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Evaluation of the Efficacy of Alcohol-Based Herbal Hand Rubs Against Selected Pathogens

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Abstract

Background: Hand hygiene products are designed for the application of inactivation or suppression of microorganisms.

Objective: To develop alcohol-based herbal hand rubs (ABHHRs) and to evaluate *in-vitro* and *in-vivo* antimicrobial efficacy

Methods: Four types of formulations (AF_1-AF_4) were prepared and stability testing was performed for three months at room temperature. *In-vitro* antimicrobial efficacy of prepared formulations against selected pathogens was also performed. The most active formulations were subjected to a phase II clinical trial (*in-vivo*) along with a self-administered questionnaire.

Results: The formulated ABHHRs were found to be homogenous, liquid, and milky white-pale yellow in colour with a pungent odour. Mean values of inhibition zones obtained for *in-vitro* antimicrobial efficacy test ranged from 10.00–34.67 and 11.00-37.00 mm for AF_1 and AF_3 respectively. The high values for AF_1 (34.67±1.15 and 22.67±0.58 mm) and AF_3 (37.00±1.73 and 21.33±0.58 mm) were obtained compared to positive (hand disinfectant market product, ethanol) and negative controls (distilled water, glycerin) against *C. albicans* and *S. aureus* respectively. AF_1 and AF_3 showed >96% reductions of colony-forming units (CFUs) in *in-vivo* efficacy testing. The majority of participants (>90%) had no hesitation, itching, irritation, rashes, or skin damage while >85% was no skin-drying and >65% of participants responded with a moisturizing effect.

Conclusion: The formulated ABHHRs have promising *in-vitro* and *in-vivo* antimicrobial activity against the tested pathogens with clinically proven safety, low skin sensitivity and compatibility on human volunteers.

Keywords: *Antimicrobial efficacy, Clinical trial, Herbal hand rubs, Pathogens*

Background

Hand hygiene is an important measure to prevent cross-transmission of microorganisms from one patient to another which demonstrating a reduction in infection rates after improvement in hand-hygiene practices [1]. The recommendations and guidelines state that healthcare professionals should clean their hands with an effective hand hygiene product before and after health-related work or patient contact [2]. Poor hand-hygiene practices are reported due to the lack of scientific knowledge, unawareness of risks, misconceptions, unavailability of hand hygiene facilities, understaffing, and patient overcrowding [3].

Hand hygiene may be accomplished by using alcohol-based hand rubs (ABHR), hand scrubs, soap, and running water [4]. Such preparations contain one or more types of alcohol, other active ingredients with excipients, and humectants. According to the revised guidelines for hand-hygiene, the use of an ABHR is the preferred method of hand hygiene [3]. Though the activity of ABHR is well explained, it is reported with adverse effects such as irritancy, dryness, redness, itching, and eczema [3]. These side effects can be mitigated by using a hand rub with natural moisturizing agents like medicinal aloe [5] which contains a vast amount of essential nutrients and vitamins giving excellent moisturizing properties while soothing dry skin. Utilizing the benefits of amino acids, the natural humectant retains moisture with the presence of sugar, water and polysaccharides. Therefore, combining an essential oil or natural plant extract play a vital role in the reduction of side effects and enhance antimicrobial activity. Studies have shown that certain essential oils extracted from plant extracts have promising antimicrobial activity against bacterial species and fungi [6]. Therefore, this study was aimed to develop alcohol-based herbal hand rubs and to evaluate *in-vitro* and *in-vivo* antimicrobial efficacy against selected pathogens.

Methods

Study design and setting

An experimental laboratory-based study was conducted at Medical Research Institute (MRI), Colombo and Faculty of Allied Health Sciences (FAHS), University of Ruhuna (UoR).

Materials

Glycerin, clove oil, cinnamon oil, ethanol, polysorbate 20, a commercially available hand disinfectant, Muller Hinton Agar and Blood agar were used for this study.
Aerial parts of the leaves of *Aloe vera* were collected in January 2020 from Ganegama, Baddegama, Galle. The herbarium specimen of medicinal aloe was authenticated by the Bandaranayke Memorial Ayurvedic Research Institute, Nawinna (14.02.2019.2030). The standard cultures [(*Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 23355), *Acinetobacter baumannii* (ATCC 19606), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 70603), *Proteus mirabillis* (ATCC 12453), *Streptococcus pyogenes* (ATCC 12384), *Enterococcus faecium* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (ATCC 10231)] and clinical isolates (*Salmonella enterica* Typhi and *Shigella sonnei*) were obtained from the Medical Research Institute.

Data collection methods and tools

After obtaining the informed consent, a set of employees [Medical Laboratory Technologists (MLTs) and Research Officers (ROs)] who were working in MRI were selected as the test group. Based on the references, this was a randomized control trial and there were 120 (30 volunteers per formula) volunteer participants in the experiment [7].

Extraction of Aloe vera gel

Defective leaves were discarded and the remaining leaves were used for the preparation of the extract. The extract of *A. vera* was prepared by blending flesh (250.0 g) in a small volume (3.0 mL) of distilled water [8-9].

Formulation of ABHHRs ($AF_1 – AF_4$)

Four ABHHRs (AF_1 - AF_4) were prepared and the compositions are given in Table 01.

Table 01: Compositions of ABHHRs

Ingredients	AF_1/ mL	AF_2/ mL	AF_3/ mL	AF_4/ mL
10% ethanol	70	70	70	70
Glycerin	05	05	-	-
Aloe extract	10	10	15	15
Clove oil	10	-	10	-
Cinnamon oil	-	10	-	10
Polysorbate 20	02	02	02	02
Distilled water	03	03	03	03
Total volume	100	100	100	100

Determination of physical stability

The appearance, colour and odour were measured at 0, 7, 30 and 90 days at room temperature. The pH was determined using 10.0 mL of formulations dissolved in 100.0 mL of distilled water separately and stored for 2 h over a period of 90 days [10].

Evaluation of in-vitro antimicrobial efficacy

Standard Agar well diffusion method was performed against selected pathogens to evaluate the *in-vitro* antimicrobial efficacy of prepared ABHHRs [9, 11-13]. An aliquot of 50.0 µl of each formulation (AF_1, AF_2, AF_3, AF_4), negative (distilled water - N_1, glycerin - N_2), and positive controls (hand disinfectant market product - P_1, ethanol - P_2) were seeded and incubated plates were calculated for inhibition zones using a calibrated Vernier caliper.

Evaluation of in-vivo antimicrobial efficacy

A sample size of 120 (30 per each formula) with a sampling method of randomized control trial [7, 9] was used. Inclusion criteria were, human volunteers, both sex and over 18 years was used and participants who have allergies, and other skin conditions were used as exclusion criteria. AF_1/AF_3 (most active formulations) was applied on the right thumb (n=30) of a human volunteer. At the same time positive (hand disinfectant market product) or negative control (water) was applied on left thumb (n=15) of human volunteers. Each formulation was used to evaluate for intervention before application, 10s and 15s after application of formula on the fingertip. Following incubation at 35 ± 2^0C for 16-18h, the colony counts were counted using a bacterial colony counter [8-9, 14].

As above, this clinical trial had been conducted on 1st day of preparation, after 15th and 30th days. After obtaining the written informed consent, it was distributed a self-administered questionnaire to identify skin safety, sensitivity and compatibility [9].

Data analysis

All experimental measurements were conducted in triplicates and results were expressed as mean (±SD). Significant levels ($p \leq 0.05$) in 95% confidence intervals were analyzed by multiple comparisons paired samples t-test using SPSS version 16.0.

Ethical considerations

Ethical approval (32/2015/11.09.2015 & 32/2015/08.02.2019) was granted by the Ethics Review Committee, MRI. Clinical trial approval (SLCTR/2019/016) was granted by the Sri Lanka Clinical Trial Registry (SLCTR).

Results

Determination of physical stability

The developed ABHHRs were found to be liquid, homogeneous, milky white to pale yellow in colour with a pungent odor. The pH of the developed formulations (AF_1-AF_4) ranged from 6.68 to 7.02.

Evaluation of in-vitro antimicrobial efficacy

Table 02: Zones of inhibition against selected pathogens

Formula	**AF_1**	**AF_2**	**AF_3**	**AF_4**	**N_1**	**N_2**	**P_1**	**P_2**
Pathogen	**M±SD**							
E. coli	10.30 ± 0.58	0	11.30 ± 0.58	0	0	0	0	0
E. cloacae	14.00 ± 1.00	0	13.00 ± 2.00	0	0	0	0	0
A. baumannii	20.00 ± 2.00	10.00 ± 0.00	22.30 ± 2.52	0	0	0	0	0
P. aeruginosa	10.00 ± 0.00	0	11.00 ± 0.00	0	0	0	0	0
K. pneumoniae	0	0	11.00 ± 1.70	0	0	0	0	0

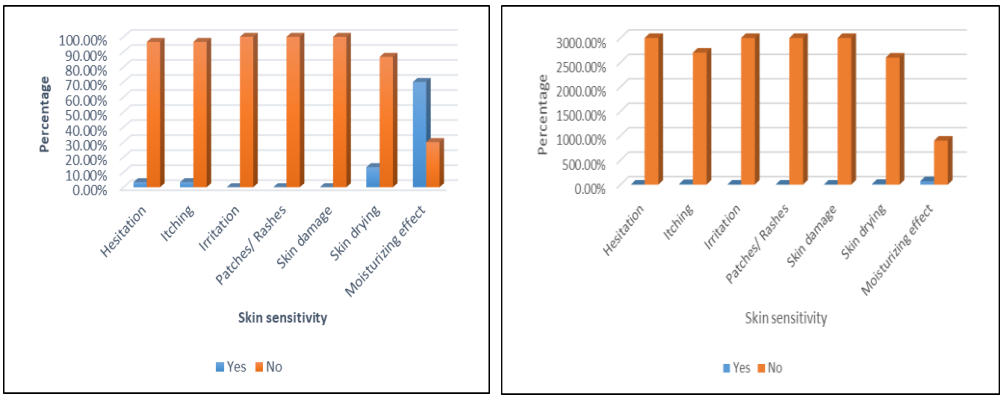
S. enterica Typhi	16.00 ± 3.20	0	18.00 ± 5.30	0	0	0	0	0
S. sonnei	17.00 ± 3.00	10.30 ± 0.58	16.30 ± 5.13	8.00 ± 0.00	0	0	11.30 ± 2.31	0
P. mirabillis	20.30 ± 1.53	9.33 ± 1.15	20.00 ± 1.00	10.70 ± 1.16	0	0	0	0
C. albicans	34.67 ± 1.15	18.00 ± 1.00	37.00 ± 1.73	19.00 ± 0.00	0	0	12.00 ± 1.73	0
S. pyogenes	20.33 ± 0.58	10.33 ± 0.58	21.00 ± 1.00	11.33 ± 0.58	0	0	10.33 ± 0.58	0
E. faecium	13.00 ± 0.00	0	12.33 ± 0.58	0	0	0	11.00 ± 1.00	0
S. aureus	22.67 ± 0.58	12.00 ± 0.00	21.33 ± 0.58	12.33 ± 0.58	0	0	13.67 ± 0.58	0

AF: Alcohol-based Formula, ATCC: American type culture collection; N: Negative control; M: Mean; P: Positive control SD: Standard deviation

Evaluation of in-vivo antimicrobial efficacy

Table 03: Mean log_{10} reduction values and reduction factors (RF)

	AF_1		**AF_3**	
	Mean (±SD) Log $_{10}$ reduction of CFUs	**Mean RF**	**Mean (±SD) Log $_{10}$ reduction of CFUs**	**Mean RF**
1st day				
Before vs 10 s after application	*1.69 ± 0.33*	99.90%	*1.21 ± 0.47*	99.59%
Before vs 15 s after application	*1.42 ± 0.33*	99.42%	*1.51 ± 0.47*	99.62%
15th day				
Before vs 10 s after application	*1.66 ± 0.53*	99.95%	*1.50 ± 0.35*	99.83%
Before vs 15 s after application	*1.36 ± 0.53*	99.86%	*1.43 ± 0.35*	99.57%
30th day				
Before vs 10 s after application	*1.02 ± 0.49*	95.90%	*1.43 ± 0.46*	99.92%
Before vs 15 s after application	*1.16 ± 0.49*	99.78%	*1.04 ± 0.46*	99.80%



(a) **(b)**

Figure 01: Skin sensitivity of human volunteers against (a) AF_1 and (b) AF_3

Discussion

Alcohol solutions containing 60–80% alcohol are usually considered to have efficacious microbicide activity, with concentrations higher than 90% less potent. Alcohol-based hand rubs with optimal antimicrobial efficacy usually contain 75 to 85% ethanol, isopropanol, n-propanol, or a combination of these products. WHO-recommended formulations contain either 75% v/v isopropanol, or 80% v/v ethanol. They also have excellent activity against *Mycobacterium* spp, a variety of viruses, including respiratory viruses (e.g., severe acute respiratory syndrome coronavirus [SARS- CoV], influenza), blood-borne viruses [15-16].

The selection of a hand hygiene product is an important factor according to the New Centers for Disease Control and Prevention Guidelines. Among the hand hygiene products, alcohol-based hygiene products (60-85% ethanol, 60-80% isopropanol and 60-80% n-propanol) are available at the market [15-16]. Although alcohol-based rubs are known to be more effective than others they are reported with many adverse effects such as irritancy, dryness, itchy etc. Therefore, this study was aimed to develop herbal hand rub formulations having antimicrobial activity, no irritation, no skin drying or damage in prolonged use, and have a moisturizing effect for a soothing effect.

The tested formulations they were showed no remarkable changes of the physical parameters tested during 90 days. The higher inhibition zones were observed for AF_1 and AF_3 compared to AF_2 and AF_4 (Table 02). Formulations of AF_1 and AF_3 which contain clove oil showed the highest activity against all tested pathogens compared to AF_2 and AF_4 which contain cinnamon oil. Seventy percent (70%) ethanol and negative controls (distilled water and glycerin) showed zero zones of inhibition against tested pathogens while the market product showed a zone of inhibition only against *S. sonnei*, *C. albicans*, *S. pyogenes*, *E. faecium* and *S. aureus*. A similar study conducted to formulate a poly-herbal soap and hand sanitizer using the leaf and bark extracts of *Cassia fistula*, *Ficus religiosa*, and *Milletia pinnata* had been evaluated for antimicrobial activity by using the Agar well diffusion method against the pathogens of *E. coli*, *S. aureus*, and *P. aeruginosa*. The results showed the zones of inhibition ranging from 18.0 to 26.0 mm which was far better than the zones of inhibition of individual extracts. This enrichment of antimicrobial

properties had been contributed to the synergistic effect produced by the combinations of extracts [10]. Further, another research study evaluated the antimicrobial efficacy of four different hand sanitizers against *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *E. coli*, and *E. faecam* and compared the antimicrobial effectiveness among four different hand sanitizers. Maximum inhibition (22.0 ± 6.0 mm) was found with one hand sanitizer against all the tested organisms [17]. *In-vitro* testing of antimicrobial agents is beneficial in screening antimicrobial agents in product formulations because such agents that tested both *in-vitro* and *in-vivo* activity may have reduced antimicrobial effects when formulated into a hand clean perspective [18].

Mean $\log_{10}$ reductions of CFUs were high before versus after 10 s as well 15 s application for AF_1 and AF_3 on 1st, 15th, and 30th days. The RFs were nearly 100% before versus after 10 s and 15 s applications for both AF_1 and AF_3 on 1st, 15th and 30th days. ABHHR formulations specifically showed high mean $\log_{10}$ reductions of CFUs and RFs for novel formulations than the positive control according to the results given in Table 03. In agreement with the findings of a research study carried out in Canada, a 100% reduction of colonies for fingertip colony count for alcohol-based hand rubs was observed [14]. Efficacy had been evaluated for different brands of hand sanitizers against standard cultures of *E. coli*, *S. aureus*, and *P. aeruginosa* as per the European Norms in a similar study and the logarithmic RF were assessed at baseline and after treatment, and the results showed that the four hand sanitizers had a 5.9 RF on all three bacteria strains [19].

According to self-administered questionnaire, the majority of human volunteers are female (56.67%, and 63.33%) MLTs (93.33%, and 93.33%) between 18–30 years (86.67%, and 80.00%) age group for AF_1, and AF_3 respectively. With regards to Figure 01, all participants (> 90%) responded that there was no hesitation, itching, irritation, rashes, or skin damage while > 85% responded that there was no skin-drying condition for AF_1, and AF_3. More than 65% of participants responded that there was a moisturizing effect in all novel formulations.

In the medical environment, the use of ABHHR now represents the preferred method of performing hand hygiene when delivering non-surgical care. The ABHHR protocol is less costly and less time-consuming when compared to traditional hand washing [20]. Therefore, a hand rub with moisturizer should be there to minimize the adverse effects on the skin. According to the results, the newly prepared hand rubs are beneficial; since these contain essential oils that enhance the antimicrobial activity and address the moisturizing effect of medicinal aloe [8]. Thus, the majority of participants prefer to use these types of hand rubs in the daily working environment as they regularly complain of the irritant effects of the present products in the healthcare setting [21-22].

Conclusion

It is concluded that the formulated alcohol based herbal hand rubs are having antimicrobial effects against the tested pathogens and clinically proven skin sensitivity, safety, and compatibility in human volunteers. The findings revealed that the prepared formulations are efficacious, safe, and effective to be used in the healthcare setting.

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