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In-vitro Evaluation of the Antimicrobial Properties of Various Hand Sanitizers

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ABSTRACT

Background: Hand hygiene is vital in controlling the spread of infections in both hospital and community settings. This study evaluates the antimicrobial effectiveness of four hand sanitizers—Imported, Local chemical, Local herbal, and Multinational—against bacterial strains including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterococcus faecalis*.
Objective: The objective was to determine the comparative efficacy of these products in an in-vitro environment.
Methods: This in-vitro study employed the agar disk diffusion method using Mueller-Hinton agar to assess antimicrobial efficacy. Bacterial suspensions were standardized to McFarland 0.5 turbidity, and 50 µL of each sanitizer was introduced into pre-drilled agar wells. Sterile water served as the control. After 24 hours of incubation at 37°C, zones of inhibition were measured with a digital caliper. The results underwent statistical analysis using ANOVA and post hoc testing.
Results: Imported exhibited the largest zones of inhibition, with mean diameters (in mm) significantly greater than the other sanitizers: Imported (22 ± 6), Local Herbal (7.7 ± 0.5), Local Chemical (9.5 ± 1.5), and Multinational (8 ± 1). The statistical significance of these differences was confirmed ($P < 0.001$). Among all products, Imported demonstrated the highest efficacy against both Gram-positive and Gram-negative bacteria.
Conclusion: Imported was the most effective hand sanitizer for maintaining hand hygiene, followed by Local Chemical, Multinational, and Local Herbal. This study highlights the critical need for regulatory oversight to ensure product claims align with verified antimicrobial performance.

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Introduction

Hand hygiene remains one of the most critical measures to prevent the transmission of infections in healthcare and community settings. Hands are a primary route for spreading pathogens, including bacteria, viruses, and fungi, contributing to healthcare-associated infections (HAIs) and community outbreaks. Infections transmitted via hands, including gastrointestinal and respiratory illnesses, can lead to significant morbidity and mortality worldwide [1].

The World Health Organization (WHO) and the Center for Disease Control and Prevention (CDC) emphasize the importance of proper hand hygiene to reduce HAIs. Evidence suggests that washing hands with soap and water can remove transient flora, while alcohol-based hand sanitizers provide an alternative when soap and water are unavailable. Sanitizers have gained

prominence due to their convenience and ability to provide rapid antimicrobial action [2].

Traditionally, microorganisms present on hands are categorized into resident and transient flora. Resident flora typically includes *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Enterococcus faecalis*, which inhabit the deeper layers of the skin and are resistant to mechanical removal. In contrast, transient flora comprises *S. aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, which temporarily colonize the superficial skin layers. For this study, these organisms were selected to evaluate their susceptibility to various hand sanitizers [3].

Research has demonstrated that up to 80% of individuals retain some pathogenic bacteria on their hands even after washing [4]. While handwashing effectively removes dirt and some microorganisms, it also strips the skin of its natural fatty acids, potentially causing cracks that serve as entry points for pathogens [5]. To address the limitations of traditional handwashing, hand sanitizers were introduced, offering not only efficacy against harmful microorganisms but also benefits for maintaining skin health through the inclusion of emollients [3].

Despite their widespread use, not all hand sanitizers are equally effective. Several products claim to eliminate 99.9% of germs, yet studies have revealed variability in their efficacy. This raises concerns about the reliability of such claims and the need for scientific validation. Alcohol-based sanitizers are typically preferred due to their rapid bactericidal activity, but the effectiveness of herbal formulations remains debated.

This study was designed to assess and compare the antimicrobial efficacy of four commercially available hand sanitizers—Imported, Local Chemical, PureHands, and Multinational—against five common bacterial strains. The results aim to provide insights into the effectiveness of these products and guide informed choices for personal and clinical use.

Methods

Study Design: An in-vitro experimental approach was utilized. Ethical clearance was obtained from the Institutional Review Committee.

Test Organisms: Clinical isolates of *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *E. coli*, and *E. faecalis* were used.

Sanitizers Evaluated: Details are given in the Table 1.

Table 1: Details of tested hand sanitizers

Sanitizer Type	Ingredients	Make
Imported	Propan-2-ol, Propan-1-ol, Mecetronium ethyl sulfate, Glycerol, Tetradecan-1-ol, fragrances, Patent blue V, Purified water	Germany
Multinational	Denatured Alcohol- 69.4% w/w, Water PEG/PPG-17/6 copolymer, Propylene glycol, Acrylate/C10-30 alkyl acrylate, cross polymer, Tetrahydroxpropyl ethylenediamine, Perfume.	Pakistan
Local Chemical	Ethyl alcohol 95% v/v IP 55% w/w, Isopropyl alcohol 10% IP w/w, Tocopheryl acetate IP 0.05% w/w, Perfumed gel base: qs to 100% w/w	Pakistan
Local Herbal	Hrivera, Coriander, Lime, Ushira, Neem	Pakistan

Experimental Procedure: Bacterial suspensions were standardized to McFarland 0.5 turbidity. Mueller-Hinton agar plates were inoculated, and wells of 6 mm diameter were drilled. Each well received 50 µL of sanitizer, with central well containing sterile water as a control. Plates were incubated at 37°C for 24 hours, and zones of inhibition were measured. The experiment was repeated five times, and the average of the measurements was calculated to determine the zone of inhibition for each case. The zones of inhibition were measured using a digital caliper (in millimeters).

Personal Protective Equipment (PPE) and care were strictly observed during the experiment to ensure safety and prevent contamination. Laboratory personnel wore disposable gloves, lab coats, face masks, and safety goggles while handling bacterial cultures, sanitizers, and agar plates.

Table 2: Zone of inhibition (mm) by sanitizer and organism

Organism	Imported	Local Herbal	Local Chemical	Multinational	ANOVA	p-value
S. aureus	26.7 ± 1.4	3.5 ± 4.9	8.5 ± 0.7	7.5 ± 0.7	30.982	0.002
S. epidermidis	21.9 ± 1.4	7.1 ± 0.0	8.5 ± 0.7	7.5 ± 0.7	137.8	0.001
P. aeruginosa	19.6 ± 0.7	7.7 ± 0.7	10.5 ± 0.7	8.5 ± 0.7	119.9	0.001
E. coli	15.8 ± 0.7	7.4 ± 0.7	9.5 ± 0.7	8.5 ± 0.7	52.1	0.001
E. faecalis	16.7 ± 0.7	7.1 ± 0.0	8.5 ± 0.7	7.5 ± 0.7	105.9	0.001

All work surfaces were disinfected before and after the experiment to maintain a sterile environment. These precautions were essential to protect individuals from exposure to chemicals and biological materials, and to ensure the integrity of the study by minimizing the risk of cross-contamination.

Statistical Analysis: Data was entered and analyzed by using Statistical Package for Social Sciences (SPSS) software. ANOVA was performed to compare groups and paired t-test was used to observe significance with cut off p-value <0.05.

Results

Table 2 shows that imported sanitizer produced the largest zones of inhibition against all test organisms, with mean diameters ranging from 15.5 mm to 27 mm. local herbal, a herbal sanitizer, demonstrated the least efficacy. The differences among the sanitizers were statistically significant ($P < 0.001$).

Detailed Results by Test Organism

S. aureus: Imported showed the highest inhibition (26.7 ± 1.4 mm), followed by Local Chemical (8.5 ± 0.7 mm), Multinational (7.5 ± 0.7 mm), and Local Herbal (3.5 ± 4.9 mm).

S. epidermidis: Zones of inhibition were 21.9 ± 1.4 mm for Imported, 8.5 ± 0.7 mm for Local Chemical, 7.5 ± 0.7 mm for Multinational, and 7.1 ± 0.0 mm for local herbal.

P. aeruginosa: Imported showed 19.6 ± 0.7 mm, Local Chemical 10.5 ± 0.7 mm, Multinational 8.5 ± 0.7 mm, and Local Herbal7.7 ± 0.7 mm.

E. coli: Imported inhibited growth with a 15.8 ± 0.7 mm zone, followed by Local Chemical (9.5 ± 0.7 mm), Multinational (8.5 ± 0.7 mm), and Local Herbal (7.4 ± 0.7 mm).

Enterococcus faecalis: The highest inhibition was observed with Imported (16.7 ± 0.7 mm), compared to Local Chemical (8.5 ± 0.7 mm), Multinational (7.5 ± 0.7 mm), and Local Herbal (7.1 ± 0.0 mm).

Discussion

The results reaffirm the superior performance of alcohol-based sanitizers, particularly Imported, which contains 75% propanol. Its liquid formulation allows deeper penetration into skin folds, unlike gel-based alternatives [6]. Local herbal, despite being herbal, lacked the antimicrobial potency of alcohol-based products [7].

Numerous studies have evaluated the antimicrobial efficacy of hand sanitizers independently, yet there is limited literature comparing the effectiveness of various disinfectants and hand sanitizers. Disinfectants are chemical agents designed for immediate and sustained activity, effectively eliminating microorganisms to levels required for hygienic and surgical purposes. In contrast, hand sanitizers act rapidly to reduce microbial counts to safe levels, meeting public health standards. Disinfectants often utilize a more potent alcohol, such as propanol, which achieves greater bacterial reduction compared to the ethanol commonly used in sanitizers. Both agents, however, are effective in reducing bacterial presence upon contact [8].

Alcohol is well-documented for its antimicrobial properties, disrupting microbial membranes and denaturing proteins. The combination of propanol and mecetronium ethyl sulfate in Imported likely contributes to its enhanced efficacy, offering both immediate and residual antimicrobial effects. In contrast, herbal components in local herbal, such as neem and coriander, may lack sufficient potency to combat robust bacterial strains.

Previous studies have similarly highlighted the effectiveness of alcohol-based sanitizers over non-alcoholic or herbal formulations. For instance, research

by Mondal and Kolhapure showed limited efficacy of herbal products against Gram-negative bacteria. Additionally, formulations with higher alcohol concentrations have demonstrated superior bactericidal activity, emphasizing the role of active ingredient concentration and composition [9]. While Local Chemical and Multinational showed moderate antimicrobial effects, their gel-based formulations may have influenced performance. Gel sanitizers often create a superficial layer on the skin, limiting penetration into folds and creases where bacteria reside. The observed variability in efficacy underscores the importance of selecting appropriate sanitizers for specific applications.

The findings of the current study align with those reported by Reena Rajkumari, who observed that Sterillium demonstrated superior efficacy against *Candida albicans*, *E. coli*, and *Klebsiella pneumoniae* [10]. Similarly, a study by Oke et al. found that Dettol hand sanitizer was effective solely against P. aeruginosa but showed no significant activity against *S. aureus* and *E. coli* [11]. Although multinational hand sanitizer exhibited antimicrobial properties against the tested organisms in this study, a precise and comprehensive comparison with other studies remains challenging due to the limited availability of scientific literature on this topic.

Further research should investigate other microbial species, long-term usage effects, and the potential benefits of combining herbal and alcohol-based components. Additionally, regulatory frameworks must enforce stringent testing and verification of sanitizer claims to ensure public trust and safety.

Conclusion

Imported outperformed other hand sanitizers, demonstrating robust antimicrobial efficacy against diverse bacterial strains. Local Chemical and Multinational showed moderate effectiveness, while Local Herbalexhibited the least. These findings underscore the importance of evidence-based recommendations for sanitizer usage in clinical and community settings.

Recommendations

1. Regulatory validation of sanitizer claims is essential to ensure public safety.
2. Further studies should explore application duration, microbial spectrum, and long-term effects.

Public awareness campaigns should emphasize the importance of proper hand hygiene practices.

Authors' contributions

ICMJE criteria	Details	Author(s)
1. Substantial contributions	Conception, OR Design of the work, OR Data acquisition, analysis, or interpretation	1,2 1,2 3
2. Drafting or reviewing	Draft the work, OR Review critically for important intellectual content	2,3 1
3. Final approval	Approve the version to be published	1,2,3
4. Accountable	Agree to be accountable for all aspects of the work	1,2,3

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Ethical clearance was obtained from the Institutional Review Committee.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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