

## **Potential of *Azadirachta indica* A. Juss., *Eucalyptus globulus* Labill. and *Ocimum sanctum* L. Phytochemicals as Herbal Hand Sanitizer: A Review**

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### **ABSTRACT**

Hand hygiene is a fundamental and essential practice in infection prevention, control, and reduction. The hands serve as the most common and rapid mode of germ and infection transmission. Hand sanitizers are effective in eliminating microorganisms present on the skin and reducing the spread of pathogens from one person to another. This review aims to develop a plant-based herbal hand sanitizer that can eradicate microbes while preserving skin health. Unlike alcohol-based sanitizers, the plant-based herbal hand sanitizer has no adverse effects on the skin due to the absence of harmful chemicals and alcohol. It does not strip away the natural oils that retain moisture on the skin, thus preventing dryness. The non-flammable nature of the alcohol-free sanitizer ensures its safety, making it suitable for use by children and individuals with sensitive skin. The proposed sanitizer is cost-effective and aims to include plant leaf extracts such as Neem (*Azadirachta indica*), Eucalyptus (*Eucalyptus globulus*), and Tulsi (*Ocimum sanctum*). The study intends to evaluate the antimicrobial activity of these plant extracts and the formulated hand

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sanitizer. Additionally, the evaluation of physical parameters such as color, odour, and pH of the sanitizer will also be assessed.

**Keywords:** Hand sanitizer, Neem (*Azadirachta indica*), Eucalyptus (*Eucalyptus globulus*), Tulsi (*Ocimum sanctum*), Antimicrobial activity.

## 1. INTRODUCTION

The transmission of microorganisms and diseases is most commonly facilitated through the hands. Hand cleanliness is the primary means to prevent the transmission of harmful diseases and infections.

Appropriate hand washing and hand sanitization are essential measures for maintaining healthy skin and preventing the dissemination of diseases. Clean hands are particularly vital in healthcare, research, and clinical settings, as individuals need to avoid contracting illnesses (51). The World Health Organization (WHO) advises washing our hands before, during, and after food preparation, before eating, when dealing with a wound, after blowing our nose, coughing, or sneezing, after treating pets, their food, or their treats, and after touching trash. The concept of disinfecting hand washing probably appeared in the 19th century, but hand washing with soap and water has been recognised as a personal hygiene practice for generations (1). Plants were the primary treatment of many diseases before to the development of modern medicine. As microorganisms develop resistance to various antibiotics, researchers are looking at plants with antibacterial capabilities and their secondary metabolites to identify potent and sustained actions against a variety of microbes (2). The primary goal of hand sanitizers is to kill all microorganisms, leading to a rapid reduction of skin microbes. Hand sanitizers serve as both disinfectants and cleaning agents, with the primary purpose of maintaining cleanliness in the environment. Similar to disinfectants, hand sanitizers chemically destroy bacteria on hands. Hand sanitizers are available as gel-based liquids, foams, or fluids, with alcohol-based gel sanitizers being particularly popular in some markets (48). While alcohol-based sanitizers are effective and safe to use, frequent use can damage the skin, leading to the development of non-alcoholic hand sanitizers that are gentle on the skin while still effective in killing bacteria. Several non-alcoholic herbal hand sanitizers have been developed to meet the demands of the market and effectively combat microorganisms (44,48). Essential oils and sebum, which act as a natural barrier against bacterial contamination and can cause protein to precipitate, can be removed from the skin by alcohol. This increases the risk of harm when applied to wounds, and may also result in the formation of a coagulum in which bacteria can thrive. As a result, alcohol-based antimicrobial products are limited in their effectiveness and may actually increase the risk of illness (46). Conversely, medicinal plants offer a number of benefits as a treatment for various ailments, including their safety, cost-effectiveness, and accessibility. Plant secondary metabolites with antibacterial activities include tannins, terpenoids, alkaloids, and flavonoids, among others, making plant extracts a useful ingredient in the production of antibacterial hand sanitizers (7). While alcohol-based sanitizers are effective at breaking down the cell walls of germs or slowing their metabolism, they may also have negative effects, such as destroying helpful microbes and contributing to early puberty through endocrine-disrupting effects. Additionally, overuse of alcohol-based sanitizers can cause skin to lose its natural oils, leading to drying and cracking, which can serve as a portal for germs to infect the body and create contamination (48). Neem (*Azadirachta indica* A. Juss.) (Figure 1), Tulsi (*Ocimum sanctum* L.) (Figure 2), and

Eucalyptus (*Eucalyptus globulus* Labill.) (Figure 3) (Table 1) leaves are known for their various medicinal benefits. Numerous bioactive substances with antibacterial, analgesic, anti-oxidative, and anti-inflammatory properties can be found in natural sources such as herbs, trees, essences, and extracts (33). In this study, the antibacterial effects of aqueous extracts of eucalyptus, neem, and tulsi are being examined as a way of replacing chemical products with more eco-friendly alternatives (48). The link between illnesses and treatments is greatly influenced by medicinal plants and they have active roles in alleviating all types of illnesses (32,34).

Table 1. Herbs studied in the review

Scientific Name	Family	Local Name	English Name	Plant part used
<i>Azadirachta indica</i>	Meliaceae	Neem	Neem	Leaves
<i>Ocimum sanctum</i>	Lamiaceae	Tulsi	Basil	Leaves
<i>Eucalyptus globulus</i>	Myrtaceae	Eucalyptus	Eucalyptus	Leaves

## 2. NEEM (*A. indica*)

Neem leaves are known for their antifungal, antibacterial, and insecticidal properties. The neem tree is commonly grown in India and other tropical countries, and its leaves are used in the production of various products, such as soaps, shampoos, and toothpaste. The neem oil derived from the seeds of the neem tree is also used in the production of pesticides, fertilizers, and other agricultural products. Neem leaves are of high economic importance as they can be used as pesticides. Neem leaves contain compounds such as azadirachtin, which have insecticidal properties. They can also be used as animal feed as they are a rich source of protein. The leaves are fed to livestock to improve their overall health and increase milk production. Neem leaves are also used in the production of various cosmetic and personal care products such as soaps, shampoos, and toothpaste. Neem leaves have antibacterial and antifungal properties, making them ideal for use in personal care products. They have been used in the making of traditional medicines. The neem tree is also grown for its timber, which is used in the production of furniture, flooring, and construction materials. Thus, neem leaves have significant economic importance as they are used in the production of a wide range of products (5).

Figure 1. Neem (*A. indica*) Tree and leaves

### 2.1 Phytochemical composition and antimicrobial properties of *Neem* leaves:

*Neem* is a plant species belonging to the Meliaceae family of mahogany plants. It is commonly known as Indian lilac and is indigenous to the tropical and semi-tropical regions of the Indian subcontinent, including India, Nepal, Pakistan, Bangladesh, Sri Lanka, and Maldives. Numerous phytochemicals, including nimbin, nimbidin, nimbanene, 6-desacetyl-nimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol, and amino acids, are found in the leaves of the neem plant. The first polyphenolic flavonoids, Quercetin and  $\beta$ -sitosterol, with antifungal and antibacterial effects were isolated from freshly harvested neem leaves (17). *Neem* has been shown to possess anti-inflammatory, antipyretic, hypoglycemic, antigastric ulcer, antifungal, antibacterial, and antitumor properties. *Neem* is frequently used in Ayurvedic medicine to treat fever and malaria. This study aimed to prepare extracts of neem leaves using the methanol extraction method (33,40). The total phenol content and free radical scavenging activity of the neem phytochemicals were assessed using the DPPH method. The biochemical screening of neem leaf crude extracts revealed the presence of flavonoids, saponins, steroids, alkaloids, amino acids and tannins (8). Similarly, the phytochemical screening of neem extracts was also conducted, and phytochemicals such as tannin, phenol, saponin, terpenoid, and flavonoid were detected (26,35). These are just a few examples of the many biologically active compounds present in neem that may have allelopathic effects (Table 2).

Table 2. Phytochemicals present in *Neem* leaves and their properties

Phytochemicals	Properties
Azadirachtin	A tetranortriterpenoid compound that acts as a feeding deterrent and growth regulator for insects.
Nimbin	A triterpenoid compound with antifeedant and antifungal properties.
Salannin	A limonoid compound that acts as a feeding deterrent for insects.
Meliantriol	A triterpenoid compound with antifeedant and antifungal properties.
Gedunin	A tetranortriterpenoid compound with insecticidal, antifeedant, and growth-regulating properties.
Quercetin	A flavonoid compound with antioxidant and anti-inflammatory properties.
Kaempferol	A flavonoid compound with anti-inflammatory and anti-cancer properties.
Emodin	An anthraquinone compound with antifungal, antibacterial, and anti-inflammatory properties.

Notably, Nimbin is present in significant amount and is considered the main antibacterial constituents. Nimbin is a phytochemical present in *Neem* leaves, which is known to have several health benefits due to its antibacterial, antifungal, and antiviral properties.

**Antibacterial Properties:** Nimbin has potent antibacterial properties, which can help to combat bacterial infections. It inhibits the growth of several bacterial strains, including *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli*. Nimbin works by disrupting the bacterial cell wall and interfering with the DNA synthesis process, thus preventing the bacteria from replicating and spreading (26).

**Antifungal Properties:** Nimbin is also known to have antifungal properties, which can help to prevent and treat fungal infections. It has been shown to inhibit the growth of several fungal strains, including *Candida albicans*, *Aspergillus niger* and *Trichophyton mentagrophytes*. Nimbin works by disrupting the fungal cell membrane and interfering with the production of ergosterol, a key component of the membrane (35).

**Antiviral Properties:** Nimbin is also known to have antiviral properties, which can help to prevent and treat viral infections. It has been shown to inhibit the replication of several viruses, including herpes simplex virus (HSV), human immunodeficiency virus (HIV), and hepatitis B virus (HBV). Nimbin works by inhibiting the activity of the viral enzymes, thus preventing the virus from replicating and spreading (35).

In conclusion, Nimbin present in Neem leaves is a potent phytochemical that has antibacterial, antifungal, and antiviral properties. It can be used to prevent and treat a wide range of infections, making it a valuable natural remedy for maintaining overall health and well-being.

### 3. TULSI (*O. sanctum*)

Tulsi or Holy Basil leaves are used in traditional medicine for their antifungal, antibacterial, and anti-inflammatory properties. Tulsi leaves are also used in the production of various herbal teas, supplements, and personal care products. Tulsi is widely grown in India and other parts of Southeast Asia and is used in religious ceremonies as well. Eucalyptus leaves are known for their antiseptic, antifungal, and antimicrobial properties. The oil extracted from the leaves is used in the production of various medicinal and personal care products, such as cough drops, vapor rubs, and insect repellents. Eucalyptus trees are also grown for their wood, which is used in the production of furniture, flooring and paper products. These leaves are economically significant since they are used to make a variety of goods that are sold on both domestic and foreign markets. Additionally, they help maintain the livelihoods of those who cultivate and gather these plants, particularly in areas where they are native.



Figure 2. Tulsi (*O. sanctum*) Tree and leaves

Tulsi leaves, also known as Holy Basil, have several economic benefits due to their medicinal and commercial applications. Some of the economic importance of Tulsi leaves include the production of herbal medicines and supplements to treat various ailments such as fever, cold, cough, and respiratory problems. Tulsi leaves contain compounds that have antifungal, antibacterial, and anti-inflammatory properties. They are also used in cosmetics and personal care products. The oil extracted from Tulsi leaves is used in aromatherapy and

as a natural remedy for various ailments. Tulsi oil has antimicrobial and antiviral properties that make it effective in treating respiratory infections and skin conditions. Tulsi is considered a sacred plant in Hinduism and is grown in many households and temples. Tulsi leaves are used in religious ceremonies and rituals, and there is a significant demand for Tulsi plants and leaves during religious festivals (11,49).

Thus, Tulsi leaves have several economic benefits due to their medicinal and commercial applications. The demand for Tulsi leaves in the food, cosmetics, and healthcare industries is increasing, which creates income opportunities for farmers and supports rural economies.

### 3.1 Phytochemical composition and antimicrobial properties of Tulsi leaves:

Tulsi is a highly esteemed and sacred herb renowned for its medicinal properties, primarily found in the Oriental region. It is commonly referred to as "Incomparable One," "Queen of Herbs," and "Incomparable One of Herbs." This plant is native to the Indian subcontinent and is a member of the Lamiaceae family. In Ayurveda therapy, *Ocimum sanctum* is widely used to treat various ailments due to its potent anti-stress properties. The plant's various components can be used to remedy different health conditions. Tulsi, also referred to as Holy Basil in English and Rama Tulsi and Krishna Tulsi in Sanskrit, is a widely cultivated plant (22,25,40).

Tulsi leaves contain several potential allelochemicals, which are natural compounds that can affect the growth and development of other plants. Some of the potential phytochemicals present in tulsi leaves include (Table 3) (28,37,49,50,53).

Notably, Rosmarinic acid is present in significant amount and is considered the main antibacterial constituents (29,30). Rosmarinic acid is a polyphenol compound that is found in several plant species, including rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), and basil (*Ocimum basilicum*). It has been shown to have antibacterial, antifungal, and antiviral properties, making it a potentially useful natural compound for medicinal and pharmaceutical applications (36,59).

Table 3. Phytochemicals present in Tulsi leaves and their properties

Phytochemicals	Properties
Eugenol	This compound is responsible for the strong aroma of tulsi leaves and has been shown to have allelopathic effects on other plants. Eugenol inhibits the growth of several plant species, including lettuce, tomato, and corn.
Rosmarinic acid	This compound has been found to have allelopathic effects on several plant species, including lettuce, wheat, and ryegrass. Rosmarinic acid inhibits the germination and growth of these plants.
Ursolic acid	This compound has been shown to have allelopathic effects on several plant species, including lettuce, wheat, and ryegrass. Ursolic acid inhibits the germination and growth of these plants.
Oleanolic acid	This compound has been found to have allelopathic effects on several plant species, including lettuce, wheat, and ryegrass. Oleanolic acid inhibits the germination and growth of these plants.
Linalool	This compound has been shown to have allelopathic effects on several plant species, including wheat, lettuce, and ryegrass. Linalool inhibits the germination and growth of these plants.

There are some studies that have investigated the antibacterial, antifungal, and antiviral properties of rosmarinic acid:

**Antibacterial properties:** Rosmarinic acid has been shown to inhibit the growth of several bacteria, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. A study published in the Journal of Agricultural and Food Chemistry found that rosmarinic acid extracted from rosemary had potent antibacterial activity against *S. aureus*, with an MIC (minimum inhibitory concentration) value of 128 µg/mL (14,33).

**Antifungal properties:** Rosmarinic acid has also been shown to have antifungal activity against several fungal species, including *Candida albicans*, *Aspergillus flavus*, and *Aspergillus niger*. According to a study in the Journal of Medicinal Food, rosmarinic acid from sage demonstrated a high level of antifungal activity against *Candida albicans*, having a minimum inhibitory concentration (MIC) of 125 g/mL (54,55).

**Antiviral properties:** Rosmarinic acid has been shown to have antiviral activity against several viruses, including influenza virus, herpes simplex virus, and HIV-1. A study published in the Journal of Natural Products found that rosmarinic acid extracted from rosemary had potent antiviral activity against influenza virus, with an EC<sub>50</sub> (effective concentration 50) value of 0.8 µM (25,37).

Overall, these studies suggest that rosmarinic acid has potential as a natural antibacterial, antifungal, and antiviral agent (58).

#### 4. EUCALYPTUS (*E. globulus*)

*Eucalyptus*, a member of the Myrtaceae genus, is an Australian native and comprises a wide variety of flowering trees and shrubs (3,23). *Eucalyptus* species are commonly planted in tropical and temperate areas including the Americas, Europe, Africa, the Mediterranean Basin, the Middle East, China, and the Indian subcontinent. The plant itself serves as a rich source of terpenoids and polyphenols, and cineole or eucalyptol is the major ingredient of the essential oil derived from the leaves approximately constituting seventy to eighty mg/ml (12,14). Microbiologists, horticulturists, and scientists studying global improvement agree that some *eucalyptus* species stand out because of their beneficial traits, such as being a rapidly expanding resource for timber, generating oil which is useful for cleansing, acting as an insect repellent, also showing activities against microorganisms (54,40). *Eucalyptus* is known to contain several allelochemicals, which are secondary metabolites produced by plants that can have allelopathic effects on other organisms. Here are some examples of potential allelochemicals present in *Eucalyptus* leaves, along with their allelopathic effects: (27,42,43). *Eucalyptus* leaves have significant economic importance due to their numerous medicinal and commercial applications. Some of the economic benefits of *eucalyptus* leaves include the use of essential oils as *eucalyptus* leaves are used in the production of essential oils, which are used in various industries, such as cosmetics, pharmaceuticals, and aromatherapy. *Eucalyptus* oil has antiseptic, antimicrobial, and anti-inflammatory properties and is used in the treatment of respiratory problems and muscle pain. They are also used in the production of paper. They are also used as animal feed for livestock, particularly in regions where *eucalyptus* trees are abundant. The leaves are a rich source of nutrients and can improve the overall health and productivity of livestock. *Eucalyptus* leaves and wood are used as a source of fuel in many countries. The high energy content of *eucalyptus* wood makes it an ideal source of biomass energy.

Therefore, *Eucalyptus* leaves have significant economic importance due to their various medicinal and commercial applications. Overall, the objective of this review is to provide a comprehensive study of the effectiveness of herbal hand sanitizers and to highlight their potential advantages as well as to provide recommendations for their use.



Figure 3. Eucalyptus (*E. globulus*) Tree and leaves

#### 4.1 Phytochemical composition and antimicrobial properties of Eucalyptus leaves:

**Eucalyptol (1,8-cineole):** This is a major component of Eucalyptus essential oil and has been shown to have allelopathic effects on several plant species. A study published in the Journal of Chemical Ecology found that eucalyptol inhibited seed germination and root growth of several weeds, including Barnyardgrass (*Echinochloa crusgalli*), Johnsongrass (*Sorghum halepense*), and Velvetleaf (*Abutilon theophrasti*) (34,35). **Phenolic acids:** Eucalyptus leaves contain several phenolic acids, including caffeic acid, chlorogenic acid, and gallic acid, which have been shown to have allelopathic effects on other plants. A study published in the Journal of Plant Nutrition and Soil Science found that aqueous extracts of Eucalyptus leaves containing phenolic acids inhibited the germination and growth of lettuce (*Lactuca sativa*) and radish (*Raphanus sativus*) seeds (22,37).

**Terpenoids:** *Eucalyptus* leaves contain several terpenoids, including  $\alpha$ -pinene,  $\beta$ -pinene, limonene, and camphene, which have been shown to have allelopathic effects on other plants. A study published in the Journal of Plant Physiology found that  $\alpha$ -pinene and  $\beta$ -pinene inhibited the germination and growth of *Arabidopsis thaliana* seedlings (49).

**Flavonoids:** *Eucalyptus* leaves contain several flavonoids, including quercetin, kaempferol, and luteolin. A study published in the Journal of Agricultural and Food Chemistry found that quercetin inhibited the growth of lettuce seedlings and reduced the chlorophyll content of their leaves.

Overall, these studies suggest that Eucalyptus leaves contain several allelochemicals that can have allelopathic effects on other plants. Eucalyptol, also known as 1,8-cineole, is a major constituent of the essential oils of many Eucalyptus species. It has been studied for its antimicrobial properties, including antibacterial, antifungal, and antiviral effects. Here are some examples of studies on the properties of eucalyptol:

**Antibacterial:** Eucalyptol has been shown to have antibacterial effects against a variety of pathogenic bacteria, including *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. A study published in the Journal of Agricultural and Food Chemistry found that eucalyptol inhibited the growth of these bacteria by disrupting their cell membranes (30,31).

**Antifungal:** Eucalyptol has also been shown to have antifungal effects against several fungal species, including *Candida albicans*, *Aspergillus niger*, and *Trichophyton mentagrophytes*. A study published in the Journal of Medical Microbiology found that eucalyptol inhibited the growth of these fungi by disrupting their cell membranes and altering their cellular metabolism (40,50,52).

**Antiviral:** Eucalyptol has been shown to have antiviral effects against several viruses, including herpes simplex virus type 1 (HSV-1), influenza virus, and human rhinovirus. A study published in the Journal of Medical Virology found that eucalyptol inhibited the replication of these viruses by interfering with their attachment to host cells and inhibiting viral protein synthesis.

Overall, these studies suggest that eucalyptol has broad-spectrum antimicrobial properties, making it a potential candidate for the development of new antimicrobial agents (15,53).

##### 5. Quantification of Phytochemicals present in Neem, Tulsi and Eucalyptus Leaves

There are different techniques by which the quantification of phytochemicals present in the leaf extracts can be quantified. Gas chromatography-mass spectrometry (GCMS) and high-performance liquid chromatography (HPLC) can be used to identify and measure the secondary metabolites found in Neem, Tulsi and *Eucalyptus* leaves (18,28).

By GCMS, the following steps can be taken: (39,56)

- **Sample Preparation:** Collect the leaves of Neem, Tulsi, and Eucalyptus and dry them in shade. Once dried, grind them into a fine powder using a mortar and pestle. Weigh 1 gram of each powdered sample and transfer them into separate glass vials.
- **Extraction of phytochemicals:** Add 10 mL of methanol to each vial containing the powdered sample. Sonicate the vials for 20 minutes to extract the phytochemicals. Filter the extract through a filter paper and transfer the filtrate to a new vial. This extract can be used for GCMS analysis.
- **GCMS Analysis:** Inject 1  $\mu$ L of the sample extract into the GCMS instrument equipped with a capillary column. Use a temperature program ranging from 50°C to 280°C. Use helium as the carrier gas. The GCMS will separate the different compounds in the sample and identify them by their mass spectra (21).
- **Quantification:** The abundance of each compound can be quantified by comparing its peak area with that of an internal standard. An internal standard is a known compound added to the sample before extraction to account for variations in

extraction efficiency and injection volume (26). The concentration of each compound can be calculated using the formula:

$$\text{Concentration (mg/g)} = \left( \frac{\text{Peak area of compound}}{\text{Peak area of internal standard}} \right) \times \text{Concentration of internal standard}$$

A study shows the GCMS analysis of phytochemicals in the methanolic extract of the leaves of Neem where the chemical constituents and its activity of some of the phytochemicals identified in the methanolic extracts of the leaves of Neem have been identified (4) (Figure 4, Table 4).

Similarly, the GCMS analysis of phytochemicals in the methanolic extract of the leaves of Tulsi have been studied (4) (Figure 5, Table 5).

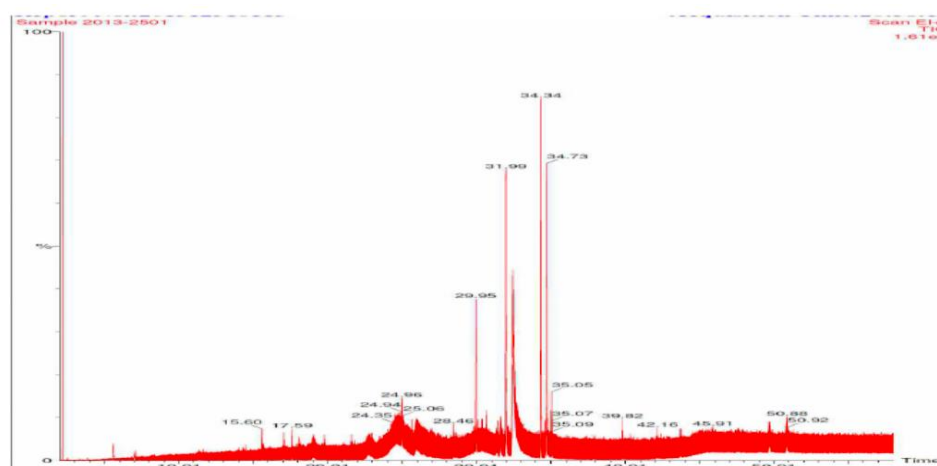


Figure 4. GC-MS chromatogram of the methanolic extract of the leaves of Neem

Table 4. Chemical constituents and its activity of some of the phytochemicals identified in the methanolic extracts of the Leaves of Neem (*Azadirachta indica*) by GCMS

Retention Time	Name of Compounds	Molecular Formula	Molecular Weight	Activity
34.34	3,7,11,15-tetramethyl-2-hexadecen-1-ol	C <sub>20</sub> H <sub>40</sub> O	296.53	Cancer-Preventive, Antimicrobial, Anti-inflammatory, Anti-diuretic, Antioxidant
34.73	9,12,15-Octadecatrienoic acid,(z,z,z)-	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	278.43	Antibacterial, Antifungal
34.73	8.11.14-Eicosatrienoic acid	C <sub>20</sub> H <sub>34</sub> O <sub>2</sub>	306.4	Astringent, Anti-inflammatory Anticoagulant
31.99	N-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256.423	Antioxidant, Nematicide 5-Alpha-Reductase-Inhibitor Flavour, Hemolytic Hypercholesterolemic, Pesticide, Antiallopecic, Antiandrogenic, Antifibrinolytic
31.99	Tridecanoic acid	C <sub>13</sub> H <sub>26</sub> O <sub>2</sub>	214.34	No activity reported

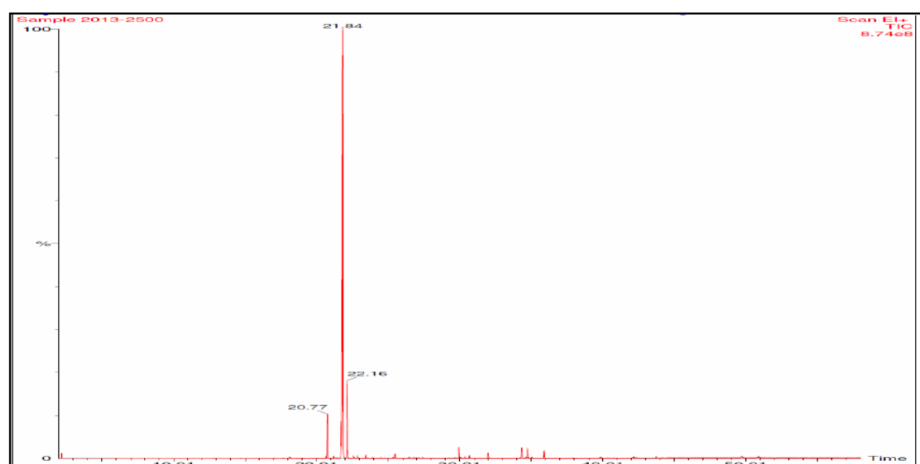


Figure 5. GC-MS chromatogram of the methanolic extract of the leaves of Tulsi

Table 5. Chemical constituents and its activity of some of the phytochemicals identified in the methanolic extracts of the Leaves of Tulsi (*Ocimum sanctum*) by GCMS

S. No.	Retention Time	Name of Compounds	Molecular Formula	Molecular Weight	Activity
1.	21.84	Benzene, 1, 2-dimethoxy-4-(1-propenyl) -	C <sub>11</sub> H <sub>14</sub> O <sub>2</sub>	178.2	Antibacterial, Nematicide, Insect-attractant, Perfumery, Flavour
2.	22.16	Caryophyllene	C <sub>15</sub> H <sub>24</sub>	204.3	Anti-tumor, Analgesic, Antibacterial, Anti-inflammatory, Fungicide
3.	20.77	Eugenol	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	164.2	Acaricide, Antibacterial, Anti-inflammatory, Antioxidant, Cancer-Preventive, Antispasmodic, Antiviral, Insecticide

By HPLC, the following steps can be taken: (19).

- **Sample Preparation:** Collect the leaves of Neem, Tulsi, and Eucalyptus and dry them in shade. Once dried, grind them into a fine powder using a mortar and pestle. Weigh 1 gram of each powdered sample and transfer them into separate glass vials.
- **Extraction of phytochemicals:** Add 10 mL of methanol to each vial containing the powdered sample. Sonicate the vials for 20 minutes to extract the phytochemicals. Filter the extract through a filter paper and transfer the filtrate to a new vial. This extract can be used for HPLC analysis.
- **HPLC Analysis:** Inject 10-20  $\mu$ L of the sample extract into the HPLC instrument equipped with a C18 reverse-phase column. Use a mobile phase consisting of water and acetonitrile, with a gradient elution. The HPLC will separate the different

compounds in the sample and identify them by their retention times and UV spectra (45).

- **Quantification:** The abundance of each compound can be quantified in a similar way as done in GCMS method by comparing its peak area with that of an internal standard. The concentration of each compound can be calculated using the similar formula:

Concentration (mg/g) = (Peak area of compound/ Peak area of internal standard) × Concentration of internal standard (47)

Thus the phytochemicals present in Neem, Tulsi, and Eucalyptus leaves can be identified and quantified using GCMS and HPLC. The concentrations of these compounds can be compared to determine which plant contains higher amounts of phytochemicals.

## 6. Efficacy and Safety of Alcohol-Free and Alcohol-Based Herbal Hand Sanitizers.

### Author please give all details in table form?

Research studies have been conducted to investigate the effectiveness of herbal hand sanitizers with and without alcohol (Table 6). The analysis of phytochemicals present in Neem, Tulsi and Eucalyptus leaves have been studied (Table 7). Various studies on the herbs, Neem, Tulsi and Eucalyptus have been done. (Table 8) (9).

Table 6. Comparative Study of Alcohol-Free and Alcohol-Based Herbal Hand Sanitizers

S. No.	Constituents	Properties	Reference
1.	Alcoholic extracts of <i>Mentha arvensis</i> , <i>Ocimum sanctum</i> , <i>Eucalyptus globules</i> , <i>Cuscuta reflexa</i> and <i>Azadirachta indica</i>	Have intense antibacterial effect.	(38)
2.	Non-Alcoholic extracts of <i>Ocimum sanctum</i> , <i>Eugenia caryophyllus</i> , and <i>Cymbopogon flexuosus</i>	Leaf extracts performed better than commercially available hand sanitizers when tested against <i>Staphylococcus aureus</i> and <i>Pseudomonas aeruginosa</i> .	(52)
3.	Non-Alcoholic Cinnamon oil	Exhibited superior antibacterial action against other herbal oils.	(5)
4.	Non-Alcoholic Multi-herbal hand wash gel that contains extracts from <i>Ocimum sanctum</i> , <i>Azadirachta indica</i> , and <i>Citrus lemon</i>	Highly efficient against <i>Staphylococcus aureus</i> , <i>Salmonella</i> and <i>Escherichia coli</i> .	(48)
5.	Non-Alcoholic Alemlaq (Alkyl Dimethyl Benzyl Ammonium Chloride) and Alcoholic Lux (Sodium Laurate)	Alemlaq, a non-alcoholic sanitizer, was more effective against <i>Staphylococcus aureus</i> than other alcoholic sanitizers, while Lux was superior to other alcoholic sanitizers against <i>E. coli</i> .	(2)
6.	Non-Alcoholic extracts of <i>Cymbopogon flexuosus</i> , <i>Eugenia caryophyllus</i> and <i>Ocimum sanctum</i>	Formulated a herbal hand sanitizer to study the antibacterial effects.	(47)

Table 7. Phytochemical screening result

Test Name	Neem	Tulsi	Eucalyptus
Alkaloids	-	+	+
Flavonoids	+	+	+
Saponins	-	+	+
Tannins	+	+	+
Phenolic compound	+	+	+
Glycosides	+	+	+
Carbohydrates	-	-	+
Acidic compound	+	+	+

Table 8. Study on Plant Herbs, Neem, Tulsi and Eucalyptus

Herb	Effective part	Antimicrobial Property
Eucalyptus	Eucalyptus oil	Inhibit the development of specific bacterial strains including <i>Staphylococcus aureus</i> as well as <i>Escherichia coli</i> .
Neem	Neem extracts	Effective against a range of fungi like <i>Aspergillus niger</i> and <i>Candida albicans</i>
Tulsi	Tulsi extracts	Demonstrated to work against a variety of bacteria and fungi.

## 7. Preparation of Herbal Hand Sanitizer

### Preparation of Extract

- **Plant Material Collection:** In order to produce a sanitizer, it is necessary to collect leaves from three distinct plant species that have demonstrated potent antimicrobial properties. The leaves will be obtained from a local nursery and transported to the laboratory for processing (6).
- **Drying and Grinding:** Once collected, the leaves must be carefully washed and sorted to eliminate any unwanted materials and aged leaves. The leaves will be placed under green light for a period of five days to dry completely and remove any remaining moisture. The dried leaves will then be finely powdered using a blender, followed by a sieving process to obtain a fine dust. The resulting powder will be stored in an air-tight plastic bag, in a dark area, until ready for use.
- **Preparation of Methanolic Extracts:** To prepare the extract, 15 grams of the coarse plant material powder will be separately weighed and added to 150 mL of 99.8% methanol. The mixture will be subjected to extraction via a Soxhlet extractor for 6 hours per sample, maintaining a temperature of 65°C, the boiling point of methanol (1).

**Separation of Extract from Solvent:** The resulting extract will be separated from the solvent using a water bath. The solvent will then be evaporated, leaving the extract to be oven-dried.

### Preparation of Hand Sanitizer

In order to prepare the hand sanitizer, extracts of Eucalyptus, Neem and Tulsi that have been sterilized using an autoclave must be mixed in equal volumes of 10 ml each, along

with 0.1 ml of disinfected glycerin in sterile conditions in a cleaned container. In order to get a homogeneous solution, the resulting mixture needs to be vortexed (24).

### **8. Screening of Antimicrobial Activity**

Methods such as the Finger Imprint method and the Agar well diffusion method can be used to evaluate the antimicrobial activity of the Eucalyptus, Neem and Tulsi extracts.

- **The Finger Imprint method**

It involves placing sterilized nutrient agar into sterile petri plates in an aseptic environment. Four fingers, except for the thumb, are gently placed on the solidified plates while the machine is in operation to serve as the control. Eucalyptus, neem, and tulsi extracts are placed into separate beakers and autoclaved. The same four fingers are then treated with the eucalyptus extract and imprinted onto the surface of the solidified plates. The plates are then incubated for 24 hours at 37°C and imprinted again at 2, 5, 10, and 15-minute intervals before being inspected and the number of colonies counted using a colony counter. Neem and Tulsi extracts are evaluated similarly (57).

- **The Agar Well Diffusion method**

It involves the use of laminar air flow to prepare nutrient agar sterile plates in an aseptic environment (41). To ensure uniformity, each agar plate has 0.1ml of culture spread out across it. Next, a tip is used to make a hole that is 6 to 8 mm in diameter. Subsequently, 0.1ml of eucalyptus, neem and tulsi extract are poured into each well, and the plates are labeled and incubated for 24 hours at 37°C. The tested microbial strain's growth is hindered during incubation by the antimicrobial agent, which diffuses in the agar medium leading to the observation of a clear zone or zone of inhibition (54,33,51).

Similarly, these two techniques can be used to assess the antimicrobial properties of hand sanitizers.

### **9. Assessment of Physical Parameters of the Formulated Herbal Hand Sanitizer**

An examination of the physiochemical properties of the formulated hand sanitizer will be conducted. This will entail an evaluation of its odour, colour, appearance, and pH (52,25).

- **Visual Examination of Physical Appearance**

The physiochemical characteristics of the newly developed hand sanitizer will be assessed in the food chemistry laboratory. Based on its visual appearance, the hand sanitizer's colour and design will be determined. Furthermore, its sanitizing odour will be detected.

- **Determination of pH Value**

To determine the pH value of the produced formulation, a digital pH meter will be utilized. The pH meter will be calibrated at room temperature (25°C) before conducting the measurement.

### **10. Importance of Herbal Hand Sanitizer**

The significance of herbal hand sanitizer is notable as alcohol-based products can strip the skin's natural oils and sebum, which serves as a barrier against bacterial infections and also encourages the precipitation of proteins. This can lead to an increased risk of damage and create a coagulum where bacteria can thrive, making it unsuitable for treating open sores or irritated, abraded skin (29). Furthermore, the fast effectiveness of alcohol-based antimicrobial products is limited due to their drawbacks, which increases the risk of disease spread (20). Therefore, medicinal plants are gaining importance as they offer safety, affordability, effectiveness, and accessibility in treating various ailments (13). These plants

include secondary metabolites that have been shown to have antibacterial effects in vitro, including tannins, terpenoids, alkaloids, and flavonoids. Herbal hand sanitizers are made from plants due to their antibacterial qualities (16). The efficacy of herbal hand sanitizer has been compared with commercial synthetic hand sanitizer using microorganism suspensions, and the results have shown that herbal hand sanitizers have enhanced antibacterial activity and potency, making them suitable for commercialization. Overall, herbal hand sanitizers offer a natural and effective alternative to alcohol-based sanitizers and can help reduce the risk of bacterial infections.

## **11. CONCLUSION**

The review findings suggest that herbal hand sanitizers offer several advantages over alcohol-based hand sanitizers. Notably, herbal sanitizers are cost-effective and free from any unpleasant odours. On the other hand, the alcohol smell in sanitizers is often disliked by individuals, which can discourage its frequent use. Additionally, herbal sanitizers have a prolonged effect, are gentle on the skin, and are non-flammable. The presence of phytochemicals in various herbs has been shown to exhibit antimicrobial properties, which makes them ideal for making herbal sanitizers. Scientific studies have identified Neem, Tulsi, and Eucalyptus leaves as having significant phytochemicals that can effectively kill microorganisms without damaging the skin. Therefore, herbal hand sanitizers made from natural plant extracts offer a promising alternative to alcohol-based sanitizers for maintaining hand hygiene and preventing the spread of infections.

### **ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

This research work is insilico, hence it doesn't contain any human and animal related data.

### **CONFLICTS OF INTEREST**

The authors declare no conflict of interest.

### **COMPETING INTERESTS**

We declare no competing financial or non-financial interests.

### **DECLARATION**

We declare that all authors of this manuscript have made substantial contributions. We have not excluded any author that substantially contributed to this manuscript. We have followed our ethical norms established by our respective institutions.

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## REFERENCES

1. Acharya, B., Ghosh, S., Yadav, G., Sharma, K., and Joshi, S., (2018). Formulation, evaluation and antibacterial efficiency of water-based herbal hand sanitizer gel. *bioRxiv* 373928 **10**: 1101/373928.
2. Ali, Y.A., (2015) "To study the effect of Hand Sanitizers used In Kingdom of Saudi Arabia Against the common Bacterial Pathogens. *International research journal of natural and applied sciences* **2(2)**: 17-27.
3. Ayepola, O., and Adeniyi, B., (2008). The antibacterial activity of leaf extracts of Eucalyptus. *Journal of Applied Sciences Research* **4(11)**: 1410-1413.
4. Balasubramanian, S., Ganesh, D., and Narayana, S., (2014). GC-MS analysis of phytocomponents in the methanolic extract of *Azadirachta indica* (Neem). *International Journal of Pharma and Bio Sciences* **5(4)**: 258-262.
5. Baliga, S., Jimmy, R., Bhat, R., and Arora, R., (2013). "Ocimum sanctum L. (Holy Basil or Tulsi) and Its Phytochemicals in the Prevention and Treatment of Cancer." *Journal of Nutrition and Cancer* **65(1)**: 26-35.
6. Balouiri, M., Sadiki, M., and KoraichiIbnsouda, S., (2016). "Methods for in vitro evaluating antimicrobial activity"- *Journal of Pharmaceutical Analysis* **6**: 71-79.
7. Chakroborty, A., Mukhopadhyay A., Sur, A., and Ghosh, S., (2021). Production of cheap hand sanitizer with herbal ingredients, *Journal of Physics: Conference Series*. **1797(1)**: 1742-1797.
8. Chandak, N., and Patankar, R.S., (2018). Formulation of Herbal Sanitizers and Determining Their Antimicrobial Activities Against Skin Pathogens. *International Journal of Innovative Science and Research Technology* **3(8)**: 169-177.
9. Chandravanshi, J.S., Rawat, A., and Ganesh, N., (2018). Formulation of Hand Sanitizers from Herbs: A Review. *International Journal of Science and Research* **9(6)**: 1399-1400.
10. Das, K., Tiwari, R. and Shrivastava, D.K., (2010). Techniques for evaluation of medicinal plant <sup>1</sup> products as antimicrobial agent: Current methods and future trends. *Journal of Med Plants Res* **4(2)**: 104-111.
11. Debas, H.T., Ramanan, L., and Straus, S.E., (2006). *Disease Control Priorities in Developing Countries*. 2nd edition. Chapter 69 Complementary and Alternative Medicine.
12. Dhahi, A.N., and Arasu, M.V., (2015). Quantification of rosmarinic acid and flavonoids, and *in-vitro* antioxidant activity of various tissues of three different colors of *Ocimum tenuiflorum* L. *Industrial Crops and Products* **66**: 166-176.
13. Duke, S.O., and Dayan, F.E., (2012). Allelopathy as a basis for plant competition. *Journal of Crop Improvement* **26(3)**: 386-407.
14. Falahati, M., and Jahaniani, F., (2005). Anti dermatophyte activities of *Eucalyptus camaldulensis* in comparison with Griseofulvin. *Iranian Journal of Pharmacology & Therapeutics* **4(2)**: 80- 83.
15. Gertsch, J., and Viveros-Paredes, J. M., (2020). The effects of plant-derived essential oils on the immune system and the control of viral infections. *Pharmaceuticals* **13(10)**: 261.
16. Gorlenko, C.L., Kiselev, H.Y., Budanova, E.V., Zamyatnin, A.A., and Ikryannikova, L.N., (2020). Plant secondary metabolites in the battle of drugs and drug-resistant bacteria: new heroes or worse clones of antibiotics. *Antibiotics Journal* **9(4)**: 170.
17. Govindachari, T.R., Suresh, G., Gopalakrishnan, G., Banumathy, B., and Masilamani, S., (1998). Identification of antifungal compounds from the seed oil of *Azadirachta indica* *Phytoparasitica* **26(2)**: 109–116.
18. Gupta, V.K., and Agarwal, S., (2011). Quantitative HPLC analysis of bioactive compounds in tulsi (*Ocimum sanctum* L.) from different parts. *International journal of pharmacy and pharmaceutical sciences* **3(3)**: 203-205.
19. Jaiswal, V., DerMarderosian, A., and Porter, J.R., (2010). Detection and quantification of neem alkaloids in neem oil and cosmetics using high-performance liquid chromatography with tandem mass spectrometry. *Journal of agricultural and food chemistry* **58(10)**: 6895-6901.
20. Jumaa, P.A., (2005). Hand hygiene: simple and complex. *International Journal of Infectious Diseases* **9(1)**: 3-14.
21. Kaushik, P., and Chauhan, N.S., (2016). Comparative GC-MS analysis of essential oils of Tulsi and Ajwain. *International Journal of Research in Ayurveda and Pharmacy* **7(5)**: 19-24.
22. Kavooosi, G., and Rowshan, V., (2013). Antifungal activity of the volatile oil of *Eucalyptus camaldulensis*. *Journal of Medical Microbiology* **62(12)**: 1878-1884.
23. Kayastha, B.L., (2014). Queen of herbs tulsi removes impurities from water and plays disinfectant role. *Journal of Medicinal Plants Studies* **2(2)** : 01-08.
24. Khatoon, Q.S., Khatoon, M.Q., Mohammed, S., Mohammed, T., Begum, I.S., (2020). Preparation and evaluation of herbal formulation-hand sanitizer. *ir.aiktlibrary.org* **5(1)**: 40-43.

25. Kishore, N., Wani, S., Bhalerao, A.K., Ranaware, V.P., and Zanje, R., (2013). Formulation and Evaluation of Herbal Sanitizer. *International Journal of Pharm Tech Research* **5(1)**: 40-43.
26. Kolhapure, S.A., and Sunanda, M., (2004). Evaluation of the antimicrobial efficacy and safety of Pure Hands herbal hand wash gel in hand hygiene and on inanimate objects, *The Antiseptic* **101(2)**: 55-57.
27. Koul, O., Walia, S., and Dhaliwal, G.S., (2008). Essential oils as green pesticides: potential and constraints. *Biopesticides international* **4(1)**: 63-84.
28. Kumar, B., and Sivakumar, T., (2014). Allelopathic effects of aqueous extracts of different parts of tulsi on germination and seedling growth of some agricultural crops. *International Journal of Agriculture, Environment and Biotechnology* **7(2)**: 295-299.
29. Kumar, R., Pratibha, M.D., and Kolhapure, S.A., (2005). Evaluation of the antimicrobial efficacy and safety of Pure Hands as a hand sanitizer: *Indian Journal of Clinical Practice* **15(10)**: 19-27.
30. Kumar, S., and Pandey, A.K., (2013). GC-MS analysis and allelopathic potential of lemongrass oil. *Natural product research* **27(10)**: 925-928.
31. Lu, Y., YeapFoo, L., and Polyak, S.J., (2016). Rosmarinic acid inhibits hepatitis C virus replication in vitro through suppression of cyclooxygenase-2 and inducible nitric oxide synthase. *PLoS One* **11(3)**: e0152022.
32. Magaldi, S., Mata, S., and Capriles, C.H., (2004). Well diffusion for antifungal susceptibility testing, *International Journal Infection Disease* **8**: 39-45.
33. Makino, T., Ono, T., Muso, E., Honda, G., and Sasayama, Y., (2003). Antibacterial activity of some essential oils against some pathogenic bacteria. *Journal of Plant Medicine* **69(7)**: 692-695.
34. Marín, D.M., and Ferrero, A.A., (2015). Allelopathic effects of eucalyptol on the germination and growth of selected weed species. *Journal of Chemical Ecology* **41(2)**: 182-190.
35. Mishra, R., Verma, S., Pandey, P., and Khan, F., (2021). "Evaluation of Antifungal Efficacy of Cinnamomum Zeylanicum Bark Oil" *Ambient Science, National Cave Research and Protection Organization, India* **8(1)**: 24-27.
36. Mishra, R., Verma, S., Pandey, P., and Khan, F., (2021). "In Vitro Antibacterial Properties of Cinnamomum Zeylanicum Essential Oil Against Enteric Bacteria". *Journal of Natural Remedies* **21(1)**: 26-31.
37. Mishra, R., Verma, S., Rustagi, S., Pandey, P., and Khan, F., (2021). A Comparative Study of Antimicrobial Efficacy of Cinnamomum Zeylanicum Leaf Oil and Cinnamomum Tamala Leaf Oil. *PalArch's Journal of Archaeology of Egypt/Egyptology* **18(7)**: 1477-1484.
38. Natarajan, S., Anand, P., and Selvaraj, K., (2021). Review on the importance of herbal hand sanitizers with the emergence of COVID-19. *International Journal of Pharmaceutical Research and Applications* **6(3)**: 702-705.
39. Orhan, I.E., and Suntar, I., (2016). *Rosmarinus officinalis* L.: an update review of its phytochemistry and pharmacological activities. *Journal of Medicinal Plants Research* **10(14)**: 189-212.
40. Pacheco, B.C., and Robbie, K., (2007). Nanomaterials and nanoparticles: sources and toxicity. *Biointerphases Journal* **2(4)**: 17-71.
41. Pareek, A., Suthar, M., and Rathore, G.S., (2013). Allelopathic effect of *Ocimum sanctum* L. on germination and growth behaviour of chickpea (*Cicer arietinum* L.). *Annals of Plant Sciences* **2(12)**: 541-548.
42. Ramesh, T., Maheswari, M.U., and Kumaravel, S., (2014). GC-MS analysis of bioactive components of *Cassia auriculata* flowers. *International Journal of Current Microbiology and Applied Sciences* **3(4)**: 981-990.
43. Rizvi, S.J.H., and Rizvi, V., (2015). Allelopathic effects of *Eucalyptus globulus* Labill. Leaf litter on germination and early seedling growth of lettuce (*Lactuca sativa* L.) and radish (*Raphanus sativus* L.). *Journal of Plant Nutrition and Soil Science* **178(3)**: 460-466.
44. Sanchez-Mata, M.C., Camara, M., and Díez-Marques, C., (2012). Identification and quantification of phenolic compounds in aromatic herbs and their potential use as inhibitors of *Fusarium oxysporum*. *Industrial crops and products* **37(1)**: 41-48.
45. Shultz, E.B., (1992). *Neem: A Tree for Solving Global Problems*. National Academies Press, Board on Science and Technology for International Development, 1-152.
46. Siddika, S.K., (2022). Preparation of alcohol based herbal hand sanitizers and assessment of antimicrobial efficacy against surface microorganisms. *Chattogram Veterinary & Animal Sciences University*
47. Singh, H.P., Batish, D.R., and Kohli, R.K., (2003). Allelopathic effect of *Ocimum* species on germination and growth of wheat. *Biochemical Systematics and Ecology* **31(6)**: 659-663.
48. Singla, D., and Saini, K., (2019). Formulation of an Herbal Substitute for Chemical Sanitizer and its Evaluation for Antimicrobial Efficiency. *International Journal of Chem Tech Research* **12(3)**: 114-120.

49. Tembe-Fokunang, EA., Charles, F., Michael, A., and Bonaventure, N., (2019). The potential pharmacological and medicinal properties of neem in the drug development of phytomedicine. *Journal of Complementary and Alternative Medical Research* **7(1)** : 1-18.
50. Thombare, M.A., Udugade, B.V., Mulik, M.B., and Pawade, D.A., (2015). Formulation and evaluation of novel herbal hand sanitizer. *Indo American Journal of Pharm Research* **5**: 483-488.
51. Tripathi, A.K., Prajapati, V., and Aggarwal, K.K., (2010). Chemical composition and allelopathic effect of essential oil from *Ocimum tenuiflorum* (Lamiaceae) on germination, growth, and yield of soybean (*Glycine max*). *Journal of Agricultural and Food Chemistry* **58(6)**: 3357-3362.
52. Tundis, R., Menichini, F., Conforti, F., and Loizzo, M.R., (2014). Recent knowledge on medicinal plants as source of antimicrobial agents against bacterial and fungal infections. *Natural Product Communications* **9(1)**: 5-14.
53. Tyagi, R., Sharma, G., Jasuja, N.D., and Menghani, E., (2016). Indian medicinal plants as an effective antimicrobial agent. *Journal of Critical Reviews* **3(2)**: 69-71.
54. Valgas, C., Souza, S.M., and Smânia, E.F.A., (2007). Screening methods to determine antibacterial activity of natural products, *Brazilian Journal of Microbiology* **38**: 369-380.
55. Vazirian, M., Nabavi, S.F., Jafari, M., Manayi, A., and Berenjian, A., (2016). Rosmarinic acid: a new potential medicinal plant against fungal infections. *Current Medicinal Chemistry* **23(14)**: 1500-1514.
56. Yackovich, F., Cheryl, A., and John, E., (1989). Validation of the agar patch test with an antibacterial liquid soap and comparison with the finger imprint method. *J. Soc. Cosmet. Chem* **40**: 265-271.
57. Yadav, A., Kumar, R., Kumar, S., and Kumar, A., (2017). Analysis of secondary metabolites from neem seed kernel using GC-MS. *Journal of pharmacognosy and phytochemistry* **6(6)**: 1569-1575.
58. Zhang, Y., and Smuts, J.P., (2017). Diverse roles of ursolic acid in cancer. *Oncology Letters* **14(5)**: 5171-5180.
59. Zhou, Y., Yang, J., and Jiang, S., (2017). Rosmarinic acid ameliorates lipopolysaccharide-induced acute lung injury via regulation of RhoA/Rock1. *Life Sciences*, **182**: 93-101.

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