

# Hand Sanitizer With Natural Ingredients Exhibits Enhanced Antimicrobial Efficacy

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## Research Article

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# Abstract

Hand sanitizers are amongst the foremost preventive defence against coronavirus. Ever since the outbreak of this virus in late 2019 the hand sanitizers has become an essential part of our day-to-day life in fight against this contagious disease. The global demand for hand sanitizers has suddenly peaks and reaches record high with ever-increasing market. However, the regular and excessive usage of hand sanitizers, which contains chemical additives and synthetic fragrances, do have serious health and environmental hazards. Thus, due to the evident role of natural phytoconstituents and essential oils against various infectious microorganisms including viruses, they appeared as prospective and viable option for toxic and hazardous chemicals frequently used in hand sanitizers.

Here we have developed an alcohol based hand sanitizer formulation supplemented with natural and sustainable ingredients like tea extract and lemon grass essential oil enriched with phenolics and natural flavours respectively. The antimicrobial efficacy of developed hand sanitizer was evaluated and compared with alcohol based hand sanitizers and commercial hand sanitizers. The results of well diffusion assay method and time-kill test have clearly revealed the enhanced effectiveness of hand sanitizer with natural ingredients compared to commercial sample and World Health Organization recommended formulation. Moreover, the herbal origin of these ingredients is very significant since the left over residues on hands after repeated usage might not have any toxicity issues. Hence, it has established that usage of tea extract and lemon grass essential oil in alcoholic hand sanitizer formulation may have positive effects on health and environment.

## 1. Introduction

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection distinguished as COVID 19 begins from Wuhan province of China. In the last 14 months, this infectious disease has grabbed the whole world with more than 122 Million infections and claimed lives of over 2.7 Million (World Health Organization Coronavirus Disease Dashboard). Although recently in some countries of world, the vaccination program has been started but vaccinating the huge populations has its own repercussions (Dai et al. 2020). The preventive measure to avoid viral infection, reinfection and containment are the only options available with humankind to cope with COVID 19 and associated problems (Gorassi et al. 2020; He et al. 2021; Sharma et al. 2021). The high endurance rate of coronavirus in air, different surfaces, water bodies and wastewater streams is also disturbing (Sharma et al. 2020). Therefore, for containment of COVID 19 frequent chemical sanitization of possible COVID 19 spreading sources has carried out extensively (Golin et al. 2020). However, the regular and excessive use of soaps, hand sanitizers and other chemical surface disinfectants releases tonnes of non-degradable bio contaminants, which have deleterious health and environmental hazards (Jing et al. 2020).

It was noticed that the demand of hand sanitizers has increased manifold since the inception of COVID 19. The alcohol (ethanol or isopropanol) based hand sanitizers were recommended by scientist, doctors and health agencies across the world. However, the alcohol content in majority of commercial hand sanitizers remained 60–65 % contrary to studies, which suggests greater effectivity at concentration of 75–80 %

(Suchomel et al. 2012; 2020). Moreover, excess occurrence of chemicals like triclosan, phthalates, benzalkonium, and synthetic fragrances in hand sanitizers formulations also have huge health and environment issues over prolonged usage (Dinwiddie et al. 2014; Pereira and Tagkopoulos 2019; Daverey and Dutta 2020).

Therefore substituting these toxic chemicals additives and synthetic fragrances in hand sanitizer formulations with natural and sustainable ingredients with evident safety and efficacy data is highly commendable. Hence, we have developed an alcohol based hand sanitizer formulation with tea extract and lemongrass essential oil as natural ingredients. This hand sanitizer exhibits enhanced antimicrobial effectivity and offers additional benefit of free from toxic ingredients, which might be quite beneficial for health and environment as well.

## **2. Experimental**

### **2.1. Material and methods**

The studied organisms were obtained from Microbial Type Culture Collection (MTCC). Gram-positive bacteria (*Bacillus subtilis* MTCC 121, *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MTCC 96), and Gram-negative bacteria (*Salmonella typhi* MTCC 733, *Escherichia coli* MTCC 43, *Klebsiella pneumoniae* MTCC 109) were used. All analytical grade chemicals were purchased from Merck Specialities Ltd and commercial hand sanitizer was procured from local market.

### **2.2. Chemical evaluation by liquid and gas chromatographic analysis**

The hydroalcoholic extract of tea was prepared by percolation extraction of tea (1:20) following two subsequent extractions. The pooled extract was vacuum concentrated followed by spray drying. The essential oil was obtained by hydro-distillation at  $60 \pm 5^\circ\text{C}$  from the dry leaves of lemon grass. The phytochemical screening of tea extract and essential oil was performed with reverse phase high-pressure liquid chromatography with diode array detector and gas chromatographic mass spectral analysis respectively.

### **2.3. Preparation of formulation**

The hand sanitizer formulation was developed as per the recommendation of World Health Organization. Briefly, 80% of ethanol (99.9%) was taken and supplemented using varying content of ingredients viz. 1.45% glycerol, 0.125%  $\text{H}_2\text{O}_2$ , 0.1 % tea extract, 0.1% of lemongrass essential oil and remaining distilled water. This mixture was stirred properly for up to 10 minutes using stirrer. The final solution was filtered and stored in clean airtight container away from direct light. Subsequently, other formulations with 60 % and 70 % ethanol along with above-mentioned ingredients were also prepared for comparative analysis.

### **2.4. Antimicrobial studies**

#### **2.4.1. Antibacterial assay**

Ampicillin and streptomycin each 10 µg per disc were used as positive controls against gram-positive and gram-negative bacteria respectively, while sterile water was used as the negative control. The susceptibility of the test organisms to the hand sanitizers was evaluated by well diffusion method (Valgas et al. 2007). The bacterial strains were cultured overnight in Mueller Hinton Broth at 37 °C until a suspension of 0.5 McFarland standard corresponding to  $1.0 \times 10^8$  Colony Forming Unit per mL was achieved. A lawn of 100 µL bacterial inoculum was prepared on the Mueller Hinton Agar plates with the help of a sterile cotton swab. Holes of 6 mm diameter were aseptically bored on the agar surface with a sterile tip, and 50 µL of the hand sanitizers was introduced into each well. The plates were incubated at 4 °C for 2–3 h to allow diffusion of test sanitizers into the agar medium, followed by incubation at 37 °C for 12-24h. The inhibition zones were measured that indicate the degree of susceptibility or resistance of an organism to the test sanitizer. The test was performed in triplicates and the average of readings was considered.

## 2.4.2. Time-kill test

The time-kill test against three bacteria, viz. *S. aureus* MTCC 96, *P. aeruginosa* MTCC 2453 and *S. typhi* MTCC 733 was performed according to a previous method (Kampf and Hollingsworth 2003). Bacterial inoculum was prepared by culturing the bacteria in nutrient broth overnight at 37 °C. Nine hundred microlitre of IHBT-hand sanitizer was dispensed into a sterile flask containing stir bar. A control flask was prepared by dispensing distilled water in place of the test sanitizer. One hundred microlitre of bacterial test suspension was added to both the control and test flasks and the exposure of 30 s was given, after which an aliquot of hundred microlitre was withdrawn and transferred into nine hundred microlitre of D/E (Dey-Engley, Difco) neutralizing broth. Serial tenfold dilution in normal saline was carried out. Appropriate dilutions were plated on nutrient agar in triplicates. The plates were incubated at 37°C for 12–14 h, and number of colony-forming units per ml was calculated.

## 3. Results And Discussion

Ever since the beginning of COVID 19 pandemic the frequent hand disinfection preferably with alcohol-based hand sanitizers and washing with soap and lots of water continued as preferred option to prevent the spread of this infectious disease (Golin et al. 2020). Since excessive and repeated hand washing with soap and water is slight unpractical and non-environment friendly, as more soap-leather water drives into waste streams containing numerous chemical additives and contaminants which doesn't degrade easily (Daverey and Dutta 2020). Therefore, use of hand sanitizers especially alcohol based are preferred due to their instantaneous disinfectant property and ease of application unless otherwise hand washing with soap is extremely required. Although wide range of hand sanitizers are available in the market with diverse composition as varied from region to region. The alcohol based hand sanitizers contains different emollients, fragrances and colours, whereas non-alcoholic hand sanitizers contains antiseptic chemicals such as triclosan, ammonium compounds and chlorohexidine etc along with synthetic fragrances and colours which may cause acute to severe dermal ailments on continuous usage (Jing et al. 2020). Moreover, the frequent use of these formulations that leaves toxic residues may also have serious damaging effects on human health and environment over long-term usage.

The phytochemicals (volatile and non-volatile) have long history of usage as effective antimicrobial agents against diverse array of infectious agents along with skin protecting properties (Minami et al. 2003; Clarke et al. 2016; Lee et al. 2018). Both tea and lemon grass are rich source of bioactive constituents with antimicrobial and skin emollient properties (Minami et al. 2003; Shin et al. 2012). The tea constituents have shown to exhibit potent antiviral property against several viruses and are extremely efficient compared to various synthetic chemical (Lee et al. 2018; Mhatre et al. 2020). We also reported in our recent studies the SARS-CoV-2 main protease inhibitor potential of tea bioactives (Bhardwaj et al. 2020). Studies have also reported the effect of ethanol on skin and cases of irritation and allergic reactions due to persistent exposure that results into dryness and skin cracking (Mahmood et al. 2020), hence alcohol needs to be supplemented with natural and sustainable phytoconstituents.

The green tea polyphenols enriched extract was prepared from secondary grade of made tea followed by quality evaluation using high-pressure liquid chromatography analysis (Figure S1). The extract showed presence of all major tea constituents such as; epigallocatechin, epicatechin, epigallocatechin gallate, catechin and caffeine. The quality of lemon grass oil was evaluated by gas chromatography analysis. The results have showed occurrence of limonene, 2-beta-pinene and citral as major constituents in the sample (Table 1). The content of natural ingredients tea extract (0.1%) and lemongrass oil (0.1 %) were also selected based on earlier studied results of ours and Minami et al. 2003.

Table 1

Gas chromatography mass spectral data showing major volatile constituents in lemon grass oil used in hand sanitizer formulation

Sr. no.	Name	R. Time	% Area
1	Camphene	5.8	2.13
2	6-Methyl-5-hepten-2-one	6.6	3.73
3	l-Limonene	8.0	0.34
4	4-Naonanone (CAS) Propyl amyl ketone	9.3	2.18
5	Linalool l	10.4	1.63
6	1,4-Hexadiene,3,3,5-trimethyl	11.8	0.26
7	2,2-Dimethyl-ota-3,4-dienal	12.1	0.43
8	Citronella	12.2	0.32
9	Bicyclo[3.1.0]hept-3-en-2-ol, 4,6,6-trimethyl-	12.5	0.58
10	2-(2',3'-Epoxy-3'-methylbutyl)-3-methylfuran	12.8	0.59
11	Verbenol	13.2	1.21
12	Z-Citral	15.3	33.7
13	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-(CAS) Piperitone	15.9	0.66
14	E-Citral	16.4	43.9
15	Geranyl acetate	20.1	1.94
16	trans-Caryophyllene	21.6	0.92
17	gamma-Cadinene	24.7	2.98
18	delta-Cadinene	24.8	0.48
19	(-) Caryophyllene oxide	26.9	1.56

The formulation was optimized by using varying alcohol (ethanol) content such as 60%, 70 % and 80% ethanol v/v. These results showed 80 % alcohol formulation exhibits higher effectiveness against different microorganisms (Fig. 1). These results are in correspondence to earlier study by Suchomel et al. 2012, which also showed better results with 80 % alcohol as compared to 60 %. Further, the antimicrobial potential of the selected formulation was evaluated and compared with World Health Organization recommended formulation and commercial sample as well. Well diffusion assay revealed that our hand sanitizer formulation was effective against all the tested Gram-positive and Gram-negative bacteria (Table 2). This formulation also showed enhanced antibacterial activity than the World Health Organization recommended formulation. *S. aureus* MTCC 96 was the most susceptible to the effect of hand sanitizer formulation, while *B. subtilis* MTCC 121 was the most resistant. It was observed that this sanitizer

exhibited almost equal antibacterial activity towards all the Gram-negative bacteria tested, with highest activity against *S. typhi* MTCC 733 (Table 2).

Table 2  
Comparative inhibition study of IHBT hand sanitizer with WHO Formulation and commercial sample against three Gram-positive and three Gram-negative bacterial pathogens

	GRAM-POSITIVE			GRAM-NEGATIVE		
Samples	<i>Bacillus subtilis</i> MTCC 121	<i>Staphylococcus aureus</i> MTCC 96	<i>Micrococcus luteus</i> MTCC 2470	<i>Salmonella typhi</i> MTCC 733	<i>Escherichia coli</i> MTCC 43	<i>Klebsiella pneumoniae</i> MTCC 109
IHBT Hand Sanitizer	4 ± 0	21.66 ± 0.5	9 ± 0	13.33 ± 0.5	11 ± 0	9.66 ± 0.5
WHO Formulation	3 ± 0	19.33 ± 0.5	5 ± 0	10.33 ± 0.5	8.66 ± 1.5	7.66 ± 0.5
Commercial sample	6 ± 0	9.33 ± 0.5	10.33 ± .05	4 ± 0	7.66 ± 0.5	5.33 ± 0.5
Positive Control	22.33 ± 0.5	25 ± 1	28.66 ± 0.5	13.33 ± 0.5	15.66 ± 1.15	8.66 ± 0.5
Positive Control = Ampicillin 10 mcg/disc in case of Gram-positive bacteria and Streptomycin 10 mcg/disc in case of Gram-negative bacteria						
MTCC - Microbial Type Culture Collection						

The time-kill test of hand sanitizer formulation was found to cause reduction of all the three tested bacteria by more than 5 log<sub>10</sub>-steps within an exposure time of 15 seconds. The results demonstrated a log<sub>10</sub> reduction factor (RF) above 5 against all the tested bacteria (Table 3). The efficacy of the sanitizer in a short application time provides benefits for prevention of many kinds of infections.

Table 3  
Activity of IHBT hand sanitizer against one Gram-positive and two Gram-negative opportunistic bacterial pathogens (15 seconds exposure time) by presentation of the minimum log<sub>10</sub> reduction factor (RF)

Bacterial species	Minimum RF
<i>Pseudomonas aeruginosa</i> MTCC 2453	5.90
<i>Salmonella typhi</i> MTCC 733	5.86
<i>Staphylococcus aureus</i> MTCC 96	5.92
MTCC - Microbial Type Culture Collection, RF- Reduction Factor	

## 4. Conclusion

Due to the emergence of COVID 19 hand sanitizers has become an essential commodity in home, workplace and public places. The SARS-CoV-2 is an enveloped virus that can be certainly inactivated by ethanol at concentration ranging from 60–80 %. Hence, alcohol based hand sanitizers are considered as quite effective against its transmission as suggested by health practitioners and medical agencies around the globe. However, taking into consideration the impact of frequent usage of hand sanitizer's formulations with chemical ingredients on human health and environment is quite worrisome. Since synthetic chemical ingredients, fragrances may be responsible for various dermal syndromes like allergic contact dermatitis and irritant contact dermatitis with mild to severe reactions. Therefore, the effectivity of natural and sustainable ingredients in the formulations has been scrutinized. The tea polyphenols and essential oils clearly improved the antimicrobial potency of hand sanitizer formulation. Hence, it has established that these natural ingredients are quite beneficial for health as well as environment over long-term usage.

## Declarations

## Acknowledgments

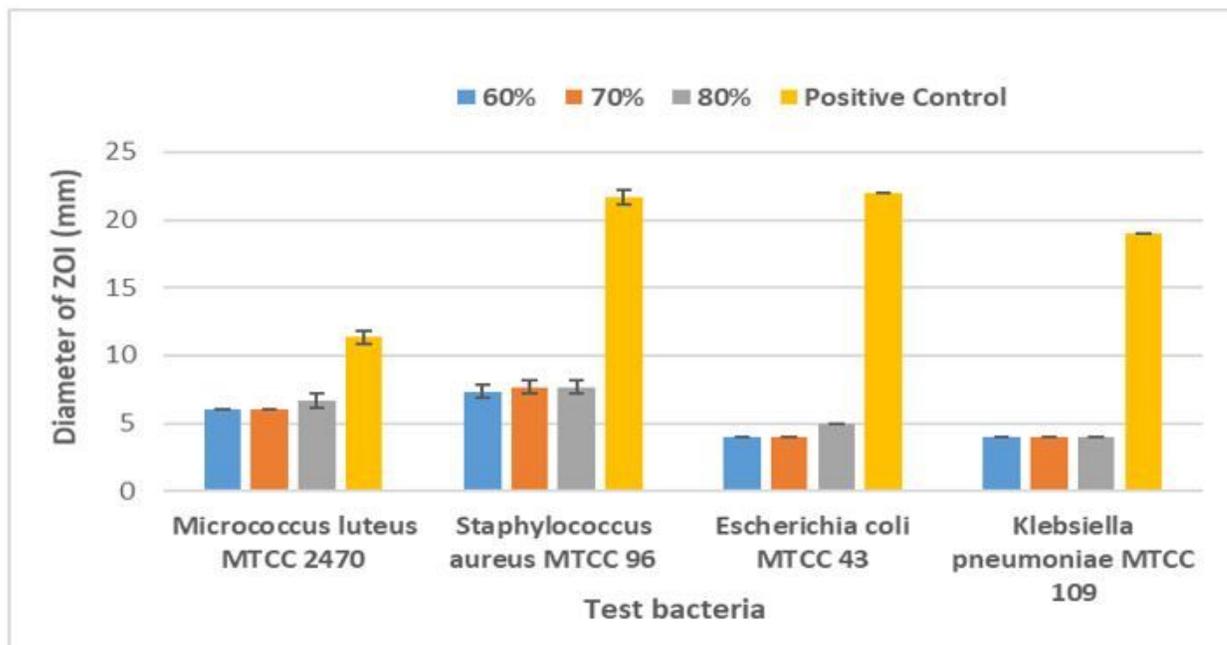
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## Figures



Positive Control: Streptomycin 1mg/ml, MTCC - Microbial Type Culture Collection

### Figure 1

Zone of inhibitions shown by hand sanitizer formulation with varying alcohol percentage at 60 %, 70 % and 80 % ethanol against *M. luteus*, *S. aureus*, *E. coli* and *K. pneumoniae*

## Supplementary Files

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