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Preparation, Effectiveness and Antimicrobial Activity of Polyherbal Hand Sanitizer



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ABSTRACT

Introduction: Hand Hygiene, particularly hand sanitizing, is essential in reducing infectious disease transmission. The recent outbreak of corona in world increased public awareness of practice of hand sanitizing in order to introduce new products of hand sanitizer to the world. **Objective:** Thus, the present study was to preparation of polyherbal hand sanitizer which contains antimicrobial efficiency. **Material:** The present study was carried out by mixing the extract of Aloe vera, Ginger and Lemon with isopropanol, carbapol and glycerin instead of chemical ingredients. **Method:** The anti-microbial activity of the polyherbal hand wash gel was tested against *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella* by Kirby-Bauer well diffusion method and the results obtained were compared with commercial hand sanitizer. The efficiency of polyherbal hand wash gel was checked on volunteers. The physical parameters of hand sanitizer were studied as well. **Result:** According to the zone of inhibition and by bacterial load, the prepared hand sanitizer was more effective than other marketed hand sanitizer. **Conclusion:** An attempt has been made to prepare herbal hand sanitizer by using plant extract with no side effects on human tissue. The prepared hand sanitizer was cheaper than other marketed hand sanitizer and gave more yield in less quantity and time. It was safer to use due to its natural ingredients.

INTRODUCTION




Hands hygiene is well known as one of the most significant of activities essential for the reduction of transmission of infectious diseases, particularly in hospitals (Pittet *et al.*, 2006, Zapka *et al.*, 2017). Hand hygiene generally refers to different method of eliminating or killing microorganisms which may be present on hands, by either hand washing or sanitizing. Though the concept of hand sanitization has been in place right from the start of the hand hygiene campaign by Semmelweis (WHO 2009, Pires *et al.* 2017), majority of early reports focused primarily on the role of handwashing as an infection control measure (Garner and Favero 1986). Since the beginning of the coronavirus outbreak, health authorities have been urging us to pay attention to our hand hygiene and for good reason. WHO has recommended all people should wash hands before entering at home, during and after preparing food, before eating food, before and after caring for someone who is sick, before and after treating a cut or wound, after using the toilet and changing diapers or cleaning up a child who has used the toilet. After blowing your nose, coughing, or sneezing and after touching garbage [3]. Washing hands with soap and water is one of our cheapest forms of infection control and also one of the most effective. It significantly helps to prevent and slow the spread of infection. When soap and water are not readily available, alcohol based hand sanitizers or rubs are acceptable. Hand sanitizers are effective against bacterial and fungal infections, as well as enveloped viruses, such as the common cold and flu viruses and in preventing nosocomial infections caused by different opportunistic microorganisms. Thus, occurrence of nosocomial infections is alarmingly increasing and has emerged as a serious concern in hospital care outcome; which results in prolonged hospitalization, a lot of disease and mortality and excessive costs [1]. Alcohol rub sanitizers containing at least 70% alcohol kill 99.9% of the bacteria on hand 30 seconds after application and 99.999% in 1 minute [2]. Historically, plants have provided a good source of antimicrobial activity [4]. Plant extract have a potential as antimicrobial compounds against several pathogenic microorganisms which cause infections disease and resistance towards synthetic drugs [5]. The main advantage of using natural source is that they are easily available cheap & harmless compared to chemical products [6]. In this study we used, *Aloe barbadensis miller*, *Zingiber officinale*, *citrus limon* due to their individual benefits. *Aloe barbadensis miller*, it belongs to Asphodelaceae (Liliaceae) family, and is a shrubby or arborescent, perennial, xerophytic, succulent, pea-green color plant. It is having anti-microbial, antiviral, antitumor, anti-inflammatory, laxative effects and healing properties [7]. An anti-inflammatory constituent, *Zingiber officinale*, commonly known as ginger, is a spice consumed

worldwide for culinary and medicinal purposes. The plant has a number of chemicals responsible for its medicinal properties, such as antiarthritis, anti-inflammatory, antidiabetic, antibacterial, antifungal, anticancer, etc [8]. *Citrus limon* is an important medicinal plant of the family Rutaceae. It is cultivated mainly for its alkaloids, which are having antibacterial potential in crude extracts of different parts (viz, leaves, stem, root and flower) of lemon against clinically significant bacterial strains has been reported [9]. Citrus flavanoid has a large spectrum of biological activity including antibacterial, antifungal antidiabetic, anticancer and antiviral activities [10]. Considering this ultimatum, an attempt has been made to screen classical literature for the herbs with antimicrobial properties and found that, *Aloe barbadensis miller* (Aloe vera), *Zingiber officinale* (Ginger), *citrus limon* (lemon) has those antimicrobial activities. The result from the present work suggest and support the incorporation and utilization of herbs in formulation to give better effect.

MATERIALS AND METHODS:

MATERIAL:

Table No. 1: Function and Ingredients to make hand sanitizer

Sr. No.	Ingredients	Function (Quantity)
1.	Aloe vera 	Anti-microbial efficiency (10ml)
2.	Ginger 	Anti-microbial efficiency (10ml)
3.	Lemon 	Anti-microbial efficiency (10ml)
4.	Isopropanol	Disinfectant (40ml)
5.	Carbapol-940	Thickening agent (1ml)
6.	Glycerine	Humecants (5ml)
7.	Perfume	Imparts smell (1ml)
8.	Vitamin E Tablet	Skin conditioning agent (1ml)
9.	Almond oil	Emulsifier (2ml)
10.	Water	Solvent (20ml)

COLLECTION OF SAMPLE:

Fresh leaves of *Aloe barbadensis miller* (Aloe vera), *Zingiber officinale* (Ginger), *citrus limon* (lemon) were collected from Nagpur, Maharashtra region. Leaves were sorted, washed, dried at room temperature.

EXTRACT PREPARATION:

Aloe vera extract:

10 gram of gelly fluid sample in 100ml of distilled water & left undisturbed for 24 hrs. After 24 hours, the suspension was shaken and this mixture was filtered through filter paper to obtain the aqueous extract [11].

Lemon extract:

10ml of lemon juice were extracted.

Ginger extract:

10 grams of ginger were sorted, washed and then were boiled in 100ml distilled water till the volume reduces to one third its initial volume. The extract was cooled, filtered and stored in an airtight container.

Preparation of Hand Sanitizer:

Sterilized (autoclaved) extracts of Aloe vera, Ginger, Lemon were mixed in equal volume (10 ml each) and mix all the ingredients as shown in Table No.1 as per the given quantity under aseptic condition in a sterilized container. The mixture was vortex in order to get homogenous solution.

ANTIMICROBIAL ACTIVITY BY KIRBY-BAUER METHOD:

The screening of antimicrobial efficacy of polyherbal hand sanitizer was performed on various microorganisms by using well diffusion method (Kirby- Bauer method) as per standard procedure. The pure cultures were used in these procedure were *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia*. The broth cultures of above organisms were prepared. Sterile petri plates of Muller Hinton agar were prepared. After solidification of the media, 0.1 ml of test organisms (24 hrs old) spreaded over separate MHA plate by using sterile

spreader. Three wells were punctured with sterile borer (2mm size) into the media. In one well formulated sample were added, in second well marketed sanitizer were added and third well is set as control. Plates were kept in the Incubater at 37°C for 24 hours.

Determination of minimum inhibitory concentration (MIC) of polyherbal hand sanitizer:

The MIC is defined as the lowest concentration that completely inhibits the growth of microorganisms for 24 hours incubation. Determination of minimum inhibitory concentration of polyherbal hand sanitizer was determined by following method. Prepare different concentrations of polyherbal hand sanitizer 200µg, 400µg, 600µg, 800µg, 1000µg in different test tubes respectively. Take five different test tubes and labelled it as 1 to 5. Add 5ml of steriled nutrient broth in each test tubes. Now add 1ml of cultures in each test tubes. In 1st test tube add 200ug of polyherbal hand sanitizer in 2nd test tube, add 400 µg of sample and so on. Now incubate the tubes at 37°C for 24 hrs.

Antibacterial efficiency of polyherbal hand sanitizer:

The antibacterial efficiency was performed by spread plate technique. Sample were collected from the five different volunteers showing no clinical signs of dermal infection. Approximately 500ul of polyherbal hand sanitizer was applied to both hands. After washing the hands with polyherbal hand sanitizer, the hand was put on nutrient agar plate similarly done with Life and Smartway hand sanitizer (Market products) also. Then incubate it at 37^oc for 24 hours.

RESULTS AND DISCUSSION

By the above procedure the formulated polyherbal hand sanitizer are as follows:



Figure No. 1: Polyherbal hand sanitizer in sterilized plastic bottle

Evaluation Parameters:

Sr. No.	Parameters	Observation
1.	Colour	Light yellow
2.	Odour	Fragrant
3.	pH	6.9

Table No. 2: Antimicrobial activity of formulated hand sanitizer against microorganisms

Sr. No.	Organisms	Zone of inhibition
1.	<i>E. coli</i>	20mm
2.	<i>S. aureus</i>	16mm
3.	<i>Klebsiella</i>	15mm

Table No. 3: Antimicrobial activity of marketed hand sanitizer (smartway sanitizer) against microorganisms

Sr. No.	Organisms	Zone of inhibition
1.	<i>E. coli</i>	17mm
2.	<i>S. aureus</i>	15mm
3.	<i>Klebsiella</i>	14mm

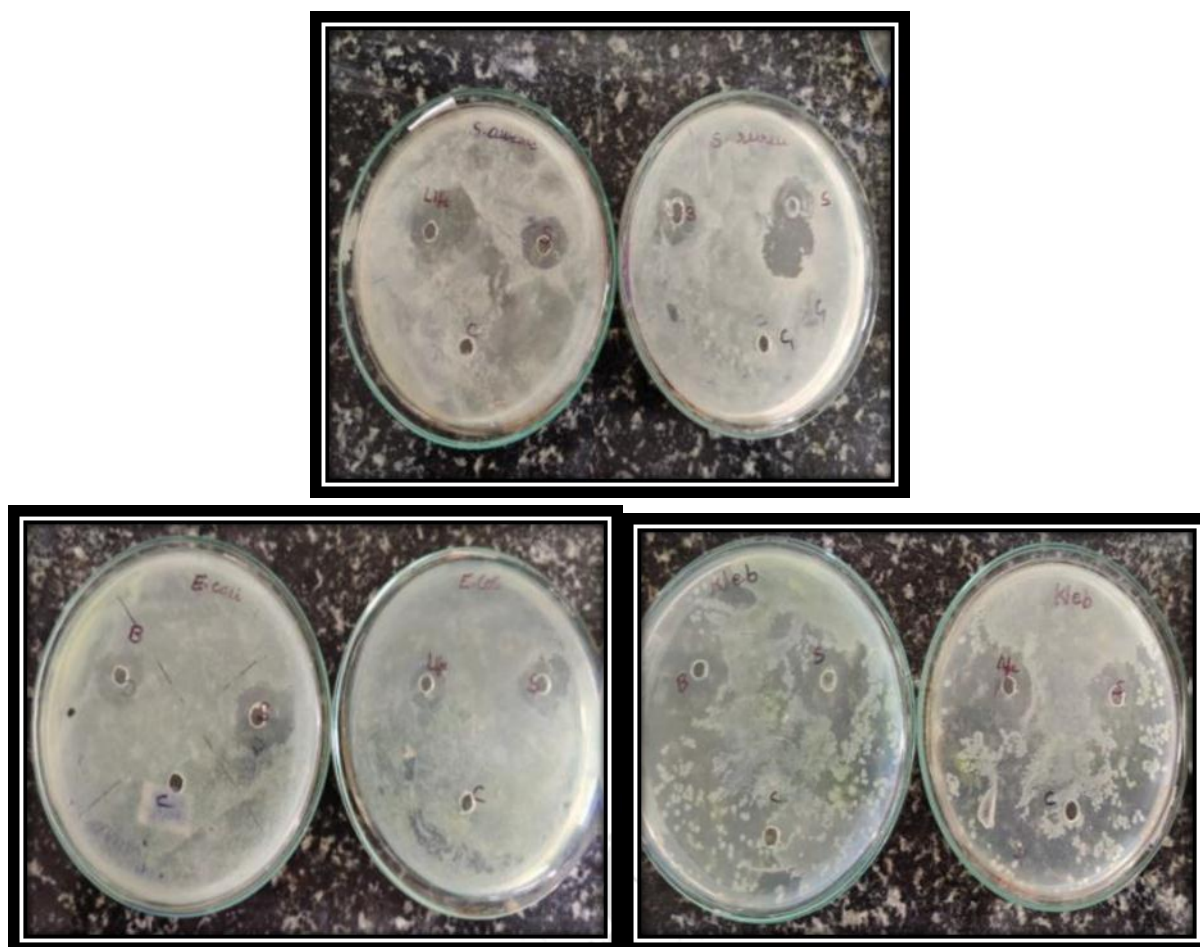
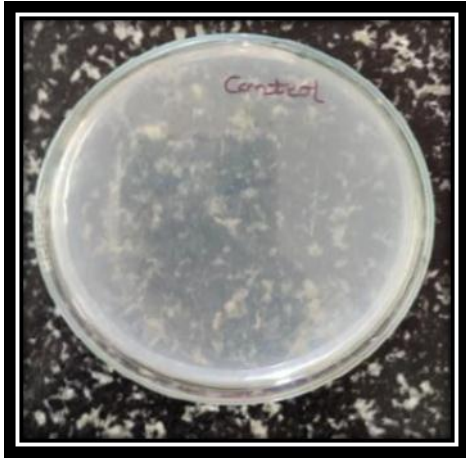


Figure No. 2: Result of antimicrobial study of Hand-sanitizer against various pathogens.

According to zone of inhibition formed resulting the polyherbal hand wash against different bacterial isolates showed that hand sanitizer prepared with aloe vera, ginger and lemon has great activity. Statistical analysis findings showed that polyherbal hand sanitizer is the broad spectrum antibacterial agent with different response for different bacterial kinds tested. According to antimicrobial activity, the zone formed by formulated hand sanitizer on *E. coli* plate was 20mm which is more than other two marketed hand sanitizer *i.e* Life sanitizer show 13mm zone and Smartway sanitizer show 15mm zone. On *S. aureus* plate formulated hand sanitizer show 17mm zone while life sanitizer show 16mm zone and smartway sanitizer show 15mm zone that means in *S. aureus* also, formulated hand sanitizer show more antimicrobial activity. On Klebsiella plate formulated hand sanitizer show 15mm zone while life sanitizer show 15mm zone which is similar to formulated hand sanitizer zone and Smartway hand sanitizer show 14mm zone. From the investigation it was clear that aloe vera, ginger and lemon are more effective against bacteria and the formulated hand sanitizer show more antimicrobial activity.

The antibacterial efficiency of formulated hand sanitizer with other two marketed hand sanitizer (Life and Smartway).



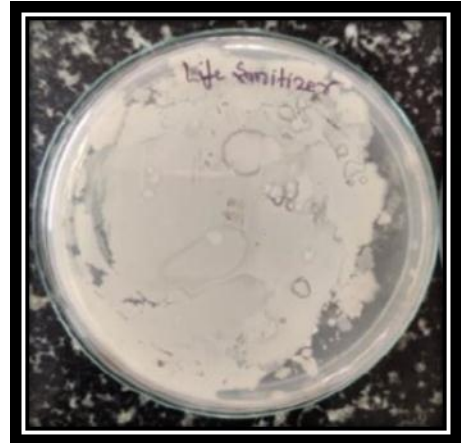
CONTROL PLATE



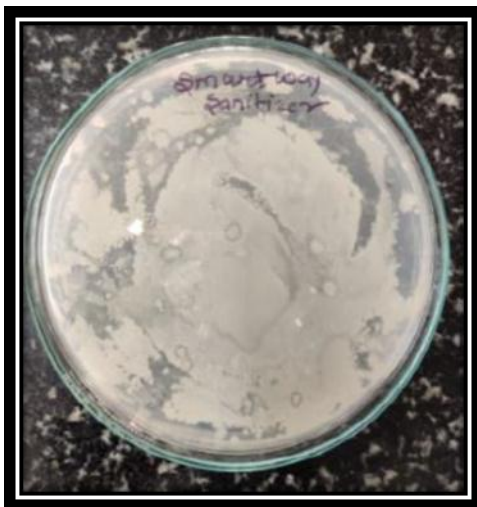
(POLYHERBAL) SAMPLE SANITIZER



WITHOUT SANITIZER



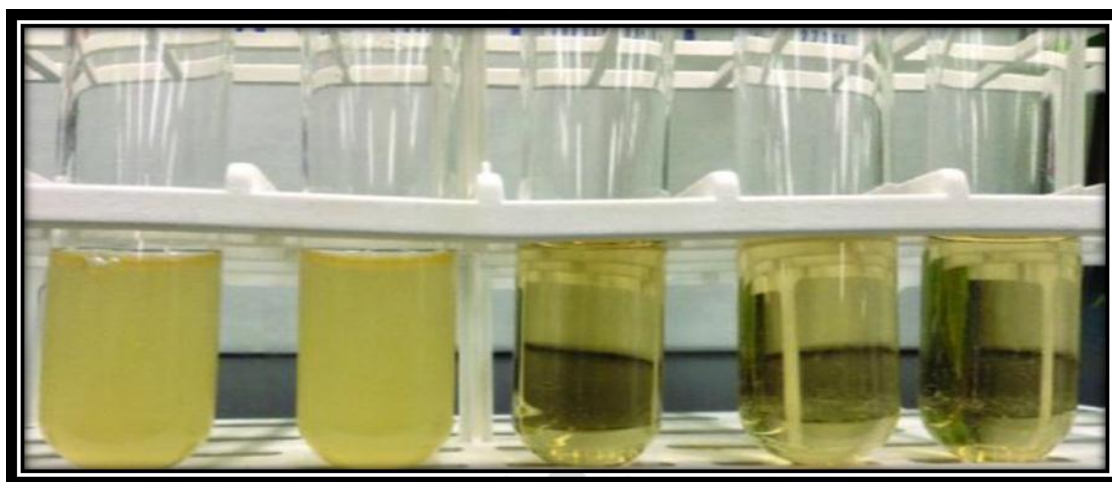
LIFE SANITIZER



SMARTWAY SANITIZER

By performing swab test of formulated hand sanitizer along with other marketed hand sanitizer on different volunteers shows that formulated sanitizer shows less microbial load on the nutrient agar plate while the other two hand sanitizer shows more microbial load. The microbial load difference between before and after of hand sanitizer was varied. After using hand sanitizer the microbial load was less than that of before using hand sanitizer.

The minimum inhibitory concentration of formulated hand sanitizer is as follows:



1ST TUBE

200 µg

2ND TUBE

400 µg

3RD TUBE

600 µg

4TH TUBE

800 µg

5TH TUBE

1000 µg

By performing minimum inhibitory concentration it was proved that tube containing 600 µg of sample has lowest inhibition concentration that is taking approximately 600 µg of sample will inhibit maximum growth microorganisms.

The main ideology behind combining the plant materials is to observe the additive effect of constituents of different herbal ingredients. The combination proves to be beneficial and hence it is used in preparation of herbal hand sanitizer it is far more active than the marketed synthetic formulation. The method of formulation of hand sanitizer was very cheap, easy and fast process.

CONCLUSION

Hand hygiene can also be problem in between the people. Prevention and control of infection are the activities designed to limit the spread of infection and provide a safe environment for all people, regardless of setting. In light of emergence of antibiotic resistant organisms,

effective infection control measure such as hand sanitizing are essential to prevention. Hand sanitizer are used for purpose of cleaning hands. The above preparation can be used as a good hand sanitizer. It is concluded that from the result the formulation of hand sanitizer is good in appearance and homogeneity. This preliminary *in vitro* study demonstrated that aloe vera, ginger and lemon extract active against hand swab sample. Polyherbal hand sanitizer was effective against pathogenic bacteria in volunteer sample with no side effects on human tissue. The prepared formulation showed significant results against three bacteria species. *E. coli*, *S. aureus* and *Klebsiella* compared to marketed synthetic hand sanitizer. We can conclude that the active compounds in the herbal hand sanitizer are more effective in killing and removing organism than the synthetic chemicals used in marketed formulation. Thus these compounds can be extracted and incorporated in herbal formulation. Thus these compounds can be extracted and incorporated in herbal formulation with less or no side effects. Thus a new way can be found to provide safe and healthier living through germ free hands. Although the removal is not 100% but a major number can be reduced.

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