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# Development of novel chlorhexidine gluconate hand scrub with *Aloe vera* and *in-vitro* antimicrobial activity against selected bacteria and fungi

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***Abstract:*** Healthcare professionals are constantly challenged by the need to practice good hand hygiene to alleviate nosocomial infections whilst protect the skin from irritants. This study aimed to develop and evaluate of antimicrobial efficacy of chlorhexidine gluconate hand scrub (CGHS) against selected pathogens. The CGHS was prepared by mixing chlorhexidine gluconate and *Aloe vera* and the stability was studied for 90 days at room temperature. The in-vitro test was performed against selected microbes [*Streptococcus pyogenes* (ATCC 12384), *Enterococcus faecium* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923), *Candida albicans* (ATCC 10231), *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 23355), *Acinetobacter baumannii* (ATCC 19606), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 70603), *Proteus mirabilis* (ATCC 12453), Salmonella Typhi (clinical isolates), *Shigella sonnei* (clinical isolates)] to evaluate in-vitro antimicrobial efficacy followed by in-vivo antimicrobial efficacy test using fingertip method. The formulation was found to be homogenous, liquid, and reddish with a pleasant odour. The mean values of inhibition zones for CGHS were 23.10±1.00, 21.67±0.58, 20.00±1.00, 22.67±0.58, 17.67±0.58, 25.00±1.00, 23.50±0.71, 18.00±1.00, 34.33±0.58, 23.67±0.58, 24.33±0.58, and 27.33±0.58 mm against *E. coli, E. cloacae, A. baumannii, P. aeruginosa, K. pneumonia,* Salmonella Typhi, *S. sonnei, P. mirabillis, C. albicans, S. pyogenes, E. faecium* and *S. aureus,* respectively. Zero values of inhibition zone were obtained for distilled water (negative control) while the market product (positive control) showed the lower zones of inhibition against *E. cloacae*, Salmonella Typhi, *C. albicans, S. pyogenes, E. faecium*, and *S. aureus.* The formulated CGHS has promising antifungal and antibacterial efficacy against the tested pathogens and the in-vivo test was confirmed the skin compatibility of the formulation.

**Keywords:** Antibacterial; Antifungal; Chlorhexidine; Hand scrub

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## INTRODUCTION

Chlorhexidine gluconate (CHG), a cationic bis-biguanide, was developed in the United Kingdom in the 1950s. The antimicrobial activity of this chemical compound appears to be related to the attachment and subsequent disruption of cytoplasmic membranes, resulting in the precipitation of cellular contents (Rotter, 1984). The antimicrobial activity of CHG is mainly directed toward vegetative Gram-positive and Gram-negative bacteria; it is inactive against bacterial spores except at elevated temperatures, and acid-fast bacilli are inhibited but not killed by aqueous solutions. Yeasts (including *C. albicans*) and dermatophytes are usually sensitive, although, as with other agents, CHG's fungicidal action, in general, is subjected to species variation (Public health agency of Canada, 2012). Chlorhexidine has been reported with in-vitro activity against enveloped viruses, such as cytomegalovirus, herpes simplex virus, human immunodeficiency virus, influenza, and respiratory syncytial virus, but significantly less activity against nonenveloped viruses, such as adenovirus, enteroviruses, and rotavirus (Platt & Bucknall, 1985).

Hand hygiene aims to move the world towards the goal which supports the most vulnerable communities with the means to protect their health and environment (World Health Organization (WHO), 2020a). Types of hand hygiene products are hydro-alcoholic liquid rubs which contain alcohols, such as ethanol, 1-propanol, and 2-propanol have a broad spectrum of antimicrobial activity, alcohol-based liquid/gel rubs, non-alcohol-based hand scrubs and antiseptic soaps which contain chlorhexidine and triclosan (Batalla et al., 2012). Several comparative studies have shown that chlorhexidine to be a better antiseptic than povidone-iodine because of its faster action and greater residual antimicrobial activity (Batalla et al., 2012). Chlorhexidine gluconate 4% (CHG) is recommended as a broad-spectrum antiseptic which is used as a surgical hand scrub (De Bengoa Vallejo et al., 2018), particularly effective against bacteria, viruses, fungi and more suitable for pre and post-operative skin antisepsis as well as routine hand washing (Vet-way, 2021).

There are few reasons for lack of compliance such as time limitations, skin irritation. Skin irritation by hygiene products is affecting less compliance by the health professionals (Batalla et al., 2012). A combination of moisturizing agents can be used to reduce skin irritations, dryness, itchiness and redness. *Aloe vera* can be used as a natural moisturizing agent in skincare products and can be suitable for any skin type. According to the research published by the National Institutes of Health (NIH), *A. vera* is also effective to address specific skin conditions, including acne, psoriasis, burns and wounds.

## MATERIALS

*Plant materials:* The aerial parts of the leaves of *Aloe vera* were collected in January 2019 in the morning from Galle District (geographical coordinates; latitude: 6.053519; longitude: 80.220978) in Southern Province of Sri Lanka and were authenticated by the Bandaranaike Memorial Ayurvedic Research Institute, Nawinna, Maharagama, Sri Lanka (14.02.2019.2030) (Karunanayaka et al., 2020; Karunanayaka et al., 2021)

*Chemical materials:* All experiments were performed at the Department of Bacteriology, Medical Research Institute, Colombo 08, Sri Lanka. Four percent (4%) Chlorhexidine gluconate, and the market product were purchased from Colombo Chemicals (Pvt) Ltd, Sri Lanka. Muller Hinton Agar (Oxoid, Hampshire, UK) and

Blood agar (Oxoid, Hampshire, UK) were purchased from Hemsons International (Pvt) Ltd, Colombo, Sri Lanka (Karunanayaka et al., 2020; Karunanayaka et al., 2021).

*Microorganisms: Streptococcus pyogenes* (ATCC 12384), *Enterococcus faecium* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923), *Candida albicans* (ATCC 10231), *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 23355), *Acinetobacter baumannii* (ATCC 19606), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 70603), *Proteus mirabilis* (ATCC 12453), *Salmonella typhi* (clinical isolates), and *Shigella sonnei* (clinical isolates) were obtained from the Quality Control Laboratory, Department of Bacteriology, Medical Research Institute, Colombo 08, Sri Lanka (Karunanayaka et al., 2021).

*Development of formulations of novel chlorhexidine gluconate hand scrub:* The leaves of *A. vera* in good condition were used for the preparation of the extract. The extract was formulated by using *A. vera* flesh (250 g) in a small volume (3 ml) of distilled water. Chlorhexidine gluconate hand scrub was prepared by adding chlorhexidine gluconate 4% (70 ml), *Aloe vera* extract (20 ml) and distilled water (10 ml).

*Evaluation of physical stability, in-vitro and in-vivo antimicrobial efficacy of chlorhexidine gluconate hand scrub:* The physical stability parameters such as pH, odor, appearance, and color of the formulations developed were tested for 90 days [7, 30, and 90 d (s)] at room temperature in a transparent borosilicate type II glass container. Agar well diffusion in-vitro method was performed by following the methods described (Kunicka & Kalemba, 2003; Wani et al., 2013; Karunanayaka et al., 2020; and Karunanayaka et al., 2021). The CGHS formulation was subjected to in-vivo testing on healthy human volunteers as the study sample to evaluate the skin sensitivity using the fingertip method published (Jenkins & Belu, 2009; Karunanayaka & Parahitiyawa, 2013; Karunanayaka et al., 2020; Karunanayaka et al., 2021) with slight modifications. The study sample (n=30) was randomly (Kac et al, 2005) recruited. A self-administered questionnaire was given to assess adverse effects such as skin sensitivity, itching, irritation, dryness, or rashes. Statistical analysis was performed by multiple comparisons paired sample T-test using SPSS software version 16.0 (SPSS Inc., Chicago, USA) at significance levels ($p \leq 0.05$) in 95% confidence intervals.Ethical approval for the *in-vivo* antimicrobial efficacy and post-analysis on the skin sensitivity, safety, and compatibility was obtained from Sri Lanka Clinical Trial Registry (SLCTR) of Sri Lanka Medical Association (Sri Lanka Clinical Trial Registry, 2016) and Medical Research Institute, Colombo 08.

## RESULTS AND DISCUSSION

The prepared CGHS was physio chemically homogenous and liquid during the tested period of 90 d (s) and it was pleasant in odour and reddish. The pH values showed around 7 with no remarkable changes. The results showed favourable antibacterial and antifungal activity of CGHS against the tested pathogens (Table 1). The highest zone of inhibition (34.33±0.58) was revealed by the formulation prepared against *C. albicans*. Gram-negative and Gram-positive bacteria showed higher in-vitro antibacterial efficacy with zone of inhibitions more than 17.00 mm. It was showed no zone of inhibition against the tested pathogens for the negative control. Though the market product showed inhibition zones, higher antimicrobial efficacy was revealed by the novel CGHS, presenting higher inhibition zones.

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**Table 1: Zone of inhibition (mm) against selected pathogens for in-vitro testing**

| Formulation/ Control → | CGHS | N | P |
|---|---|---|---|
| Pathogens ↓ | M±SD | M±SD | M±SD |
| *Streptococcus pyogenes* (ATCC 12384) | 23.67±0.58 | 0.00 | 10.00±0.00 |
| *Enterococcus faecium* (ATCC 29212) | 24.33±0.58 | 0.00 | 10.00±0.00 |
| *Staphylococcus aureus* (ATCC 25923) | 27.33±0.58 | 0.00 | 12.33±1.15 |
| *Candida albicans (ATCC 10231)* | 34.33±0.58 | 0.00 | 11.33±1.53 |
| *Escherichia coli* (ATCC 25922) | 23.00±1.00 | 0.00 | 0.00 |
| *Enterobacter cloacae* (ATCC 23355) | 21.67±0.58 | 0.00 | 8.00±0.00 |
| *Acinetobacter baumannii* (ATCC 19606) | 20.00±1.00 | 0.00 | 0.00 |
| *Pseudomonas aeruginosa* (ATCC 27853) | 22.67±0.58 | 0.00 | 0.00 |
| *Klebsiella pneumoniae* (ATCC 70603) | 17.67±0.58 | 0.00 | 0.00 |
| Salmonella Typhi (clinical isolates) | 25.00±1.00 | 0.00 | 9.33±1.15 |
| *Shigella sonnei* (clinical isolates) | 23.50±0.71 | 0.00 | 0.00 |
| *Proteus mirabillis* (ATCC 12453) | 18.00±1.00 | 0.00 | 0.00 |

ATCC: American type culture collection; P: Positive control; N: Negative control; CGHS: Chlorhexidine gluconate hand scrub; M: Mean; SD: Standard deviation

It is reported in the literature that CHG is active against Gram-positive (GP) and Gram-negative (GN) bacteria, as well as yeast and some viruses. Further, it has been reported that the daily skin cleansing with CHG has been demonstrated to reduce the density of potential pathogens, including *methicillin-resistant Staphylococcus aureus* (MRSA) and *vancomycin-resistant Enterococcus*, rendering it is particularly useful in preventing healthcare-associated infections (HAIs) (Johnson J et al., 2019). Thus, the tested CGHS proved the higher activity against potential pathogens including *S. aureus* and *Enterococcus* species. It showed 100% mean $\log_{10}$ reductions and reduction factors before and after the application of CGHS (Fig. 1) on the 1$^{st}$, 15$^{th}$, and 30$^{th}$ day from the preparation compared to the controls (Table 2, 3). Similar to our findings, it showed that the mean of the colony-forming unit counts of conventional chlorhexidine was 0.5±0.2 after surgical hand disinfection. Thus, it was recommended as a standard method for perioperative hand antisepsis (Tsai JC et al., 2017). Another research study showed that hand antisepsis for chlorhexidine gluconate 4%, 1.1 ± 0.3 colony-forming units (CFU) per millilitre of sampled fluid (Rotter et al., 2006).

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**Table 2: Mean Log $_{10}$ reduction values of colony-forming units (CFUs)/ml for CGHS**

| | Mean Log $_{10}$ reduction of CFUs/ml | Mean reduction Factor |
|---|---|---|
| 1$^{st}$-day application | | |
| 10 s after vs Before | 1.4627 ± 0.52 | 100% |
| 15 s after vs Before | 1.4627 ± 0.52 | 100% |
| 15$^{th}$-day application | | |
| 10 s after vs Before | 1.2347 ± 0.46 | 100% |
| 15 s after vs Before | 1.2347 ± 0.46 | 100% |
| 30$^{th}$-day application | | |
| 10 s after vs Before | 1.4152 ± 0.26 | 100% |
| 15 s after vs Before | 1.4152 ± 0.26 | 100% |

CGHS: Chlorhexidine gluconate hand scrub; CFUs: Colony-forming units; s: second; vs: versus

**Table 3: Mean Log $_{10}$ reduction values of colony-forming units (CFUs) controls**

| | P | | N | |
|---|---|---|---|---|
| | Mean Log $_{10}$ reduction of CFUs | Mean reduction Factor | Mean Log $_{10}$ reduction of CFUs | Mean reduction Factor |
| 15 s after vs Before application | 0.7768 ± 0.43 | 94.69% | 0.0406 ± 0.48 | - 3.06% |
| 10 s after vs Before application | 0.9015 ± 0.43 | 98.85% | 0.0785 ± 0.48 | 4.72% |

CFUs: Colony-forming units; P: Positive control; N: Negative control; s: second; vs: versus

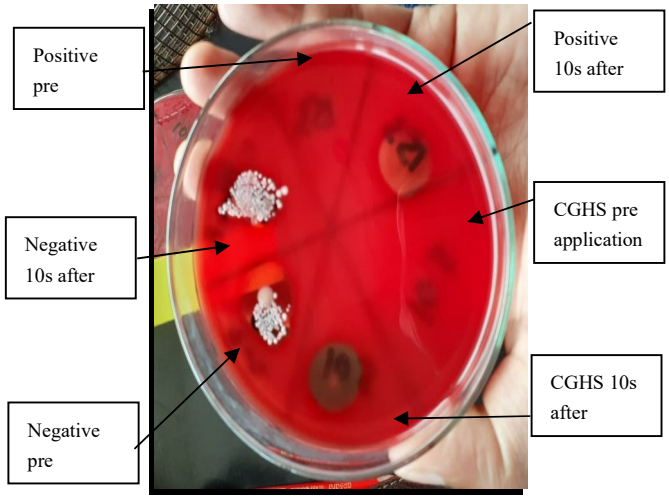


**Fig. 1:** Results of fingertip method for chlorhexidine gluconate hand scrub and controls in before application and 10s after application

It was shown 100% results of no irritation, itching, rashes, or skin damages, skin drying and hesitation of using CGHS. The majority of participants (70%) responded that there was a promising moisturizing effect of CGHS. The majority (60%) of participants agreed that CGHS was moderately safe to use in practice in the healthcare setting.

## CONCLUSION

The novel chlorhexidine gluconate and *A.vera* hand scrub showed promising antibacterial and antifungal activity against the tested bacteria and fungi, concerning the positive and negative controls. The prepared CGHS has clinically proven skin compatibility against healthy human volunteers from the clinical trials.

## ACKNOWLEDGEMENT

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## DECLARATION OF CONFLICT OF INTEREST

We hereby declare that the authors alone are responsible for the content and writing of this article as well the study does not encompass any conflict of interest.

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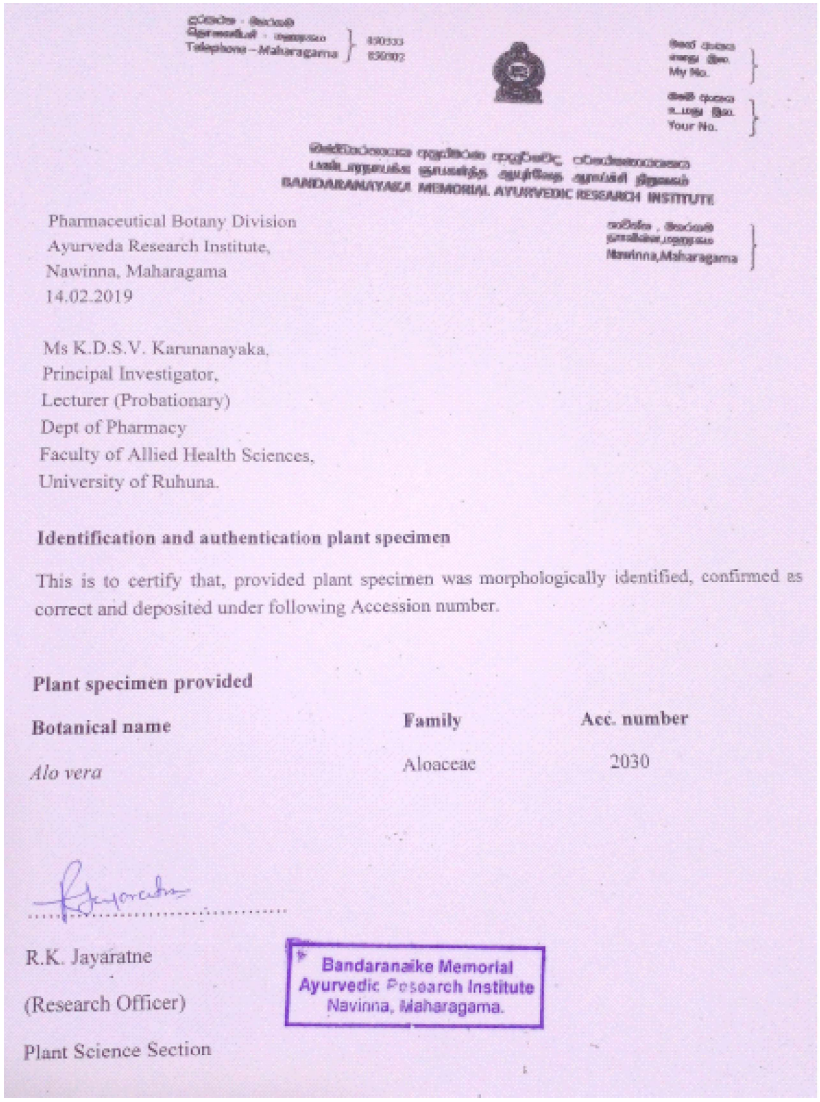
## Annexure 1.: Authentication letter for *Aloe vera* from Bandaranaike Memorial Ayurvedic Institute, Nawinna, Sri Lanka



Telephone – Maharagama 430333 836002

My No.

Your No.

BANDARANAYAKA MEMORIAL AYURVEDIC RESEARCH INSTITUTE

Pharmaceutical Botany Division
Ayurveda Research Institute,
Nawinna, Maharagama
14.02.2019

Nawinna,Maharagama

Ms K.D.S.V. Karunanayaka,
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Faculty of Allied Health Sciences,
University of Ruhuna.

**Identification and authentication plant specimen**

This is to certify that, provided plant specimen was morphologically identified, confirmed as correct and deposited under following Accession number.

**Plant specimen provided**

| Botanical name | Family | Acc. number |
|---|---|---|
| *Alo vera* | Aloaceae | 2030 |

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