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# Bio-botanical products for human hygiene and sustainable environment: effectiveness of herbal hand sanitizer

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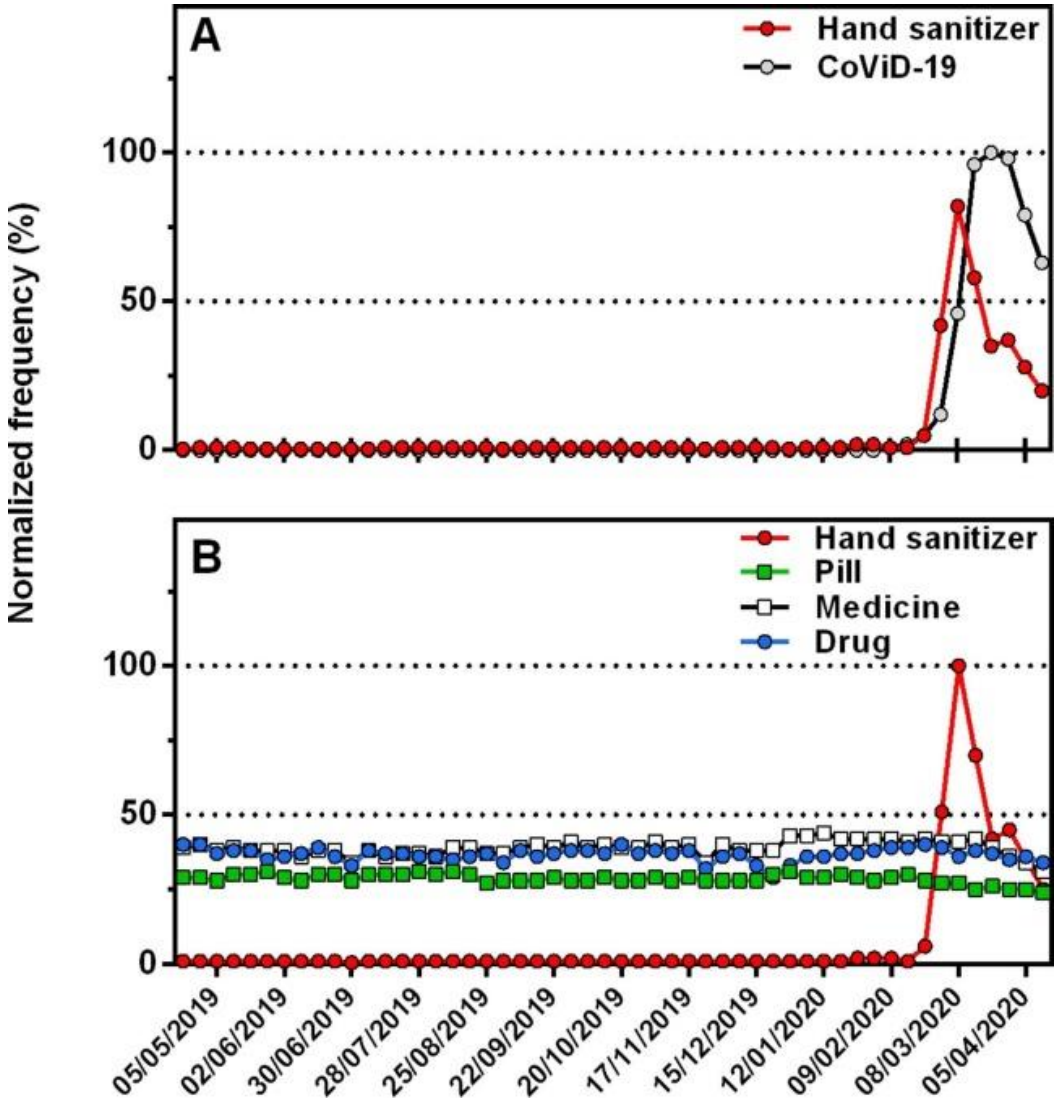
**Abstract**

Human hygiene is an important concept and practice in preventing, controlling, and reducing healthcare-acquired infections. The ideal way of achieving it is by proper hand washing and drying methods that break the chain of transmission of deadly pathogens from hands to other parts of the body. The usage of effective hand sanitizer reduces nosocomial infections occurring due to various bacteria. Most healthcare products in the category comprise harmful chemicals and polymer derivatives from petroleum. The long-term use of sanitizers containing chemical antimicrobial agents may pose the hazards like the development of resistant microbes, adverse effects on the human immune system, and skin infections. Customary additives used for fragrance like aldehydes and phthalates can cause disruption and imbalance in endocrine secretions. During pre- and post-COVID times, the need for more hand sanitizer use around the world made it important and opportune to develop a recipe for hand sanitizers that is sustainable and free of derivatives from fossil fuels. One argument for reducing the usage of fossil fuels and consequently, the amount of greenhouse gases released into the atmosphere to stop climate change is sustainability. Herbal hand sanitizer solutions produced from plant extracts and natural oils seem to be the perfect answer. These healthcare products are free from harsh chemicals and are termed natural disinfectants. These herbal products do not provoke an allergic reaction and have no negative side effects, are biodegradable, skin-friendly, and cause less irritation and dryness.
The goal of this study is to make a herbal hand sanitizer with leaves extracts of Ocimum sanctum (Tulsi) and Azadirachta indica (Neem) and Zingiber officinale (Ginger) and Citrus limon (Lemon). The study tests are concentrated on testing its antimicrobial efficacy and hand safety against E. coli, and S. aureus.
The research suggests and supports the use of natural herbs in the formulation for a better tomorrow.
**Keywords:** - Sustainability, pathogens, antimicrobial, and hand sanitizers

## 1. Introduction

Skin hygiene, particularly of hands, is considered to be one of the basic mechanisms to prevent the risk of transmission of infectious agents. Effective hand sanitizers reduce vulnerability to bacterial and fungal infections, as well as enveloped viruses. [1-2]. Hand sanitizer is a disinfectant and complements hand washing with soap and water [3-4]. Most of the available hand rubs used as sanitizers are comprised of isopropyl alcohols, H2O2, and ethanol in different combinations. Misuse of these provisions may lead to toxicity in human well beings and to the environment. Alcohol-based hand sanitizers are more effective at killing microorganisms than soap.[5-6] All hand sanitizer products require a designation known as the "National Drug Code" in the United States.[7] Furthermore, the widespread use of these anti-bacterial hand sanitizers has resulted in the accumulation of a variety of toxic emerging contaminants like triclocarban, triclosan, hydroxychloroquine, etc. in treated sludge and discharged wastewater effluent, posing significant threats to ecosystems. Besides being toxic and harmful to our skin, these are flammable and hazardous to our environment. It has also been suggested that frequent usage of hand sanitizers may raise the risk of developing anti-microbial resistance and other viral

infections. A Google search reveals the general public's newfound interest in hand sanitizers. The graph below depicts the search trend for the term "hand sanitizer." The volume of Google searches on this issue remained quite consistent until February 2020, when a large spike of a 100-fold rise in interest in "hand sanitizer" arose. This finding was associated with a rise in searches for the phrase "CoViD-19," implying an obvious association between the two terms. To put these figures into context, a search for "hand sanitizer" was compared to searches for the words "pill", "drug", and "medicine" in the graph. According to the findings, prior to the emergence of the new coronavirus, these terms were regularly googled 25-50 times more frequently than "hand sanitizers," while during the peak of the pandemic (March 2020), "hand sanitizers" was searched nearly twice as frequently as those keywords. Although the astonishing peak in "hand sanitizer" searches is moderately tapering off, it is expected that interest in this topic will remain much higher than pre-pandemic levels because, until a vaccine against CoViD-19 is available, hand sanitization will remain at the forefront of infection prevention measures. Furthermore, it is reasonable to assume that the current public knowledge of the need for hand disinfection will be integrated and will become an intrinsic part of people's lives.[8]




Today, there are numerous amounts of hand sanitizer formulations produced and marketed by various popular pharmaceutical companies. Some of them are listed:

1) Sterellium

2) Dettol

3) Savlon

4) Lifebuoy

5) Multani

6) Godrej

7) Dabur

8) Corvil

9) Trust

10) Cipla

Traditionally, the crude extracts of different parts of medical plants, including root, stem, flower, fruit, and twigs were widely used for treatments of some human diseases. Flavonoids, alkaloids, tannins, and terpenoids are some of the phytochemicals found in medicinal plants that have antibacterial and antioxidant activities [9].
Numerous studies have been conducted on certain plant species 'antibacterial properties for instance, the raw extracts of several herbs, such as those from cinnamon, garlic, basil, curry, ginger, sage, and mustard family have antibacterial characteristics that are effective against a variety of Gram–positive and Gram-negative bacteria. [10-13].
In addition, it has been reported that the extracts from Chinese basil and lemon [14- 15] can effectively reduce the growth of Escherichia coli and other bacteria during the storage of meat juices, and milk.[16] Moreover, reported that neem oil extract could decrease the growth of C.albicans and Pseudomonas aeruginosa.
Thus, it becomes vital and timely to develop a formula for hand sanitizers that is sustainable and free of derivatives from fossil fuels in light of the global focus on increasing the usage of hand sanitizers [17].
This leads us to the objective to synthesize a herbal hand sanitizer from commonly available plant extracts and fundamentally assess its efficacy. Neem [18-21], Tulsi, and Lemon [22] extracts are an important source of compounds having anti-microbial, anti-oxidant, anti-malarial, anti-fungal, anti-inflammatory, and anti-viral properties.

## 2. Criteria for Selection of Microorganisms for the Experiment

- Ability to grow in culture
- Genetic stability
- Ability to efficiently produce colonies in the short time period
- Limited need for additional growth factors
- Utilization of low-cost and readily available Carbon sources
- Non-Pathogenicity
- Amendable to the culture techniques
- Simpler Purification

## 3. Reasons for Selecting E. Coli and S. Aureus as Model Organisms

An organism suitable for studying a specific trait, disease, or phenomenon, due to its short generation time, characterized genome, or similarity to humans is known as Model Organism.
Escherichia coli has been a key model organism from the beginning of molecular genetics research and continues to play a vital role to this day. E. coli investigations have contributed significantly to our understanding of key principles in molecular biology including replication, gene expression, and protein synthesis. When compared to our genome (almost 3 billion bp), the E. coli genome is comparatively tiny (4.5 to 5.5 Mbp) and simple. E. coli is now being studied for its ability to act as a vector, a host for genetic elements, and the manufacture of proteins of interest.[23] E. coli, is a Gram-negative, facultatively anaerobic, rod-shaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms. The harmless strains can benefit their hosts by producing vitamin K2 (which helps blood to clot) [24] and preventing colonization of the intestine with pathogenic bacteria, having mutualistic relationships.
E. coli and other facultative anaerobes account for around 0.1% of the gut microbiota.
E. coli is the best-studied prokaryotic model organism and a vital species in biotechnology and microbiology. It just takes 20 minutes to recreate under ideal conditions.
Our second model organism, Staphylococcus aureus is a Gram-positive, round-shaped bacterium, frequently found in the upper respiratory tract and on the skin. S. aureus does not form spores. It appears as staphylococci and has large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plates. S. aureus reproduces asexually by binary fission. S. aureus, a part of the normal microbiota, can exist in humans in the skin, gut mucosa, and upper respiratory tract.

## 4. Material and Methods

### Characteristics of components of Herbal Sanitizer:

1. Lemon extract - Strong antiviral properties.

2. Neem + Tulsi extract – Antibacterial properties.
3. Ginger extract – Antimicrobial properties.
4. Isopropyl alcohol – Acts as a diluent, a good antibacterial agent
5. Hydrogen peroxide – Kills germs after drying.
6. Glycerol – Lowers the evaporation of alcohol, a thickening agent.
7. Polyethylene glycol – pH adjuster, emulsifier.
8. Perfume – Fragrance.

| S.NO. | INGREDIENTS | CONCENTRATION | QUANTITY |
|---|---|---|---|
| 1. | Lemon extract | 10% | 5ml |
| 2. | Neem + Tulsi extract | 10% | 3+2=5ml |
| 3. | Ginger extract | 10% | 5ml |
| 4. | Isopropyl alcohol | 75% | 75ml |
| 5. | Hydrogen peroxide | 0.125% | 2ml |
| 6. | Glycerol | 2.30% | 5ml |
| 7. | Polyethylene glycol | 1,25% | 2ml |
| 8. | Perfume | 10% | 1ml |

**5. Methodology**

In this work, four plant elements in dried forms were selected based on their traditional usage as folk medicine, like dried Citrus limon (Lemon), Azadirachata indica (Neem), Ocimum sanctum (Tulsi,) and Zingiber officinale (Ginger). Then grind it well to make a homogenous powder. [25].
For Soxhlet extraction, 20g of powder of each tested plant material was taken in a filter paper, covered well, and inserted into the thimble of the apparatus. The solvent used for this purpose is ethanol.[26]
Extracts were taken in a round-bottom flask and stored in a cool place for 24 hours. Isopropyl alcohol, hydrogen peroxide, and glycerol were embraced in a beaker using a mechanical stirrer. Now to the solution made, the prepared extracts and Polyethylene glycol were comprehended. After that, perfume was added and mixed well.

**5.1 Physical and Chemical Analysis**

The pH of the spray was tested by a pH–meter. Color and homogeneity were observed visually and the odour was also recorded.
The laboratory-formulated spray was tested for the percentage of ethanol as an active ingredient

| S.NO. | PARAMETER | ANALYSIS |
|---|---|---|
| 1. | pH | 6.40 |
| 2. | Color | Light Green |
| 3. | Odour | Lemon fragrance |
| 4. | Homogeneity | Homogenous |
| 5. | Appearance | Clear spray |

### 5.2 Microbiological Analysis

Antibacterial activity by Agar well diffusion Assay Bacterial strains used:
Escherichia coli (Gram negative) ATCC10531
Staphylococcus aureus (Gram positive) ATCC6538
The cultures were maintained on nutrient media agar. For the preparation of inoculums, isolated colonies of each bacterial culture were selected from 18-24 hours incubated agar plates and inoculated in nutrient agar.
0.1ml of each bacterial culture suspension was evenly spread with a sterile inoculating loop. 6 mm wells were cut with a sterile gel borer and 10-50 µl of formulated spray and commercial brand sanitizers were added to the wells. All plates were allowed to settle for 5 min and incubated at 370C for 18-24 hours.
After incubation, inhibition zones surrounding the wells created by each sanitizing gel were recorded on an automatic colony counter in inhibition zone mode.

## 6. Determination of Minimum Inhibitory Concentration

MIC is the lowest concentration of an antibacterial agent expressed in mg/L or (µg/mL) which, under strictly controlled in vitro conditions, completely prevents visible growth of the test strain of an organism.

### 6.1 Methods

#### 6.1.1 Broth dilution assay

The MIC is determined by examining tubes containing the microbe and a dilution series of antimicrobial agents for turbidity.
This assay requires three major reagents: medium, an antimicrobial agent, and the bacterium being examined. Because of its capacity to support the growth of most pathogens, Mueller Hinton Broth is commonly used. The media can be changed and altered depending on the pathogen and antibiotics being studied. By mixing stock antimicrobial with medium, the antimicrobial concentration is adjusted to the correct concentration. To create a gradient, the modified antimicrobial is serially diluted into numerous tubes (or wells). The dilution rate can be modified based on the breakpoint. The microbe or inoculating agent must come from the same colony-forming unit and be at the appropriate concentration. This can be changed by adjusting the incubation period and dilution. Microbes are inoculated into the tubes (or plate) and cultured for 16-20 hours. Turbidity is commonly used to calculate the MIC. [27]

#### 6.1.2 E-Test

E-Tests are an alternative approach for determining the minimum inhibitory doses of a wide variety of antimicrobial drugs against various species. They've been utilized extensively in microbiology labs all around the world. E-Tests is a non-porous plastic reagent strip that has a predetermined gradient of antibiotics that covers a continuous concentration range.
MIC was determined by inoculating 0.1 ml of sample from each tube onto Nutrient agar plates by spread plate technique. The MIC was determined as the lowest concentration of hand sanitizer gel that totally eradicates the investigated bacterial strains after plates were incubated at 370C for 18 to 24 hours.[28]
The absence of colony formation on these plates indicates that the sanitizer concentration had killed the bacterial cells and they were no longer viable to grow on nutritive media without antibiotics.[29]

## 7. Results

The herbal sanitizer was produced to use as a preventive measure to avoid infection naturally, and the best way to prevent illness is to avoid being exposed to the virus and wash hands with soap and water and hand sanitizer that contains at least 60% alcohol.

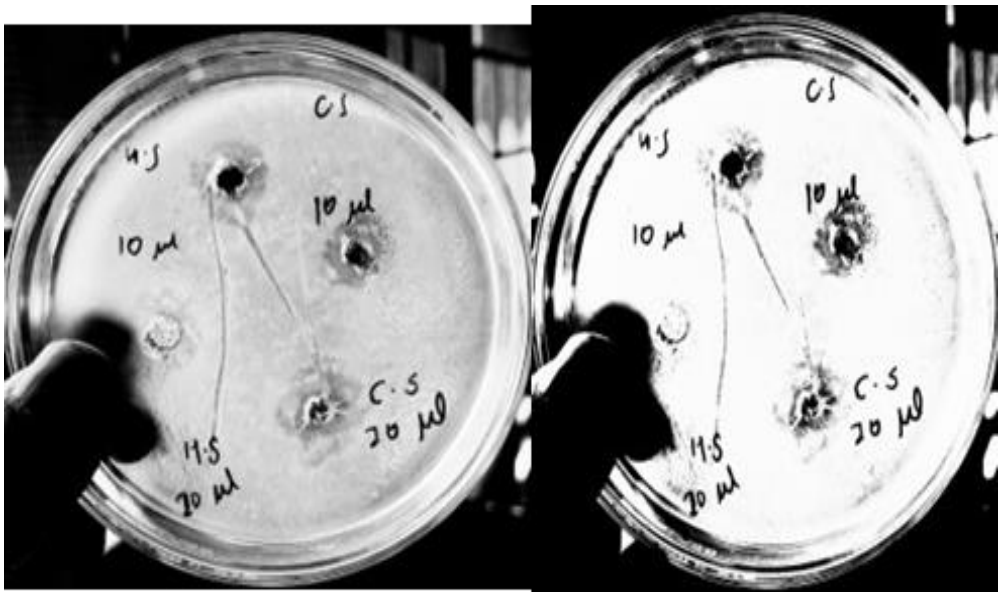
### 7.1 Verbalizing the Results

The ZOI of Herbal sanitizer against E. coli and S. aureus are 20.2 mm and 19.4 mm respectively. Whereas the Commercial sanitizer has a ZOI of 17.5mm and 18.1mm respectively against E. coli and S. aureus.

### 7.2 Zone of Growth Inhibition of Sanitizers Against the Test Microorganisms

| Conc. of Drug (g/ml) Organisms | Zone of Inhibition (diameter mm) | | | | |
|---|---|---|---|---|---|
| | **800** | **400** | **200** | **Control** | **STD** |
| E. coli [Herbal Sanitizer] | 19+-2.0 | 20+-1.2 | 20+-0.5 | 0 | 20+-1.1 |
| S. aureus [Herbal Sanitizer] | 17+-1.8 | 19+-2.0 | 18+-1.9 | 0 | 19+-1.2 |
| E. coli [Commercial Sanitizer] | 17+-1.4 | 16+-1.8 | 17+-1.5 | 0 | 17+-1.3 |
| S. aureus [Commercial Sanitizer] | 18+-1.1 | 18+-1.3 | 17+-1.8 | 0 | 18+-1.1 |

| **Sanitizers** | **Test Organism** | **Zone of Growth Inhibition (mm)** |
|---|---|---|
| Herbal Sanitizer | Escherichia coli | 20.2mm |
| | Staphylococcus aureus | 19.4mm |
| Commercial Sanitizer | Escherichia coli | 17.5mm |
| | Staphylococcus aureus | 18.1mm |




Inhibition zone on E. coli Inhibition zone on S. aureus

Conferring to the MICs, Herbal sanitizer has 15% and 16.6% MIC against E. coli and S. aureus respectively. Whereas the Commercial one has a MIC of 17.4% and 19.5% against E. coli and S. aureus. Higher the MIC, the more antibacterial activity of sanitizers.

**7.3 Minimum Inhibitory Concentrations (MICs) of Sanitizers Against Test Microorganisms**

To determine the value of MICs of Herbal and Commercial hand sanitizers, we use the following formula :

MIC % = Powder weight of antibiotics (mg) × solvent volume of

sanitizer (ml) × concentration of solvent (gm/l) ÷ antibiotic potency (g/mg)

MIC% of E. coli (Herbal Sanitizer) = 100mg * 10ml * 75% / 50 = 15.01%

MIC% of S. aureus (Herbal Sanitizer) = 100mg * 10ml * 75% / 45 = 16.61%

MIC% of E. coli (Commercial Sanitizer) = 100mg * 10ml * 75% / 43 = 17.40%

MIC% of S. aureus (Commercial Sanitizer) = 100mg * 10ml * 75% / 38 = 19.50%

| **Hand Sanitizer** | **Test Organism** | **Concentration %** |
|---|---|---|
| Herbal Sanitizer | Escherichia coli | 15% |
| | Staphylococcus aureus | 16.6% |
| Commercial Sanitizer | Escherichia coli | 17.4% |
| | Staphylococcus aureus | 19.5% |

## 8. Discussion

The prepared formulation of herbal hand sanitizer showed significant results against two bacterial species. In comparison to the accepted reference, it was discovered that the importance was greater. The composition (Ocimum tenuiflorum, Azadirachata indica, Citrus limon, and Zingiber officinale) has been attributed with properties like free radical scavenging, anti-helminthic, antimicrobial [30] anti-inflammatory and analgesic, etc. More concentrations may be needed to get a broad-spectrum activity of the test drug. The herbal sanitizer has excellent, rapid (within seconds) germicidal activity against vegetative bacteria, fungi, and many viruses, and antimicrobial activity is based on the protein denaturation of microorganisms. Alcohol-based sanitizers are highly effective against mycobacteria (the bacteria most resistant to the disinfection process) and multidrug-resistant pathogens. They are almost 100 times more effective than any type of hand washing at preventing viruses. Sanitizers offer numerous advantages over non-alcoholic hand disinfectants, rubbing sanitizers onto both hands & until it completely evaporates, usually requires only 15 to 30 seconds. Whereas vigorous friction, rinsing with water, and drying with a towel are not needed like hand disinfectants or soaps.[31]

## 9. Conclusion

The most frequent way that germs are conveyed to patients is through their hands, yet good hand cleanliness can reduce the risk of healthcare-associated infections and the spread of antibiotic resistance. When providing patient care, alcohol-based hand sanitizers are recommended because of their effectiveness and simplicity of use. It may be concluded that with the exception of Ps. aeruginosa and S. cerevisiae, herbal hand sanitizer significantly inhibits the growth of the specified pathogens. As a result, there is tremendous potential for developing the use of herbal antimicrobial treatments as a strategy to manage multidrug-resistant bacteria and keep track of their hand-borne transmission from one location to another. Thus, it follows that the extraction yield is effectively increased by the Soxhlet method. Alcoholic extracts from particular plants that have antibacterial characteristics may prevent the growth of test microorganisms. In cells exposed to a herbal sanitizer, a breakdown of the cell wall was seen, pointing to a potential antibacterial action mechanism. These results suggest that the plant extracts utilized in this study may be utilized as natural preservatives in sanitizers to prevent the growth of pathogenic germs or to remove them.

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