

## ANTIBACTERIAL EFFICACY OF COMMERCIALY AVAILABLE HERBAL AND ALCOHOL BASED HAND SANITIZERS- AN IN VITRO STUDY

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### ABSTRACT

#### Introduction

Hand sanitizers significantly increase the chance of keeping the hands clean and aseptic. Traditionally, microbes habitation on hands is divided into resident and transient floras. Involved resident floras are commonly Staphylococcus aureus and Enterococcus faecalis that colonize the deeper skin layers and are resistant to mechanical removal. Therefore, the aim of the present study is to evaluate and compare the antimicrobial efficacy of four different hand sanitizers against Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli

#### Materials and methods:

The present study is an in vitro study to evaluate antimicrobial efficacy of Palmolive, Lifebuoy, Spar and Margo hand sanitizers against clinical isolates of the aforementioned test organisms. The well variant of agar disk diffusion test using Mueller-Hinton agar was used for evaluating the antimicrobial efficacy of hand sanitizers. After incubation, antimicrobial effectiveness was determined using a digital caliper (mm) by measuring the zone of inhibition.

#### Results

Assessment of antimicrobial effectiveness among herbal and alcohol based hand sanitizers revealed that in all cases herbal based hand sanitizers (group III Spar-20mm and IV Margo-16mm) showed maximum efficacy against bacteria as much as the alcohol based sanitizers.

## Conclusion

Herbal hand sanitizers possessed maximum antimicrobial effect against all the Bacteria's used in the study. Despite the claims of efficacy and 99.9% bacterial reduction by hand sanitizer manufacturers, there still exists a need for verification of these claims by regulatory bodies and higher authorities for the enforcement of good-quality measures

**Key words:** Hand sanitizers, Hygiene, Organisms, efficacy, Innovative analysis

## INTRODUCTION

Hospital and community-acquired infections are escalating and pose a serious public health problem worldwide [1]. Hands are considered to be the primary route for transmitting microbes and infections to the individuals[2]. The importance of hygiene is universally recognized and evidence-based. It is well known that hand hygiene is crucial to prevent and minimize healthcare-associated infections [3]. Several studies have shown the importance of proper hand hygiene in reducing the incidence of nosocomial infections[4].

Hand sanitizers significantly increase the chance of keeping the hands clean and aseptic. Traditionally, microbes habitation on hands is divided into resident and transient floras. Involved resident floras are commonly Staphylococcus aureus and Enterococcus faecalis that colonize the deeper skin layers and are resistant to mechanical removal[5]. The transient floras consist of S. aureus and Escherichia coli that colonize the superficial layers of skin in a short period of time[6]. To overcome the limitations of plain hand washing, hand sanitizers were introduced claiming to be effective against those pathogenic micro-organisms as well as to improve skin condition due to the addition of emollients in it[7]. Several studies suggested that sanitizers with at least 70% alcohol were suggested to kill 99.9% of the bacteria on hands [8].

Alcohol-based hand sanitizers exist in liquid, foam, and easy-flowing gel formulations. Sometimes combined with quats (quaternary ammonium cations) such as benzalkonium chloride quats are added at level up to 200 parts per million to increase antimicrobial effectiveness[9]. Before the discovery of modern medicine, plants were the main remedy for treating various diseases. With the advent of different antibiotics microbes also gradually develop resistance to these substances. These bring researchers interest towards the plants having antimicrobial properties. They try to exploit the unique ability of different secondary metabolites to show persistent and prolonged activity against a broad spectrum of microbes [10]. To protect the skin from harmful microorganisms and to prevent spreading of many contagious diseases, hand washing is absolutely an important precaution.

Food production workers and food service personnel must be taught to use correct hand and fingertip washing by management in preparation for work[11]. Contaminated hands can serve as vectors for the transmission of microorganisms. Pathogenic microorganisms accountable for outbreaks are spread from the hands of the food handler to others when the food handler contaminates his/her hands and then passes these microorganisms to consumers via hand contact with food or drinks [12].

Our team has extensive knowledge and research experience that has translate into high quality publications[13–21],[22],[23],[24,25],[26],[27],[28–32] The aim of the study is to evaluate the antimicrobial efficacy of four different hand sanitizers against Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli as well as to compare the antimicrobial effectiveness among four different hand sanitizers.

## MATERIALS AND METHODS

The present study is an in vitro study conducted at the Department of Nanotechnology, Saveetha Dental College, Chennai, India. Ethical clearance for the study was obtained from the Saveetha Institutional Review board. Four different brands of hand sanitizers were selected out of many available in the market based on their popularity and maximum usage in Chennai City.

Selected hand sanitizers to test their antimicrobial efficacy were Spar (Trisis Ventures Pvt Ltd., India), Palmolive (Colgate-Palmolive Co Pvt. Ltd., India), Margo (Accra pac Pvt. Ltd., India), and Lifebuoy (Hindustan Unilever Pvt. Ltd., India) [Figure 1]. Recently manufactured and packed sanitizers have been purchased based on their popularity from the local retail outlet.

The culture media used in the present study were Mueller-Hinton agar for agar diffusion method while nutrient broth and nutrient agar medium for bacterial isolate preservation. The clinical isolates of S. aureus, E. coli, and P.

aeruginosa were obtained from the culture plates of the respective micro-organisms preserved on the nutrient agar slants and were stored at 4°C in the Department of Microbiology, Saveetha Dental College, Chennai, India.

McFarland standards were taken as a reference to adjust the turbidity of bacterial suspensions. The McFarland 0.5 turbidity standard was prepared by adding 0.5 ml of 1.175% w/v barium chloride dihydrate ( $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ ) solution to 99.5 ml of 15 w/v sulfuric acid ( $\text{H}_2\text{SO}_4$ ). A sterile loop was used to pick a loopful of inoculum from a pure culture of the test organism. This was then transferred and suspended into a tube containing sterile normal saline (NaCl 8.5 g, distilled water 1 L). The tube was compared with the turbidity standard, and the density of the organism was adjusted by adding more bacteria or sterile saline until standardization was attained.

## RESULTS AND DISCUSSION

Herbal hand sanitizers were effective against all the test organisms. The antimicrobial effectiveness was assessed by measuring the zone of inhibition against the particular test organism. Maximum inhibition (in mm) seen in Spar which shows the antibacterial effectiveness against *S. Aureus* is 20mm, *P. Aeruginosa* is 10mm and *E. Coli* is 9mm. Margo shows the antibacterial effectiveness against *S. Aureus* is 16mm, *P. Aeruginosa* is 11mm and *E. Coli* is 12mm. Minimum inhibition seen in Lifebuoy which shows the antibacterial effectiveness against *S. Aureus* is 11mm, *P. Aeruginosa* is 9mm and *E. Coli* is 8mm. Palmolive shows the antibacterial effectiveness against *S. Aureus* is 9mm, *P. Aeruginosa* is 7mm and *E. Coli* is 6mm. [Figure 4]

To overcome the negative impact of microbial contamination in health-care settings, hand sanitizers are recommended as an adjunct to plain hand washing. Most commonly and easily available hand sanitizers in Chennai city were selected for the study. Among the four hand sanitizers used in this study, Palmolive and Lifebuoy were alcohol-based and Spar and Margo was herbal, i.e., non-alcohol-based hand sanitizer [33]. Many studies have been conducted to assess the antimicrobial effectiveness of hand sanitizers alone, but very few literature is available to assess the difference between various disinfectants and hand sanitizers. Disinfectants are chemical agents with an immediate and sustained activity which destroys micro-organisms to such a level mandated for hygienic and surgical indications [34].

Sanitizers, on the other hand, are agents with an immediate activity that reduce the number of micro-organisms to a safe level to meet the public health requirements. A study conducted by Oke et al. revealed that Dettol hand sanitizer was effective only against *P. aeruginosa* whereas herbal hand sanitizers are effective against *S. aureus* and *E. coli* [35]. A study conducted by Mondal and Kolhapure showed that Herbal hand sanitizers were effective against *E. coli*, *Proteus mirabilis*, *Shigella sonnei*, *S. aureus*, and *S. epidermidis*. [36]

Lifebuoy hand sanitizer also showed antimicrobial activity against the tested organisms; however, the exact and valid comparison could not be done with other studies due to lack of scientific literature. The present study also showed antimicrobial efficacy of Alcohol based hand sanitizers against tested organisms; however, it was the least effective among all the hand sanitizers which may be probably due to low antimicrobial potency of alcohol. Further studies are required to find the exact cause of least effectiveness of alcohol based hand sanitizer against the tested organisms. [37]



Figure 1: Different hand sanitizers used in the study.



Figure 2: Analysis of zone of inhibition to evaluate antimicrobial efficacy of Herbal hand sanitizers.



Figure 3: Analysis of zone of inhibition to evaluate antimicrobial efficacy of Alcohol hand sanitizers.

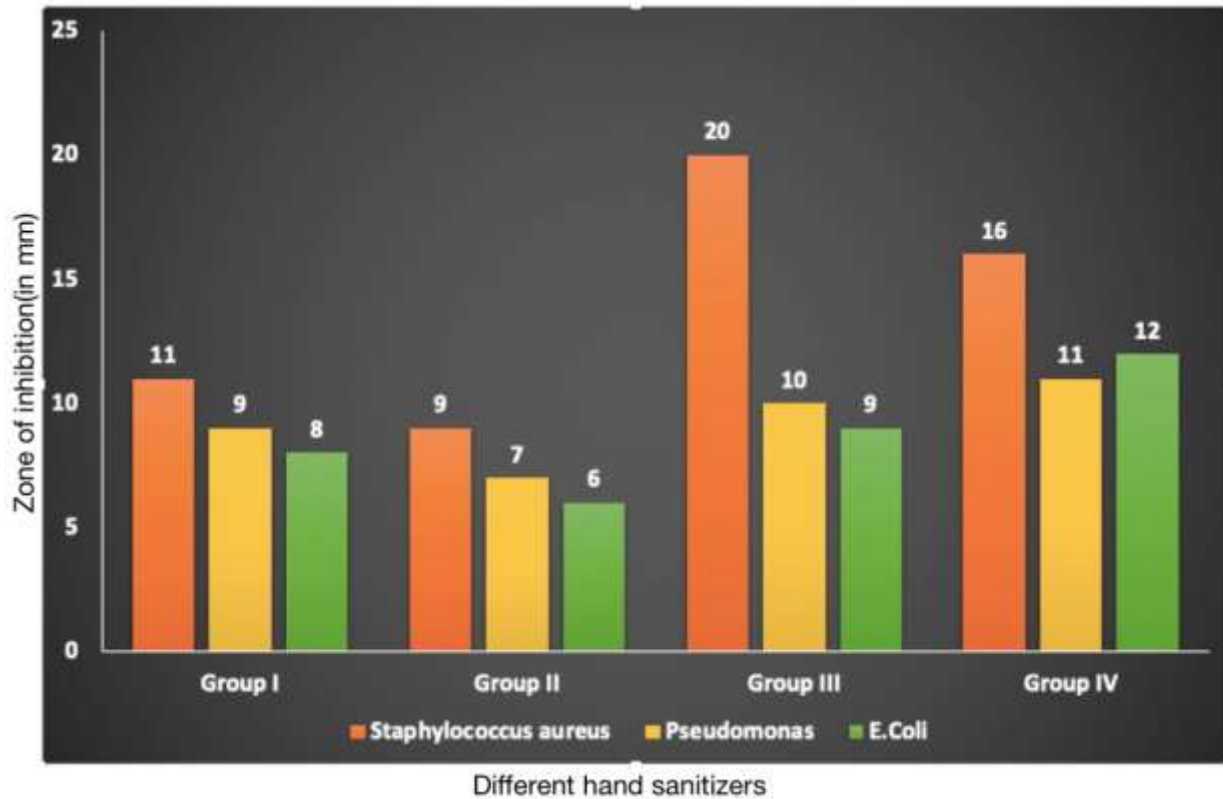


Figure 4: Bar graph depicting herbal based hand sanitizers Group III & IV (Spar & Margo) and the alcohol based hand sanitizers Group I & II (Lifebuoy & Palmolive) . X axis represents the different hand sanitizers used in this study and Y axis represents the zone of inhibition(in mm). Orange colour denotes S. Aureus and yellow colour denotes Pseudomonas Aeruginosa and green colour denotes E.Coli.

## CONCLUSION

Herbal hand sanitizers possessed maximum antimicrobial effect against all the Bacteria's used in the study. Despite the claims of efficacy and 99.9% bacterial reduction by hand sanitizer manufacturers, there still exists a need for verification of these claims by regulatory bodies and higher authorities for the enforcement of good-quality measures.

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## CONFLICTS OF INTEREST

There are no conflicts of interest.

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## REFERENCE

1. Pond K, World Health Organization, United States. Environmental Protection Agency. Water Recreation and Disease: Plausibility of Associated Infections : Acute Effects, Sequelae, and Mortality. World Health Organization; 2005. 239 p.
2. Kalaivani DR, Kalaivani R, Bakiyalakshmi MSV, Arulmozhi P, PG and Research Department of Botany and Biotechnology, Bon Secours College for Women, et al. A Study on Evaluation and Effectiveness of Herbal

- Hand Sanitizer and its Anti Bacterial Activity [Internet]. Vol. -2, International Journal of Trend in Scientific Research and Development. 2018. p. 325–30. Available from: <http://dx.doi.org/10.31142/ijtsrd12922>
3. R MHM, Mr. Harsha M R, Research Officer, Research & Development Centre, Inno Vision Healthcare Ltd. No. P 6(B), Floor 1st, et al. Evaluation of Fungicidal Activity of Herbal Hand Sanitizer [Internet]. Vol. 2, JOURNAL OF RESEARCH IN TRADITIONAL MEDICINE. 2016. p. 70–4. Available from: <http://dx.doi.org/10.21276/jrtm.2016/136>
  4. Acharya SB, Ghosh S, Yadav G, Sharma K, Ghosh S, Joshi S. Formulation, Evaluation and Antibacterial Efficiency of water-based herbal Hand Sanitizer Gel [Internet]. Available from: <http://dx.doi.org/10.1101/373928>
  5. Dicken RD, Gallagher T, Perks S. Overcoming the Regulatory Hurdles for the Production of Hand Sanitizer for Public Health Protection: The UK and US Academic Perspective. J Chem Health Saf. 2020 Jul 27;27(4):209–13.
  6. Erasmus V, Kuperus MN, Richardus JH, Vos MC, Oenema A, van Beeck EF. Improving hand hygiene behaviour of nurses using action planning: a pilot study in the intensive care unit and surgical ward. J Hosp Infect. 2010 Oct;76(2):161–4.
  7. Pittet D, Boyce JM, Allegranzi B. Hand Hygiene: A Handbook for Medical Professionals. John Wiley & Sons; 2017. 456 p.
  8. Son C, Chuck T, Childers T, Usiak S, Dowling M, Andiel C, et al. Practically speaking: rethinking hand hygiene improvement programs in health care settings. Am J Infect Control. 2011 Nov;39(9):716–24.
  9. Sickbert-Bennett EE, DiBiase LM, Schade Willis TM, Wolak ES, Weber DJ, Rutala WA. Reduction of Healthcare-Associated Infections by Exceeding High Compliance with Hand Hygiene Practices [Internet]. Vol. 22, Emerging Infectious Diseases. 2016. p. 1628–30. Available from: <http://dx.doi.org/10.3201/eid2209.151440>
  10. Widmer AF, Frei R. Evaluating antimicrobial effectiveness in environmental microbiology. Clin Infect Dis [Internet]. 2021 Jan 10; Available from: <http://dx.doi.org/10.1093/cid/ciab013>
  11. Tambekar DH, Khante BS, Panzade BK, Dahikar SB, Banginwar YS. Evaluation of phytochemical and antibacterial potential of *Helicteres isora* L. Fruits against enteric bacterial pathogens [Internet]. Vol. 5, African Journal of Traditional, Complementary and Alternative Medicines. 2008. Available from: <http://dx.doi.org/10.4314/ajtcam.v5i3.31285>
  12. Larson EL, Hughes CA, Pyrek JD, Sparks SM, Cagatay EU, Bartkus JM. Changes in bacterial flora associated with skin damage on hands of health care personnel. Am J Infect Control. 1998 Oct;26(5):513–21.
  13. Mathew MG, Samuel SR, Soni AJ, Roopa KB. Evaluation of adhesion of *Streptococcus mutans*, plaque accumulation on zirconia and stainless steel crowns, and surrounding gingival inflammation in primary molars: randomized controlled trial. Clin Oral Investig. 2020 Sep;24(9):3275–80.
  14. Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? Int J Paediatr Dent. 2021 Mar;31(2):285–6.
  15. Samuel SR, Kuduruthullah S, Khair AMB, Al Shayeb M, Elkaseh A, Varma SR, et al. Impact of pain, psychological-distress, SARS-CoV2 fear on adults' OHRQOL during COVID-19 pandemic. Saudi J Biol Sci. 2021 Jan;28(1):492–4.
  16. Samuel SR, Kuduruthullah S, Khair AMB, Shayeb MA, Elkaseh A, Varma SR. Dental pain, parental SARS-CoV-2 fear and distress on quality of life of 2 to 6 year-old children during COVID-19. Int J Paediatr Dent. 2021 May;31(3):436–41.
  17. Samuel SR, Acharya S, Rao JC. School Interventions-based Prevention of Early-Childhood Caries among 3-5-year-old children from very low socioeconomic status: Two-year randomized trial. J Public Health Dent. 2020 Jan;80(1):51–60.
  18. Vikneshan M, Saravanakumar R, Mangaiyarkarasi R, Rajeshkumar S, Samuel SR, Suganya M, et al. Algal biomass as a source for novel oral nano-antimicrobial agent. Saudi J Biol Sci. 2020 Dec;27(12):3753–8.
  19. Chellapa LR, Rajeshkumar S, Arumugham MI, Samuel SR. Biogenic Nanoselenium Synthesis and Evaluation of its antimicrobial, Antioxidant Activity and Toxicity. Bioinspired Biomim Nanobiomaterials. 2020 Jul 23;1–6.
  20. Samuel SR, Mathew MG, Suresh SG, Varma SR, Elsubeihi ES, Arshad F, et al. Pediatric dental emergency management and parental treatment preferences during COVID-19 pandemic as compared to 2019. Saudi J Biol Sci. 2021 Apr;28(4):2591–7.
  21. Barma MD, Muthupandiyani I, Samuel SR, Amaechi BT. Inhibition of *Streptococcus mutans*, antioxidant property and cytotoxicity of novel nano-zinc oxide varnish. Arch Oral Biol. 2021 Jun;126:105132.
  22. Muthukrishnan L. Nanotechnology for cleaner leather production: a review. Environ Chem Lett. 2021 Jun

- 1;19(3):2527–49.
23. Muthukrishnan L. Multidrug resistant tuberculosis - Diagnostic challenges and its conquering by nanotechnology approach - An overview. *Chem Biol Interact.* 2021 Mar 1;337:109397.
  24. Sekar D, Auxzilia PK. Letter to the Editor: H19 Promotes HCC Bone Metastasis by Reducing Osteoprotegerin Expression in a PPP1CA/p38MAPK-Dependent Manner and Sponging miR-200b-3p [Internet]. *Hepatology.* 2021. Available from: <http://dx.doi.org/10.1002/hep.31719>
  25. Gowhari Shabgah A, Amir A, Gardanova ZR, Olegovna Zekiy A, Thangavelu L, Ebrahimi Nik M, et al. Interleukin-25: New perspective and state-of-the-art in cancer prognosis and treatment approaches. *Cancer Med.* 2021 Aug;10(15):5191–202.
  26. Kamala K, Sivaperumal P, Paray BA, Al-Sadoon MK. Author response for “Identification of haloarchaea during fermentation of *Sardinella longiceps* for being the starter culture to accelerate fish sauce production” [Internet]. Wiley; 2021. Available from: <https://publons.com/publon/47375106>
  27. Ezhilarasan D, Lakshmi T, Subha M, Deepak Nallasamy V, Raghunandhakumar S. The ambiguous role of sirtuins in head and neck squamous cell carcinoma. *Oral Dis* [Internet]. 2021 Feb 11; Available from: <http://dx.doi.org/10.1111/odi.13798>
  28. Sridharan G, Ramani P, Patankar S, Vijayaraghavan R. Evaluation of salivary metabolomics in oral leukoplakia and oral squamous cell carcinoma. *J Oral Pathol Med.* 2019 Apr;48(4):299–306.
  29. R H, Hannah R, Ramani P, Ramanathan A, Jancy MR, Gheena S, et al. CYP2 C9 polymorphism among patients with oral squamous cell carcinoma and its role in altering the metabolism of benzo[a]pyrene [Internet]. Vol. 130, *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology.* 2020. p. 306–12. Available from: <http://dx.doi.org/10.1016/j.oooo.2020.06.021>
  30. J PC, Pradeep CJ, Marimuthu T, Krithika C, Devadoss P, Kumar SM. Prevalence and measurement of anterior loop of the mandibular canal using CBCT: A cross sectional study [Internet]. Vol. 20, *Clinical Implant Dentistry and Related Research.* 2018. p. 531–4. Available from: <http://dx.doi.org/10.1111/cid.12609>
  31. Wahab PUA, Abdul Wahab PU, Madhulaxmi M, Senthilnathan P, Muthusekhar MR, Vohra Y, et al. Scalpel Versus Diathermy in Wound Healing After Mucosal Incisions: A Split-Mouth Study [Internet]. Vol. 76, *Journal of Oral and Maxillofacial Surgery.* 2018. p. 1160–4. Available from: <http://dx.doi.org/10.1016/j.joms.2017.12.020>
  32. Mudigonda SK, Murugan S, Velavan K, Thulasiraman S, Krishna Kumar Raja VB. Non-suturing microvascular anastomosis in maxillofacial reconstruction- a comparative study. *Journal of Cranio-Maxillofacial Surgery.* 2020 Jun 1;48(6):599–606.
  33. Winnefeld M, Richard MA, Drancourt M, Grob JJ. Skin tolerance and effectiveness of two hand decontamination procedures in everyday hospital use. *Br J Dermatol.* 2000 Sep;143(3):546–50.
  34. Lauharanta J, Ojajärvi J, Sarna S, Mäkelä P. Prevention of dryness and eczema of the hands of hospital staff by emulsion cleansing instead of washing with soap. *J Hosp Infect.* 1991 Mar;17(3):207–15.
  35. ANONIMO, World Health Organization Staff, J. V, Verhaegen J, World Health Organization, K. E, et al. *Basic Laboratory Procedures in Clinical Bacteriology.* World Health Organization; 2003. 167 p.
  36. Rosenthal M, Goldberg D, Aiello A, Larson E, Foxman B. Skin microbiota: microbial community structure and its potential association with health and disease. *Infect Genet Evol.* 2011 Jul;11(5):839–48.
  37. Jain VM, Karibasappa GN, Dodamani AS, Prashanth VK, Mali GV. Comparative assessment of antimicrobial efficacy of different hand sanitizers: An study. *Dent Res J .* 2016 Sep;13(5):424–31.