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Research Article

Formulation and Evaluation of Herbal Sanitizers and Comparative Assessment of Antimicrobial Efficacy of Herbal and Commercially Available Hand Sanitizers

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Abstract

The present research was aimed to formulate the herbal sanitizer, from Neem, Lemon juice and Tulasi. Phytochemical properties of neem extract, lemon juice, juice and Tulasi were analysed. Their zone of inhibition was checked against standard culture. Zone of inhibition of all the formulations was found. Formulated sanitizer was compared with sterillium sanitizer and it was found that sterillium was effective against E. coli organism. Hand sanitizers were effective against the test organism. The antimicrobial effectiveness was assessed by measuring the zone of inhibition against the test organism. Maximum inhibition (in mm) was seen in group A (Sterillium), *i.e.,* 10.5 ± 0.707 and minimum in group B (Tulasi), *i.e.,* .0 ± 0.707. The difference in the values of the different sanitizers was statistically significant. From the present study, it was concluded that sterillium was most effective and sanitizers prepared from herbal extracts was close and comparable.

Keywords: Neem, Lemon, Tulasi, Hand Sanitizers, E. coli, Sterillium

Abbreviations: IUPAC: International Union of Pure and Applied Chemistry; ml: Milli litre; mm: Milli metre; Fig: Figure; qs: Quantity sufficient; g: Gram; min: Minute; w/v: Weight/volume; E. coli: Escherichia coli; *Viz*.,: Namely or which is

INTRODUCTION

Hand sanitizer (also known as hand antiseptic, hand disinfectant, hand rub, or hand rub) is a liquid, gel or foam generally used to kill many viruses/bacteria/microorganisms on the hands. In most settings, hand washing with soap and water is generally preferred. Hand sanitizer is less effective at killing certain kinds of germs, such as nor virus and Clostridium difficult, and unlike hand washing, it cannot physically remove harmful chemicals (Sharma, 2000). People may incorrectly wipe off hand sanitizer before it has

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dried, and some are less effective because their alcohol concentrations are too low (Beckett, et al., 2002).

Alcohol based hand sanitizer that is at least 60% (w/v) alcohol in water (specifically, ethanol or isopropyl alcohol/isopropanol (rubbing alcohol)) is recommended by the Centers for Disease Control and Prevention (CDC), United States, but only if soap and water are not available nosocomial infections area unit people who originate or occur in a very hospital or health care setting. The incidence of medical building infections is alarmingly increasing and has emerged as a crucial issue in medical aid outcome; leading to extended hospitalization, substantial morbidity and mortality, and excessive value.

Use of hand sanitizers has gained quality within the recent past years that has light emitting diode to the event, production of many hand sanitizers by numerous corporations. With immense amounts spent for advertisements and false claims created by makers, clinicians and customary man don't have any clue concerning the effectiveness of those commercially out there hand sanitizers. This study was distributed to assess and compare the antimicrobial efficacy of herbal hand sanitizers.

Most of the out there hand rubs used as sanitizers composed of isopropyl alcohols, H_2O_2 and fermentation alcohol in several mixtures. Misuse of those provisions might ends up in the toxicity in human well beings and to atmosphere (Willard, et al., 1986).

Adaptation of different preparations of hand sanitizers supported natural and plant resources are often the doable answer to induce ride away toxicity downside. Throughout Covid-19, reported meditative plants with glorious anti-viral and medical aid properties are often used as alternatives of alcohol based mostly hand rubs. Till date, varied plants are reported and revealed with broad spectrum anti-viral properties (**Figure 1**).

Figure 1. Face mask wearing rate predicts country's COVID-19 death rates.

Sterile cotton swab sticks were used to take swabs from both hands and swabs were inoculated on the part of the petri dishes marked before application (before) in both aerobic and anaerobic media. Approximately 0.5 ml of pure hands herbal hand sanitizer was squeezed out on the palms of the subjects and they were asked to rub the gel thoroughly on the palms, back of the hands, fingernails until the hands became dry and inoculation was done on the respective dishes, in the part marked. The same procedure was repeated for seven consecutive days on all subjects (Bassett, et al., 1986).

For evaluating the efficacy and safety of Pure Hands Herbal Hand Sanitizer on inanimate objects Preparation similar procedure was followed. All detected isolates *Escherichia coli*, *Proteus Mirabilis, Shigella* someone, *Staphylococcus aurous* and *Staphylococcus epidermis* were eliminated, from the hands of all volunteers over the period of 7 days and also from surface of inanimate objects. Pure hands herbal hand sanitizer was found to be effective, safe, less and likely to cause adverse reactions skin and saves time and human resources. At present, washing hands with appropriate soap followed by applying hand antiseptics are two important hand hygiene methods in clinical practice. Hand sanitizers significantly increase the chance of maintaining the hands clean and aseptic. Traditionally, microbes habitation on hands is divided into resident and transient floras.

Involved resident floras are commonly *Staphylococcus aureus, Staphylococcus epidermis*, and *Enterococcus faecalis* that colonize the deeper skin layers and are resistant to mechanical removal. The transient floras consist of *Escherichia aureus*,

Escherichia coli, and *Pseudomonas aeruginosa* that colonize the superficial layers of skin in a short period of time. Therefore, we selected these organisms to determine their susceptibility to different hand sanitizers tested in this study. Scientific studies have shown that after hand washing, as many as 80% of individuals retain some pathogenic bacteria on their hands. Hand washing removes body's own fatty acids from the skin, which may result in cracked skin that provides an entry portal for pathogens. To overcome the limitations of plain hand washing, hand sanitizers were introduced claiming to be effective against those pathogenic micro-organisms as well as to improve skin condition due to the addition of emollients in it (Hohat Willard, et al., 1999).

Hand sanitizers were also effective in reducing gastrointestinal illnesses in households, respiratory tract infections, and skin infections, in curbing absentee rates in elementary schools, and in reducing illnesses in university dormitories. Furthermore, to reduce infections in healthcare settings, alcohol based hand sanitizers are recommended as a component of hand hygiene. Many hand sanitizers are available in the market with varying degree of effectiveness that is registered in the national agency for food and drugs administration and control. Moreover, in outreach programs, screening procedures in day to day practice, water scarcity areas, and bed side and chair-side clinical examination, hand sanitizers could be an alternative to achieve asepsis (Gurdeep Chatwal, et al., 2008).

However, clinicians and common man face the dilemma while choosing the best among the lot. Some products marketed to the public as antimicrobial hand sanitizers are not effective in reducing bacterial counts on hands. In fact, despite a label claim of reducing "germs and harmful bacteria" by 99.9%, some studies have observed an apparent increase in the concentration of bacteria in handprints impressed on agar plates after cleansing. Hence, there still exists a need for verification of these claims by the regulatory authorities for the enforcement of good-quality measures (David, et al., 1994).

To overcome this ambiguity, the present study was carried out to assess the antimicrobial effectiveness of four different hand sanitizers against the test organisms. In response to the coronavirus disease 2019 (COVID-19) pandemic, hand hygiene has taken on a prominent role in efforts to mitigate SARS-CoV-2 transmission and infection, which has led to a radical increase in the number and types of hand sanitizers manufactured to meet public demand.

To our knowledge, no studies have evaluated or compared the antimicrobial performance of hand sanitizers that are being produced under COVID-19 emergency authorization. Tests of 46 commercially available hand sanitizers purchased from national chain brick and mortar stores revealed considerable variability in their antibacterial performance toward two bacterial pathogens of immediate health care concern, *S. aureus* and *E. coli*. Expanded testing of a subset of hand sanitizers revealed no direct correlation between antibacterial performance of individual sanitizers and their activity toward SARS-CoV-2. These results indicate that as the pandemic subsides, there will be a need to validate the antimicrobial efficacy of sanitizers being produced, it is well recognized that hand hygiene is essential to reducing microbial burden, transmission, and infection. The density and species of bacteria that colonize the hands of individuals are highly variable and can be influenced by a number of factors including age, sex, ethnicity, and profession (reviewed in reference. Health care workers have been of particular interest, as they may provide a reservoir for the circulation and transmission of drug resistant bacteria within the hospital setting. Indeed, studies have revealed that 10.5% to 78.3% of health care workers are colonized with up to 107 per hand of the bacterial pathogen *Staphylococcus aureus* and may be a source of nosocomial *S. aureus* infections (reviewed in reference. Fortunately, conventional hand washing using water, soap, and friction is an effective means of reducing microbial burden, which when combined with other infection control practices (*i.e.,* glove usage, compliance, and education) has significantly reduced microbial transmission, hospital acquired infections, reduced gastrointestinal and respiratory illness, and improved overall health (Chatten, et al., 1996).

In situations in which an individual does not have access to soap and water, the Centers for Disease Control and prevention (CDC) and World Health Organization (WHO) have recommended the use of alcohol rubs (also known as hand sanitizers) comprised of either 80% ethanol or 75% isopropyl alcohol to reduce microbial burden. Alcohol based sanitizers have proven to deliver rapid bactericidal activity toward gram-positive and gram-negative bacterial pathogens as well as excellent veridical activity toward both enveloped and no enveloped viruses of immediate health care concern, including influenza A virus, Severe Acute Respiratory Syndrome Corona Virus (SARSCoV).

Middle Eastern Respiratory Syndrome (MERS) virus, Zika virus, Ebola virus, and SARS coronavirus 2 (SARS-CoV-2). In response to the SARS-CoV-2 pandemic, there has been increased recognition of the importance of hand hygiene, which has led to an overwhelming increase in hand sanitizer demand. To meet this need, the FDA has released guidance regarding the production of hand sanitizers and temporarily relaxed production restrictions provided that manufacturers follow strict guidelines. This has resulted in an increase in number of products available for public use. Although data are sparse, previous reports have indicated there can be substantial variability in the antimicrobial performance of individual commercially available hand sanitizers both in *in vitro* testing and when

applied to the hands of individuals. Yet to our knowledge, there has never been a comparison of the antibacterial properties of a large collection of hand sanitizers; such a study may be timely given the recent onslaught of new sanitizers available on the market. Moreover, to our knowledge, there are not established *in vitro* antimicrobial testing guidelines for sanitizers (Day, et al., 1986).

Accordingly, herein, we used conventional antibacterial assays to compare the antibacterial performance of 46 commercially available hand sanitizers toward *Escherichia coli* and *Staphylococcus aureus*, prototypical gram negative and gram positive pathogens that are well recognized to contaminate skin surfaces (**Figure 2**).

Figure 2. Cartoon cute coronavirus, covid-19, woman and alcohol gel wash hands.

We also performed standard antiviral assays to evaluate activity of a subset of sanitizers that were either highly active or had weak activity against *S. aureus* and/or *E. coli* toward SARS- CoV-2. Results revealed that there are significant differences in the antibacterial properties of hand sanitizers in the assays used here and also a poor correlation between the antibacterial and antiviral activity of the sanitizers evaluated. From these perspectives, as the current pandemic and corresponding demand for hand rubs subsides, it may be wise to implement formal requirements for antimicrobial efficacy testing of hand sanitizers that have been introduced to the market under emergency COVID-19 authorization to better understand their variability in antibacterial/antiviral efficacy (Ravi Sankar, et al., 2001).

Hand hygiene is now regarded as one of the most important element of infection control activities. In the wake of the growing burden of health care associated infections, the increasing severity of illness and complexity of treatment, superimposed by multi-drug resistant pathogen infections, health care practitioners are reversing back to the basics of infection preventions by simple measures like hand hygiene. This is because enough scientific evidence supports the observation that if properly implemented, hand hygiene alone can significantly reduce the risk of cross-transmission of infection in healthcare facilities.

Washing hands with soap and water is the best way to reduce the number of microorganisms (germs) on hands. 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. 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. Cleansing products powered by natural essences like witch hazel, bitter orange peel extract, thyme, lavender (a popular one), and even organic alcohol are on the rise. Medicinal plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines (Michael, et al., 2004).

Pharmacological studies have accepted the value of medicinal plants as potential source of bioactive compounds. Phytochemicals are secondary metabolites, which are produced by medicinal plant.

So, different studies and sanitizer formulation were carried out. Sanitizer with complex chemicals as well herbal sanitizer was formulated and their efficacy were checked against various groups of organisms. Like, in this research three herbal sanitizers were formulated from neem and lemon. As, Neem *Azadirachta indica* extract is an important source of compounds having anti-microbial, anti-oxidant, anti-tumour, anti-malarial, anti-fungal, anti-inflammatory and anti-viral properties and Lemon *Citrus lemon* is an important medicinal plant, antibacterial potential in crude extracts of different parts (*viz.,* leaves, stem, root, fruit and flower) of lemon against clinically significant bacterial strains has been reported. Hence in this studies neem leaves and lemon juice is been use their antimicrobial properties were studied and accordingly sanitizers were formulated (David Harvey, et al., 1997) (**Figure 3**).

Figure 3. Sanitizer with complex chemicals as well herbal sanitizer.

LITERATURE REVIEW

M Chojnacki, et al., used zone of growth inhibition and kill curve assays to evaluate the antibacterial activity of 46 commercially available hand sanitizers obtained from national chain big box stores, gas stations, pharmacies, and boutiques against prototypical gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacterial pathogens. The results revealed significant variation in the efficacy of many sanitizers tested as a spray on medical devices or as a floor cleaner.

Rutuja Sunil Patanka, et al., has formulated the herbal sanitizer from neem and lemon juice premised sanitizer. The phytochemical and antioxidant properties of neem extract was investigated. Their MIC was compared to standard culture and hand isolates from lab assistance. A MIC of neem extract and lemon juice was discovered. Three different sanitizers were developed: lemon, lemon neem, and neem sanitizer. When formulated sanitizer was compared to sterillium sanitizer, it was discovered that sterillium was only effective against standard organisms and not against hand isolates, whereas formulated sanitizer was effective against both. As a hand sanitizer, it can be used as a spray on medical devices or as a floor cleaner.

Rahul Guptha assessed and compared the antimicrobial efficacy of different hand sanitizers. His *in vitro* studies revealed that lemon and aloe vera sanitizers possessed anti-microbial effect against both gram positive and gram-negative bacteria (Anushagandi, et al., 2002).

Vaibhav Rajendra Suryawanshi, et al., has prepared and characterized different alcohol based hand sanitizers using eucalyptus oil as an active pharmaceutical ingredient. Among five formulations, F3 is selected based upon its anti-bacterial activity. Then, F3 formulation is subjected to different evaluation parameters like odour, colour, clarity test, pH and skin irritancy characteristics.

Aim of the research work

Aim of the present study:

- The aim of present work is to formulate and evaluate herbal hand sanitizers.
- To compare the anti-microbial efficacy with the commercially available hand sanitizers

Objective:

- To prepare the extracts from Neem, Tulasi leaves and juice from lemon.
- To perform phytochemical analysis.
- To prepare the hand sanitizers from the above extracts.
- To evaluate the prepared hand sanitizers.
- To study anti-microbial activity of extract against standard organism.
- To compare the anti-microbial activity with commercially available sanitizers.

Plan of work:

The plan of the proposed work includes the following steps:

- Literature review of the selected plants.
- Preparation of extracts from neem, lemon, tulasi.
- Phytochemical analysis of prepared extracts
- Formulation of hand sanitizers with

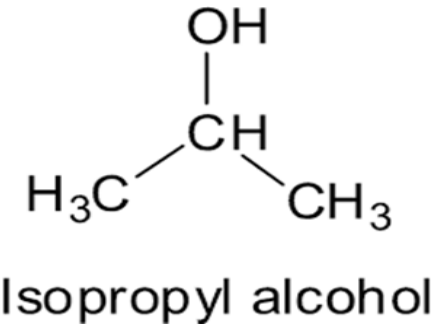
prepared extracts.

- Evaluation of prepared herbal sanitizers.
- Determination of antimicrobial efficacy of prepared sanitizers.
- Comparison of antimicrobial efficacy with commercially available hand sanitizer (Sterillium sanitizer).

MATERIALS

Isopropyl alcohol:

- Isopropyl alcohol (IUPAC name propan-2-ol and also called isopropanol or 2- propanol) is a colourless, flammable chemical compound with a strong odour.
- Isopropyl Alcohol is an isomer of propyl alcohol with antibacterial properties (**Table 1**).



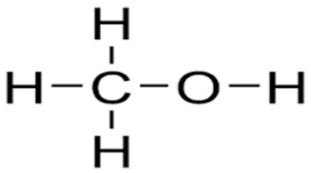
Isopropyl alcohol

Table 1. Materials used to prepare Sanitizers.

S.No.	Materials
1	Isopropyl alcohol
2	Methanol
3	Glycerol
4	Rose water
5	Neem extract
6	Lemon extract
7	Tulasi extract

Methanol:

- It is also known as Methyl alcohol, with the formula CH_3OH. It is light, volatile, colourless, flammable liquid with distinctive alcoholic odour.
- Methanol used as an industrial solvent.



Glycerol :

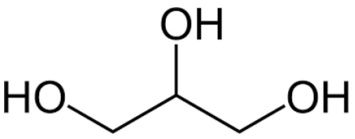
It is a simple polyol compound.

It is

- colourless
- odourless
- viscous liquid

It is sweet tasting and non-toxic.

- Glycerol is used as an emollient, humectant, solvent, and lubricant in many products in the personal care industry such as toothpaste, mouthwashes, shaving cream, and soaps.



Rose water:

Useful to hydrate your skin naturally, provide antioxidant protection, diminish the look of fine lines and wrinkles (Shakirbasha, et al., 2019).

Lemon

Synonyms: Lemon Peel, Limonis Fructus, Limonis Cortex

Botanical source: Lemon is the fruit of Citrus limon, a small tree of the family Rutaceae.

Geographical source: Lemons are widely cultivated in many countries of the world including Africa, the Mediterranean countries, Australia, many European and North and South American countries (**Figure 4**).

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Figure 4. Lemon.

Chemical constituents: β-Pinene, Limonene, Linalool, α-Terpineol, linalyl acetate, Acetate geranyl, Nerolidol, Acetate neryl and Farnesol.

Neem

Synonyms: margosa, nim tree.

Botanical Source: *Azadirachta indica* commonly known as neem, is a tree in the mahogany family Meliacea (**Figure 5**).

Figure 5. Neem leaves.

Geographical source: It grows in tropical and semi tropical regions and is widely found in Burma, India and Pakistan.

Chemical constituents: Quercetin, β-sitosterol and poly phenolic flavonoids.

Tulasi

Synonyms: Holy basil, Tulsi

Botanical source: Tulasi is an aromatic shrub in the basil family Lamiaceae.

Geographical source: It is thought to have originated in north central India and now grows native throughout the eastern world tropics (**Figure 6**).

Figure 6. Tulasi leaves.

Chemical constituents: Eugenol, ursolic acid and rosmarinic acid, carvacrol (**Table 2**).

Table 2. Composition of Sterillium Hand Sanitizer (100 Grams).

S.No.	Ingredient	Quantity
1	Propan-2-ol	45.0 g
2	Propan-1-ol	30.0 g
3	Mecetroniumetilsulfate	0.2 g
4	Glycerol	85,
5	Tetradecan-1-ol	Qs
6	Fragrances	Qs

Phytochemical screening

Test for carbohydrates (Fehling's test), amino acid (Ninhydrin Test), phenols (Ferric chloride test), tannins (Ferric chloride test), Flavonoids (Lead acetate test), Saponins (Foam test), Terpenoids (Salkowski's test), steroids (Libermann Burchard test) were performed as per given reference (Shaiba, et al., 2010).

Fehling's test:

- Take 2 ml of given sample solution in a clean test tube.
- Add 2 ml of Fehling solution A and B to it. Keep the solution in a boiling water bath for about 10 mins.
- If there is a formation of red precipitate then the presence of carbohydrate is confirmed.

Ninhydrin test:

- Take 1 ml of test sample in dry test tube.
- Add a few drops of ninhydrin reagent to tube tube.
- Place the test tube in the water bath for 5 mins and then allow cooling to room temperature.
- If it turns to purple colour then the presence of amino acid is confirmed.

Foam test:

- 1 ml of extract solution was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 mins.
- Development of stable foam suggests the presence of saponins.

Salkowski test:

- 5 ml of extract was mixed with 2 ml of choloroform.
- Then 3 ml of concentrated sulphuric acid was carefully added to form a layer.
- If reddish brown colour forms then presence of terpenoids is confirmed.

Ferric chloride test:

- Treat the extract with 3 to 4 drops of ferric chloride solution.
- Formation of bluish black colour indicated the presence of phenols.

Lead acetate test:

- Treat the extract with few drops of lead acetate solution.
- Formation of yellow precipitate indicates presence of flavonoids.

Ferric chloride test:

- 5 ml of extract was added to 20 ml of water in a test tube.
- Add few drops of ferric chloride to it.
- Development of a brownish green colour or a bluish black colour indicates the presence of tannins.

Libermann burchard's test:

- Small quantity of extract dissolved in 5 ml of chloroform.
- The chloroform extracted solution was treated with few drops of acetic anhydride.
- Boil and cool the solution.
- Add concentrated sulphuric acid.
- Formation of bluish green colour confirms the presence of steroids.

Procedure

Lemon hand sanitizer

Extraction of juice from lemon:

Lemons were washed and cut into half and then squeezed out to get juice.

Preparation of lemon hand sanitizer:

- Add 19 ml of 70% Isopropyl alcohol in flask.

- In that add 2 ml glycerol and 4 ml rose water.
- Then add 25 ml of lemon juice into it (**Figure 7**).

Figure 7. Materials used for sanitizer preparation.

Neem hand sanitizer

Preparation of neem extract:

- Neem leaves were dried in oven at 50°c and coarsely grinned to get a powder.
- For methanol extraction, 10 g of leaves powder and 90% methanol was added in 250 ml flask (maceration) and kept on shaker for 48 hours. After maceration, sample was filtered by Whatman no.1 filter paper.
- This filtrate was placed in water bath at 60°C till thick sticky, dark coloured extract is obtained.

Preparation of neem hand sanitizer:

- 18 ml of 70% isopropyl alcohol was added in flask.
- In that 2 ml glycerol and 4 ml rose water are added.
- Add 1 g of neem extract to the flask.

Tulasi hand sanitizer

Preparation of tulasi extract:

- Tulasi leaves were dried in oven at 50°c and coarsely grinded to get a powder.
- For methanol extraction, 10 g of leaves powder and 90% methanol was added in 250 ml flask (maceration) and kept on shaker for 48 hours. After maceration, sample was filtered by Wattman no.1 filter paper.
- This filtrate was placed in water bath at 60°C till thick sticky, dark coloured extract is obtained.

Preparation of tulasi hand sanitizer:

- 18 ml of 70% isopropyl alcohol was added in flask.
- In that 2 ml glycerol and 4 ml rose water are added.
- Then 1 g of tulasi extract was added to it (**Figure 8**).

Figure 8. Lemon, tulasi, neem sanitizers.

Evaluation test for herbal hand sanitizer

Appearance:

Odour: It was determined manually.

Colour: It was determined visually.

Determination of pH: The pH of herbal hand sanitizer was determined by using digital pH meter.

Clarity test: Clarity test was determined to evaluate presence of particulate matter visually.

Skin Irritation Test: Skin Irritancy of hand sanitizer was evaluated by taking small amount of formulation on palm. Checked for local irritation or any inflammatory reactions

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Preparation of agar medium

- Suspend 28 g of nutrient agar powder in 1 liter of distilled water.
- Heat this mixture while stirring to fully dissolve all components.
- Autoclave the dissolved mixture at 121 degrees Celsius for 15 minutes.
- Once the nutrient agar has been autoclaved, allow it to cool but not solidify.

Pour nutrient agar into plate and leave plate on the sterile surface until the agar has solidified Sterile agar medium plate was inoculated with *E.coli* by dipping a cotton swab containing inoculum and the swab was streaked over the surface of the medium. The swab was also passed around the edge of the agar surface. The inoculum was left to dry for a few minutes at room temperature with the lid closed.

With the aid of a sterile 6 mm cork borer, 4 equally spaced holes were bored in the agar plate with a fifth whole in the center of the plate. The agar plugs were discarded using a sterile needle. Then hand sanitizers (sterrillium, lemon, neem, tulasi) was introduced into each of the 4 wells while the central well was filled with an equal volume of sterile water to serve as control.

The plate was incubated for 24 hours at 37°C in an upright position. It was then examined for zones of inhibition which indicated the degree of susceptibility or resistance of the test organism to the antimicrobial agent.

RESULTS

The results shows Tables 3-7 and Figures 3-6.

Table 3. Results of phytochemical analysis.

Test	Lemon juice	Neem extract	Tulasi extract
Carbohydrates	-	-	+
Amino acids	-	-	-
Saponins	-	+	+
Terpenoids	-	+	+
Phenols	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Steroids	-	-	-

Table 4. Results of evaluation parameters (lemon sanitizer).

S.No.	Evaluation parameter	observation
1	Colour	Slightly yellowish
2	Odour	Mild
3	pH	6.5 ± 0.1
4	Clarity testing	Opaque
5	Skin irritation test	No irritation observed

Table 5. Results of evaluation parameters (neem sanitizer).

S.No.	Evaluation parameter	Observation
1	Colour	Dark Green
2	Odour	Mild
3	pH	6.3 ± 0.1
4	Clarity testing	Opaque
5	Skin irritation test	No irritation observed

Table 6. Results of evaluation parameters (tulasi sanitizer).

S.No.	Evaluation parameter	Observation
1	Colour	Light green

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2	Odour	Mild
3	pH	6.5 ± 0.1
4	Clarity testing	Opaque
5	Skin irritation test	No irritation observed

Table 7. Zone of inhibition (in mm) measured at the end of 24 hours of different hand sanitizers against test organism.

Organism	Sterillium (A)	Tulasi (B)	Neem (C)	Lemon (D)
E. coli	10.5 ± 0.707	8.0 ± 0.707	9.5 ± 0.707	8.5 ± 0.707

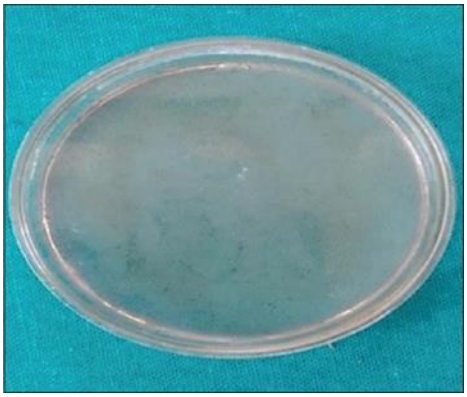

Figure 3. Sterilized agar plates inoculated with test organism (*E.coli*).

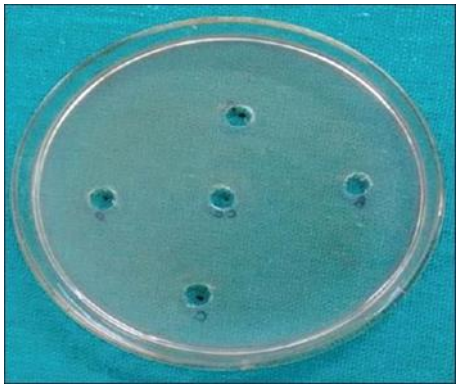

Figure 4. Four equally spaced holes in the agar plate with the 5th hole in the center.

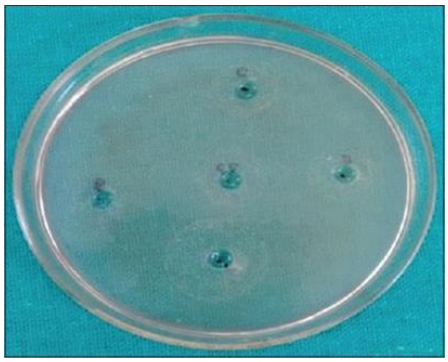

Figure 5. Four hand sanitizers were introduced into each of the 4 wells and central well filled with sterile water.

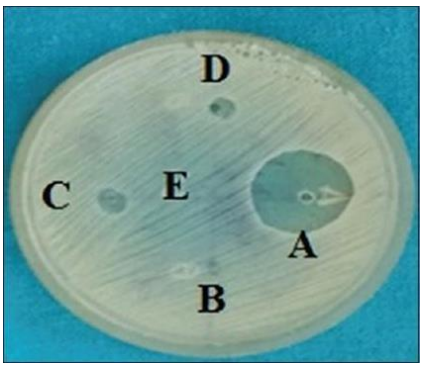

Figure 6. Analysis of zone of inhibition to evaluate antimicrobial efficacy of different hand sanitizers. Labeling on the side of respective zone of inhibition as A, B, C, D.

CONCLUSION

Hand sanitizers were effective against the test organism. The antimicrobial effectiveness was assessed by measuring the zone of inhibition against the test organism. Maximum inhibition (in mm) was seen in group A (Sterillium), *i.e.,* 10.5 ± 0.707 and minimum in group B (Tulasi), *i.e.,* .0 ± 0.707. The difference in the values of the different sanitizers was statistically significant.

From the present study, it was concluded that sterillium was most effective and sanitizers prepared from herbal extracts was close and comparable.

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