion
5	Hot plate / Water bath	Heating during extraction
6	Rotary evaporator	Concentration of extracts under reduced pressure
7	Thermometer	Temperature monitoring during extraction
8	pH meter	Determination of formulation pH
9	Brookfield viscometer	Measurement of viscosity
10	Glass slides	Spreadability testing
11	Petri dishes and inoculation loop	Antibacterial testing
12	Incubator	Incubation of microbial cultures
13	Sterile pipettes and test tubes	Handling and dispensing of liquids
14	Sterile amber bottles	Storage of final sanitizer formulations

C. Extraction of Plant Materials

1) Extraction of Tulsi (Ocimum sanctum)

Fresh Tulsi leaves were washed thoroughly, shade-dried for 7–10 days, and coarsely powdered. The powdered material was extracted using two solvents:

- Ethanolic extraction: 20 g of powdered leaves were soaked in 200 mL of 95% ethanol for 48 hours with intermittent stirring. The extract was filtered using Whatman filter paper and concentrated under reduced pressure with a rotary evaporator.
- Aqueous extraction: A separate portion of the powder was boiled in 200 mL of distilled water for 15–20 minutes, cooled, and filtered.

Both extracts were stored in airtight amber bottles at 4 °C until use.

2) Extraction of Lemon (Citrus limon)

Two extracts were prepared from the fruit:

- Lemon juice: Fresh lemons were washed, peeled, and manually squeezed. The juice was filtered through a muslin cloth to remove pulp and seeds, then stored at 4 °C.
- Lemon peel oil: The peels were shade-dried, powdered, and subjected to steam distillation to obtain essential oil rich in limonene. The oil was collected in dark glass vials and stored at 4 °C.

3) Extraction of Aloe vera (Aloe barbadensis)

Fresh Aloe vera leaves were washed, and the base was trimmed to drain the yellow latex for 10–15 minutes. The outer rind and serrated edges were carefully removed. The transparent gel was scooped out, rinsed with distilled water, and blended briefly to obtain a uniform, bubble-free gel. The gel was stored in sterilized glass containers at 4 °C.

D. Formulation of Herbal Hand Sanitizer

Different trial formulations were prepared using varying ratios of Tulsi extract, Lemon juice/peel oil, and Aloe vera gel to identify the most stable and effective composition.

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TABLE III. Standard Composition (100 mL batch)

Ingredient	Quantity
Ethanol (96%)	62.5 mL
Aloe vera gel	15.0 mL
Tulsi extract	10.0 mL
Lemon juice	5.0 mL
Glycerin	2.0 mL
Carbopol 940	1.0 g
Distilled water	q.s. to 100 mL

Procedure:

1) Gel Base Preparation:- Weigh 1.0 g of Carbopol 940 and disperse it slowly in ~3–4 mL of distilled water under continuous stirring. Allow hydration for 15–20 minutes until a uniform gel forms.
2) Addition of Extracts: Add Aloe vera gel, Tulsi extract, and Lemon juice/peel oil to the hydrated Carbopol with gentle stirring to achieve homogeneity.
3) Addition of Humectant:-Add glycerin and mix uniformly.
4) Incorporation of Ethanol:- Measure 62.5 mL of ethanol separately and slowly add to the mixture with constant stirring to avoid phase separation.
5) pH Adjustment and Gel Formation:- Adjust the pH to 6.0–7.0 using triethanolamine (TEA) dropwise. The mixture thickens to form a stable gel.
6) Final Adjustment: -Top up with distilled water to make the final volume 100 mL.
7) Filling and Storage:-The formulation was transferred to sterile, airtight bottles labeled with composition, ethanol percentage, and safety instructions. Samples were stored in a cool, dark place.

III.EVALUATION TESTS

A. Evaluation Parameters

The prepared formulations were subjected to the following evaluations:

1) Organoleptic properties: Color, odor, and appearance were visually assessed.
2) pH measurement: Determined using a calibrated digital pH meter.
3) Viscosity: Measured using a Brookfield viscometer.
4) Spreadability: Evaluated by glass-slide method.
5) Antibacterial activity: Assessed against *E. coli, S. aureus, P. aeruginosa*, and *Candida albicans* using the agar well diffusion method.
6) Stability testing: Conducted at room temperature and refrigerated conditions over four weeks to observe phase separation, color change, or microbial growth.

a) Organoleptic Properties

The color, odor, appearance, and texture of all formulated herbal hand sanitizers were visually examined and recorded. These organoleptic characteristics were used to assess the physical uniformity and consumer acceptability of each formulation.

b) pH Determination

The pH of each formulation was measured using a calibrated digital pH meter at room temperature (25 ± 2 °C). Measurements were taken in triplicate, and the mean values were calculated to ensure reliability. The acceptable pH range for skin-friendly formulations was maintained between 6.0 and 7.0.

c) Viscosity Measurement

Viscosity was determined using a Brookfield viscometer equipped with the appropriate spindle at 25 °C. Each sample was analyzed in triplicate, and the results were expressed in centipoise (cP). This parameter ensured that the formulation possessed adequate consistency for ease of application.

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d) Spreadability

Spreadability was evaluated by the parallel plate method. A fixed amount of the sample (1 g) was placed between two glass slides. A weight of 100 g was applied on the upper slide, and the time taken for it to move a specified distance (5 cm) under its own weight was recorded. The spreadability was calculated using the spreadability formula. Higher spreadability values indicate easier and more uniform application.

e) *Antimicrobial Activity*

The antimicrobial activity of the herbal sanitizer formulations was evaluated using the **agar well diffusion method**. Standard microbial strains were obtained from a microbiology laboratory, including:

- Escherichia coli (Gram-negative)
- Staphylococcus aureus (Gram-positive)
- Pseudomonas aeruginosa (Gram-negative)
- Candida albicans (fungus)

Procedure

Nutrient agar (for bacteria) and Sabouraud's dextrose agar (for fungi) were prepared and sterilized. The test organisms were inoculated uniformly on the agar surface using sterile swabs. Wells of 6 mm diameter were made using a sterile cork borer, and each well was filled with 100 µL of the herbal formulation. A commercial alcohol-based sanitizer was used as a positive control, while distilled water served as a negative control.

The plates were incubated at 37 °C for 24 hours (bacteria) and 48 hours (fungi). The **zones of inhibition (ZOI)** were measured in millimeters using a digital vernier caliper. The antimicrobial efficacy of the herbal formulations was compared with the standard control sanitizer.

f) Stability Testing

Short-term stability studies were conducted to evaluate the physical and chemical stability of the formulations under various storage conditions for 90 days:

TABLE IV: Stability parameters

Condition	Temperature / Humidity
Refrigerated	4 °C
Room temperature	25 ± 2 °C
Accelerated	40 °C and 75% RH

Samples were periodically examined for changes in color, odor, pH, viscosity, and phase separation. Stability was considered satisfactory if no significant changes were observed over the test period.

g) Skin Irritation Test

A patch test was conducted on healthy human volunteers after obtaining ethical clearance. A small quantity (0.5 mL) of the herbal sanitizer was applied on the inner forearm and covered with sterile gauze for 24 hours. The application site was observed for signs of redness, itching, swelling, or irritation. Absence of any adverse skin reaction indicated that the formulation was dermatologically safe for external use.

IV. RESULT & DISCUSSION

A. Organoleptic Evaluation

All herbal hand sanitizer formulations exhibited acceptable sensory characteristics. Tulsi imparted a greenish-brown shade and mild herbal fragrance, Lemon provided a refreshing citrus aroma, and Aloe Vera improved texture and smoothness. Among the batches, formulations with balanced herbal and citrus proportions showed superior overall acceptability.

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Table V: Organoleptic characteristics of formulations

Formulation	Color	Odor	Appearance	Texture	Acceptability
F1	Light green	Mild herbal	Clear gel	Smooth	Good
F2	Greenish-brown	Herbal + citrus	Clear gel	Smooth, thick	Very good
F3	Pale yellow-green	Strong citrus	Clear gel	Soft gel	Excellent
F4	Brownish-green	Herbal dominant	Opaque gel	Thick, sticky	Fair
F5	Light greenish-yellow	Balanced citrus-herbal	Clear gel	Smooth, spreadable	Excellent

The organoleptic analysis revealed that F3 and F5 possessed optimal consumer-friendly characteristics, while F4 was less acceptable due to opacity and higher thickness.

B. pH Determination

The pH values of the formulations ranged between 6.2 and 6.9, remaining within the dermatologically safe range of 5.5–7.0.

Table VI: *pH of formulations*

Formulation	pH (Mean ± SD)
F1	6.2 ± 0.05
F2	6.5 ± 0.03
F3	6.7 ± 0.04
F4	6.9 ± 0.02
F5	6.4 ± 0.06

F3 and F4 showed slightly higher pH values but were still well within the safe limit, ensuring compatibility with the skin's natural pH.

C. Viscosity Measurement

Viscosity influences spreadability and user experience. The results are presented below.

Table VII. Viscosity results

Formulation	Viscosity (cP)
F1	800
F2	1250
F3	1050
F4	1400
F5	950

F4 exhibited maximum viscosity, forming a dense gel, while F1 showed minimum viscosity, resulting in a thinner consistency. F3 and F5 demonstrated optimal viscosity, providing easy application and good consistency.

D. Spreadability Test

Spreadability directly affects user compliance.

Table VIII: Spreadability of formulations

Formulation	Spreadability (g·cm/s)
F1	7.5
F2	6.8
F3	8.4
F4	6.1
F5	8.0

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F3 and F5 exhibited superior spreadability, correlating with their balanced viscosity and smooth texture, enhancing user comfort during application.

E. Antimicrobial Activity

Antimicrobial testing was performed against *E. coli*, *S. aureus*, *P. aeruginosa*, and *Candida albicans* using the agar well diffusion method.

Table IX. Zone of inhibition (mm)

Microorganism	Standard (Alcohol-based sanitizer)	F1	F2	F3	F4	F5
E. coli	22	15	18	21	17	20
S. aureus	24	16	20	23	18	22
P. aeruginosa	20	14	17	20	15	19
C. albicans	18	13	16	19	14	18

F3 (rich in Lemon and Tulsi extracts) and F5 (containing balanced Aloe Vera, Lemon, and Tulsi) demonstrated strong antimicrobial efficacy, comparable to the standard alcohol-based sanitizer, particularly against *S. aureus* and *E. coli*.

F. Stability Studies

Formulations were stored under various conditions for 90 days.

Table X: Stability results

Formulation	Condition	Color Change	Odor Change	pH Stability	Phase Separation	Overall Stability
F1	4 °C	No	No	Stable	None	Good
F2	25 °C	Slight	No	Stable	None	Good
F3	25 °C	No	No	Stable	None	Excellent
F4	40 °C / 75% RH	Moderate	Slight	Slightly decreased	Slight	Fair
F5	25 °C	No	No	Stable	None	Excellent

F3 and F5 were the most stable under both normal and accelerated conditions, with negligible changes in physicochemical parameters.

G. Skin Irritation Test

Patch testing on 10 healthy volunteers showed no signs of erythema, itching, or irritation after 24 hours of application. Aloe Vera contributed to the soothing and moisturizing properties of the formulations, confirming their dermatological safety.

The results showed that F3 and F5 demonstrated superior organoleptic, rheological, and antimicrobial characteristics. The pH remained within the safe skin range for all formulations. Antimicrobial efficacy of F3 and F5 was nearly equivalent to the alcohol-based standard and Stability and irritation studies confirmed long-term safety and skin compatibility. The Formulations F3 and F5 were identified as the most effective, stable, and consumer-acceptable herbal hand sanitizers, offering a potential natural alternative to conventional alcohol-based products.

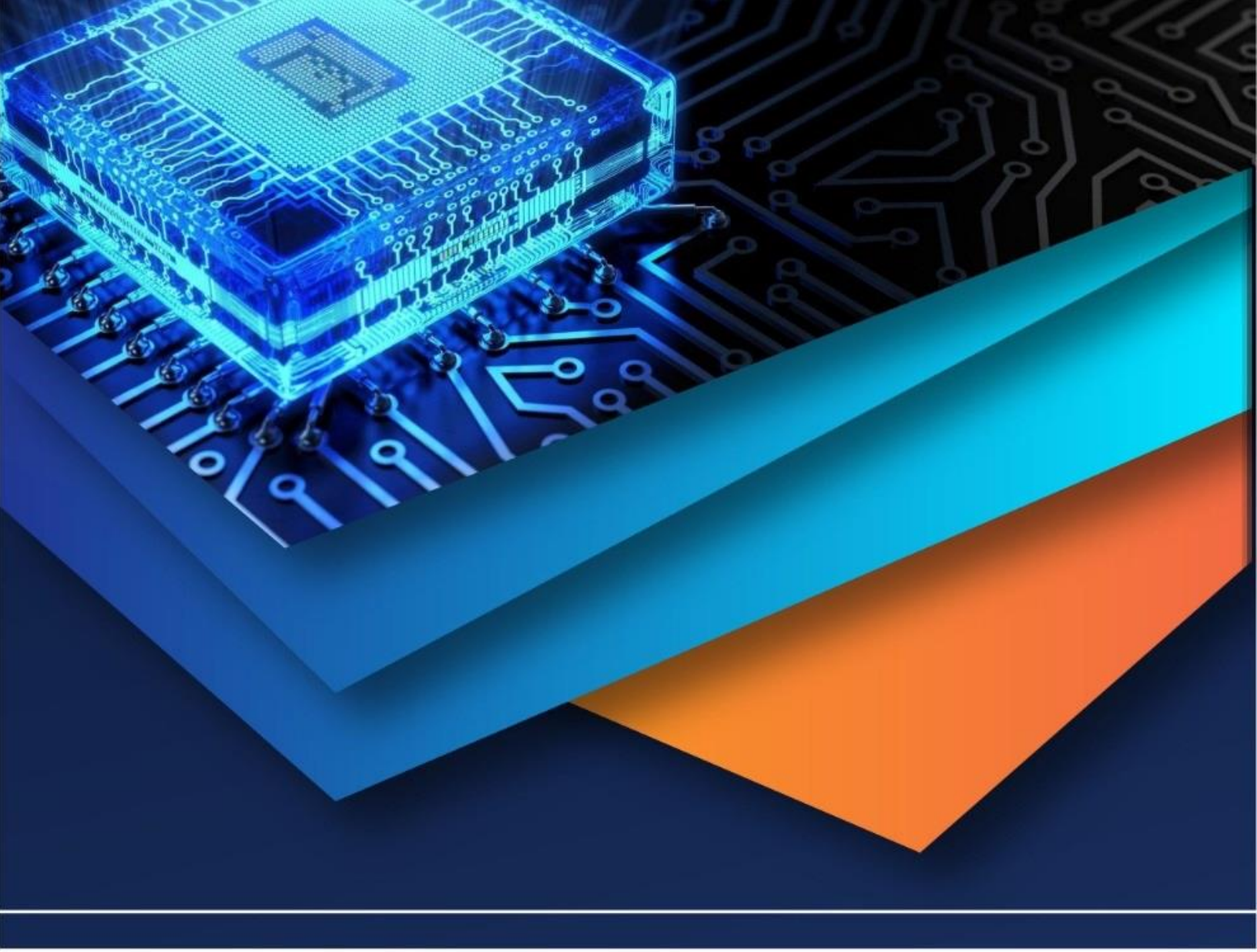
V. CONCLUSION

The study successfully formulated and evaluated herbal hand sanitizer gels using extracts of *Ocimum sanctum* (Tulsi), *Citrus limon* (Lemon), and *Aloe vera*. All formulations showed satisfactory physical and antimicrobial properties, with pH values suitable for skin application. Among the tested batches, formulations F3 and F5 exhibited optimal viscosity, good spreadability, appealing sensory characteristics, and strong antimicrobial activity comparable to standard alcohol-based sanitizers. Stability testing confirmed that these formulations remained physically and chemically stable under various storage conditions for 90 days, and skin irritation studies verified their safety for topical use.The results demonstrate that combining Tulsi, Lemon, and Aloe vera extracts provides a synergistic effect, producing an effective, safe, and eco-friendly hand sanitizer formulation. This research highlights the potential of herbal sanitizers as viable, skin-friendly alternatives to conventional synthetic products, supporting the broader move toward sustainable and natural healthcare solutions.

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