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Research Article

EVALUATION OF BACTERICIDAL ACTIVITY OF HERBAL HAND SANITIZER

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ABSTRACT

Nosocomial infection (NI), also known as hospital-acquired infections, has had increased attention due to the significant morbidity and mortality caused by NI worldwide. Transmission of NI is believed to occur predominantly via the mode of pathogen exchange to and from contaminated hands. Thus, maintaining clean and microbe free hands gains a lot of scientific and clinical scope. Accordingly, hand hygiene techniques (using hand sanitizers particularly) to eliminate disease-causing microbial bugs have been considered one of the primary-most infection blocking methods. Several scientific studies have validated the clinical efficacy of hand sanitizers against the most commonly involved microbial strains in the pathogenesis of NI. The goal of this study was to evaluate the bactericidal activity of the study material Clean Hand Gel, a specialized herbal hand sanitizer with bio-actives enriched with the goodness of Camphor, Cumin seeds, Vetiver, Citrus and Neem. The study material was evaluated for its bactericidal activity against the specified microorganisms (Bacteria - *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Enterococcus hirae* species) by time kill assay. Data indicated that the tested product at 20% concentration when tested for 120 seconds of contact time against specified bacterial species resulted in considerable logarithmic reductions of 0.03, 0.125, 0.097 and 0.091 in the bacterial viable counts of *Staphylcoccus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus hirae,* respectively. It can be concluded that InnoVision's Clean Hand Gel with its tested in vitro effects against clinically important NI causing bacterial organisms *S. aureus*, *P. aeruginosa*, *E. coli and E. hirae,* can be used as an effective herbal hand sanitizer in controlling the transmission of disease causing microorganisms (specified bacteria) from hand to hand and can have potential implications in controlling measures against the spread of associated diseases.

KEYWORDS: Nosocomial infection, herbal hand sanitizer, anti-microbial, bactericidal, *Staphylococcus aureus. P. aeruginosa.*

INTRODUCTION

Nosocomial infections (NI) or healthcare associated infections - are disease-causing infections through which pathogenic microbes are transmitted from different sources in a hospital or healthcare environment such as, hospital personnel, patients or inanimate environmental objects to a new immune-compromised host [1]. NI affect millions of people and has been reported as one of the leading causes of death worldwide, killing more number of people than cancer, road accidents etc [2]. Of all the types and modes of spread of NI, the single most, frequent and easy vehicle for the transmittance of infectious microbes is via direct/indirect routes of physical contact involving hands [3]. Microbiologically contaminated hand surfaces can act as natural reservoirs of pathogenic microorganisms and are the primary cause of contact transmission in the pathogenesis of NI. Thus, inactivation and elimination of both, transient and residing microbes on the hands, is the best way and the mainstay of preventing NI [4]. However, the lack of awareness about the significance of hand hygiene and improper hand cleansing practices has been significantly responsible for increased rate of NI. Subsequently, hand hygiene techniques have gained much importance and have become crucial and daily practices in infection control measures contributing to decline in spread of NI by several folds.

Normally the human skin is colonized by bacterial counts as high as 1000 colony forming units (CFU)/cm^2 on parts of hands [5]. The main types of hand-carriage microbial flora which can persist for long periods on the hands and facilitate contact mediated transmission of NI include bacterial strains of *Staphylococcus aureus*, *Enterococcus spp*., *Escherichia coli, Pseudomonas aeruginosa*, to mention the important few[6]. There is ample amount of scientific evidence to support the fact that appropriate hand hygiene practices result in significant reduction of acquiring contact with these bacterial strains and have also demonstrated a significant decrease in the spread of NI [7].

Medically recommended hand-hygiene methods pertain to either of the two main practices - washing hands with soap and water, or, disinfecting the hands with a waterless, alcohol-based hand rub. When subjecting a hand hygiene product to test for its efficiency and acceptability for potential use, the following factors need

to be given due consideration: antimicrobial potency; easy accessibility; smell/odor; color; consistency; effects on skin dryness/irritation[8]. Hand sanitizers have been demonstrated to be safe and effective over their counterpart soap-based hand washing agents with regard to the all of the afore mentioned factors. Additionally, several scientific studies have confirmed the increased efficiency of hand-sanitizers in reducing bacterial count when compared to soap-based hand washing methods [9]. The general notion goes like, soap is good, antimicrobial soaps are better and hand rubs (hand sanitizers) are best at killing bacteria. Although frequent use of alcohol-based hand sanitizes can result in mild skin dryness and irritation, these adverse effects can be eliminated by using herbal-based hand sanitizers that are equally effective against bacteria and other infectious germs.

In the present study, evaluation of a herbal based hand sanitizer containing a combination of extracts of *Vetiveria zizaniodes, Cuminum cyminum, Cinnamomum camphor, Citrus lemon, Vetiveria zizanioides* and *Azadirachta indica* was carried out for its anti-microbial (bactericidal) effects.

MATERIALS AND METHODS

Details of the materials and methods specified in this study are in accordance with standard operating procedures based on the European standard guidelines for evaluation of antimicrobial efficacy (EN 1276 and EN 1650).

Test material: The current study deals with the evaluation of bactericidal activity of herbal hand sanitizer, InnoVision's Clean Hand Gel (each gram) containing a combination of herbal extracts of, *Karpura* (*Cinnamomum camphor*) – 0.5 mg; *Svetajiraka* (*Cuminum cyminum*) Fruit – 0.5 mg; *Ushira* (*Vetiveria zizaniodes*) Root – 0.7 mg; *Nimbu* (*Citrus Limon*) Fruit peel – 0.5 mg; *Neem* (*Azadiratcha indica*) Leaves - 10 mg; Base-IPA and perfume.

Microbial cultures: *Pseudomonas aeruginosa*- ATCC 15442, *Staphylococcus aureus* - ATCC 6538, *Escherichia coli* - ATCC 10536, *Enterococcus hirae* - ATCC 8043 were procured from American Type Collection Center (ATCC), United State of America (USA).

Media and Chemicals: Bovine Serum Albumin (BSA), Tween-80, Lecithin, Tryptone, Soya bean Casein Digest Medium (SCDM) and Sabouraud Dextrose Agar (SDA) were purchased from Hi Media.

EXPERIMENT

Preparation of sample

In 0.6 mL of sterile water, 0.2mL of Clean Hand Gel was directly added to vial containing 0.1ml of BSA and 0.1mL of bacterial inoculum.

Preparation of working cultures

A loopful of inoculum from the respective bacterial stock culture was streaked on different Soyabean Casein Digested Agar (SCDA) plates and plates were incubated at 37˚C for 24 hours. After the incubation time, the second subculture was prepared on SCDA plates from 24 hours old culture and incubated at 37˚C for 24 hours.

Preparation of bacterial inoculums

A loopful of working bacterial culture was inoculated to 10 ml of sterile diluents and incubated at 37˚C for 24 hours. After incubation, the test bacterial suspension was spectrophotometrically. adjusted to 0.2 absorbance units at 600 nm (corresponding to approximately 108 Colony Forming Unit (CFU)/ml and McFarland Standard 1).

Time kill assay of bacteria

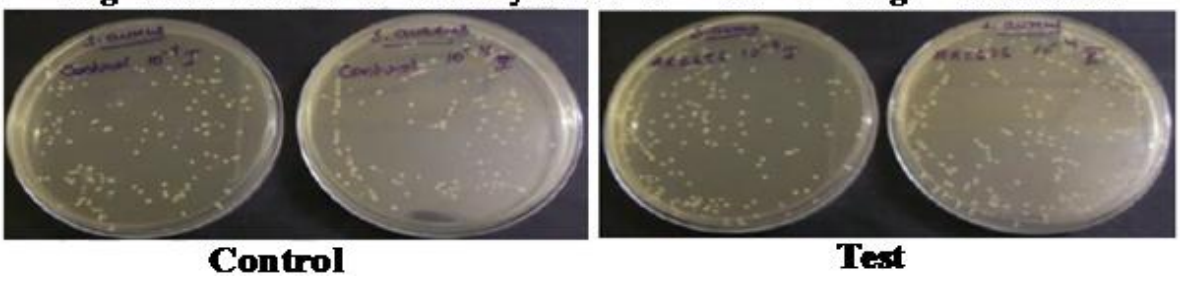
1. Prior to experimentation, all the reagents used were equilibrated to the test temperature of 20 °C.
2. To the test tube containing 0.1ml of 0.03% BSA solution, 0.1ml of bacterial inoculum was added and tube was incubated at 20˚C for 2 minutes.
3. After incubation, 0.2 ml of study material dissolved in 0.6ml of sterile water was added to the above solution. Test tube was further incubated for 2 minutes at 20˚C.
4. Immediately after 2 minutes, 0.1ml of test mixture was transferred to a test tube containing 0.8ml of neutralizer (3 ml Tween 20 + 0.3mg lecithin) and 0.1 ml of sterile water.
5. After 5 minutes of incubation, test mixture was serially diluted from 10-0 to 10-5 dilutions and 0.1 ml test mixture from each dilution was spread on SCDA plates.
6. Plates were incubated at 37˚C for 24 hours.
7. Control experiment was prepared by replacing test sample with 0.2ml of sterile water.
8. After 24 hours of incubation, CFU/ml of both test and control plates were calculated.
9. Logarithmic (Log) reduction in viable count of test organism was calculated by comparing CFU/ml of the test with CFU/ml of control.

Calculation

$$\text{Colony Forming Unit (CFU/mL)} = \frac{\text{Average number of colonies} * \text{Dilution factor}}{\text{Amount of sample plates}}$$

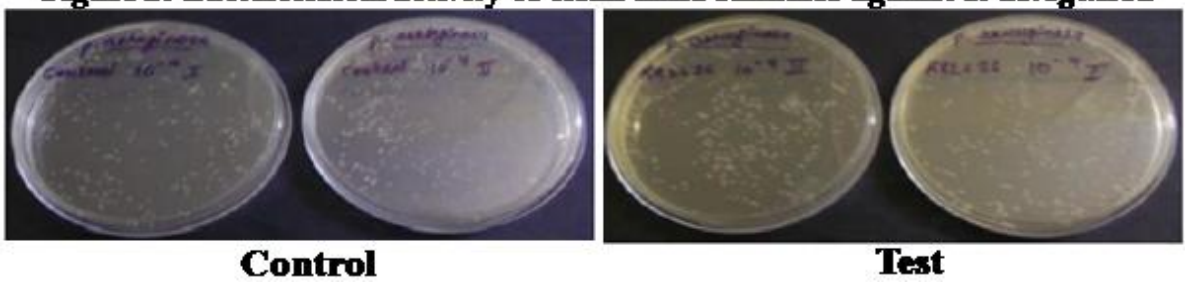
Log reduction = Log (CFU/mL of Growth Control) – Log (CFU/mL of Test Sample)

Figure 1: Bactericidical activity of clean hand sanitizer against *S. aureus*



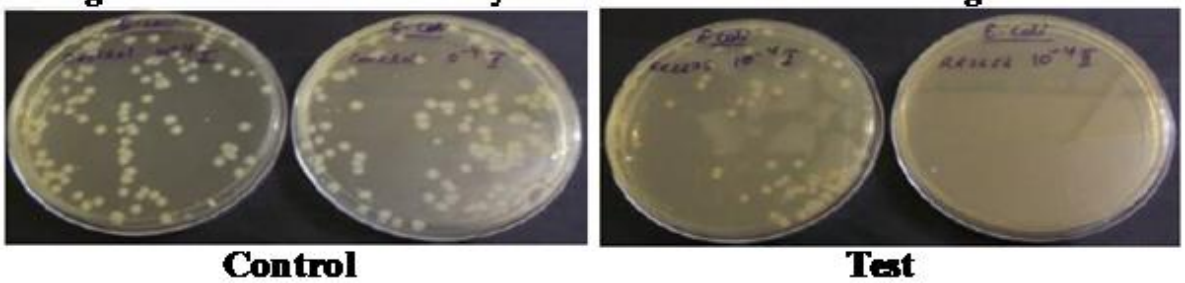
Control Test

Figure 2: Bactericidical activity of clean hand sanitizer against *P. aereginosa*



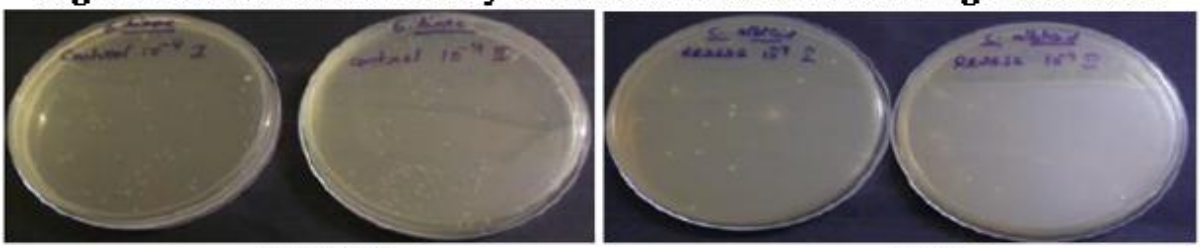
Control Test

Figure 3: Bactericidical activity of clean hand sanitizer tested against *E. coli*



Control Test

Figure 4: Bactericidical activity of clean hand sanitizer tested against *E. hirae*



Control Test

RESULTS

Table 1: Time kill assay of clean hand sanitizer tested under clean conditions

Test organism	Conc. of product	Log reduction		
		Colony forming units/mL in clean condition (0.3% BSA)		
		Control (bacterial) suspension	Test (bacterial) suspension	Logarithmic reduction units
S. aureus	20 %	1.2×10^8	1.1×10^8	0.03
P. aeruginosa	20 %	1.6×10^8	1.2×10^8	0.125
E. coli	20 %	1.2×10^8	1.0×10^8	0.097
E. hirae	20 %	1.2×10^8	1.0×10^8	0.091

+Four bacterial species, *Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus hirae* were selected in the current investigation to screen the anti-microbial efficacy of the study material Clean Hand Gel, a herbal hand sanitizer. Results of the bactericidal tests using time kill assay are expressed as logarithmic reduction in the density of viable count of specified bacteria. Table 1 shows that the clean hand sanitizer tested at a concentration of 20% for 2 minutes contact time demonstrated 0.03, 0.125, 0.097 and 0.091 units logarithmic reduction in the viable counts of *Staphylococcus aureus, Pseudomonas aeruginosa*, *Enterococcus hirae* and *Escherichia coli,* respectively. Macroscopic observations are photographed and shown in Fig. 1-4.

DISCUSSION

A strong body of scientific research categorically suggests that the hand surface contact pathway is one of the leading causes of NI, and thus implies the vitality of hand hygiene practices in protection against diseases. Hand hygiene can in fact save lives and is regarded as one of the most important infection control elements against the transmission of pathogenic microorganisms that cause lethal diseases. Waterless hand hygiene rubs are proven to be more efficacious agents in reducing the number of bacteria from contaminated hand surfaces as compared to that of soap based hand hygiene preparations. Herbal based hand sanitizers formulated with natural emollients and moisturizers are equally effective and also have the additional benefit of safety with negligible rates of skin dryness/irritation, and hence keep the skin free from germs, and also leaving the skin clean, smooth and safe [10]. Another pros aspect of herbal based hand sanitizers is, unlike exclusively chemical based hand rubs and their ingredients that provide room for antimicrobial resistance and emergence of new resistant bacterial strains, in case of herbal formulations, bacteria, viruses and other microbes fail to develop resistance to natural/herbal products and their ingredients **[11]**.

Other than acceptability features of hand hygiene products such as cost, smell, feel and allergenic potential, another important attribute that needs to be considered is the antimicrobial profile of the product. Hand hygiene products should have considerable bactericidal activity, and an additional activity against fungi (yeasts), since the hands of health care associated personnel are most frequently colonized and contaminated by these classes of microbes. The type and species of microorganisms (specific species of bacteria, fungi etc.) is one of the crucial determinants of a successful outbreak of NI. By systematic reviews, it has been explored that, conventionally, bacterial infections account for more than 50% of acquired NI. Gram-positive *Staphylococcus* and gram-negative *Pseudomonas*, *Escherichia* species have been found to be the most commonly identified clinical isolates from individuals in the scope of NI. Furthermore, it has also been noted that *S. aureus* is emerging as a highly potent antimicrobial resistant bacteria that colonizes and survives for prolonged periods up to several months by consistent transmittance and is predominantly involved in the pathogenesis of NI **[12]**.

Scientific studies have revealed the clinical potency of hand sanitizers as germ killing agents to be most effective, particularly against bacterial strains that are most involved in NI. The antimicrobial efficacy of a hand hygiene product is measured in terms of logarithmic reduction in the density of viable microbial count, and it has been established and proven the superior efficacy of hand-rub sanitizers over soap based hand wash with respect to the logarithmic reduction of microbial count. In addition, findings suggest that there have been enough scientific evidence to ascertain that soap based hand washing has been unsuccessful in eliminating the said predominant NI causing bacterial species of *S. aureus*, *Enterococcus* and others strains, which in turn favors the application of hand-sanitizers in such cases **[13]**.

Having the above discussed excerpts as an inclination, the results of this study provide supporting microbiological evidence for the antimicrobial effects of the tested polyherbal hand sanitizer against different NI causing, clinically important bacterial organisms consisting strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli* and *Enterococcus hirae*. It is well known that a wide range of active phytochemicals such as polyphenols, alkaloids, essential oils/lipids, terpenoids, peptides etc., are abundantly synthesized by the plant/herbal constituents present in a natural, plant-based product formulation. These diverse classes of bioactive molecules have also been reported to possess wide-spectrum anti-bacterial activities with a multitude of mechanisms of action. Mode of action is related to both direct mechanisms such as cytoplasmic membrane damage, inhibition of nucleic acid, cell wall syntheses and; indirect mechanisms including interference with and modulation of bacterical virulence factors including enzymes, toxins and signal receptors **[14]**. The various herbal ingredients and their biomolecular composition in the tested Clean Hand Gel formulation might have offered a synergistic, multi-compound therapeutic action responsible for the observed bactericidal effects against specified bacterial species. Additionally, all of the natural/herbal ingredients that make up the composition of this product have been established to possess significant broad-spectrum antimicrobial activity and a plethora of other biological activities that should vouch for the enhanced synergistic efficacy and safety aspects of the test substance **[15-27]**. It is worth mentioning here that our group has also published another report with regard to the antimicrobial efficacy of the same study material wherein we have demonstrated its potent fungicidal activity against the most common fungal species involved in the pathogenesis and spread of NI **[28]**. Overall, these findings should strongly advocate the efficacy of the tested Clean Hand Gel as a promising hand-sanitizing agent with noteworthy anti-bacterial and anti-fungal effects.

CONCLUSION

Hand sanitizers are slowly but steadily emerging as the best way to abate the transmission of hand-borne pathogenic microorganisms. In view of this, and owing to the results of the present study, it may be concluded that the test substance, InnoVision's Clean Hand Gel, can be used as a highly effective herbal hand-sanitizer with its tested bactericidal and fungicidal activities.

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