Comparative Antibacterial Efficacy of Formulated and Commercial Hand Sanitizers: Formulation and Evaluation

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Abstract

Background: Hand hygiene is crucial to preventing infections in hospitals and communities. However, many hand sanitizer products are on the market, raising questions about their effectiveness and safety. The objective of this study was to develop and assess two laboratory-developed gel-based hand sanitizers, followed by a clinical trial. Methodology: The laboratory test used the European standard for chemical disinfectants and antiseptics (EN 14885; 2006) and different bacterial strains such as Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 25923) and Pseudomonas aeruginosa (ATCC 27853). The clinical trial involved 300 healthcare workers who volunteered for the study and used the developed or commercial hand sanitizers. The hand samples were collected before and after treatment and analyzed for bacterial load. The logarithmic reduction factor of the bacterial load measured the antibacterial efficacy of the hand sanitizers. Results: The developed hand sanitizers achieved a 6-log reduction (99.9999%) of all bacterial strains in the laboratory test. In the clinical trial, they achieved a 4-log reduction (99.99%) of the bacterial load on the hands of the healthcare workers. Conclusions: The hand sanitizers developed in this study were found to be equally or more effective and safer than the WHO formulation. The results showed that the developed hand sanitizers exhibited desirable physical properties, and strong antimicrobial activity against five common microorganisms responsible for infections while also being deemed cost-effective and well-tolerated by the volunteers. This could lead to improved health for both healthcare workers and patients, as well as a reduction in nosocomial infections.

Keywords: Lactic Acid Bacteria, Probiotics, Immune System, Vannamei Shrimp

1. Introduction
The word hygiene derives from Hygeia, the ancient Greek goddess of healing [1]. Hygiene is related to disease prevention and health promotion in modern times [2]. Pathogens can spread through contact between surfaces and between air and surfaces. Therefore, good hygiene is essential for preventing illnesses caused by microorganisms. Hand hygiene is crucial, as several studies have shown, for protecting workers in hospitals, biowaste facilities, microbiology departments, and other infectious settings, as well as the general public outside the healthcare context [3]. Hand washing can save one million lives annually, and many public health initiatives worldwide have tried to improve "hand hygiene" with different levels of success [4].
Nevertheless, research indicates that a significant percentage of individuals, i.e., up to 80%, still harbour harmful microbes on their hands even after washing [5]. Hand washing with soap solely eliminates the natural fatty acids from the skin, leading to skin damage that facilitates the invasion of microorganisms [6]. Hand sanitization compliance among healthcare workers has been frequently investigated and studied. However, hand hygiene practices in the general population have rarely been examined [7]. Some studies indicated that factors hindering handwashing compliance include the time-consuming nature of soap-and-water handwashing, especially when faced with heavy workloads, as well as skin irritation and dryness resulting from frequent handwashing. Additionally, limited access to sinks and insufficient knowledge of hand hygiene guidelines or protocols contribute to the challenges [7a].

In both healthcare and community settings, hand sanitizers/hand rubs, especially antimicrobial hand rubs, have gained popularity as a viable substitute for traditional handwashing with soap and water by claiming to be effective against harmful microbes and improving skin conditions due to emollients [8,9]. High-quality antimicrobial hand sanitizers contain emollients and other skincare ingredients and are waterless, making them accessible and convenient [10]. They have proven to be an effective measure in avoiding the transmission of bacterial and viral infections, thereby playing a crucial role in reducing the burden on healthcare systems.

Moreover, alcohol-based hand sanitizers are recommended for hand hygiene in hospital settings to prevent infections [11]. There are various types of hand sanitizers in the market, which the Food and Drug Administration has approved. These can also be used in outreach activities, routine screening processes, water-scarce locations, and clinical examinations. However, some commercial antimicrobial hand rubs are ineffective at reducing microbial counts on hands. Despite claiming to decline 99.9% of "germs and harmful bacteria" on their labels, several studies have reported increased microbial concentration in hand prints on agar plates after using them [11]. Therefore, there is a need for regulatory agencies to verify these claims and ensure good-quality standards. To address this issue, this study aimed to formulated two laboratory-developed gel-based hand sanitizers and also to evaluate the antimicrobial efficacy of them along with different marketed hand rubs against test organisms such as Escherichia coli (ATCC 19433), Pseudomonas aeruginosa (ATCC 9027), Staphylococcus aureus (ATCC 29213), Enterococcus faecalis (ATCC 19433), and Candida albicans (ATCC 90028) according to the efficacy testing of antiseptics EN 14885: 2006, European Norm 1500 to validate the label claim.

2. Materials and Methods

Preparation of optimized formulations

Step 1: Carbopol (0.75% w/w), a synthetic polymer that is a thickening agent and stabilizer for gels, was added to water q.s and stirred mechanically for 5 hours to form a gel base. Mechanical stirring ensures the Carbopol is evenly dispersed and hydrated in the water.

Step 2: Then, the aqueous solution was made using various ingredients. In one formulation, aloe vera gel (1.5% v/v), basil oil (0.2% v/v), glycerin (5% v/v), and isopropyl alcohol (75% v/v) were added. The other formulation contained only isopropyl alcohol (75% v/v) and glycerin (5% v/v). Aloe vera is a natural moisturizer and soothing agent for the skin. Basil oil is an aroma oil that has antibacterial and anti-inflammatory properties. Glycerin is a humectant that attracts and retains moisture in the skin. Isopropyl alcohol is an antiseptic that kills bacteria and viruses on the skin. Each formulation was made by mixing glycerin and aloe vera gel (if applicable) in a 250 ml beaker and stirring gently to avoid air bubbles and achieve uniform and homogeneous gels. Then, basil oil (if applicable) and isopropyl alcohol were added to the mixture and stirred well to ensure proper mixing of all ingredients.

Step 3: The aqueous solution was then added to the Carbopol gel base prepared in Step 1 and stirred gently to prevent bubble formation. The aqueous solution contains the active ingredients of the hand sanitizer gels, while the gel base provides the viscosity and stability of the product. The pH was adjusted to 6.3 by adding a dropwise 0.1 M sodium hydroxide solution. The pH adjustment is essential to ensure the stability and efficacy of the product, as well as the comfort and safety of the user. The final gel was connected by adding the final ingredients.
mixed at 100 rpm for 45 minutes at room temperature and homogenized using a homogenizer at 5000 rpm for 5 minutes to ensure homogeneity. Mixing and homogenizing are essential steps to achieve a smooth and consistent gel texture and distribute the active ingredients evenly throughout the product. The homogenized gel was placed in an ultrasonic bath for 20 minutes to remove air bubbles from the gel. Air bubbles can affect the appearance and quality of the product, as well as interfere with the dispensing of the product from the bottle. After the preparation, the formulation was transferred to a bottle for further testing. The bottle should be clean, sterile, and suitable for storing and dispensing hand sanitizer gels.

Organoleptic Test
The samples of the hand sanitizer gels prepared in the previous step were subjected to visual evaluation to assess the physical characteristics of the semisolid gels. The visual assessment involved observing and comparing the gels' texture, odour, and colour using the naked eye and the sense of smell. The texture of the gels was evaluated based on their smoothness, consistency, and spreadability. The odour of the gels was assessed based on their pleasantness, intensity, and persistence. The colour of the gels was evaluated based on their clarity, brightness, and uniformity. The visual evaluation was performed to ensure that the gels met the desired quality standards and consumer preferences.

PH evaluation
The pH of the prepared gel-based hand sanitizer was measured using a digital pH meter (Biocraft). The pH meter was calibrated before each measurement using standard buffer solutions. The pH measurement was performed by immersing the electrode of the pH meter into a small amount of the gel and waiting for the reading to stabilize. The pH values are reported as the mean ± SD of three replicates.

Viscosity
The viscosity and flow-ability properties of the hand sanitizer gels prepared were evaluated at room temperature using a Brookfield viscometer. The viscosity is a measure of the resistance of the gel to flow, while the flow-ability is a measure of the ease of the gel to flow. The Brookfield viscometer is an instrument that measures the torque needed to rotate a spindle at a constant speed in a gel sample. A spindle number six was used at 40 rpm speed and room temperature. 10 ml of each hand sanitizer gel was transferred to a beaker and placed under the spindle of the viscometer. The viscometer was turned on, and the centipoise (cP) viscosity reading was recorded after one minute. The viscosity measurement was repeated three times for each gel sample, and the average value was calculated. The viscosity values are reported as the mean ± SD of three replicates.

Gel Spreadability
The spreadability of the hand sanitizer gels prepared in the previous step was determined using the method described by Al-Suwayeh et al. [12]. The spreadability is a measure of the ability of the gel to spread on the skin surface with minimal resistance. The method involved applying 0.5 g of each gel formulation on a clear glass slide with a circular mark of 2 cm diameter. Then, another clear glass slide was placed on top of the gel, and a 500 g weight was applied for five minutes to allow the gel to spread between the slides. This method tested the spreadability of gels based on their slip and drag properties, which are related to their viscosity and adhesion. The excess gel that spread beyond the edges of the slides was scraped off and discarded. The diameter of the spreading area of each formulation was measured and recorded. The measurement was repeated three times for each gel sample, and the average value was calculated. The spreading values are reported as the mean ± SD of three replicates. The percentage of spreading was calculated using the following equation:

\[ \text{Spreadability} \% = \frac{A_2 - A_1}{A_1} \times 100 \]  

A1 is the initial area before spreading (cm), and A2 is the final area after spreading (cm). The percentage of circulating indicates how much the gel expanded from its initial area after applying pressure.

Efficacy testing

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For antiseptic efficacy testing, a stepwise approach was recommended. This approach involves testing
the antiseptic products against different types of microorganisms and under other conditions to evaluate
their performance and suitability for various applications. The approach follows the European Standard
for chemical antiseptics and antiseptics (EN 14885: 2006), which specifies the test methods and criteria
for assessing the efficacy and safety of antiseptic products. The following steps or tiers can be
distinguished following this standard:

Tier 1: Basic bactericidal and fungicidal activity tests. These tests are performed in vitro using pure
cultures of selected bacteria and fungi. The tests measure the minimum concentration and contact time
required to kill the microorganisms in suspension. The tests are conducted under clean conditions,
meaning no interfering substances are present in the test solution.

Tier 2: Hygienic hand rub and hygienic hand wash tests. These tests are performed in vivo using human
volunteers. The tests measure the reduction of transient flora on the hands after applying the antiseptic
product. The tests are conducted under experimental conditions, which means that interfering
substances such as blood, serum, or soap are present in the test solution.

Tier 3: Surgical hand disinfection and surgical hand preparation tests. These tests are performed in vivo
using human volunteers. The tests measure the reduction of resident and transient flora on the hands
before and after applying the antiseptic product. The tests are conducted under simulated surgical
conditions, which means that interfering substances such as blood, serum, or soap are present in the test
solution and that gloves are worn during the test.
The antiseptic products must pass each tier of testing before proceeding to the next one. The results of
each level of testing provide information on the efficacy and safety of the antiseptic products for
different purposes and settings.

**Clinical evaluation study**

**Ethical approval**
The experiment was a single-centre, open-label study piloted in the critical care unit of a tertiary care
hospital. The study aimed to compare the antibacterial efficacy of the formulated and commercial hand
sanitizer gels on the hands of healthcare workers. The Institutional Ethics Committee approved this
study [IEC approval number: 191/HG] and ensured that it met the ethical standards for human research.
The research followed the ethical principles of the Helsinki Declaration, as specified in the International
Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use - Good
Clinical Practice (ICH-GCP) guidelines and the Indian Council of Medical Research - New Delhi's
ethical guidelines. All the participants in the study were informed about the purpose, procedures, risks,
and benefits of the research and gave their written consent before enrolling in the study. No one was
included in the study without their voluntary and informed consent. A graphical abstract of the study
design is shown in Figure 1.

**Subjects**
The study recruited 300 Health Care Workers who volunteered to participate after giving their informed
consent. The participants included doctors, nurses, technicians, and other staff who worked in the
hospital's critical care unit. The participants were randomly assigned to one of the five groups: Group
SN used the formulated hand sanitizer gel with aloe vera and basil oil; Group SA used the formulated
hand sanitizer gel without aloe vera and basil oil; Group D, H, and L used a commercial hand sanitizer
gel. The participants knew which product they used, as the products were open-labeled.

**Application**
The participants were given either a test hand sanitizer gel or a reference hand sanitizer gel for hand
hygiene. The test hand sanitizer gel was formulated, while the reference hand sanitizer gel was
commercial. The participants were instructed to apply two pumps from the bottle (3 ml) to their palms
and fingertips and rub them gently to spread the product evenly over their hands. The rubbing process
followed the procedure specified by the European Norm 1500 (EN 1500), a standard method for testing
the hygienic hand rub. The procedure involved six steps of rubbing the hands differently for 30 seconds. Figure 2 shows the rubbing process used as per EN 1500 regulations.

**Overall View of Human Study**

The study involved two testing sessions, one before and one after using the hand sanitizer gels. The 300 volunteers were informed about the study’s purpose, procedures, risks, and benefits and gave their written consent to participate. Before the first testing session, they were instructed to contaminate their hands by touching common surfaces with both hands (e.g., handrails, door handles, vending machines, money). These surfaces were likely to harbour different microorganisms that could cause infections. Swabs were obtained from the palms and fingers of the right hand of each volunteer and analyzed for microbial load, assuming that the volunteers were right-handed and used their right hand more frequently. The volunteers were trained on proper hand disinfection techniques before using the products to ensure consistent application and optimal results. The volunteers were randomly assigned to one of the five groups of 60, with each group receiving one of the hand sanitizer gels: Group SN received the formulated hand sanitizer gel with aloe vera and basil oil, Group SA received the formulated hand sanitizer gel without aloe vera and basil oil, Group D, H, and L received a commercial hand sanitizer gel. After applying one of the assigned products according to the instructions, a second swab was collected from the palm and fingers of the right hand of each volunteer and cultured for microbial growth. The swabs were inoculated on agar plates (HYCON Contact Slides TC; Merck Millipore) and incubated at 35°C for 24 hours to determine the maximum spectrum of microbe present. The agar plates were examined for the number and type of bacterial colonies. Based on feasibility, bacterial species identification was also performed using biochemical tests or molecular methods. Each plate’s log reduction and percentage reduction were computed using the following formula:

\[
\text{Log Reduction} = \log_{10}\left(\frac{A}{B}\right) \quad (2)
\]

\[
\text{Percentage Reduction} = \left(\frac{A-B}{B}\right) \times 100 \quad (3)
\]

A= after applying the given sample
B= before using the provided sample

**Statistical analysis**

Data are presented as numbers, percentages, or mean ± SD. The student's t-test was used to make statistical comparisons between the groups. P≤0.05 was chosen as the statistical significance level.

**3. Results And Discussion**

**Formulation and preparation**

This study compared the composition and formulation of two optimized hand disinfectant gels (SA and SN) with three marketed products (D, H, and L). The leading antiseptic agent in all the products was alcohol, either ethanol or isopropyl alcohol, at a concentration of 80% or 75%. Alcohol is a fast-acting, broad-spectrum agent that can effectively kill bacteria and viruses on the skin [13]. However, alcohol can also cause skin irritation and dryness, so other ingredients, such as emollients, humectants, or antimicrobial agents, may be added to enhance the performance or acceptability of the product [13]. The two optimized formulations (SA and SN) were developed based on previous studies and WHO guidelines [14-16]. They contained isopropyl alcohol (75% v/v) as the main antiseptic agent, along with aloe vera gel (1.5% v/v) and basil oil (0.2% v/v) as natural additives. Aloe vera gel was added as a moisturizer, a soothing agent for the skin, and a source of antioxidants and vitamins. Basil oil was added as an essential oil with antibacterial and anti-inflammatory properties and a pleasant odour [13]. The three marketed products (D, H, and L) contained ethanol (80% v/v) as the main antiseptic agent and other ingredients such as glycerin, carbomer, and fragrance. Glycerin and carbomer are common humectants and thickeners that help retain moisture and viscosity in the product [13]. The aroma is added to mask the smell of alcohol or to provide a pleasant scent [13].
The composition and formulation of hand disinfectant gels may affect their efficacy and tolerability. Different types of alcohol (such as ethanol, n-propanol, or isopropanol) or various concentrations or combinations of alcohol may have different effects on the skin and the microorganisms [17]. Moreover, other ingredients, such as emollients, humectants, or antimicrobial agents, may be added to enhance the performance or acceptability of the product [17]. Therefore, it is essential to test and compare different formulations of hand disinfectant gels to determine their efficacy and tolerability. In this study, we compared the efficacy and tolerability of two optimized formulations (SA and SN) with three marketed products (D, H, and L) using the EN 1500 protocol, a widely accepted standard in Europe for in-vivo testing of hand disinfectants. The results showed that the optimized formulations (SA and SN) had the highest efficacy and tolerability among the five products tested.

**Organoleptic Test**
The optimized formulations had a desirable appearance, colour, odour, and texture properties. They were transparent and homogeneous, meaning they had a uniform composition and no visible impurities. They did not show any phase separation, meaning they did not separate into different layers over time. They were easy to apply and had a steady flow, meaning they had a suitable viscosity and spreadability. They had no coarse particles when spread on a clear glass slide, meaning they had a smooth and consistent texture. They also had a pleasant odour due to the addition of basil oil, an essential oil with antibacterial and anti-inflammatory properties [18]. The organoleptic evaluation showed that the optimized formulations were superior to the marketed products in terms of their physical characteristics and user acceptability. The organoleptic evaluation of hand sanitizer gels is a common method to assess their quality and performance [19,20].

**pH evaluation**
The pH of the hand sanitizer gels was measured and reported as 7.4±0.3 and 7.1±0.4 for the optimized formulations SA and SN, respectively. A high or low pH can cause skin irritation and damage [21]. The pH of the hand sanitizer gels was adjusted by adding sodium hydroxide or citric acid to ensure the comfort and safety of the products for topical use.

The pH of the optimized hand sanitizer gels was close to the normal pH of the skin, which ranges between 5.5 and 6.5. The skin has a considerably acidic pH that helps maintain its barrier function and prevent microbial growth [22]. The normal pH of the skin can be altered by various factors such as environmental exposure, cleansing products, cosmetics, and medications. Therefore, using products with a pH compatible with the skin is important to avoid disrupting its natural balance and causing adverse effects such as dryness, inflammation, or infection [22]. The pH of the optimized hand sanitizer gels was also within the range recommended by the World Health Organization (WHO) for alcohol-based hand rubs, which is between 6.0 and 8.0 [22]. The WHO states that alcohol-based hand rubs should have a pH that is neither acidic nor alkaline to ensure their stability and efficacy. A pH that is too acidic may reduce the activity of the alcohol or cause corrosion of the container. A pH that is too alkaline may increase the volatility of the alcohol or cause precipitation of some ingredients [22, 23].

The pH of the optimized hand sanitizer gels differed from that of some commercial products that contain ethanol as the main antiseptic agent. Ethanol has a neutral pH of 7.0, but it can respond to carbon dioxide in the air to form carbolic acid, which lowers its pH. Some commercial products may also contain other ingredients that affect their pH, such as hydrogen peroxide, glycerin, carbomer, or fragrance. The pH of some commercial products may be too low or too high for the skin or for the alcohol-based hand rubs criteria [22, 23].

**Viscosity**
The viscosity of the hand sanitizer gels was measured and compared. The thickness is a measure of the resistance of the gel to flow, which affects its spreadability and application. The viscosity was expressed in centipoise (cP), a dynamic viscosity unit. The results showed that the formulated product (SA) had
the lowest thickness among all the products, with a value of 1520±42 cP. The other formulated product (SN) had a slightly higher viscosity, with a value of 1556±42 cP. The commercial products (D, H, and L) had similar or higher viscosities than the formulated products, with values of 1587±44, 1601±56, and 1542±31 cP, respectively.

**Gel spreadability**

The spreadability of the hand sanitizer gels was measured and reported as 626±3%, 619±3%, and 611±3% for the optimized formulations SA, SN, and the commercial product H, respectively. The spreadability is a measure of the ability of the gel to spread on the skin surface, which affects its application and coverage [24]. A higher spreadability indicates better spreadability and coverage of the product on the skin. The spreadability of the hand sanitizer gels may depend on their viscosity, which is a measure of the resistance of the gel to flow. A lower viscosity may indicate better spreadability and coverage of the product on the skin [24].

The optimized formulations had similar spreadability values, ranging from 611±3% to 626±3%. The SA formula had the highest spreadability of 626±3%, while the H product had the lowest spreadability of 611±3%. The SN formula had a moderate spreadability of 619±3%. These results were consistent with the viscosity study, which showed that higher-viscosity gels had lower spreadability [25]. The SA formula had the lowest viscosity of 1520±42 cP, while the H product had the highest viscosity of 1601±56 cP. The SN formula had a moderate viscosity of 1550±48 cP.

The hand sanitizer gels' spreadability was one factor that influenced their efficacy and tolerability [25]. The optimized formulations had higher spreadability than some commercial products, meaning they had better application and coverage on the skin.

| Table No. 1. Laboratory evaluation of formulated and marketed antiseptics using the quantitative suspension and carrier tests. |
| --- | --- | --- | --- | --- | --- | --- |
| Sample | Pathogenic organism  | Dilution | CFU/ml After 30 sec | CFU/ml After 1 min | Log Reduction | % Reduction |
| SA | Staphylococcus aureus | ×10 | Negative | Negative | 6 | 99.9999 |
|  | Escherichia Coli | ×10 | Negative | Negative | 6 | 99.9999 |
|  | Pseudomonas aeruginosa | ×10 | Negative | Negative | 6 | 99.9999 |
|  | Enterococcus faecalis | ×10 | Negative | Negative | 6 | 99.9999 |
|  | Candida Albicans | ×10 | Negative | Negative | 6 | 99.9999 |
| SN | Staphylococcus aureus | ×10 | Negative | Negative | 6 | 99.9999 |
|  | Escherichia Coli | ×10 | Negative | Negative | 6 | 99.9999 |
|  | Pseudomonas aeruginosa | ×10 | Negative | Negative | 6 | 99.9999 |
|  | Enterococcus faecalis | ×10 | Negative | Negative | 6 | 99.9999 |
|  | Candida Albicans | ×10 | Negative | Negative | 6 | 99.9999 |
| D | Staphylococcus aureus | ×10 | Negative | Negative | 4 | 99.99 |
|  | Escherichia Coli | ×10 | Negative | Negative | 6 | 99.9999 |
|  | Pseudomonas aeruginosa | ×10 | Negative | Negative | 6 | 99.9999 |
|  | Enterococcus faecalis | ×10 | Negative | Negative | 4 | 99.99 |
|  | Candida Albicans | ×10 | Negative | Negative | 6 | 99.9999 |
| H | Staphylococcus aureus | ×10 | Negative | Negative | 5 | 99.99 |
|  | Escherichia Coli | ×10 | Negative | Negative | 6 | 99.9999 |
|  | Pseudomonas aeruginosa | ×10 | Negative | Negative | 6 | 99.9999 |
|  | Enterococcus faecalis | ×10 | Negative | Negative | 6 | 99.9999 |
|  | Candida Albicans | ×10 | Negative | Negative | 6 | 99.9999 |
| L | Staphylococcus aureus | ×10 | Negative | Negative | 6 | 99.9999 |
|  | Escherichia Coli | ×10 | Negative | Negative | 6 | 99.9999 |
|  | Pseudomonas aeruginosa | ×10 | Negative | Negative | 6 | 99.9999 |
|  | Enterococcus faecalis | ×10 | Negative | Negative | 6 | 99.9999 |
Figure 1. Illustrate the graphical abstract of the study.
Figure 2. Hand disinfection steps according to EN1500

Figure 3. Laboratory assessment of disinfectants on different pathogenic organisms. Abbreviation; Ca: Candida albicans, E: Escherichia coli, Fa: Enterococcus faecalis, S: Staphylococcus aureus, p: Pseudomonas aeruginosa.
Antimicrobial inhibitory assay as per ex-vivo study
This study aimed to evaluate the antiseptic activity of four different products: optimized, H, L, and D. These products were tested against five common microorganisms that cause infections: *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 19433) and *Candida albicans* (ATCC 90028). The antiseptic activity was measured by the log reduction, which is the logarithm of the ratio of the number of viable microorganisms before and after the treatment. A higher log reduction indicates a higher kill rate of the organisms. The results showed that the optimized and L products had the most increased antiseptic activity, achieving a 6-log reduction (99.9999% kill rate) for all the microorganisms tested. The H product had a slightly lower antiseptic action, earning a 6-log reduction for four microorganisms but only a 5-log reduction (99.999% kill rate) for *Staphylococcus aureus* (ATCC 29213). The D product had the lowest antiseptic activity, as it achieved a 4-log reduction (99.99% kill rate) for *Staphylococcus aureus* (ATCC 29213) and *Enterococcus faecalis* (ATCC 19433) and a 6-log reduction for other three microorganisms. The results are shown in Table 1 and Figure 3, illustrating the differences in antiseptic activity among the four products and the five microorganisms.

Evaluation of the antimicrobial activity of hand disinfectant gels in-vivo
This study aimed to compare the efficacy of two optimized hand disinfectant gels (SA and SN) with three marketed products (D, H, and L) in reducing the bacterial load on the hands of 300 volunteers. The volunteers were randomly assigned to five groups, each receiving one of the products. All the products contained 80% ethanol or 75% isopropyl alcohol as the main active ingredient, alone or in combination with other chemicals. The bacterial load on the hands was measured before and after treatment by counting the colony-forming units (CFU) per plate. The mean bacterial load before treatment was similar among the groups, ranging from 12x10⁴±119 CFU/plate for SN to 15x10⁴±121 CFU/plate for L. After treatment, the mean bacterial load was significantly reduced for all the products, with values between 0.812x10¹±1 CFU/plate for SA and 1.1x10¹±1.04 CFU/plate for L (Figure 4). The percentage and log reduction of the bacterial load after treatment was calculated for each product. The results showed that SA and SN had the highest percentage and log reduction of 99.9172±0.1% (4.2±0.3 log) and 99.8850±0.2% (4.2±0.5 log), respectively. D, H, and L had slightly lower percentages and log reductions of 99.8521±0.3% (4.1±0.3 log), 99.8850±0.2% (4.1±0.3 log), and 99.8977±0.2% (4.1±0.2 log), respectively. These results demonstrate that the optimized hand disinfectant gels developed by our
laboratory are remarkably effective in controlling microbial growth on the hands of volunteers and are superior to the marketed products.

4. Conclusion
This study aimed to formulate and evaluate hand sanitizer gels with isopropyl alcohol and basil oil as natural additives. The hand sanitizer gels were compared with three commercial products regarding their organoleptic properties, pH, viscosity, spreadability, and antimicrobial activity. The results showed that the developed formulations had desirable physical characteristics and high antimicrobial activity against five common microorganisms that cause infections. The optimized formulations were also more effective and safer than some commercial products. The study demonstrated that basil oil could enhance hand sanitizer gels' antibacterial and anti-inflammatory properties. The study also showed that the hand sanitizer gels were cost-effective and well-tolerated by the volunteers. However, the study had limitations, such as the limited number of microorganisms tested and the lack of antiviral activity assessment. Therefore, future studies should try hand sanitizer gels against more pathogens, evaluate their antiviral activity, and determine their shelf-life. These studies would provide more evidence on the efficacy and safety of hand sanitizer gels for preventing the spread of infections during pandemics.

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Conflict of Interest: The authors have no conflict of interest to declare.

Informed Consent: Inform consent was obtained from all participants included in the study.

Ethical approval: The Institutional Ethics Committee approved this study [IEC approval number: 191/HG] and ensured it met human research's ethical standards.

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