



Research Article

STUDY ON THE ANTIMICROBIAL EFFICACY OF HAND SANITIZERS DEVELOPED BY THE PHARMACEUTICAL CORPORATION (I.M) KERALA LTD (OUSHADHI)

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ABSTRACT

Hand sanitizers are used to ensure hygiene in the absence of hand washing facilities or where frequent sanitizing is required especially in the wake of the Covid-19 pandemic. Handsanitizers using different combinations were prepared at Oushadhi using WHO recommendations and also to promote Kerala Government's initiative of break the chain campaign. The campaign was launched on 15th March 2020 and it aims to cut down the progress of disease transmission. The efficacy of the batches of sanitizers prepared was tested against *Escherichia coli* and *Staphylococcus aureus*. They were also tested against common air and water contaminants found in the premises which would be of more significance to the general workforce. The susceptibility test was performed by agar well diffusion method and it revealed that the antimicrobial efficacy of the prepared hand sanitizer Batch III with *Cymbopogon citratus* oil was the most effective among the three combinations. A hedonic sensory evaluation was carried out for better consumer acceptability.

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INTRODUCTION

Hand sanitizers are used to ensure hygiene in the absence of hand washing facilities or where frequent sanitizing is required especially in the wake of the Covid-19 pandemic^[1]. The COVID-19 pandemic is caused by Severe acute respiratory syndrome coronavirus 2 (SARS CoV 2). It has been purported to primarily spread between people during close contact by small droplets produced during coughing or sneezing. People have also become infected by touching contaminated surfaces and then by contact with their eyes, nose or mouth. Recommended preventive measures include hand washing, covering one's mouth when coughing, social distancing and wearing masks in public^[2]. A mass hand washing campaign has been started by the Kerala government on March 15th 2020 named 'Break the chain' and it aims to educate people about the importance of hand hygiene. Hand sanitizer can be used instead of hand washing when frequent hand sanitization is imperative as well as when soap and water are not available. In many

workplaces and office settings, washing hands at intervals may not be practical. In such settings, use of hand sanitizers has been shown to be extremely important in improving community health and hygiene behaviour. Sanitizers can be formulated as gel or liquid preparations. Sanitizers have one or more active ingredients which may be ethanol or isopropanol. Sometimes extract of plants are added in hand sanitizers which may or may not contribute to the antimicrobial effect. Further, they may be added only for aesthetic purposes.

The use of alcohol based hand sanitizers has been known to reduce the chances of spread of infections in the community. CDC recommends washing hands with soap and water whenever possible because hand washing reduces the amounts of all types of germs and dirt on hands. But if soap and water are not available, using a hand sanitizer with at least 70% alcohol can help people from getting sick and passing the germs to others^[3]. To help countries and health-care facilities to achieve system change and adopt alcohol-based

hand rubs as the gold standard for hand hygiene in health care, WHO has identified formulations for their local preparation^[4].

Ethanol used between 60% to 95% is generally considered as safe and numerous studies have confirmed its virucidal activity^[5]. There is sufficient evidence for virucidal activity of ethanol against enveloped viruses. Literature points out that ethanol concentration of 42.6 % (w/w) is effective within 30s against SARS coronaviruses^[6].

In the formulations used, the concentrations of ethanol are described in v/v unless otherwise specified. Alcohols are broad spectrum disinfectants and are known to kill bacteria and fungi.

Each of these batches was also subjected to a sensory evaluation based on the characteristic of smell. The sensory evaluation technique is basically a scientific method used to measure, analyse and interpret those responses to products as

perceived through the senses of sight, touch and smell^[7]. The study was conducted to evaluate the antibacterial activity and consumer acceptability of the different batches of hand sanitizers made by Oushadhi, R & D team and zero in onto the best sanitizer formulation for large scale production.

Material and methods

The different batches and their formulations made at Oushadhi are given as follows:

Batch I- 16-03-2020

- Isopropyl alcohol (purity 99.8%), 751.5 ml
- H₂O₂ 3%, 41.7 ml
- glycerol 98%, 14.5 ml
- Aloe vera gel, 40 gms
- Orange essence oil, 0.5ml

Top up the flask to 1000 ml with distilled water and shake the flask gently to mix the content.



Fig 1.1 Different batches of sanitizer - as per WHO

Batch II- 17-03-2020

- Isopropyl alcohol (purity 99.8%), 751.5 ml
- H₂O₂ 3%, 41.7 ml
- glycerol 98%, 14.5 ml
- Orange essence oil, 0.5ml

Top up the flask to 1000 ml with distilled water and shake the flask gently to mix the content.

Batch III-26-03-2020

- ethanol 96% v/v, 833.3 ml- as Ayurvedic ingredient *Prasanna*
- H₂O₂ 3%, 41.7 ml
- glycerol 98%, 14.5 ml
- Cymbopogon citratus* oil 0.5ml - as Ayurvedic ingredient *PulThailam*.

Top up the flask to 1000 ml with distilled water shake the flask gently to mix the content.

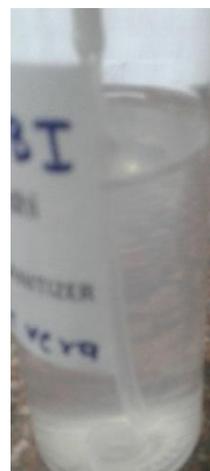


Fig 1.2 Batch I with Aloe vera gel - slightly cloudy

Two commercial brands were also tested. For the sake of anonymity, they have been labeled Brand X and Brand Y. Brand X was a liquid based sanitizer whereas Brand Y was a gel based sanitizer.

The batch number, manufacturing date, product composition were recorded before analysis of samples for antimicrobial activities. Sensory evaluation can assure that only good products are released in the market and it gives enough credence to customer satisfaction. For conducting sensory analysis, prior permission was taken from the head of R & D. The assessing of scents was done with R & D personnel, packing staff and laboratory support staff as panelists. The test was conducted as consumer testing (affective testing) with a small group of panelists due to fewer workforces as per Government guidelines during lockdown. The sensory related information based on the human perception was then analyzed and the best sample

was taken for further consideration. It was a demanding task as the description of olfactory characteristics is difficult to be expressed verbally^[8].

The presentation of the batches to the panelists for hedonic evaluation was done in a balanced manner so that each batch appeared equal number of times for evaluation^[9]. Samples were labelled as A, B and C; A- orange essence, B- distilled water and C- *Cymbopogon citratus* oil. The sanitizers with the two fragrant oils or essences and

the distilled water were presented in petriplates as cotton wetted samples in closed petriplates. Panelists had to open the petriplates and inhale the fragrance. Proper care was taken to prepare the samples by wearing protective clothing so as not to contaminate the samples. The ranking test was carried out in the following pattern: ABC-ACB-BCA-BAC-CBA-CAB

The score card given to the panelists was as follows:

Sensory Evaluation Scorecard 9 point hedonic scale

Grade	Score
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Based on the above, rate the samples given below:

Samples	A	B	C
Rating 1-9			

The score cards were made in English as well as in the local language, Malayalam, for better understanding and accuracy. Once odour ratings were completed, panelists were asked to describe each odour by describing it in terms of hedonic characteristics. Each panelist was asked to sniff each plate with sample wetted cotton and then to rate how intense and pleasant the smell was. It was rated on a scale from 1 (extremely liked smell) to 9 (extremely disliked smell). Then, after rating each odour, they were asked to describe the smell as precisely as possible^[10]. The batches were presented in the above given sequence and panelists were encouraged to describe the odour in either technical or emotional terms as per their level of understanding. In order to familiarize the panelists, a mock evaluation was carried out with a sequence of 1–3 empty plates with distilled water wetted cotton.

Samples from each batch were also taken for antimicrobial effect studies. Bacterial cultures of *Staphylococcus aureus* and *Escherichia coli* obtained from culture bank of Cooperative Lab, Kuttanellur were used for the studies. These cultures had been previously isolated from clinical sources. Common

contaminants in the air and water were also used as these would be the common microbes present in the premises. These were identified as *Sarcina lutea* and *Chromobacterium violaceum*. The cultures were maintained in nutrient broth and prepared as per standard procedures^[11].

The cultures were inoculated in 5ml of nutrient broth and incubated at 37°C for 24 hrs. The turbidity of the bacterial suspensions was checked using McFarland 0.5 turbidity standard as reference to adjust the turbidity of bacterial suspensions. The standard was prepared by adding 0.5 ml of 1.175% w/v barium chloride dihydrate (BaCl₂•2H₂O) solution to 99.5 ml of 15 w/v sulphuric acid (H₂SO₄). These were prepared in test tubes similar to those used in preparing suspensions of the test organisms. Using a sterile loop, pure culture was transferred into the saline tubes and a homogenous suspension prepared. It was then compared with the McFarland turbidity standard, and the density of the organism was adjusted by adding either more loopful of culture or sterile saline until visual similarity was obtained.

Sterile Mueller-Hinton agar plates were taken and inoculated with the test organisms by

dipping sterile cotton swabs into the culture and then swabbing onto agar surface three consecutive times at different start points after rotating the plates to an angle of 60°. The culture was also swabbed around the edge of the plate for uniform lawn growth. The culture was allowed to soak into the agar surface for a few minutes at room temperature with the lid closed. Using a sterile 6 mm cork borer, 4 equidistant holes were made in the Mueller- Hinton agar plate. The agar plugs were discarded using a sterile arrowhead needle. The three different batches of hand sanitizer as well as commercial hand rub and 1:200 diluted *Cymbopogon citratus* oil were introduced into the wells (50 -60 microlitres) according to a template with distilled water and alcohol as controls.

The setup was then allowed to incubate upright at 37°C for 24 h. After incubation, antimicrobial effectiveness was determined by measuring the zone of inhibition using HiMedia Antibiotic Zone scale. Each antimicrobial testing was performed in triplicate.

The data obtained were analyzed statistically and conclusion was drawn on the basis of analysis of variance technique.

Table 1: 9 point hedonic scale Sensory evaluation of fragrances used in sanitisers

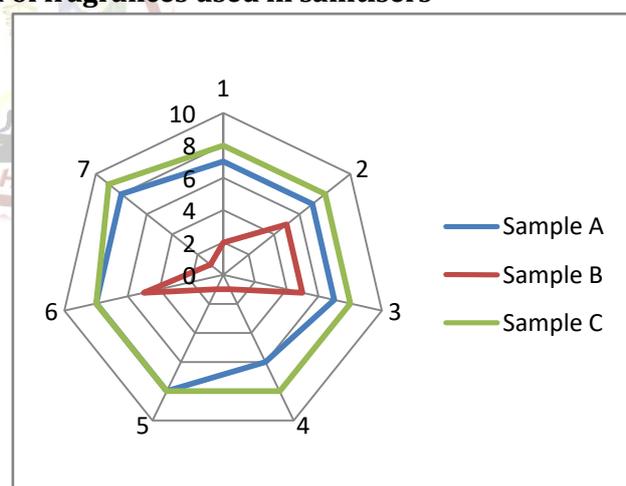
	Sensory evaluation- Scores as per 9 point hedonic scale		
Panelists	Sample A	Sample B	Sample C
1	7	2	8
2	7	5	8
3	7	5	8
4	6	1	8
5	8	1	8
6	8	5	8
7	8	1	9
Total	51	20	57
Mean	7.28	2.85	8.14

The prepared batches as well as test samples and controls were analysed for their antimicrobial effect^[12]. The antimicrobial susceptibility pattern of hand sanitizers and other samples for each organism are tabulated in tables 1 and 2. *Cymbopogon citratus* oil, 1:200 dilutions was also tested to see whether it had any antimicrobial

Results and discussion

The purpose of hand sanitizers is to establish hand hygiene and thus prevent the spread of infection. However aesthetic considerations are also important as the finished product with a pleasant fragrance ensures customer satisfaction. Also visual characteristics are of importance. Batch I was rejected as it presented a cloudy appearance. Subsequent batches were found to be clear visually. Olfactory evaluation was conducted in a planned manner. *Cymbopogon citratus* oil was found to be better than the orange essence used in the batches of hand sanitizers in terms of better lasting fragrance and freshness attribute on application. Thus hedonic analysis resulted in judging the batches on basis of olfactory rating as pleasant or unpleasant.

Interestingly majority of panelists rated Batch III to be having better hedonic attributes with smell description being “citrus like, lemony and fresh after feel”. Hence Batch III was selected to be the best in terms of sensory evaluation. Statistical analysis revealed that the result was significant at $P < .01$.



Graph 1.1 A radar graphical representation shows that Sample C is preferred over other samples

effect apart from the aesthetic enhancement of the product.

It was seen that batch III showed better antimicrobial effect as compared to the other samples. Comparable zones of inhibition were obtained while taking average of triplicate results^[13].

Table 2: Means of inhibition in mm obtained using different batches and control on bacterial strains of *Staphylococcus aureus* and *Escherichia coli*.^C- Clinical strain

Test organism	Inhibition zone(mm)		Mean +S.D		
	Batch III	Brand X	<i>Cymbopogon citratus</i> oil (1:200)	Batch II	Distilled water
<i>Staphylococcus aureus</i> ^C	11.2 ± 0.84	10.4±0.55	10.2±0.45	10.0±0.71	0.0
<i>Escherichia coli</i> ^C	11.4±0.89	10.2±0.45	9.6±0.55	9.4±1.52	0.0

Statistically significant at p< 0.05, One way ANOVA

Table 3: Means of inhibition in mm obtained using different batches and control on bacterial strains of *Sarcina lutea*^E and *Chromobacterium violaceum*^E- Environmental strain

Test organism	Inhibition zone(mm)		Mean +S.D		
	Batch III	Brand Y	<i>Cymbopogon citratus</i> oil (1:200)	Batch II	Distilled water
<i>Sarcinalutea</i> ^E	14.0 ± 0.84	13±1.87	11.6±0.55	10.2±0.45	0
<i>Chromobacteriumviolaceum</i> ^E	17.2±0.84	16.0±1.41	11.4±0.55	10.4±0.55	0

Statistically significant at p< 0.05, One way ANOVA

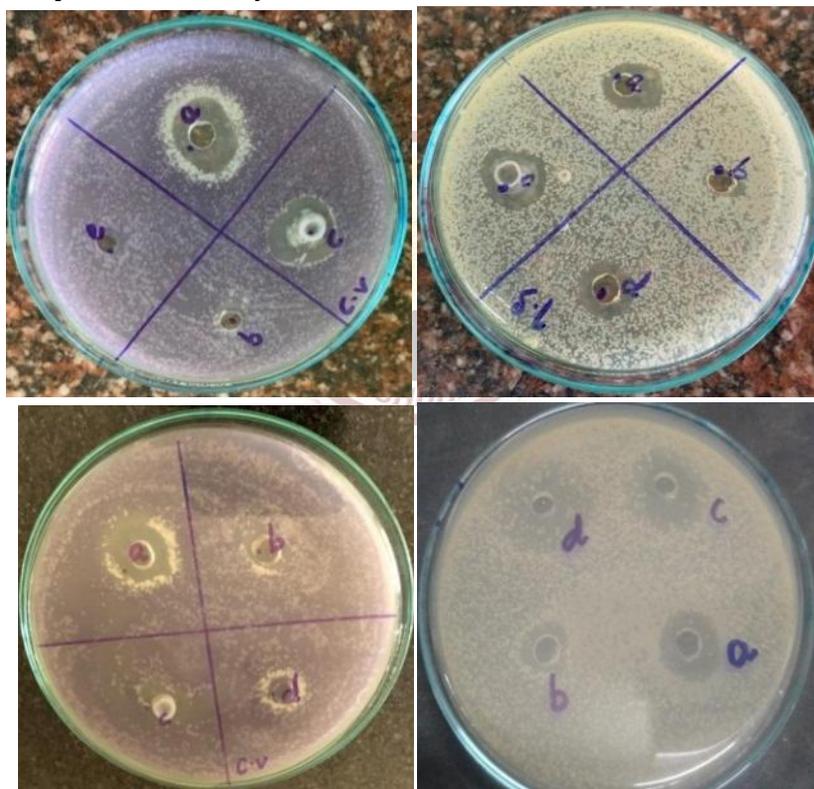


Fig 1.4 Antimicrobial effect of different batches of sanitizers and controls on test cultures

Data were statistically analyzed using analysis of variance. The data exhibited a normal and homogeneous distribution; thus, zone of inhibition (in mm) was analyzed using the mean of all the readings obtained showing that the difference in the values of different hand sanitizers was statistically significant at P < 0.05.

CONCLUSION

Batch III made was found to have a good antimicrobial effect as well as good after use feel due to the citrus smell. Hence it was suggested for

further production. *Cymbopogon citratus* oil is commonly used in many folk remedies and its antimicrobial effect at 1: 200 dilution is interesting. Interestingly, *Cymbopogon citratus* oil was found to have a beneficial effect in the management of influenza cases. Batch III was found to be the most effective. Even though alcohol is the major antimicrobial agent, addition of lemongrass oil not only improves the aesthetic characteristic but also contributes to antimicrobial effect.

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