

# Development and Assessment of Herbal Sanitizer Derived from Plant Extract

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## Abstract:-

### ➤ Introduction:

A key component in the prevention, management, and decline of healthcare-acquired illnesses is hand sanitizer. Multi-drug-resistant infections are among the persistent issues related to infections acquired while receiving medical care. Maintaining good hand hygiene can greatly lower the chance of cross-contamination. In recent times, hand sanitization has been increasingly popular as a means of reducing nosocomial infections that a variety of opportunistic bacteria can cause.

### ➤ Method:

Using a methanol solvent and a maceration method, the extract was obtained from the leaves of the neem and tulsi plants. The disc diffusion technique was used to determine and assess the antibacterial activity by measuring the diameter of the zone of inhibition. It was decided to create a herbal hand sanitizer.

### ➤ Results:

According to the study, both *Ocimum sanctum* and *Azadirachta indica* include terpenoids, alkaloids, glycosides, tannins, and saponins. All of the studied microorganisms displayed a zone of inhibition in the solvent extract within the 5-23 mm range. It was discovered that the extracts' antibacterial efficacy depended on concentration.

### ➤ Conclusion:

As a platform for further research and the creation of novel therapeutic entities, the study's findings indicate the presence of various phytochemical elements with antibacterial capabilities. We may infer that the stated organisms (*S. aureus*, *K. pneumoniae*, *P. aeruginosa*, & *E. coli*) are much less susceptible to bacterial growth when exposed to herbal sanitizer. Based on many anti-bacterial tests, it has been shown that the high-concentration formulation (40 mg/ml) is highly efficient in treating bacterial disorders.

**Keywords:-** Hand Hygiene, Herbal Sanitizer, Antibacterial, *Ocimum Sanctum*, *Azadirachta Indica*.

## I. INTRODUCTION

Maintaining cleanliness standards is known as hygiene, and it is crucial for preserving one's health. Maintaining personal hygiene and using cleaning products are necessary for a healthy lifestyle.

The most crucial step in preventing the spread of dangerous germs and illnesses is to practice good hand cleanliness, as hands are the main site of infection and microbe transmission. The single most effective, least complicated, and affordable way to avoid nosocomial infections is to practice good hand hygiene [1].

Hands that are contaminated may act as carriers of germs. When a person handling food contaminates their hands and subsequently comes into touch with food or beverages on their hands, they transfer pathogenic bacteria that cause outbreaks to other people.

After ingesting these germs, the customer is exposed, which may result in gastrointestinal distress. One crucial way for infections to reach the food chain is through hand contact with prepared meals. Food handlers who handle unwrapped food that will be ingested raw or without additional cooking or preparation have been designated as a specific risk category [2].

Hand washing is an essential precaution to keep the skin safe from dangerous microorganisms and to stop the spread of many infectious illnesses. Management must provide proper hand and fingertip cleaning instructions to employees in the food manufacturing and service industries before they begin work [3].

### ➤ Hand Sanitizer:

Hand sanitizer is a liquid, gel, or foam that is often used to destroy various viruses, bacteria, or other microbes on the hands. It is also referred to as hand antiseptic, hand disinfection, hand rub, or hand rub. Hand cleaning with soap and water is typically favoured in most contexts. In addition to being less efficient than hand washing in eliminating some types of bacteria, such as the nor virus and *Clostridium difficile*, hand sanitizer is unable to physically eliminate

hazardous substances. It is possible for people to mistakenly remove hand sanitizer before it has dried, and certain types are less effective due to low alcohol concentrations [4].

Hand sanitizer with an alcohol basis that contains at least 60% (w/v) alcohol in water; this includes rubbing alcohol, ethanol, and isopropyl alcohol/isopropanol. is advised by the US Centers for Disease Control and Prevention (CDC), but only in situations when soap and water are not readily accessible. nosocomial infections are diseases that start or develop in a hospital or other healthcare facility.

#### ➤ *Important Features of Sanitizer* [4,7]

- **Alcohol:** The active element in most effective hand sanitizers is alcohol, either isopropyl or ethanol. Alcohol content is significant; it is often recommended to consume at least 60% alcohol for optimal effects.
- **Herbal Ingredients:** - The antibacterial properties of herbal ingredients vary. The efficacy of the specific herbal extracts or oils in the sanitizer against germs and viruses should be evaluated through research.
- **Antimicrobial Efficacy:** - Determine how effective the herbal hand sanitizer is overall against different types of infections, including bacteria and viruses. Studies can assess the antibacterial activity using conventional methods.
- **Skin Compatibility:** Examine the effects of the herbal ingredients on your skin's state. An excellent herbal hand sanitizer should be gentle on the skin and potent against germs to encourage frequent usage.
- **Comparable to Conventional Sanitizers:** Consider the potential for bacterial resistance with prolonged usage. Consider whether regular usage of the herbal hand sanitizer would lead it to lose its effectiveness over time.

#### ➤ *Herbal Sanitizer:*

Herbal sanitizers are a type of hand sanitizer formulated using natural ingredients derived from plants, aiming to provide antimicrobial protection while being gentler on the skin compared to traditional alcohol-based sanitizers.

Herbal hand sanitizers are becoming more and more well-liked as substitutes for conventional alcohol-based hand sanitizers, particularly in light of worries about the possible health risks connected to heavy alcohol consumption. By using the antibacterial qualities of different plants, these herbal compositions effectively disinfect while being kinder to the skin and the environment.

#### ➤ *Ingredients and Composition of Herbal Sanitizer* [5,6]

Medicinal herbs with antiseptic and antibacterial qualities are commonly used in herbal sanitizers,

- **Neem (*Azadirachta indica*):** Known for its antiviral and antibacterial properties.
- **Lemon (*Citrus limon*):** Rich in vitamin C and has antibacterial effects.
- **Tulsi (*Ocimum sanctum*):** Recognized for its broad-spectrum antimicrobial properties.

- **Chamomile (*Matricaria chamomilla*):** Contains essential oils with effective antimicrobial action

Extracts including flavonoids, alkaloids, and polyphenolic chemicals are frequently included in these formulations, which enhances their capacity to disinfect.

#### ➤ *Scientific Classification of Plants/Herbs Used:*

- *Neem* [8]
  - ✓ **Kingdom:** Plantae
  - ✓ **Division:** Angiosperm
  - ✓ **Order:** Rutales
  - ✓ **Family:** Meliaceae
  - ✓ **Genus:** *Azadirachta*
  - ✓ **Species:** *indica*
  - ✓ **Common Names:** Nim, Nimb, Arista, Nimba, Nimbah, Picumarda, Indian Lilac
  - ✓ **Applications:** Antitumor, anti-spermatogenic, antitumor, analgesic, anthelmintic, antibacterial, anti-yeast, antiulcer, antifertility, antifungal, antihyperglycemic, antiviral, antimalarial, diuretic.

- *Tulsi* [9]

- ✓ **Kingdom:** Plantae
- ✓ **Division:** Angiosperms
- ✓ **Order:** Lamiales
- ✓ **Family:** Lamiaceae
- ✓ **Genus:** *Ocimum*
- ✓ **Species:** *sanctum*
- ✓ **Synonym:** *Ocimum sanctum*
- ✓ **Common names:** Holy Basil, Tulsi, Vishnu Priya
- ✓ **Application:** as a sedative and expectorant. hepatoprotective, hypotensive, hypolipidemic, antistress, anticancer, antiasthmatic, antiemetic, diaphoretic, antidiabetic, and antifertility.

#### ➤ *Statement of Problem* [10,11]

The health of the world is gravely threatened by the rising incidence of antibiotic resistance. Recent years have seen a significant rise in antibiotic resistance, which is creating an ever-growing therapeutic challenge.

A growing number of harmful microorganisms are resistant to the recent generation of near- or contemporary antibiotics, which may be misused due to their toxicity and lack of safety. Antibiotic resistance is responsible for the deaths of at least 1.27 million people worldwide and approximately 5 million in 2019. In the United States, more than 2.8 million antibiotic-resistant infections occur annually, killing over 35,000 individuals [12].

Despite this, people continue to practice inadequate hand hygiene since the hands are the main source of germ transmission. Because of this, the prevalence of nosocomial infections is rising and has become a major worry for hospital year-end outcomes, leading to extended hospital stays, illness and mortality, and exorbitant expenditures.

In actuality, poor hygiene has been linked to global illnesses. Hand washing can be supplemented or replaced with hand sanitizer. But complete hand washing cannot be substituted with hand sanitizer. Epidemiological studies have not conclusively shown that using hand sanitizers reduces sickness. The results of this study may inspire the creation of fresh natural treatments for a range of illnesses.

➤ *Rationale of the Study* <sup>[12,13]</sup>

Medicinal plant species with antibacterial qualities are used to treat various bacterial diseases. Thus, the purpose of the study was to examine these qualities. In Nepal, few plants

are tested for their antimicrobial properties, but not much research has been done to assess their antibacterial potential.

Our study's primary goal is to create an antibacterial chemical using various plant extracts and formulate it into a useful herbal sanitizer. The primary goal in creating the herbal hand sanitizer is its high effectiveness, ability to combat resistant germs, and lack of adverse effects as compared to synthetic hand sanitizers with an alcohol base.

➤ *Conceptual Framework*

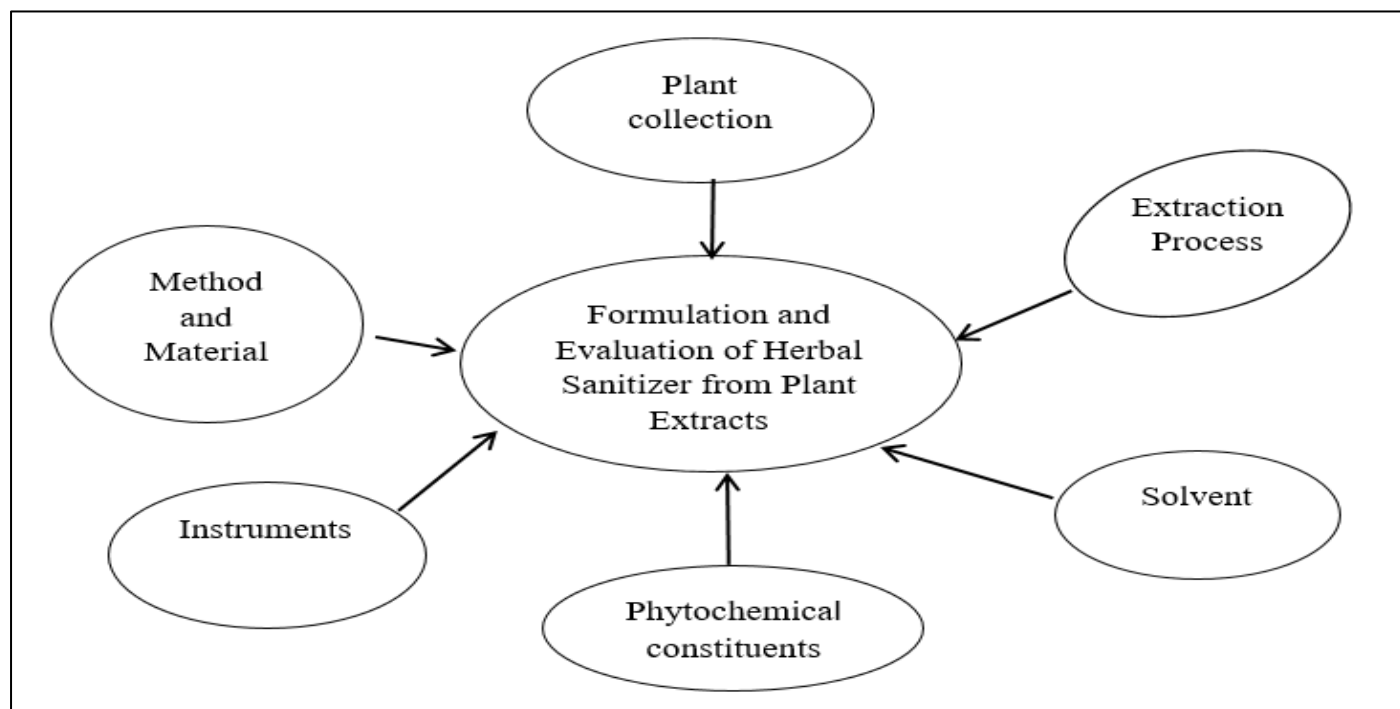


Fig 1 Conceptual Framework

➤ *Objectives:*

The principal objectives are to create a polyherbal hand sanitizer, screen plant extracts for phytochemicals, and assess the antibacterial properties of common plants (Tulsi & Neem).

## II. MATERIALS AND METHODS

➤ *Plant Material Collection:*

Azadirachta indica and Ocimum sanctum leaves were gathered in Lalitpur and Bara, Nepal. All debris, dust, or insects found on the leaf surfaces were removed, and only fully developed, healthy leaves were gathered.

➤ *Herbal Specimen Preparation:*

The plant specimen that had been gathered was compressed and stored in a newspaper. Every day until the plant specimen was completely dry, the newspaper was replaced. Subsequently, the desiccated specimen was placed onto a herbarium sheet that measured 43 by 29 cm<sup>2</sup>. The specimen at the herbarium has the correct label.

➤ *Drying of Plant Materials:*

After cleaning, the plant materials were placed in the shade for a week. To minimize the size of the leaves, the dried leaves were crushed by hand after they had fully dried. Plant materials that had been completely dried were employed for extraction.

➤ *Extraction from Plant Materials* <sup>[14]:</sup>

The maceration method was used to extract the dried sample after it had been ground into a powder using a mechanical grinder.

➤ *Procedure* <sup>[15,16]</sup>

To extract Ocimum sanctum, 80 grams of ground material were soaked in 1000 millilitres of methanol, and to extract Azadirachta indica, 80 grams of ground material were soaked in 1000 millilitres of methanol for a whole day before being filtered through regular filter paper.

After soaking the residue for a second time in 1000 millilitres of new methanol each for Ocimum sanctum and Azadirachta indica, it was filtered after a day. Following three treatments with 1000 millilitres of methanol, the extract was

filtered. After being poured into beakers, the filtrate was let to evaporate until it was totally dry.

➤ *Phytochemical Test of Herbal Extract* <sup>[17,18]</sup>:

Standard protocols were used to conduct chemical tests on the plant material to determine the different constituents like,

- Test for alkaloids
- Test for glycosides
- Test for saponins
- Test for tannins
- Test for terpenoids

➤ *Antimicrobial Studies of Herbal Extracts* <sup>[19,20]</sup>

Using the disc diffusion technique, the extracts' antibacterial efficacy against microorganisms was tested. Following preliminary antimicrobial activity, the extracts would undergo further antimicrobial screening using cefuroxime, cefixime, azithromycin, and ciprofloxacin as standards.

For antimicrobial screening, both Gram-positive and Gram-negative microorganisms were used.

- Gram positive organisms: *Staphylococcus aureus*
- Gram negative organisms: *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*

A screening for microorganisms was done on the methanol extract. Each plant extract was made at concentrations of 20 mg/ml, 30 mg/ml, and 40 mg/ml by dissolving it in an appropriate solvent, such as 50% Dimethyl Sulfoxide (DMSO).

➤ *Procedure* <sup>[21,22]</sup>

Mueller-Hinton Agar medium was made (14 grams in 500 millilitres) and autoclaved for 15 minutes at 121 degrees Celsius to sanitize it. 20 to 25 millilitres of the hot-sterilized medium were placed into each sterile petri dish. The plates were placed in an incubator and allowed to cool for fifteen to

twenty minutes. By inoculating a loop full of bacteria into the nutritional broth, bacterial suspension was created. Using a sterile cotton swab, the pure form of the bacterial solution was swabbed onto the medium and left to dry. The sterile petri plate was stacked with a disc containing plant extracts, a control, and a standard on opposite sides.

Plant extract at concentrations of 20 mg/ml, 30 mg/ml, and 40 mg/ml was added to separate petri plates, along with a standard of 30 mg of azithromycin, 30 mg of ciprofloxacin, 5 mg of cefixime, and 30 mg of cefuroxime (DMSO). For 18 to 24 hours, all of the plates were incubated at 37°C. Measurements of the zone of inhibition were made and compared to standards.

• *Setting up Suspended Microorganisms in Order* <sup>[23]</sup>:

Distilled in 80 millilitres of distilled water, 1.04 grams of nutritional broth was autoclaved at 121 degrees for 15 minutes. Four distinct test tubes each held 20 millilitres of the prepared nutrition broth. Following that, each test tube contained four distinct bacteria, which were then incubated for a full day.

• *Zone of Inhibition Determination* <sup>[24]</sup>

After a day, the incubated petri plates were removed from the incubator and the zone of inhibition was determined using a scale. Zones of inhibition were measured and compared for the sample and standard.

➤ *Formulation of Sanitizer:*

Deionized water was combined with carbopol while being continuously stirred. Tri-ethanol amine was added after the mixture had been thoroughly mixed and slowly stirred to prevent any potential air bubbles from forming, and the mixture was left for 24 hours. Glycerine and all of the extracts were added to denatured alcohol, and the aqueous phase was combined with Tween 20. Lastly, Fragrance agents and preservatives were applied, and the HDPE containers were sealed. A 50 ml batch of the herbal sanitizer formulation composition was created, and the procedure was done twice with varying plant extract concentrations.

Table 1 Formulation Composition of Herbal Sanitizer

S. No	Excipients	Roles	Quantity
1.	Distilled water	Vehicle	15.50ml
2.	Alcohol Denatured	Anti-infective	30.00ml
3.	Tween 20	Surfactant	0.25ml
4.	Rose Water	perfume	0.25ml
5.	Carbopol	Thickening, Suspending, Stabilizing, Emulsifying	0.25gm
6.	Triethanol amine	Surfactant, emulsifier	0.35ml
7.	Glycerine	Humectant	1.15ml

Table 2 Concentrations of Plant Extract

S. N	Plant Extract	Concentration
1	Neem	1gm 1.5gm 2gm
2	Tulsi	1gm 1.5gm 2gm

➤ *Evaluation Studies of Herbal Sanitizer*

• *Appearance:*

- ✓ Odour: It was determined manually.
- ✓ Color: It was determined visually.

• *Determination of pH:*

The herbal hand sanitizer's pH was measured with a digital pH meter.

• *Clarity Test:*

To determine whether there were any deposits or particles in the liquid hand sanitizers, particle tests were conducted. The presence or lack of a black-and-white backdrop was used to conduct the test.

• *Skin Irritation Test:*

A small quantity of the formulation was applied to the palm to test the skin irritability of the hand sanitizer, examined for signs of inflammation or localized discomfort (whether present or not).

• *Hair Dryer Test (Test for Alcohol Content):*

Initially Fill a dish with hand sanitizer. For around 30 seconds, use a hair dryer to dry the sanitizer.

A decent hand sanitizer should dry fast (within five seconds). If it takes much longer, there can be an excessive amount of water and not enough alcohol.

• *Antimicrobial Test of Herbal Sanitizer* <sup>[25,26]</sup>:

Using the disc diffusion method, hand sanitizer was screened for microorganisms. A 14g/500ml Mueller Hinton Agar medium was made, and it was autoclave sterilized for 15 minutes at 121°C. 20 to 25 millilitres of the hot-sterilized medium were placed into each sterile petri dish. After letting the plates cool for ten to fifteen minutes, they were refrigerated to solidify. By inoculating a loop full of bacteria into the nutritional broth, bacterial suspension was created. Using a sterile cotton swab, the pure form of the bacterial solution was swabbed onto the medium and left to dry.

The disc containing plant extracts, control, and standard were positioned on opposite sides of the sterile Petri plate. A standard of 30 mg of ciprofloxacin and plant extract at concentrations of 20, 30, and 40 mg were added to separate petri plates, along with DMSO as a control. For 18 to 24 hours, all of the plates were incubated at 37°C. Measurements of the zone of inhibition were made and compared to standards.

• *Antioxidant Assay (DPPH Assay)* <sup>[24,26]</sup>

One millilitre of the DPPH solution was added to the test tube or sampling tube along with two millilitres of the analytical sample solution and eight hundred millilitres of the 0.1 M Tris-HCL buffer (PH 7.4). The solution was immediately combined for ten seconds using a test tube mixer. It was then kept in the dark at room temperature. The absorbance of the solution at 517 nm was measured. 800µl of Tris-HCL buffer and 1.2 mL of ethanol were combined to form a mixed solution as the placeholder. When the analytical sample was added, the absorbance was represented as the absorbance after ethanol is added as opposed to the sample as Ac, as well as the inhibition ratio (%) was acquired using the equation that follows.

$$DPPH \text{ Assay} = \frac{\text{abs of control} - \text{abs of test or standard}}{\text{abs of control}} \times 100$$

The result is shown in Table 10 below.

**III. RESULT**

➤ *Medicinal Plants' Methanol Extract Yield:*

The graphic below illustrates how much of the maceration process's crude extract of medicinal herbs was used. The maximum yield was produced by varying the amount of extract from the medicinal plants; *Azadirachta indica* came in second with 8 grams, while *Ocimum sanctum* produced the lowest yield with 6 grams.

➤ *Solubility Profile of Extract:*

The following table shows the solubility profile of the methanolic extract of *Ocimum sanctum* and *Azadirachta indica*:

Table 3 Solubility Profile of Methanolic Extract

Name of solvent	<i>Ocimum sanctum</i>	<i>Azadirachta indica</i>
Acetone	Insoluble	Soluble
Chloroform	Insoluble	Insoluble
Di-Methyl Sulfoxide (DMSO)	Highly soluble	Highly soluble
Ethyl acetate	Insoluble	Insoluble
Methanol	Soluble	soluble
Water	Insoluble	Insoluble

➤ *Phytochemical Screening:-*

The plant's phytochemical screening revealed the presence of several components in various solvent extracts. The phytochemical research revealed that *Ocimum sanctum*

and *Azadirachta indica* both included terpenoids, glycosides, saponins, tannins, and alkaloids in their plant extract.

Table 4 Chemical Constituents of Medicinal Plants

S. N	Chemicals	<i>Azadirachta indica</i>	<i>Ocimum sanctum</i>
1	Alkaloids	+	+
2	Glycosides	+	+
3	Saponins	+	+
4	Tannins	+	+
5	Terpenoids	+	+

Where (+) means positive/ present & (-) means absent

➤ Assessment of Plant Extracts' Anti-Bacterial Aspects

Table 5 Result of Antibacterial Study of Methanolic Extract of *Ocimum Sanctum*

Concn of Drug(mg/ml)/ Organisms	Zone of inhibition(diameter mm)				
	20mg/ml	30mg/ml	40mg/ml	Control	Standard
<i>S.aureus</i>	11mm	14mm	17mm	0	18mm (cipro)
<i>Ps.aeruginosa</i>	10mm	12mm	16mm	0	- (cefuroxime)
<i>E.coli</i>	11mm	13mm	14mm	0	15mm (cefixime)
<i>K.pneumoniae</i>	3mm	9mm	11mm	0	13mm (azithro)

Table 6 Result of Antimicrobial Study of Methanolic Extract of *Azadirachta Indica*

Concn of Drug(mg/ml)/ Organisms	Zone of inhibition (diameter mm)				
	20mg/ml	30mg/ml	40mg/ml	Control	Standard
<i>S.aureus</i>	14mm	16mm	19mm	0	18mm (cipro)
<i>Ps.aeruginosa</i>	11mm	10mm	12mm	0	13mm (azithro)
<i>E.coli</i>	14mm	15mm	17mm	0	18mm (cefixime)
<i>K.pneumoniae</i>	-	-	-	0	14mm (azithro)

• Alcohol Content:

Time taken by sanitizer to dry = 7 sec, which means sanitizer likely contains adequate alcohol.

• Evaluation Studies of Herbal Sanitizer

Table 7 Results of Evaluation Parameters (Neem Sanitizer).

S. No.	Evaluation parameter	Observation
1	Colour	Slightly yellowish
2	Odour	Mild
3	pH	6.8 ± 0.1
4	Clarity testing	Opaque
5	Skin irritation test	No irritation observed

Table 8 Results of Evaluation Parameters (Tulsi Sanitizer)

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

Table 9 Zone of inhibition (in mm) measured at the end of 24 hours of different hand sanitizers against test organism.

Concentration of drug(mg/ml) / Organisms	Zone of inhibition (in mm)				
	20mg/ml	30mg/ml	40 mg/ml	Control	Standard
<i>S.aureus</i>	18mm	21mm	23mm	0	21mm (cipro)
<i>Ps.aeruginosa</i>	14mm	15mm	17mm	0	18mm (cipro)
<i>pneumoniae</i>	6mm	9mm	11mm	0	14mm(cipro)
<i>E.coli</i>	17mm	14mm	18mm	0	18mm( cipro)

Table 10 Result of Antimicrobial Study of DPPH Scavenging Activity of Samples

Sample name	% inhibition
<i>Azadirachta indica</i>	9.18%
<i>Ocimum sanctum</i>	11.76%
Hand sanitizer	14.02%

#### IV. DISCUSSION

Many contemporary medications we utilize for our various illnesses are derived from plants or plant-based compounds (Abraham, 1981).

These extracts include phytochemical elements, as the observation in the study mentioned above shows. These phytochemical components comprise terpenoids, alkaloids, glycosides, tannins, and resins. Here, the presence of alkaloids, glycosides, tannins, saponins, and terpenoids is confirmed by the leaves in the methanolic extract of *Azadirachta indica*. Similarly, the existence of these compounds is confirmed in the research of *Ocimum sanctum*.

The antioxidant activity of hand sanitizer was greatest (14.02%), followed by the DPPH scavenging activity of *Ocimum sanctum* (11.76%) and *Azadirachta indica* (9.18%).

The highest zone of inhibition against *S. aureus* that the methanolic extracts of *O. sanctum* appeared to have in our investigation was 18 mm, 15 mm, and 13 mm at doses of 40 mg/ml, 30 mg/ml, and 20 mg/ml, respectively. Similarly, at 20 mg/ml, 30 mg/ml, and 40 mg/ml, *K. pneumoniae* exhibited a minimum zone of inhibition of 5 mm, 7 mm, and 10 mm, respectively. The methanolic extracts of *O. sanctum* showed modest sensitivity (5 mm) against *K. pneumoniae* and great sensitivity (18 mm) against *S. aureus*.

At 40 mg/ml, 30 mg/ml, and 20 mg/ml, respectively, the methanolic extracts of *A. indica* demonstrated the highest zone of inhibition against *S. aureus*, which was 20 mm, 17 mm, and 15 mm, and the minimum zone of inhibition against *P. aeruginosa*, which was 13 mm, 11 mm, and 10 mm. Our study also revealed that there was no zone of inhibition against *K. pneumoniae* which concludes that *K. pneumoniae* is resistant to Neem.

Rutuja Sunil Patankar and Dr. Nanya Chandak's antimicrobial investigation on hand sanitizer demonstrated the maximum zone of inhibition, 16 mm, in 40 mg/ml against *S. aureus* and the average zone of inhibition, 15 mm, against *P. aeruginosa* and *E. coli* in 40 mg/ml, respectively.

The antimicrobial investigation we conducted on hand sanitizer demonstrated an average maximum zone of inhibition of 19 mm against *S. aureus* and 17 mm against *E. coli*. However, we also found that the zone of inhibition against *K. pneumoniae* was lower, at 7 mm.

#### V. CONCLUSION

The test organism was successfully combatted by hand sanitizers. The zone of inhibition against the test organism was measured to evaluate the antibacterial efficacy. Using methanol solvent, the antibacterial activity of *A. indica* and *O. sanctum* leaves was examined. For every examined bacterium, the solvent extract revealed a zone of inhibition within the approximate 5-23 mm range. It was discovered that the extracts' antibacterial efficacy depended on concentration.

There was a statistically significant difference in the values of the various disinfectants. The results of this investigation showed that sterillium was the most efficient disinfectant, while sanitizers made from plant extracts were similar and near to it.

#### VI. LIMITATIONS OF STUDY

- The plant's bark, fruits, and seeds were not taken into consideration in favor of concentrating the study just on the leaves of the plant.
- Only four species of bacteria—*E. coli*, *P. aeruginosa*, *S. aureus*, and *K. pneumoniae*—were taken into consideration in the study; the possible impacts on additional bacterial strains were not assessed.
- There was no comparison of the plant's antibacterial activity with that of other plants or varieties of the same plant grown in other places or climates within the research.
- We were unable to cover additional aspects of plant extraction and formulation due to time constraints.
- Due to a lack of equipment, we were unable to get plant fingerprints.

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