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Research Article

Physical and Chemical Analysis and Assessment of Antimicrobial Activity of Hand Sanitizers by Time Kill Procedure

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Abstract

This study analyzed the physical and chemical analysis and assessed the antimicrobial activity of hand sanitizers by time kill procedure. Three hand sanitizer samples were evaluated for the study namely CHBS2001, CHBS2002, and CHBS2003. The physical and chemical parameters namely appearance, odor, ethanol content, hydrogen peroxide and glycerol were analyzed in the three hand sanitizers. It was noted that the appearance of the three hand sanitizers were clear colorless liquid with characteristic odor. The Time Kill procedure was carried out to evaluate the *in vitro* reduction of a microbial population of test organisms after exposure to hand sanitizers. It was found that, all the three sanitizers tested reduced 99.99% of the test microorganisms at 60 seconds contact time.

Keywords: Hand sanitizers, physical and chemical analysis, antimicrobial activity, time kill procedure.

Introduction

Hand sanitizer is a liquid, gel or foam generally used to decrease infectious agents on the hands (Boyce and Pittet, 2002). In most settings hand washing with soap and water is generally preferred *(Meadows and Le Saux, 2004)*. In most healthcare settings, alcohol-based hand sanitizers are preferable to hand washing with soap and water, because it may be better tolerated and is more effective at reducing bacteria (Bolon, 2016). Hand washing with soap and water, however, should be carried out if contamination can be seen, or following the use of the toilet (WHO, 2015). Alcohol-based versions typically contain some combination of isopropyl alcohol, ethanol or *n*-propanol, with versions containing 60% to 95% alcohol the most effective. Alcohol-based hand sanitizer works against a wide variety of microorganisms. Compounds such as glycerol may be added to prevent drying of the skin (Boyce and Pittet, 2002). Some versions contain fragrances; however, these are discouraged due to the risk of allergic reactions. Non-alcohol based versions typically contain benzalkonium chloride or triclosan; but are less effective than alcohol-based ones (Bruce *et al.,* 2015; Baki*et al.,* 2015). Alcohol has been used as an antiseptic at least as early as 1363 with evidence to support its use becoming available in the late 1800s(*Stanton, 2001).*Alcohol-based hand sanitizer has been commonly used in Europe since at least the 1980s.

The alcohol-based version is on the World Health Organization's List of Essential Medicines, the safest and most effective medicines needed in a health system (WHO, 2019).In 2010, the World Health Organization produced a guide for manufacturing hand sanitizer, which received renewed interest in 2020 because of shortages of hand sanitizer in the wake of the COVID-19 pandemic. Considering the above facts in view, this study analyzed the physical and chemical attributes and assessed the antimicrobial activity of hand sanitizers by time kill procedure.

Materials and methods

Hand sanitizer: Three different hand sanitizers (Klenze-CBHS2001, CBHS2002 and CBHS2003) from Cymbio Pharma, Bangalore, India were evaluated in this study.

Physical and chemical analysis: Physical parameters namely appearance, odor and chemical analysis like ethanol content, hydrogen peroxide and glycerol were analyzed in the three hand sanitizers.

Assessment of antimicrobial activity: The Time Kill procedure is carried out to evaluate the *in vitro* reduction of a microbial population of test organisms after exposure to hand sanitizers.

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Table 1. Physical and chemical parameters of hand sanitizers.

S.No.	Physical & chemical	Hand sanitizer		
		CBHS2001	CBHS2002	CBHS2003
1.	Appearance	Clear colorless liquid	Clear colorless liquid	Clear colorless liquid
2.	Odor	Characteristic	Characteristic	Characteristic
3.	Ethanol content	80.4% v/v	80.6% v/v	80% v/v
4.	Hydrogen peroxide	0.124% v/v	0.127% v/v	0.131% v/v
5.	glycerol	1.47% v/v	1.46% v/v	1.46% v/v

Table 2. Assessment of antimicrobial activity by time kill procedure.

Test organism	Contact time	CFU/ml	Percentage reduction compared to control at time zero	Log_{10} reduction compared to control at time zero
Staphylococcus aureus	Time zero	4.4×10^6	-	-
	30 seconds	1.4×10^3	99.97%	3.50
	60 seconds	2.4×10^2	99.97%	4.26
Escherichia coli	Time zero	3.4×10^6	-	-
	30 seconds	1.5×10^3	99.96%	3.40
	60 seconds	3.1×10^2	99.99%	4.09
Pseudomonas aeruginosa	Time zero	3.2×10^6	-	-
	30 seconds	3.6×10^3	99.89%	2.95
	60 seconds	2.6×10^3	99.99%	4.09
Aspergillusniger	Time zero	1.2×10^6	-	-
	30 seconds	6.2×10^3	99.48%	2.29
	60 seconds	1.4×10^3	99.99%	3.93
Candida albicans	Time zero	2.5×10^6	-	-
	30 seconds	6.2×10^3	99.75%	2.61
	60 seconds	2.8×10^2	99.99%	3.95

The suspension-based time-kill test has been standardized by ASTM International, as ASTM E2315-Standard Guide for Assessment of Antimicrobial Activity Using a Time-Kill Procedure. This method measures the change in the population of microorganisms within a specified sampling time after exposure to antimicrobial test materials *in vitro*. Hand sanitizer is brought into contact with a known population of microorganisms for a specified period of time at a specified temperature. Hand sanitizer is then neutralized at the target sampling time and the surviving organisms are enumerated. The percent reduction in the test microorganisms at 30 and 60 seconds contact time were recorded.

Results and discussion

Physico-chemical characteristics: The physical and chemical parameters namely appearance, odor, ethanol content, hydrogen peroxide and glycerol were analyzed in the three hand sanitizers. It was noted that the appearance of the three hand sanitizers were clear colorless liquid with characteristic odor. The major constituent of the three hand sanitizers were ethanol, hydrogen peroxide and glycerol. Ethanol (80%) content was in high in all the tested three hand sanitizers followed by glycerol (1.47%) and hydrogen peroxide (0.125%) v/v (Table 1).

Assessment of antimicrobial activity: The suspension time-kill test is excellent for topical antiseptic product developers because it is a fast, relatively inexpensive, and reproducible way to measure the biocidal potential of a liquid antimicrobial formulation. It consists of directly inoculating a liquid test substance with a high concentration of test microorganisms and then determining the percentage killed over time. The time kill procedure was carried out to evaluate *in vitro* reduction of microbial population of test organisms after exposure to hand sanitizers. It was found that all the hand sanitizers tested showed reduction of about 99.99% of the test microorganisms at 60 seconds contact time (Table 2).

Conclusion

This study analyzed the physical and chemical analysis and assessed the antimicrobial activity of hand sanitizers by time kill procedure.

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From the study it may be concluded that the tested hand sanitizers showed reduction of about 99.99% of the test microorganisms at 60 seconds contact time. In this covid-19 pandemic situation, we can see that hand sanitizers can now be found in the entrances to nursing homes and hospitals and in many public washrooms. Hence, from the present study it may be noted that the tested hand sanitizer complies with the standard physico-chemical properties and also complies with the ASTM international for assessment of antimicrobial activity using a time-kill procedure.

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