



Oregano Derived Anti-Bacterial Hand Sanitizer: Formulation and Characterization

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Authors' contributions

This work was carried out in collaboration between both authors. Author SKS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors IR and SKS managed the analyses of the study. Author IR managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

In day to day activity hands serves important role in accomplishing tasks, but the most important function that people forget while achieving task is sanitization of hands. It is the most important practice that is required in every sector while it may be food sector, pharma sector or corporate sector in order to prevent from spreading diseases caused due to pathogenic microbes which preferably includes viruses, bacteria, fungi and alike. The present research focus towards formulating a herbal sanitizer wherein an oregano herb possessing anti-bacterial activities and a baobab extract possessing anti-inflammatory properties is mixed with ethanol along with glycerin, wherein glycerin act as a humectant and emulsifying agent to aid in preparation of a anti-bacterial herbal sanitizer. Carbomer was used as a gelling agent. The prepared sanitizer is then subjected to characterization studies which includes pH, viscosity, anti-microbial analysis, anti-bacterial efficacy, anti-oxidant assay, stability, and spreadability. After analyzing the results it was found that, the sanitizer possessed specific potent activity against pathogenic bacterial growth but was not harmful to skin.

Keywords: Baobab; carbomer; oregano; sanitizer.

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1. INTRODUCTION

Hands are important part of the body as it aids in achieving day to day tasks. Mostly at workplaces, hands are utilized by work-officials to compose a report or even while working on systems hands play important role. While doing so, it evenly happens that people interact with one another either by shaking hands or by sharing items [1-2]. This sharing may sometimes be harmful as it may lead to spread of diseases or pathogenic agents from infected person to uninfected person. These contaminated germs may be so harmful that it may sometimes lead to contagious ailments. The contaminated germs, talked herein may include but not limited to viruses, bacteria and alike [3]. Ailment further leads to loss in economic growth due to which unemployment may also take place. Taking into account that 90 percent of all contaminations are transmitted by hands, it's important to place a powerful hand cleanliness program at workplaces [4-7]. With the ongoing rise of extreme intense respiratory conditions e.g. Severe Acute Respiratory Syndrome (SARS) a naive irresistible sickness that severely affects people and persists for long time, there is a an urgent need for an effective and safe hand sanitizer. Routine hand washing with cleanser and water has been referred by the World Health Organization (WHO) as being "the most significant cleanliness measure in preventing the spread of disease [8]. Some of the common symptoms caused due to respiratory related infection of the above mentioned pathogens includes:

- Fever
- Dry cough
- Breathing problem

Additional symptoms may also include aches, nasal clogs but not limited to runny nose, sore throat, diarrhoea. Thus, there is an urgent need to spread hand care awareness among people to prevent such illnesses. At present more importance is given to sanitizers as they have been recommended by WHO regarding its potential effects in preventing pathogenic growth. Sanitizers are pharmaceutical products that aid in killing the germs residing over human skin in order to prevent humans from harmful diseases. For example at present there is outburst of COVID-19 and the main reason for this is corona virus which actively spreads through touch, or contact with the infected person [9]. There are many sanitizers available in the market but most of them are alcohol based. Although alcohol-

based sanitizers are effective in killing germs, but they have certain disadvantages which include skin irritation, dermal unlayering and dryness. Also, exposure of hands to the highly concentrated alcohol may cause certain ailments. Thus in this paper focuses on formulating a herbal sanitizer by combining oregano extract and baobab extract both possessing anti-bacterial and anti-inflammatory activities. The above-mentioned extracts are mixed with ethanol, wherein ethanol concentration is kept lower in comparison to the extracts. The prepared formulation thus aids in preventing pathogenic growth and helps in preventing the life of humans. Also, whether in workplace or anywhere else, these tasks may include people interacting with one another.

2. REVIEW OF LITERATURE

Macinga and colleagues developed an alcohol based hand sanitizer for inactivating non-enveloped viruses and also their surrogates, wherein the sanitizer is made from a combination of polyquaternium polymer and an organic acid. From a food borne disease outbreak surveillance system, (1988-2002) it was noted that 33% of outbreaks and 41% of infection are caused due to viruses [10].

Wilson and co-workers conducted a comparison test between residual and non-residual hand sanitizer for finding their potency towards reducing risks caused by noroviruses. The aim behind this study was to develop tentative estimation of log₁₀ reductions of human norovirus for both residual i.e. 60% ethanol and non-residual sanitizers i.e. quaternary ammonium centered sanitizers. Estimations were made by utilizing ASTM international standard E-1838-10 technique along with adjustment. It was found that for contact time of 60 seconds the largest log₁₀ reduces in the residual and non-residual sanitizers decreased by approximately 99% and 85%, individually. After using for 4 hrs, residual sanitizer reduced the risk of illnesses by 78.5% but there was no decline in non-residual sanitizer [11].

Although the above cited sanitizers were effective in performing their activity against pathogenic germs, the main problem lies in the use of high amount of alcohol which may lead to dried and cracked skin. The large content of alcohol when frequently comes in contact with the skin withdraws natural oil from the skin and may causes skin drying. The skin drying then

leads to skin cracking which may further increase the chances of contamination with the skin by providing entry to pathogenic micro-organism via cracked skin. Therefore there is a need to develop a potent herbal formulation for eradicating contaminants such as virus, bacteria and alike from the skin and at the same time moisturizing the hand in addition to providing smoothening and comfort to the user.

3. METHODOLOGY

3.1 Design

Anti-bacterial hand sanitizer was developed from an herbal extract of oregano in combination with alcohol and herbal excipients. It is prepared in two steps which includes: i) oregano extraction, and ii) process of preparing herbal hand sanitizer by using carbomer as a gelling agent and glycerine as a humectant. Further the prepared gel formulation was subjected to characterization studies which includes pH, viscosity, anti-microbial analysis, anti-bacterial efficacy, anti-oxidant assay, stability, and spread ability.

3.2 Ingredients

The ingredients involved in sanitizer preparation includes:

- Oregano flakes as herbal anti-bacterial extract,
- Glycerine as humectant
- Ethanol
- Carbomer as gelling agent
- Baobab herb as anti-inflammatory agent
- Nutrient agar
- MacConkey agar
- Muller Hinton agar

3.3 Samples

Samples required for conducting analysis of the sanitizer includes:

Hands of subjects for analyzing the effect of sanitizer on skin which includes spreadability, dermis test, pH and alike.

Bacterial species which include *E. coli*, *S. aureus*, *P. aeruginosa*.

3.4 Process of Oregano Extraction

The oregano extract was prepared according to standard protocols. The method of extraction of oregano extract involves following steps: Firstly oregano flakes were dried under hot red sun to obtain dried flakes. After that the flakes were grinded with the help of a grinder to obtain a coarse powder. 18 gm of powder was weighed and placed in a laboratory beaker with volume upto 150 ml. The beaker was placed in shaker to dissolve the coarse powder in the water and obtain a solution. The solution was then placed in soxlet extractor for duration of 7hrs by maintaining the temperature of around 100°C to bifurcate the extract from solvent. Finally the extract was subjected to rotary evaporator to evaporate the solvent and obtain the extract following oven drying to obtain a final oregano extract [12].

3.5 Process of Preparing Herbal Hand Sanitizer

To prepare 500 ml of sanitizer, 470 ml of 70% ethanol was placed in a beaker followed by addition of 20 ml of oregano extract to obtain a solution. Further, carbomer was added to the solution to obtain a gel consistency. To the obtained gel consistency, 10 ml of glycerin was added to avoid formation of any air bubbles followed by addition of 3.4 drops of baobab and stirred slowly to obtain a homogeneous formulation. Fig. 1 represents flow chart representing method of preparation of herbal hand sanitizer.

Further, the prepared homogeneous formulation was subjected to characterization studies which includes: pH, viscosity, anti-microbial analysis, anti-bacterial efficacy, anti-oxidant assay, stability, spreadability [12].

3.6 pH

After the formation of hand sanitizer, pH was checked by digital pH meter (Labman, India), wherein 2gm of sanitizer was kept in a beaker and was subjected to pH electrode [12].

3.6.1 Viscosity

Viscosity of gel formulation was determined by utilizing viscometer at 27±1.5°C [12].

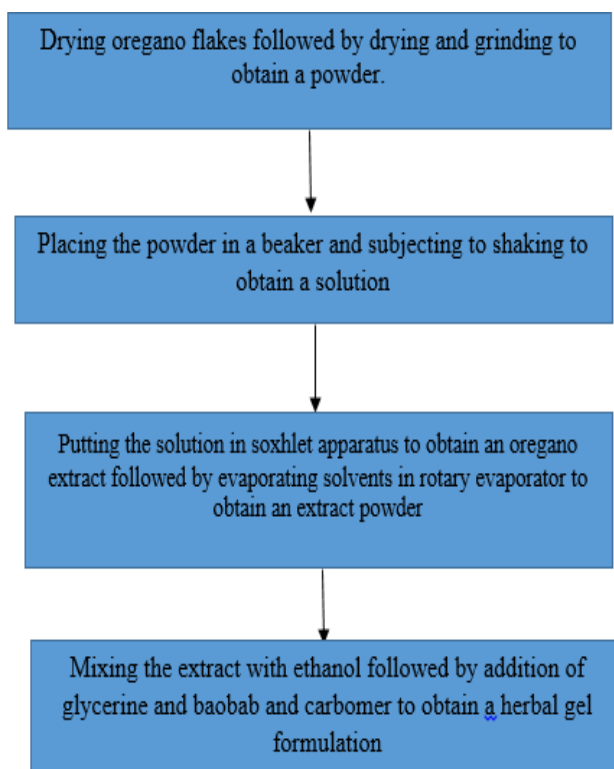


Fig. 1. Representation of methods involved in the preparation of herbal hand sanitizer

3.7 Spreadability

Spreadability of the sanitizer was performed by following the protocol which includes taking two glass slides and marking the area of 2 cm on the first slide followed by spreading sanitizer gel. Then placing the second slide over the first slide and pressing it to spread the gel between the two slides. Finally, the area of spread was noted down [13].

3.8 Extrudability

Extrudability was determined via collapsible frame. Extrudability is addressed as the amount of fluid extruded from the foil. Weight was determined that is responsible for extruding at least 0.4 cm gel within 15 sec [13].

3.9 Anti-Microbial Assay (Herbal Extract)

Specific microbes were involved to analyze the antimicrobial activity of herbal extracts. Dip well protocol was adopted for performing antimicrobial activity. Analysis of antimicrobial activity for herbal extract was taken on three separate sterile plates. The micro-organisms

involved were *E. coli*, *S. aureus*, *Salmonella*. MacConkey agar and muller hinton agar media were prepared and solidified. Further, inoculation was performed by inoculating sub-cultured micro-organism i.e. *E. coli*, *S. aureus*, *Salmonella* on nutrient agar followed by inoculating three discs with oregano extracts. Then plates were incubated for whole night at 37°C. Zone of inhibition was observed and MIC was calculated after 24 hrs of incubation [12].

3.10 Anti-Bacterial Efficacy (Sanitizer)

MIC is described as lowest concentration required to inhibit micro-organism growth. The calculation of minimum inhibitory extracts amount was calculated by preparing nutrient broth containing different concentrations of herbal extract: 300 µg, 600 µg and 900 µg, respectively. In Muller Hinton agar reservoirs were seeded with uniform inoculum of sample bacteria, 0.5 ml amount of each dilution was applied aseptically. Triplicate tests were conducted. The plates were incubated at 37°C for 28 hours. The lowest amount of MIC were considered samples showing a strong inhibition region. Spread plating was conducted for analyzing anti-bacterial

efficacy of the sample. 100 ml of sanitizer sample was applied to hands of subject. After sanitization, collected samples were allowed to grow on nutrient agar medium overnight at 37 degree celsius and colony forming units were counted [12].

3.11 Cyto-Toxicity Assay on Human Cells

For the evaluation of cytotoxicity on human cells, Human BJ cell lines of fibroblast origin which was derived from Human foreskin was maintained in Eagle's Minimum Essential Medium (EMEM) in addition with 10% Fetal Bovine Serum (FBS) in normal mammalian cell culture conditions. Cells were then treated with varying concentrations of poly-herbal extracts (0, 100, 350, 500, 750 and 1000 µg/ml) after 48h. MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was conducted to determine the percentage of viable cells.

3.12 Anti-Oxidant Assay

3.12.1 DPPH assay (1,1-diphenyl-2-picrylhydrazyl)

DPPH assay was performed for the sanitizer sample wherein 300 µl of sample and 700 µl of Tris-HCl buffer (pH 7.4) were poured in test tube followed by addition of DPPH solution and mixed by vigorous shaking for at least 15 seconds to obtain a mixed solution. The solution was then stored in dark environment at room temperature for 30 min. After that, absorbance of the solution was quantified at 517 nm. Blank used herein is a mixture of 1.3 ml of ethanol and 799 µl of Tris-HCl buffer. Experiments were conducted in triplicate. Inhibition ratio was calculated by below given formula:

$$\text{Inhibition ratio (\%)} = (\text{Ac-As})/\text{Ac} \times 100$$

3.13 Stability

To check the stability of the sample, they were stored at varying temperatures ranging from 40°C, 27°C and 37°C for a duration of 6 weeks.

4. RESULTS AND DISCUSSION

4.1 pH

After conducting the pH test, it was observed that the pH of the sanitizer was optimum which is exactly 6.6±1.01.

4.2 Viscosity

Viscosity of the sanitizer sample was performed wherein it was observed that viscosity of sanitizer sample was exactly 55c Pascal.

4.3 Spreadability

The spreadability of the sanitizer was conducted by spreading the sample on the plate followed by pressing to measure the area covered by sanitizer by spreading. Spreading co-efficient analyzed herein is 15.55. This clearly indicates that sanitizer easily spreads on the hands and thus penetrates within skin through pores thereby providing a complete protection by killing germs.

4.4 Extrudability

Sanitizer sample was subjected to extrudability test wherein gel extruded from the foil was measured. It was observed that, extrudability of the sample was 20.24 gm/cm². This may be attributed to higher viscosity i.e. higher the viscosity highest is the extrudability. Fig. 2 shows percentage result of spreadability and extrudability.

4.5 Anti-Microbial Assay

As per inhibition zone produced from sanitizer sample against various bacterial species, there has been significant activity shown by herbal sanitizer i.e. oregano sanitizer, thus stating that oregano herb possess immense anti-microbial property against pathogens. Oregano produced four zones of inhibition against *E. coli*, *S. aureus*, *P. aeruginosa*. It was observed that oregano produced the widest inhibition zone against *E. coli* with diameter of 3.4cm. Then the inhibition zone of 2.6 cm and 2.8 cm and 3.0 cm was produced by oregano for *S. aureus*, and *P. aeruginosa* respectively. After that the mean value was determined for all the three inhibition zones. It was observed that: mean value of zone of inhibition is 2.95 cm which is quite good to inhibit bacterial species. Table 1 shows zone of inhibition of different bacterial species by oregano herb.

Further, the prepared hand sanitizer showed maximum zone of inhibition against *S. aureus* which is 4.3cm followed by 4.0, 4.2, 3.8 for *E. coli*, and *P. aeruginosa* respectively. Average zone of inhibition of the sanitizer sample was 4.075 cm. This clearly indicates the anti-bacterial efficacy of the sanitizer sample. Thus, this proves that the

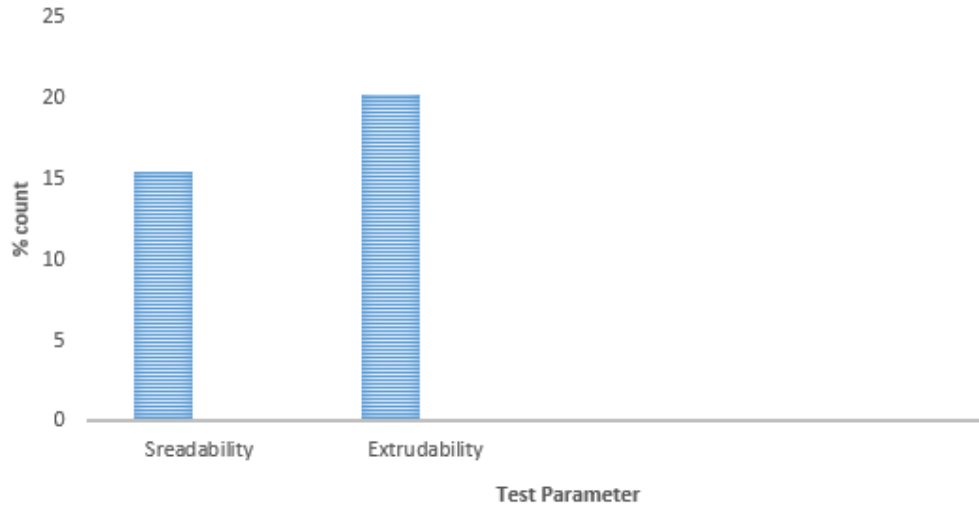


Fig. 2. Representation of percentage result of spreadability and extrudability, wherein spreadability refers to amount of formulation spreaded on the skin surface, spreadability co-efficient measured herein is 15.55 and extrudability refers to weight required to extrude the formulation from the bottle, extrudability co-efficient measured herein is 20.24

sanitizer possess potent anti-bacterial activity against these pathogens. Also the sanitizer may prove to be very effective due to anti-bacterial effect of the oregano. This may be attributed to anti-bacterial compound present in oregano i.e. carvacol which is highly effective against both human and animal viruses. Table 2 represents zone inhibition area of bacterial species by herbal sanitizer.

Table 1. Zone inhibition area of microbial species by oregano herb, Inhibition zone of *E. coli*, *S. aureus*, and *P. aeruginosa* observed herein is 3.4 cm, 2.6 cm, and 2.8 cm respectively

S.No.	Bacterial Species	Zone of inhibition
1.	<i>E. coli</i>	3.4 cm
2.	<i>S. aureus</i>	2.6 cm
3.	<i>P. aeruginosa</i>	2.8 cm

Table 2. Zone inhibition area of microbial species by herbal sanitizer, inhibition zone of *E. coil*, *S. aureus*, and *P. aeruginosa* is 4.0 cm, 4.3 cm, 4.2 cm

S.No.	Bacterial Species	Zone of inhibition
1.	<i>E. coli</i>	4.0 cm
2.	<i>S. aureus</i>	4.3 cm
3.	<i>P. aeruginosa</i>	4.2 cm

Anti-oxidant activity of the hand sanitizer was found to be 14.67% and that of oregano herb

was found to be 12.56%. Baobab used herein also possess anti-oxidant activity that was found to be 8.99% (Table 3). The existence of phenolic substances in formulation and herbal extracts confers stability to gel formulation i.e. sanitizer. Hand sanitizer application check on numerous volunteers during 5 different days randomly evoked an enthusiastic response. On the first day the formulation decreases the microbial effect for 20 minutes, and on second day, load has increased on 40 minutes. Fig. 3 shows effect of effect of sanitizer on different people in different days.

Table 3. Representation of anti-oxidant activity of hand sanitizer, oregano herb and baobab herb, wherein highest anti-oxidant activity is of hand sanitizer (14.67%)

S.No.	Bacterial Species	Percentage inhibition
1.	Hand sanitizer	14.67%
2.	Oregano herb	12.56%
3.	Baobab herb	8.99%

On third day it was seen that the microbial growth has eradicated upto 99.99% by continuous application of sanitizer on hands.

Thus it was clearly seen the effect of sanitizer on hands before and after the application of sanitizer on hand. It clearly indicated that prepared sanitizer possess immense potential to

kill the germs. As many cosmeceuticals contain materials that are harmful for skin. The newly formulated sanitizer contains extracts of natural herbs that are beneficial for skin and do not damage skin. Natural remedial steps are very safe and useful for hands.

4.6 Cytotoxicity Assay

Human BJ cells treated with varying concentrations of poly-herbal extracts for 48 h yielded negligible cytotoxicity (Fig. 4). Thereby, it can be presumed that the poly-herbal extracts being used as a sanitizer is safe to use.

The anti-bacterial sanitizer formulated in the present work was found to be effective as it inhibited bacterial species and also showed better extrudability and spreadability results with extrudable and spreadable co-efficient of 20.24 and 15.55 which is sufficient enough for utilization on commercial scale. All these results thus indicated that, the anti-bacterial hand sanitizer when applied on the skin may seriously serve as an effective means to eradicate pathogenic micro-organism residing over skin and may also protect the skin from any damage.

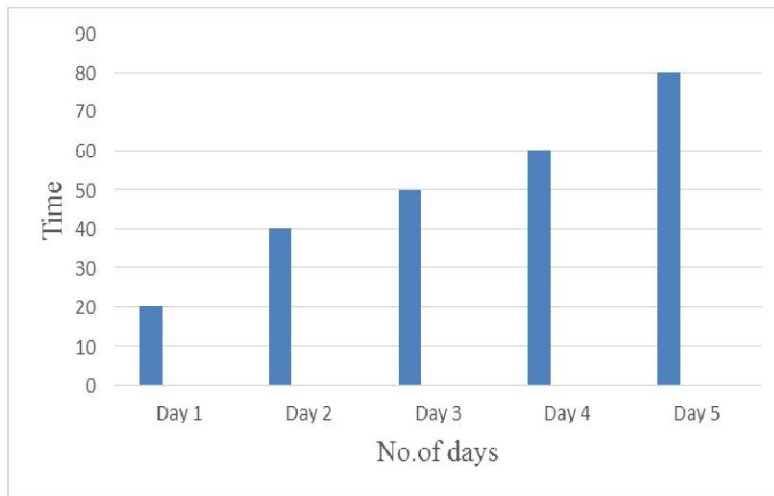


Fig. 3. Representation of effect of sanitizer on different subjects at different days. The graph represents increase in effect of sanitizer on different pathogenic species, as the days of the sanitizer increases its effect on pathogenic species also increases

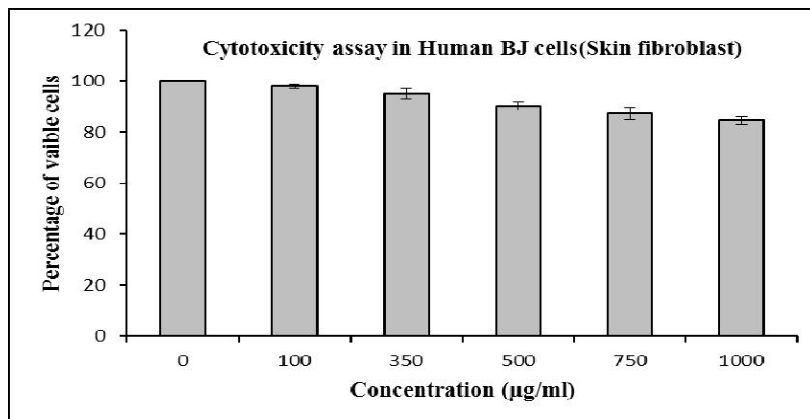


Fig. 4. Cytotoxicity assay on Human BJ cells. Human BJ cells of fibroblast origin were treated with an increasing concentrations of the poly-herbal extracts (0,100,350,500,750 and 1000 µg/ml) after 48 h. The poly-herbal extract showed negligible cytotoxicity even after 48 h

The combination of oregano herb and baobab herb used herein provides anti-bacterial and anti-inflammatory properties which may be very useful for people who utilizes it [14,15]. Moreover, glycerin used in the formulation serve as a humectant which may moisturize the skin and thus protect it from drying. Also the sanitizer is cost-effective as it involves utilization of herbs that are widely available [15-18]. This formulation in comparison to alcohol based sanitizers may seriously serve as a potent formulation in killing the pathogenic species in contact with the skin [18-21].

5. CONCLUSION

Maintaining proper hygiene of hands is an important practice that has to be practiced by everyone. Preventive techniques are formulated especially to restrict the extent of infectious agents spreading and thus prove a clean and hygienic environment to people living in surroundings. Thus to prevent from such severities there is a need to developed a formulation that instantly retards the microbial growth and thus prevent the spread of infection.

In conclusion it is said that sanitizer or herbal gel formulation is effective and has cleared all the analysis tests to prove its effectivity. The pH of gel formulation was found to be 6.6 ± 1.01 which is almost equivalent to skin pH thereby indicating that sanitizer does not cause any harmful effect to the skin. In viscosity test, it was observed that viscosity of gel formulation was found to be 55c Pascal which indicates that talks about viscous nature of the formulation. spreadability coefficient of the formulation was found to 15.55 which indicates that sanitizer gel easily adheres on the skin and covers the bacteria residing on the skin, if any. Extrudability of the sample was found to be 20.24 gm/cm². The sanitizer also showed immense potential in anti-microbial and anti-bacterial analysis i.e. it was able to inhibit growth of bacterial species which preferably includes *E. coli*, *S. aureus*, *P. aeruginosa*. Lastly, stability test conducted for sanitizer also proved that the sample was stable and can be used for long term as there was no change in physical appearance of the sanitizer sample.

Thus to conclude it is said that the prepared sanitizer possesses anti-bacterial and anti-inflammatory activities and thus is suitable for long term use without causing any harm to human skin. Thus it aids in preventing people from harmful diseases.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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