



“FORMULATION AND EVALUATION OF ALCOHOL FREE HAND SANITIZER FOR THE PREVENTION OF VARIOUS DISEASES.”

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Abstract

Hand hygiene plays a vital role in preventing the spread of infectious diseases caused by bacteria, viruses, and fungi. Conventional alcohol-based hand sanitizers, though effective, often cause skin dryness, irritation, and are flammable, limiting their frequent use. To overcome these drawbacks, alcohol-free formulations using natural or synthetic antimicrobial agents have emerged as safer alternatives. The present review focuses on the formulation, evaluation, and effectiveness of alcohol-free hand sanitizers developed using herbal extracts, essential oils, and other non-alcoholic antimicrobial components. Such formulations aim to provide broad-spectrum antimicrobial activity while maintaining skin moisture and safety. Evaluation parameters including pH, viscosity, spreadability, antimicrobial efficacy, and stability are discussed to assess product quality and performance. The incorporation of herbal ingredients like Aloe vera, Neem, Tulsi, and Tea tree oil enhances the product's skin compatibility and therapeutic value. This review highlights the potential of alcohol-free sanitizers as an effective, skin-friendly, and sustainable approach for maintaining hand hygiene and preventing disease transmission in both healthcare and community settings.

Keywords:

Alcohol-free hand sanitizer, Herbal formulation, Antimicrobial activity, Essential oils, Aloe vera, Neem, Tea tree oil, Hand hygiene, Non-alcoholic sanitizer, Skin-friendly formulation, Disease prevention, Natural ingredients.

1. INTRODUCTION:

New infectious diseases, whether bacterial or viral, continue to pose serious global public health challenges. Among these, the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) emerged as a highly contagious pathogen responsible for the Coronavirus Disease 2019 (COVID-19), which was declared a global pandemic by the World Health Organization (WHO) in early 2020 [1]. Since its initial outbreak in Wuhan, China, in December 2019, the infection rapidly spread worldwide, leading to over 150 million confirmed cases by April 2021. As no specific treatment was initially available, preventive measures such as maintaining social distancing, wearing masks, and ensuring proper hand hygiene were recognized as the most effective strategies to reduce viral transmission. Among

these, frequent and proper handwashing or sanitization plays a critical role in interrupting the chain of infection, thereby minimizing the spread of COVID-19 and other contagious diseases.

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Following the COVID-19 outbreak, alcohol-based hand sanitizers (ABHS) became a widely used alternative to conventional handwashing in both healthcare and community settings, resulting in a significant rise in global alcohol demand. Numerous hand sanitizer formulations are available on the market, varying in composition and antimicrobial efficacy. It is crucial to understand the types of sanitizers that effectively act against pathogenic microorganisms. The ABHS formulations recommended by the World Health Organization (WHO) typically contain ethanol, isopropyl alcohol, or hydrogen peroxide in different combinations, with the concentration of ethanol or isopropyl alcohol generally ranging between 60–95%. This range is recognized as the optimal bactericidal concentration for most ABHS. However, the surge in production during the pandemic led to a considerable strain on the global alcohol supply chain. To address this, the WHO proposed two standard formulations for local or small-scale production of ABHS. The first formulation contains 80% (v/v) ethanol, 0.125% (v/v) hydrogen peroxide, and 1.45% (v/v) glycerol, while the second includes 75% (v/v) isopropyl alcohol, 0.125% (v/v) hydrogen peroxide, and 1.45% (v/v) glycerol. These challenges highlighted the urgent need to develop effective alcohol-free hand sanitizer (AFHS) formulations with comparable antimicrobial efficacy, capable of mitigating both alcohol-related supply issues and potential skin irritation caused by frequent use of alcohol-based products.

In this study, alcohol-free hand sanitizer (AFHS) formulations were developed and evaluated using natural ingredients such as *Aloe vera*, vitamin E, glycerin, and various essential oils (EOs). These components are readily available in the market, making the formulation both cost-effective and accessible. *Aloe vera* gel serves as the primary base due to its natural moisturizing properties, antimicrobial potential, and soothing effect on the skin. Vitamin E and glycerin were incorporated for their respective roles in preventing rancidity caused by oxidation or hydrolysis of fats and oils, and in maintaining skin hydration. The essential oils act as the principal active agents in AFHS formulations, exhibiting broad-spectrum antimicrobial activity. Their hydrophobic nature enables the penetration of bioactive components into bacterial cell membranes and mitochondria, leading to the disruption of membrane integrity and subsequent cell death. For instance, combinations such as clove oil and cinnamon oil have demonstrated strong inhibitory effects against various microbial strains, including *Aspergillus flavus*, *Debaryomyces hansenii*, and *Staphylococcus aureus*. The natural compounds used in AFHS are widely applied in cosmetic and pharmaceutical preparations owing to their antimicrobial, antioxidant, and skin-conditioning properties. However, the concentration of these bioactive agents must be optimized carefully, as excessive amounts may cause dermal irritation or hypersensitivity reactions.

These formulated alcohol-free hand sanitizer (AFHS) gels are designed to help control the spread of co-infections, particularly during pandemics. After formulation, the prepared hand sanitizing gels were subjected to a comprehensive characterization and evaluation process. The formulations were analyzed for their organoleptic properties, pH, rheological behavior, and spreadability to ensure desirable physical and aesthetic qualities. The antimicrobial efficacy of the AFHS gels was determined using the agar well diffusion method, measuring the zones of inhibition against various bacterial strains and *Candida albicans* (C. albicans) yeast. Furthermore, an acceptability

test was performed to evaluate the safety and tolerability of the formulations by monitoring potential adverse skin reactions, such as irritation or redness, following topical application. The overall assessment aimed to confirm the efficacy, stability, and user safety of the alcohol-free hand sanitizers as a promising alternative to conventional alcohol-based formulations.

2. Materials and Methods

2.1. Materials

Aloe vera (raw purified aloe; Pure Aloe Force , Herbal), glycerin (USP vegetable), vitamin E , clove oil ,tulsi extract ,neem extract .

2.2. Methods

2.2.1. Preparation of Hand Sanitizer Gels

Several natural ingredients were utilized in the preparation of alcohol-free hand sanitizer (AFHS) gels in specific proportions, including *Aloe vera* gel, glycerin, vitamin E, and various essential oils (EOs). Each formulation was prepared by first dispersing glycerin (5% v/v) into *Aloe vera* gel (90% v/v) in a 250 mL beaker, followed by gentle stirring at room temperature to ensure proper mixing. The essential oils, at concentrations of either 2.5% v/v or 1.25% v/v, were then added dropwise with continuous stirring to prevent air entrapment and achieve a uniform and homogenous gel consistency. Subsequently, vitamin E (0.05% v/v) was incorporated into the mixture, and the final volume of each formulation was adjusted with distilled water. A control formulation was also prepared using the same base components, excluding the addition of essential oils, to serve as a reference. The composition of all prepared hand sanitizer gels, including the control, along with the concentration of each ingredient.

2.2.2. Physiochemical Characterization and Evaluation of Formulated AFHS Gels : -

Organoleptic Test

Evaluation of Formulated AFHS Gels

1. Organoleptic Evaluation

The prepared alcohol-free hand sanitizer (AFHS) gels were visually inspected to assess their physical appearance, including texture, color, and odor, under semisolid conditions. This evaluation helped ensure product uniformity and user acceptability.

2. pH Evaluation

The pH of each formulated gel was measured using a calibrated digital pH meter (Mettler Toledo, USA). Approximately 1 g of each sample was dispersed in 10 mL of distilled water before measurement. The pH values were recorded in triplicate, and the results were expressed as mean \pm standard deviation (SD).

3. Viscosity (Rheological Properties)

The viscosity of the prepared gel formulations is one of the fundamental parameters that must be carefully controlled, as it directly reflects the consistency, spreadability, and flowability of the product during application to the skin [28]. In this study, the viscosity evaluation was conducted to determine the thickness and flow behavior of the formulated alcohol-free hand sanitizer (AFHS) gels. Two different viscometers were utilized—namely, the Ostwald viscometer and the Brookfield viscometer—to assess and compare the rheological characteristics of the formulations. The Ostwald viscometer was employed to measure the viscosity of formulations with relatively low fluid resistance, whereas the Brookfield viscometer was used for

gel-based formulations exhibiting higher viscosity and non-Newtonian flow behavior. This analysis provided insights into the influence of various gel components, such as aloe vera gel, glycerin, and essential oils, on the rheological and textural properties of the prepared products.

4. Spreadability Test

The spreadability of the prepared hand sanitizers was evaluated following the method described by A 0.5 g sample of each gel was placed on a pre-marked glass plate (2 cm diameter). Another glass plate was gently placed on top, followed by the application of a 500 g weight for 5 minutes to allow uniform spreading. Excess gel was carefully removed from the edges. The diameter of the spread area was measured, and spreadability was expressed as the mean \pm SD of three replicates. The percentage of spreadability was calculated using the following equation:

$$\text{Spreadability}\% = \frac{D2}{D1} \times 100$$

Where $D1$ is initial area before spreading (cm) and $D2$ is final area after spreading (cm).

2.2.3. Microbial Suspension Preparation

To evaluate the antimicrobial efficacy of the formulated alcohol-free hand sanitizers (AFHS), both Gram-positive and Gram-negative bacterial strains, along with an opportunistic pathogenic yeast (*Candida albicans*), were utilized as test microorganisms. All reference microbial strains were obtained from the American Type Culture Collection (ATCC). The bacterial isolates included *Acinetobacter baumannii* (BAA-747), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (BAA-1705), *Pseudomonas aeruginosa* (BAA-1744 and ATCC 27853), and *Staphylococcus aureus* (ATCC 29213 and BAA-977). Additional bacterial isolates of clinical or environmental origin were also tested, including *E. coli* (isolate 1060), *Staphylococcus epidermidis* (isolate 5029), *Staphylococcus hominis* (isolate 5028), *Staphylococcus haemolyticus* (isolate 5034), and *Micrococcus luteus* (isolate SB115). The yeast *Candida albicans* (ATCC 66027) was also used as a representative fungal organism.

Microbial suspensions (inocula) were prepared in Mueller–Hinton broth, and their turbidity was adjusted to match the 0.5 McFarland standard, equivalent to approximately 1.5×10^8 CFU/mL, as described by [19]. All bacterial and yeast cultures were streaked onto Mueller–Hinton agar plates and incubated at 37 °C for 18–24 hours to ensure the growth of viable colonies for antimicrobial testing.

2.2.4. Antimicrobial Zone of Inhibition Test

The antimicrobial activity of the prepared alcohol-free hand sanitizer (AFHS) gels was evaluated using the agar well diffusion method to determine the zone of inhibition against various Gram-positive and Gram-negative bacterial strains, as well as *Candida albicans*. Three commercially available alcohol-based hand sanitizers (ABHS) were included as experimental controls for comparison.

A standardized inoculum containing approximately 1×10^6 CFU/mL of each test microorganism was evenly spread across the surface of sterile Mueller–Hinton agar plates. Sterile microbiological discs were immersed in each hand sanitizer gel sample, briefly air-dried to remove excess formulation, and then carefully placed onto the inoculated agar surface. The plates were incubated at 37 °C for 18–24 hours. After incubation, the diameter of the clear inhibition zones surrounding each disc was measured in millimeters (mm). All experiments were performed in triplicate, and the results were expressed as mean \pm standard deviation (SD).

2.2.5. Skin Irritation Study (Acceptability Test)

Based on the results of the antimicrobial activity test, the most effective AFHS gel formulation was selected for a skin irritation and acceptability evaluation. The study was conducted on 20 healthy volunteers following ethical approval from the Research Ethics Committee of King Abdulaziz City for Science and Technology (KACST) (IRB Approval No.: IRB#20007). All participants were informed about the study objectives, potential side effects, and procedures, and signed informed consent forms prior to participation.

For the test, approximately 1 mL of the selected hand sanitizer gel was applied to each volunteer's palm and left for 5 minutes. Volunteers were then provided with a questionnaire to assess the product's acceptability and any signs of dermal irritation. Participants with visible skin abrasions, infections, or trauma were excluded from the study. The formulation was evaluated based on its physical appearance, odor, texture, and any occurrence of redness, burning sensation, or irritation following application.

2.2.6. Statistical Analysis

All experimental results, including pH, viscosity, spreadability, and antimicrobial zone of inhibition measurements, were presented as mean \pm SD of at least three independent replicates. Statistical comparisons between the antimicrobial activities of the formulated essential oil-based AFHS and commercial alcohol-based hand sanitizers were conducted using the Student's *t*-test. A *p*-value of less than 0.05 was considered statistically significant.

3. Results and Discussion

3.1.1. Organoleptic Test :

The organoleptic evaluation of the formulated alcohol-free hand sanitizer (AFHS) gels was performed to assess their physical appearance and overall aesthetic quality. Visual inspection revealed that all prepared formulations exhibited desirable characteristics. The gels appeared

homogeneous and clear, with a pleasant and distinctive aroma imparted by the incorporated essential oils (EOs). No phase separation or syneresis was observed, indicating good formulation stability. The gels demonstrated smooth consistency, were easy to apply, and spread evenly on the skin surface, confirming satisfactory texture and flow behavior.

A minor bubble-like appearance was observed during overnight storage, which disappeared upon gentle shaking. This effect was likely attributed to the relatively high concentration of essential oils (1.25–2.5% v/v) incorporated during formulation. Furthermore, no coarse or particulate matter was detected when the gels were spread on a transparent glass surface, signifying uniform dispersion of the components and excellent homogeneity of the prepared formulations.

3.1.2. pH Evaluation

The pH of the formulated alcohol-free hand sanitizer (AFHS) gels was determined using a calibrated digital pH meter to ensure compatibility with the skin and to prevent irritation or dryness upon application. Maintaining an appropriate pH is a critical parameter for topical formulations, as the ideal range for skin-friendly products typically falls between 4.0 and 7.0, thereby minimizing the risk of inflammation or dermal discomfort .

All the prepared formulations exhibited slightly acidic pH values, averaging around 3.9. This mild acidity can be attributed to the high proportion of *Aloe vera* gel (90% v/v) used as the primary base, which naturally possesses a

slightly acidic pH in the range of 4.0–4.5. The results indicate that the formulations were within an acceptable pH range and are unlikely to cause irritation or adverse effects on normal skin physiology.

It has been well established that several pathogenic bacteria capable of causing skin infections exhibit optimal growth under neutral pH conditions, whereas the natural skin microbiota tends to thrive better in a slightly acidic environment. The mildly acidic condition of the skin surface (pH 4.0–4.5) promotes the attachment and stability of normal flora, which plays a crucial role in maintaining microbial balance and protecting against opportunistic pathogens. Studies have also demonstrated that the average physiological pH of the skin surface is below 5.0, which is essential for maintaining various dermal biological processes such as stratum corneum homeostasis, lipid barrier formation, and enzyme activity regulation.

Moreover, an acidic environment has been shown to enhance the activity of antimicrobial compounds against pathogenic microorganisms. For example, the endogenous antimicrobial peptide dermcidin, secreted in human sweat, exhibited significantly higher inhibitory activity against *Staphylococcus aureus* at a pH of 5.5 compared to a neutral pH of 6.5. Similarly, results from a long-term clinical study indicated that *Propionibacterium* growth was markedly higher after cleansing the skin with a neutral formulation compared to a slightly acidic one. These findings suggest that the slightly acidic nature of the formulated AFHS gels may confer an

additional antimicrobial advantage by creating an unfavorable environment for pathogenic bacteria while supporting the growth of beneficial skin flora.

3.1.3. Viscosity (Rheological Properties):

The viscosity of the prepared gel formulations is one of the fundamental parameters that must be carefully controlled, as it directly reflects the consistency, spreadability, and flowability of the product during application to the skin [28]. In this study, the viscosity evaluation was conducted to determine the thickness and flow behavior of the formulated alcohol-free hand sanitizer (AFHS) gels. Two different viscometers were utilized—namely, the Ostwald viscometer and the Brookfield viscometer—to assess and compare the rheological characteristics of the formulations. The Ostwald viscometer was employed to measure the viscosity of formulations with relatively low fluid resistance, whereas the Brookfield viscometer was used for gel-based formulations exhibiting higher viscosity and non-Newtonian flow behavior. This analysis provided insights into the influence of various gel components, such as aloe vera gel, glycerin, and essential oils, on the rheological and textural properties of the prepared products.

3.1.4. Gel Spreadability

3.1.4. Spreadability Evaluation

Spreadability is a critical parameter that determines the ease of application, user acceptance, and uniform distribution of hand sanitizer gels on the skin. It reflects the product's ability to spread smoothly without excessive effort and directly influences consumer compliance and the overall performance of topical formulations. Therefore, the spreadability test was performed to evaluate how effectively the prepared alcohol-free hand sanitizer (AFHS) gels could be distributed upon application. Ideally, a formulation exhibiting a shorter spreading time indicates higher spreadability and better application properties.

One of the major factors influencing gel spreadability is its viscosity; formulations with lower viscosity tend to exhibit greater spreadability due to reduced internal resistance. The measured spreadability values for all prepared gels ranged from 558% to 638%. Among all formulations, the gel containing 1.25% (v/v) clove oil demonstrated the highest spreadability. Conversely, the 2.5% (v/v) clove oil formulation exhibited the lowest spreadability value of 558%, which correlates with its higher viscosity value (1.1). This inverse relationship between viscosity and spreadability indicates that an increase in gel thickness reduces its ability to spread efficiently over the skin surface.

4. Conclusions

Hand sanitizer gels represent an effective and convenient alternative for maintaining hand hygiene. During the COVID-19 pandemic, the development of alcohol-free hand sanitizers (AFHS) has become increasingly important, particularly in response to the challenges in the global alcohol supply chain. In this study, AFHS gels were successfully formulated using aloe

vera, glycerin, vitamin E, and various essential oils (EOs) as active antimicrobial agents. The formulated gels demonstrated excellent organoleptic characteristics, pH values compatible with normal skin, and appropriate viscosity and spreadability profiles, confirming their suitability for topical use.

The antimicrobial evaluation revealed that different EO-based formulations exhibited variable effectiveness against Gram-positive and Gram-negative bacteria as well as *Candida* species. Among all tested formulations, the gel containing 2.5% (v/v) clove oil (F1) displayed the broadest antimicrobial spectrum, with an activity comparable to commercially available alcohol-based hand sanitizers (ABHS). However, mild skin irritation was reported in approximately 20% of volunteers. Consequently, the 1.25% (v/v) clove oil formulation was identified as the most favorable, offering strong antimicrobial activity with minimal or no adverse skin reactions and improved user acceptability.

Future research should focus on expanding the evaluation of these formulations against a wider range of bacterial, fungal, and yeast pathogens, as well as assessing their antiviral properties to validate their potential as effective ABHS alternatives. Additionally, stability studies are recommended to determine the shelf-life and long-term performance of EO-based hand sanitizers, thereby ensuring product safety, consistency, and efficacy for large-scale public health use.

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