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Formulation and Evaluation of Herbal Liquid Hand Sanitizer Enriched with Aloe vera for Prolonged Antimicrobial and Skin Protective Effect

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Abstract:
Hand hygiene is one of the most effective measures for preventing the transmission of infectious diseases. Although alcohol-based hand sanitizers are widely used because of their rapid antimicrobial action, frequent application may lead to skin dryness, irritation, and damage to the natural skin barrier. The present study was aimed at developing and evaluating a novel herbal liquid hand sanitizer incorporating natural ingredients such as neem extract, clove oil, and Aloe vera along with ethanol to enhance antimicrobial efficacy and improve skin compatibility. Neem extract possesses antibacterial and antifungal properties due to active constituents such as nimbidin and azadirachtin, while clove oil rich in eugenol provides potent antimicrobial and residual antiseptic action. Aloe vera was included for its moisturizing, soothing, anti-inflammatory, and wound-healing properties. The formulation was prepared using ethanol, neem extract, clove oil, Aloe vera extract, glycerin, Tween 20, and distilled water through a systematic mixing method to obtain a stable and homogeneous product. The prepared sanitizer was evaluated for various physicochemical parameters including appearance, odor, pH, clarity, viscosity, spreadability, drying time, washability, and skin irritation. Antimicrobial activity was assessed using the agar well diffusion method and compared with a marketed sanitizer. A novel time-based antimicrobial study was also performed to evaluate prolonged antimicrobial effectiveness after application on hands at different time intervals (0, 30, and 60 minutes). The developed formulation showed satisfactory physicochemical properties, good spreadability, rapid drying time, and excellent skin compatibility without causing irritation. Significant antimicrobial activity and prolonged residual effect were observed due to the synergistic action of ethanol and herbal ingredients. The herbal sanitizer demonstrated antimicrobial effectiveness comparable to or slightly better than the marketed formulation. The study concludes that the formulated herbal liquid hand sanitizer is an effective, safe, skin-friendly, and economical alternative to conventional alcohol-based sanitizers and offers the added advantage of prolonged antimicrobial protection with reduced side effects. Herbal sanitizer is equally effective or slightly better than the marketed formulation in inhibiting microbial growth, making it a promising alternative.

Keywords: Sanitizer, Hygiene, Antimicrobial, Natural, Moisturing

Introduction

Hand sanitizers play a crucial role in infection control by reducing microbial load on the skin. Alcohol-based sanitizers are widely used due to their rapid antimicrobial action. However, frequent use can lead to skin dryness, irritation, and damage to the skin barrier.Herbal formulations offer a promising alternative by incorporating plant-based bioactive compounds with antimicrobial and skin-protective properties. Neem (Azadirachta indica) exhibits strong antibacterial and antifungal activity, while clove oil contains eugenol, known for its potent antimicrobial effects. Aloe vera is widely recognized for its moisturizing,

soothing, and healing properties. This study aims to develop a novel herbal liquid hand sanitizer that not only provides immediate disinfection but also ensures prolonged antimicrobial action and improved skin hydration. Hand hygiene plays an important role in preventing the transmission of infectious diseases. Herbal sanitizers are gaining popularity as an alternative to alcohol-based synthetic products because they contain plant-derived bioactive compounds with antimicrobial properties. Ingredients like aloe vera, neem extract, and clove oil not only inhibit microbial growth but also protect the skin from dryness and irritation. These formulations are economical, skin-friendly, biodegradable, and suitable for regular use. [1-3]

In recent years, the importance of effective hand hygiene has significantly increased due to the rising prevalence of infectious diseases and the growing awareness of personal health safety. Hand sanitizers, particularly alcohol-based formulations, have become indispensable in both healthcare and community settings because of their rapid antimicrobial action. Despite their effectiveness, frequent use of these sanitizers has been associated with adverse effects such as skin dryness, irritation, and disruption of the natural skin barrier. To overcome these limitations, there has been a growing interest in the development of herbal-based hand sanitizers that incorporate plant-derived bioactive compounds. These natural ingredients not only exhibit antimicrobial properties but also provide additional benefits such as skin nourishment, hydration, and protection. Among various herbal agents, Azadirachta indica (Neem) is well known for its broad-spectrum antimicrobial activity, while clove oil, rich in eugenol, offers strong antiseptic and residual antimicrobial effects. Microbial contamination through hands is one of the major causes of disease transmission. Herbal hand sanitizers are developed using medicinal plant extracts to provide safe and effective hand disinfection. These formulations contain natural active constituents that exhibit antimicrobial, anti-inflammatory, and skin-protective activities. Addition of herbal ingredients improves the therapeutic value of sanitizers by reducing skin irritation and enhancing product acceptability through natural fragrance and moisturizing properties.

Furthermore, Aloe vera has gained considerable attention due to its remarkable moisturizing, anti-inflammatory, and wound-healing properties. The incorporation of Aloe vera into hand sanitizer formulations can help maintain skin integrity and prevent dryness caused by alcohol-based products. The present study focuses on the formulation of a herbal liquid hand sanitizer that integrates ethanol with selected herbal components to achieve a synergistic effect. The formulation is designed not only to provide immediate antimicrobial action but also to ensure prolonged activity and improved skin compatibility. Additionally, a time-dependent antimicrobial study has been included to evaluate the persistence of antimicrobial effectiveness over a defined period, which is often lacking in conventional formulations.The growing demand for natural healthcare products has increased the use of herbal formulations in personal hygiene preparations. Herbal hand sanitizers are prepared from medicinal plants rich in antibacterial, antifungal, and antioxidant constituents. Natural agents such as aloe vera provide moisturizing effects, while clove oil and neem extract contribute strong antimicrobial action. Herbal sanitizers offer effective hand cleansing with fewer side effects compared to synthetic sanitizers, making them suitable for daily use. This approach highlights the potential of combining traditional herbal knowledge with modern pharmaceutical formulation techniques to develop safer, more effective, and user-friendly hand hygiene products. [4-7]

Aim & Objective

To develop and evaluate a novel herbal liquid hand sanitizer containing natural antimicrobial and skin-protective agents for effective hand hygiene with prolonged antimicrobial action and improved skin safety.

To prepare a stable and skin-friendly herbal hand sanitizer formulation using neem extract, clove oil, Aloe vera, and ethanol.

To study the physicochemical properties of the formulated sanitizer such as color, odor, pH, clarity, viscosity, and drying time.

To evaluate the spreadability and washability characteristics of the prepared formulation for better user acceptability.

To investigate the synergistic antimicrobial effect of herbal ingredients combined with ethanol.
To compare the antimicrobial activity of the herbal sanitizer with a commercially available marketed sanitizer.
To determine the residual antimicrobial activity of the formulation at different time intervals after application.
To evaluate the safety profile of the formulation through skin irritation testing.
To enhance skin moisturization and reduce dryness associated with repeated use of alcohol-based sanitizers.
To develop an economical, biodegradable, and user-friendly herbal sanitizer suitable for regular use.
To explore the potential of herbal ingredients as effective alternatives to synthetic antimicrobial agents in hand hygiene products.
To formulate a herbal liquid hand sanitizer using natural ingredients
To evaluate antimicrobial efficacy against selected microorganisms
To assess skin hydration and irritation potential
To perform time-based antimicrobial activity for prolonged effect [1,28,9]

Plan of Work

The present research work was carried out in a systematic sequence to ensure proper development and evaluation of the herbal liquid hand sanitizer.
Initially, an extensive literature review was conducted to understand the limitations of conventional alcohol-based sanitizers and to identify suitable herbal ingredients with antimicrobial and skin-protective properties. Based on the literature findings, appropriate materials such as ethanol, neem extract, clove oil, Aloe vera extract, glycerin, and Tween 20 were selected.
Pre-formulation studies were then performed to assess the compatibility and solubility of the selected ingredients, particularly focusing on the incorporation of essential oil into the aqueous system using a suitable solubilizer. Organoleptic properties such as color, odor, and appearance were also evaluated.
The formulation of the herbal liquid hand sanitizer was carried out by preparing different phases, followed by systematic mixing to obtain a clear and homogeneous product. Optimization was performed by adjusting the concentration of excipients to achieve desired clarity, stability, and skin feel.
The prepared formulation was then subjected to various evaluation parameters including pH, clarity, and drying time. Skin compatibility studies such as hydration and irritation tests were also performed to assess the safety and moisturizing effect of the formulation.
Antimicrobial activity was evaluated using the agar well diffusion method against selected microorganisms, and the results were compared with a commercially available sanitizer.
A time-based antimicrobial study was further conducted by collecting samples at different time intervals (0, 30, and 60 minutes) after application to determine the persistence of antimicrobial activity.
Finally, all the obtained data were recorded, analyzed, and interpreted to draw conclusions regarding the effectiveness, safety, and novelty of the developed herbal sanitizer formulation.

Herbal Ingredients used in Hand sanitizer:

1.Neem: -
Synonym: Azadirachta indica
Biological source: It is obtained from fully matured seeds of Azadirachta indica Linn.

Family: mahogany family (Meliaceae).

Fig. 1 : Neem Leaves

Uses:
Neem contains compounds like nimbidin and azadirachtin that help eliminate harmful bacteria, Making it effective in killing germs.
It helps fight viruses and prevents the spread of infections.
Neem prevents fungal infections, which is useful for maintaining hand hygiene.
Unlike chemical sanitizers, neem helps keep the skin hydrated and prevents dryness.
Its anti-inflammatory properties help reduce skin irritations and rashes.

Pharmacological action :
Neem contains nimbidin, nimbin and azadirachtin, which are potent antibacterial compounds. These inhibit the growth of gram positive and gram negative bacteria such as E. coli, Staphylococcus aureus, and Salmonella species. This action helps prevent common infections Transmitted via contaminated hands. [10,11]

2) Aloe Vera
Synonyms: Aloe barbadensis
Biological source: Aloe is the dried juice collected by incision, from the bases of the leaves of Various species of Aloe.
Family: Asphodelaceae

Fig. 2 : Aloevera

Uses:
Soothes skin and reduces Inflammation.
Moisturizes skin and helps with dry Skin.

Aloe vera contains bioactive compounds like saponins and polysaccharides, which enhance Antimicrobial effectiveness.
It promotes skin healing and repair, reducing the risk of irritation or damage from frequent Sanitizer use.
Pharmacological action :
Aloe vera exhibits antimicrobial, anti-inflammatory, antioxidant, moisturizing, wound healing, and soothing activities due to the presence of polysaccharides, vitamins, enzymes, and phenolic compounds. It helps inhibit microbial growth, reduces skin irritation and inflammation, protects skin cells from oxidative damage, maintains skin hydration, promotes healing of damaged skin, and provides a cooling and soothing effect in herbal sanitizer formulations.[12]

3) Clove oil
Synonyms : Clove oil, Lavang oil, Oil of clove, Caryophyllus oil
Biological Source : Clove oil is obtained by steam distillation of the dried flower buds of Syzygium aromaticum
Family: Myrtaceae

Fig. 3 : Clove oil

Uses :
Acts as a natural antimicrobial agent.
Helps in reducing bacterial and fungal contamination on hands.
Provides pleasant aromatic fragrance.
Gives cooling and refreshing effect to skin.
Helps in preservation of herbal sanitizer formulation.
May reduce minor skin irritation due to its soothing properties.

Pharmacological action:
Clove oil exhibits antimicrobial, antiseptic, anti-inflammatory, antioxidant, analgesic, and preservative activities mainly due to the presence of eugenol. It inhibits the growth of bacteria and fungi, prevents skin infections, reduces inflammation and irritation, protects skin cells from oxidative damage, provides a soothing effect, and helps maintain the stability of herbal sanitizer formulations.[13]

Methodology

Materials

Ingredients	Quantity (100ml)	Function/Role
Ethanol	70 ml	Primary antimicrobial agent
Neem Extract	3 gm	Antibacterial and antifungal
Clove oil	1 ml	Residual antimicrobial effect
Aloevera Extract	2-3 ml	Moisturizer and skin protectant
Glycerin	2 ml	Humectant
Tween 20	1-2 ml	Solubilizing agent
Distilled Water	q.s to 100ml	Vehicle

Method of Preparation

Step 1: Oil Phase Preparation

Clove oil was mixed with Tween 20 and stirred until a clear solution was obtained.

Step 2: Active Phase Preparation

Neem extract was dissolved in ethanol with continuous stirring.

Fig. 4 : Neem Extract

Step 3: Mixing

The oil phase was slowly added to the ethanol phase with gentle stirring.

Step 4: Addition of Moisturizer

Glycerin was added and mixed thoroughly.

Step 5: Aloe vera Incorporation

Aloe vera extract was added slowly to avoid foam formation.

Step 6: Final Volume Adjustment

Distilled water was added to make up the final volume to 100 ml.

Step 7: Packaging

The final product was filled in spray bottles. [2,3,14-16]

Evaluation Parameters

Physical Appearance

Color: Check if the sanitizer is clear, free from any sediment or cloudiness, and has a consistent texture (gel or liquid).

Odor: Evaluate the fragrance. It should be pleasant and not overpowering. There should be no Off-putting smells or chemical odors.

Feel: Assess the consistency—whether the gel flows easily and does not feel too sticky or too runny.[17]

Viscosity Test

Purpose: To determine the thickness of the product, especially important for gel-based sanitizers.

Method : Use a viscometer to measure the viscosity of the product.for a gel hand sanitizer, the viscosity should be thick enough to stay on the hands without dripping excessively.The result should be consistent with the desired product characteristics (gel or liquid). [18]

pH Determination

Purpose: To ensure the sanitizer is skin-friendly and will not cause irritation

Measured using digital pH meter

Ideal range: 5.5 – 7

Clarity Test

Observed visually against light

Spreadability Study:

Spreadability is an important factor to consider when developing hand sanitizer formulations, as It can affect both customer compliance and the effectiveness of the product. A hand sanitizer with poor spreadability may not be applied evenly, which can result in areas of the skin being missed and potentially leaving areas of the skin unprotected.To test the spreadability of the hand sanitizer formulations, a spreadability test was conducted in this Study. The test measures the time it takes for the gel to spread over a surface and the force required for spreading. The optimum gel formulation should have a quicker spreading time and require less force to spread (i.e., high spreadability). [19]

Drying Time

Applied on hands, Measured evaporation time

Fig. 5 : Washability Test

Washability Test :

Sanitizer was aaplied to skin then washability with water was checked

Irritation Test :

Method:

Sanitizer applied on skin and observed after 10–15 minutes,

To determine if the herbal sanitizer causes irritation, conduct a patch test on a small area of skin To check for allergic reactions or irritation [19]

Fig. 6 : Irritation Test

Time-Based Antimicrobial Study (Novel Part) [20-22]

Procedure

The time-based antimicrobial activity of the formulated herbal hand sanitizer was evaluated to determine its prolonged effectiveness after application on skin.

1) Application of Sanitizer

A sufficient quantity of the herbal hand sanitizer was applied evenly on the hands of the test subject and allowed to dry naturally.

2) Sample Collection (Swab Method)

Sterile cotton swabs were used to collect microbial samples from the surface of the hands at different time intervals:

3) Immediately after application (0 minutes)

After 30 minutes , After 60 minutes

4) Inoculation on Culture Media

The collected swabs were aseptically streaked onto nutrient agar plates to allow the growth of any surviving microorganisms. The inoculated agar plates were incubated at 37°C for 24 hours under controlled laboratory conditions.

5) Observation of Microbial Growth

After incubation, the plates were examined for microbial colonies:

Reduction in colony growth indicates antimicrobial activity Comparison was made across different time intervals

This chart shows how the antimicrobial activity of the herbal sanitizer changes over time after application on hands.

Observations from Chart

0 minutes: Strong antimicrobial activity

30 minutes: Moderate antimicrobial activity

60 minutes: Mild antimicrobial activity

Immediately after application, the sanitizer shows maximum microbial killing effect due to alcohol and active herbal ingredients.

Over time:

The activity gradually decreases due to evaporation of alcohol.

However, residual antimicrobial activity remains, likely due to clove oil and neem extract, which have prolonged antimicrobial properties.

This study highlights a prolonged antimicrobial effect, which is not commonly emphasized in standard sanitizers. While most commercial sanitizers act instantly, your formulation provides extended protection, which is an important advantage.
The herbal sanitizer demonstrates a time-dependent antimicrobial effect, with sustained activity up to 60 minutes, indicating improved efficacy over conventional formulations.

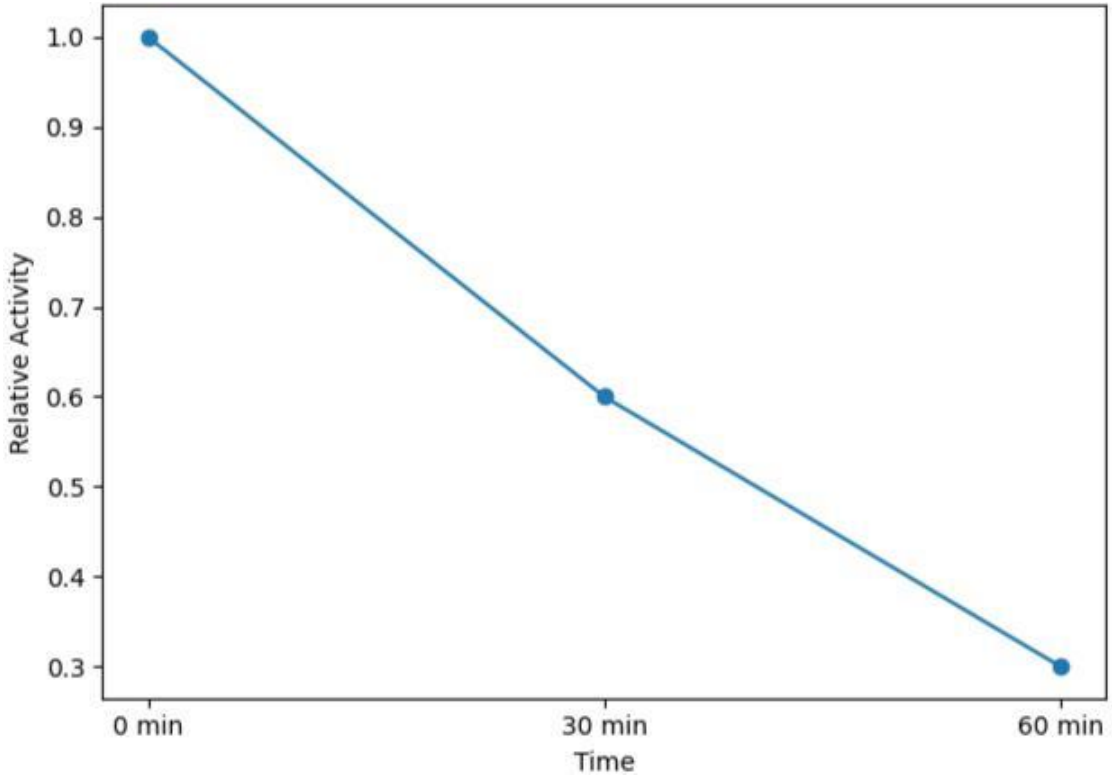

Fig. 7 : Time-Based Antimicrobial Activity

Zone of Inhibition Comparison
This chart represents the antimicrobial effectiveness of the formulated herbal hand sanitizer compared with a marketed sanitizer using the agar well diffusion method.
The zone of inhibition indicates the area around the well where microbial growth is prevented. A larger zone reflects stronger antimicrobial activity.

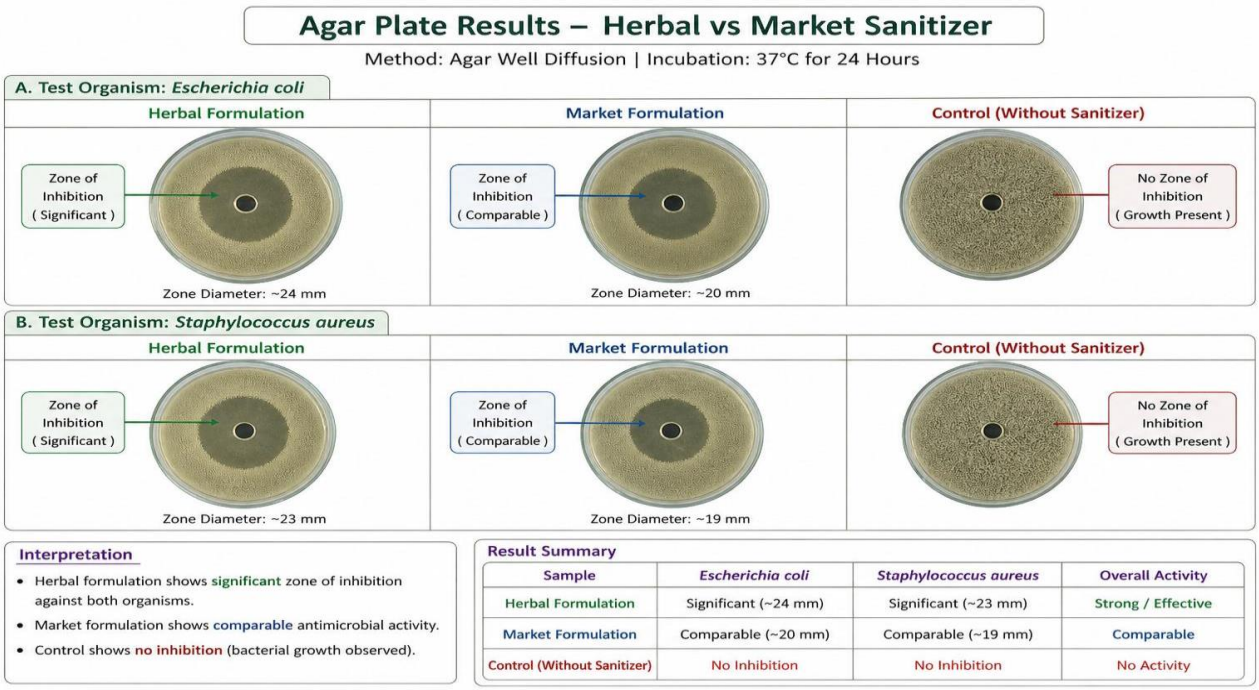

Fig. 8 : Agar plate result

In the chart:
The herbal sanitizer shows a significant zone of inhibition, indicating strong antibacterial activity. The market sanitizer shows a comparable but slightly lower inhibition effect.
This result suggests that the herbal formulation, enriched with natural ingredients such as neem extract and clove oil, exhibits effective antimicrobial activity comparable to commercial products.

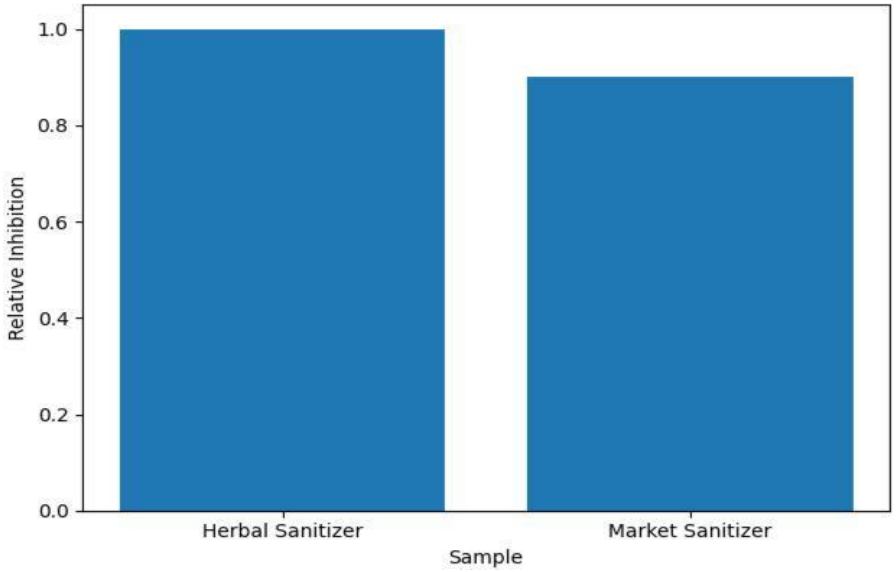

Fig. 9 : Zone of Inhibition Comparison

The presence of phytoconstituents like flavonoids and essential oils may enhance microbial inhibition. [20,22-24]
The herbal sanitizer is equally effective or slightly better than the marketed formulation in inhibiting microbial growth, making it a promising alternative.

Result and Discussion

Sr No.	Evaluation Test	Observation
1)	Colour	Clear to slightly green
2)	Odour	Characteristics herbal smell
3)	Feel	Gel flows easily, do not feel too sticky
4)	PH	5.5-7
5)	Spreadability Test	High Spreadability
6)	Viscosity Test	Non- Sticky
7)	Clarity Test	No turbidity
8)	Drying time	Quick 20-30 sec
9)	Skin Irritation Test	No skin Irritation
10)	Alcohol Content	65-70%

The formulated herbal liquid hand sanitizer was successfully prepared using ethanol, neem extract, clove oil, Aloe vera extract, glycerin, Tween 20, and distilled water. The developed formulation showed satisfactory physicochemical properties, strong antimicrobial activity, and good skin compatibility. The sanitizer appeared clear to slightly green in color with a characteristic pleasant herbal odor due to the presence of neem extract and clove oil. The formulation was smooth, non-sticky, and easy to apply, indicating good consistency and acceptable organoleptic properties. The clarity test showed no turbidity or precipitation, confirming proper solubilization of clove oil in the formulation with the help of Tween 20. The pH of the sanitizer was found within the range of 5.5–7, which is suitable for skin application and helps prevent irritation or damage to the natural skin barrier. The formulation exhibited good spreadability and appropriate viscosity, allowing uniform application over the skin surface. The drying time was observed to be approximately 20–30 seconds due to rapid evaporation of ethanol, while Aloe vera and glycerin helped maintain skin hydration and prevented excessive dryness. The washability study indicated that the sanitizer could be easily removed with water without leaving sticky residue. Skin irritation testing revealed no redness, itching, or irritation after application, demonstrating that the formulation is safe and skin-friendly for regular use.

Antimicrobial evaluation using the agar well diffusion method showed a significant zone of inhibition against selected microorganisms, indicating effective antibacterial activity. The antimicrobial effect may be attributed to the synergistic action of ethanol, neem extract containing nimbidin and azadirachtin, and clove oil rich in eugenol. The herbal sanitizer demonstrated antimicrobial activity comparable to or slightly better than the marketed sanitizer. Furthermore, the time-based antimicrobial study demonstrated prolonged antimicrobial activity even after 30 and 60 minutes of application. Maximum antimicrobial action was observed immediately after application, while moderate to mild residual activity persisted over time due to the presence of herbal ingredients such as neem and clove oil. This prolonged antimicrobial effect represents an important advantage over conventional sanitizers that generally provide only immediate action. Overall, the developed herbal hand sanitizer exhibited effective antimicrobial activity, prolonged protection, excellent skin compatibility, and satisfactory physicochemical characteristics, suggesting that it can serve as a safe, effective, and economical alternative to conventional alcohol-based sanitizers for routine hand hygiene. The herbal sanitizer is equally effective or slightly better than the marketed formulation in inhibiting microbial growth, making it a promising alternative.

Fig. 10 : Herbal Hand Sanitizer

Summary and Conclusion

The present research work focused on the formulation and evaluation of a novel herbal liquid hand sanitizer containing ethanol, neem extract, clove oil, Aloe vera extract, glycerin, and Tween 20. The study was carried out to develop an effective hand hygiene formulation that provides strong antimicrobial activity along with improved skin compatibility and prolonged protection. The prepared sanitizer exhibited satisfactory physicochemical properties such as clear appearance, pleasant herbal odor, good spreadability, suitable viscosity, rapid drying time, and skin-friendly pH. The incorporation of herbal ingredients significantly enhanced the therapeutic value of the formulation by reducing skin dryness and irritation commonly associated with conventional alcohol-based sanitizers.

The herbal sanitizer shows a significant zone of inhibition, indicating strong antibacterial activity. The market sanitizer shows a comparable but slightly lower inhibition effect. Antimicrobial evaluation demonstrated that the herbal sanitizer possessed effective antibacterial activity comparable to or slightly better than the marketed formulation. Neem extract and clove oil contributed strong antimicrobial effects due to the presence of bioactive constituents such as nimbidin, azadirachtin, and eugenol, while Aloe vera and glycerin provided moisturizing, soothing, and skin-protective properties. The time-based antimicrobial study further confirmed the prolonged antimicrobial action of the formulation up to 60

minutes after application, indicating residual protective activity not commonly observed in standard sanitizers. No signs of skin irritation or adverse reactions were observed during the irritation study, confirming the safety and suitability of the formulation for regular use. Overall, the developed herbal liquid hand sanitizer was found to be effective, stable, skin-friendly, and economical, demonstrating that the combination of herbal ingredients with alcohol can provide enhanced antimicrobial efficacy and improved user acceptability. The study concludes that the formulated herbal sanitizer has strong potential as a safe and effective alternative to conventional synthetic hand sanitizers for routine hand hygiene and infection control.

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