



FORMULATION OF HERBAL HAND WASH CONTAINING TULSI (*OCIMUM TENUIFLORUM*) METHANOLIC EXTRACT WITH ANTIMICROBIAL PROPERTY

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ABSTRACT

The demand for herbal and natural personal care products has been steadily increasing due to growing consumer awareness of the potential health risks associated with synthetic ingredients. The current study aimed to develop and evaluate a (*Ocimum tenuiflorum*) herbal hand wash formulation as an alternative to conventional chemical-based hand washes. Tulsi, also known as Holy Basil, possesses antimicrobial properties and is widely recognized for its medicinal and therapeutic benefits. Methanolic extract of *Ocimum tenuiflorum* and aqueous solution *Sapindus mukorossi* was prepared. Antibacterial activity was identified using agar diffusion method. Formulation one containing Sodium lauryl Ether Sulphate and Methylene Paraben while formulation two contains *Sapindus mukorossi* and lemon juice along with the methanolic extract of *Ocimum tenuiflorum*. Methanolic extract of *Ocimum tenuiflorum* showed maximum Zone of inhibition of 17mm

diameter against *B.subtilis* (MTCC 441) and *E.coli* (MTCC 739). Handwash formulation one showed maximum inhibition of 17mm (extract concentration of 75µg/ml and 100 µg/ml) and 16mm (extract concentration of 75µg/ml) against *B.subtilis* (MTCC 441) and *E.coli* (MTCC 739) respectively while formulation two reports to have maximum inhibitory effect at 100 µg/ml of 19mm and 17mm against *B.subtilis* (MTCC 441) and *E.coli* (MTCC 739)

respectively. The results demonstrated significant antimicrobial efficacy, indicating the potential of the formulation to effectively combat pathogens responsible for various infectious diseases by formulation one. Moreover, the hand wash exhibited excellent skin compatibility, as assessed through irritation and sensitization studies, suggesting its suitability for frequent use without causing any adverse reactions.

KEYWORDS: Ocimum tenuiflorum extract, Antimicrobial, herbal, formulation, pathogens.

INTRODUCTION

Skin is most exposed part of our body .Number of bacteria are present in human body such as skin, mouth and hands etc. Hand play important role in transmission of infection or disease. Around 2 - 10 billions of bacteria are present on fingertips. There are more than 3200 different germs present in our hand and belonging to more than 150 species. Some of bacterial species are harmful and cause infection such as *Staphylococcus aureus*, *Pseudomonas*, *E. coli* etc, Regular hand washing is on of important method to cure these type of microbial infection. Marketed hand wash are used for curing microbial infection these marketed hand wash are chemical based. Many marketed hand washes are chemical based so there frequent use can lead to skin irritation and also resist among pathogen. To overcome this use of chemical based hand wash. Now days that is focused on herbal hands wash. Plant extract and Product have been used for centuries in traditional medicine functional food etc. The main advantage of using natural source is that they are easily available, cheap and harmful compared to chemical products.^[1,2]

Tulsi handwashes can serve as a convenient option for hand hygiene when soap and water are not readily available, providing a natural and herbal alternative to conventional handwashes. As with any skincare or personal care product, it is recommended to read the label and follow the instructions provided by the manufacturer. Individuals with specific skin conditions or allergies should consult a healthcare professional before using Tulsi-infused handwashes or any new product. Herbal handwash is a natural and plant-based alternative to traditional chemical-based handwashing products. It is designed to effectively cleanse and sanitize the hands while being gentle and nourishing to the skin. Unlike conventional handwashes that often contain synthetic fragrances, harsh chemicals, and antibacterial agents, herbal handwashes harness the power of botanical ingredients to promote cleanliness and hygiene.^[3,4]

The primary objective of herbal handwash is to eliminate germs and bacteria from the hands, helping to prevent the spread of infections and diseases. It achieves this through a combination of natural ingredients known for their antimicrobial properties. These ingredients may include plant extracts such as neem, tea tree oil, aloe vera, lavender, rosemary, and eucalyptus, among others. One of the main advantages of herbal handwash is that it avoids the use of artificial fragrances and harsh chemicals, which can sometimes cause skin irritation, dryness, or allergic reactions. Instead, herbal handwashes often incorporate natural fragrances derived from essential oils, which not only provide a pleasant scent but also offer potential therapeutic benefits. Additionally, herbal handwashes are typically free from ingredients such as parabens, sulfates, triclosan, and synthetic dyes, which are commonly found in conventional handwashing products. This makes them a more environmentally friendly option, as well as suitable for individuals with sensitive skin or those who prefer natural and organic products.^[5,6]

Herbal handwashes are usually available in liquid form, similar to conventional handwashes, and can be used in a similar manner. Users simply need to dispense an adequate amount of the handwash, lather it thoroughly on their hands for at least 20 seconds, and then rinse it off with water. Some herbal handwashes may also incorporate moisturizing ingredients like glycerin or coconut oil to help keep the skin hydrated and prevent dryness. In conclusion, herbal handwash offers a natural and plant-based approach to hand hygiene. By harnessing the power of botanical ingredients, it aims to effectively cleanse and sanitize the hands while being gentle and nourishing to the skin. With their potential benefits for both personal health and the environment, herbal handwashes have gained popularity as a preferred choice for individuals seeking a more natural and holistic approach to handwashing. It is a natural alternative to conventional handwashing products. It is formulated using plant-based ingredients that have been traditionally used for their antimicrobial, antiseptic, and cleansing properties. Herbal handwashes offer a gentle and effective way to keep your hands clean and free from germs without the use of harsh chemicals. One of the key advantages of herbal handwash is its natural composition. It typically contains extracts of herbs, such as neem, tulsi (holy basil), aloe vera, tea tree, and other botanical ingredients known for their antibacterial and antifungal properties. These herbs have a long history of use in traditional medicine and are believed to have potent disinfectant properties.^[7,8]

Some of the basil is a rich source of key nutrients like Vitamin A, Vitamin C, Calcium and Phosphorus. The presence of Vitamin A helps in strengthening eyesight. Basil also contains antioxidants like beta carotene that help in preventing cell damage. "Tulsi" or the holy basil, is famous throughout the globe for its healing and other medicinal properties. Its leaves are helpful in sharpening memory and in curing fever and common cold. They also act as an anti-stress agent and also help in purifying blood. This, in turn, helps in reducing the risk of heart attacks and also lowers the cholesterol level. The leaves of the basil are also effective in reducing mouth ulcer and other infections of the mouth.^[9,10]

Tulsi leaves contain bright, yellow coloured and pleasant volatile oil (0.1 to 0.9%). The oil content of the drug varies depending upon the type, the place of cultivation and season of its collection. The oil is collected by steam distillation method from the leaves and flowering tops. It contains approximately 7.0% eugenol, carvacrol (3%) and eugenol-methyl ether (20%). It also contains caryophyllin, ursolic acid, rosmarinic acid, thymol, methyl chavicol, citral, carvacrol, β -caryophyllene. Seeds contain fixed oil with good drying properties. The plant is also reported to contain alkaloids, glycosides, saponins, tannins, an appreciable amount of vitamin C, and traces of maleic acid, citric and tartaric acid.^[11]

MATERIALS AND METHODS

The present study was focused on the *O. tenuiflorum* as antimicrobial agent collected from Bhoranj Block, Hamirpur district of Himachal Pradesh. Fresh *O. tenuiflorum* leaves were collected in a sterile container from a local area.

Preparation of methanol extract of *O.tenuiflorum*

The methanolic extract of leaves sample (40gm) of *O.tenuiflorum* were prepared by using the Soxhlet apparatus. The leaves samples were dissolved in 80% methanol (400ml). The concentrate was ready within 2-3 days. The prepared extract was filtered through the filter paper. The filtrate was collected and allowed to evaporate at 40°C, to yield thick and viscous residues as crude extract. The dried crude extract was stored at 4°C in air tight bottles till further use.^[12]

Preparation of Reetha (*Sapindus mukorossi*)

A process for the preparation of Reetha gel by heating aqueous solution of Reetha at 95°C for one hour, cooling to room temperature and keeping it overnight to allow to settle. The extract was filtered through the filter paper the Reetha extract was stored in air tight bottles till further

use. Reetha gel we will use it as a foaming agent in hand wash preparation. Reetha gel can be incorporated into herbal hand wash formulations to provide a gentle and natural cleansing experience.^[13,14]

Antibacterial Activity of Methanolic Extract

The bacterial strains were grown for 24 hours in nutrient broth (NB) at 37°C. Nutrient agar Medium was used to study the antibacterial activity by agar well diffusion assay by incubating assay plates at 37°C for 18hrs. The wells were punched with the cork borer (6mm) in the agar and the methanolic extracts of leaves with different concentration i.e. 25 µg/ml, 50µg/ml, 75µg/ml and 100µg/ml were loaded in the wells. After the incubation of 18hrs at 37°C, the zone of Inhibition (mm) was measure. Amoxicillin (10mg) was used as positive control and ethanol (solvent) was used as negative control in the antibacterial assay.^[15]

RESULTS AND DISCUSSION

The extractive yield of methanolic extract was observed 2.92g, however the percentage yield was observed 7.3.%.

Preparation of Hand wash Containing Methanolic Extract of *O.tenuiflorum*

Antibacterial Activity of methanolic extract of *O.tenuiflorum*

The antimicrobial activity of methanolic extract of leaves of *O.tenuiflorum* were checked against gram +ve (*B.subtilis*) and gram -ve (*E.coli*) by an agar well diffusion method. The result of zone of inhibition shows the significant antimicrobial activity. The zone of inhibition was measure with different concentration i.e. 25µg/ml, 50µg/ml, 75µg/ml, 100µg/ml of methanolic extract. The maximum zone of inhibition was observed 17 mm with concentration 100µg/ml against *B.subtilis* (MTCC 441) & *E.coli* (MTCC 739) as shown in Table 1 and 2 and Fig.1.

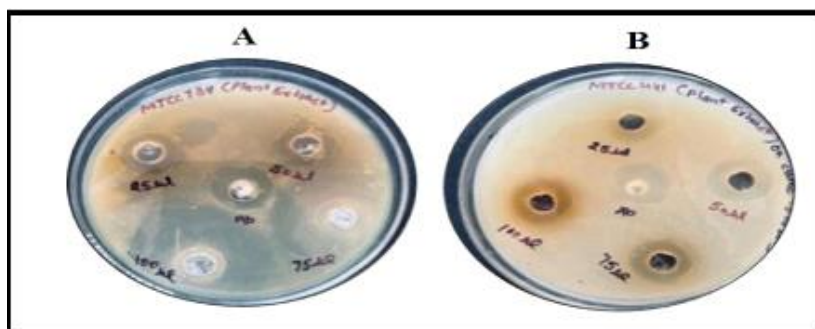


Fig. 1: Showing the zone of inhibition of methanolic extract of leaves of *O. tenuiflorum* against *B. subtilis* (MTCC 441) and *E.coli* (MTCC 739).

Table 1: Showing the zone of inhibition of methanolic extract of leaves of *O. tenuiflorum* against *B. subtilis* (MTCC 441).

Concentration of plant extract	Whole zone of inhibition (mm)	Zone of well (mm)	Zone of inhibition
25µg/ml	20	8	12mm
50µg/ml	21	8	13mm
75µg/ml	22	8	14mm
100µg/ml	25	8	17mm
Antibiotic(10g)	13	8	5mm

Table 2: Showing the zone of inhibition of methanolic extract of leaves of *O. tenuiflorum* against *E.coli* (MTCC739).

Concentration of plant extract	Whole zone of inhibition (mm)	Zone of well (mm)	Zone of inhibition
25µg/ml	17	8	9mm
50µg/ml	18	8	10mm
75µg/ml	20	8	12mm
100µg/ml	25	8	17mm
Antibiotic(10g)	20	8	12mm

Antibacterial activity of hand wash formulation 1 against (MTCC 441) *B.subtilis* & (MTCC 739) *E.coli*.

The antimicrobial activity of hand wash formulation 1 of *O.tenuiflorum* were checked against gram +ve (*B.subtilis*) and gram -ve (*E.coli*) by an agar well diffusion method. The result of zone of inhibition shows the significant antimicrobial activity. The zone of inhibition was measure with different concentration i.e. 25µg/ml, 50µg/ml, 75µg/ml, 100µg/ml of methanolic extract. The maximum zone of inhibition were observed 17 mm with concentration 100µg/ml against MTCC 441 as shown in Fig.2 and table 3 and 4.



Fig. 2: Showing the zone of inhibition of Hand wash Formulation 1 containing the methanolic extract of *O. tenuiflorum* against *B.subtilis* (MTCC 441) *E. coli* (MTCC 739).

Table 3: Showing the zone of inhibition of Hand wash Formulation 1 containing the methanolic extract of *O. tenuiflorum* against *B. subtilis* (MTCC 441).

Concentration of plant extract	Whole zone of inhibition (mm)	Zone of well (mm)	Zone of inhibition
25µg/ml	20	8	12mm
50µg/ml	22	8	14mm
75µg/ml	25	8	17mm
100µg/ml	25	8	17mm
Antibiotic(10mg)	15	8	7mm

Table 4: Showing the zone of inhibition of Hand wash Formulation 1 containing the methanolic extract of *O. tenuiflorum* against *E. coli* (MTCC 739).

Concentration of plant extract	Whole zone of inhibition (mm)	Zone of well (mm)	Zone of inhibition
25µg/ml	20	8	12mm
50µg/ml	20	8	12mm
75µg/ml	24	8	16mm
100µg/ml	20	8	12mm
Antibiotic(10mg)	20	8	13mm

Antibacterial activity of hand wash formulation 2 against (MTCC 441) *B.subtilis* & (MTCC 739) *E.coli*.

The antimicrobial activity of hand wash formulation 2 of *O.tenuiflorum* were checked against gram +ve (*B.subtilis*) and gram -ve (*E.coli*) by an agar well diffusion method. The result of zone of inhibition shows the significant antimicrobial activity. The zone of inhibition was measure with different concentration i.e. 25µg/ml, 50µg/ml, 75µg/ml, 100µg/ml of methanolic extract. The maximum zone of inhibition was observed 19mm with concentration 100µg/ml against *B.subtilis* (MTCC 441).The minimum zone of inhibition were observed 10mm with concentration25µg/ml against *E.coli* (MTCC 739) as shown in table 5 and 6 and Fig. 3.

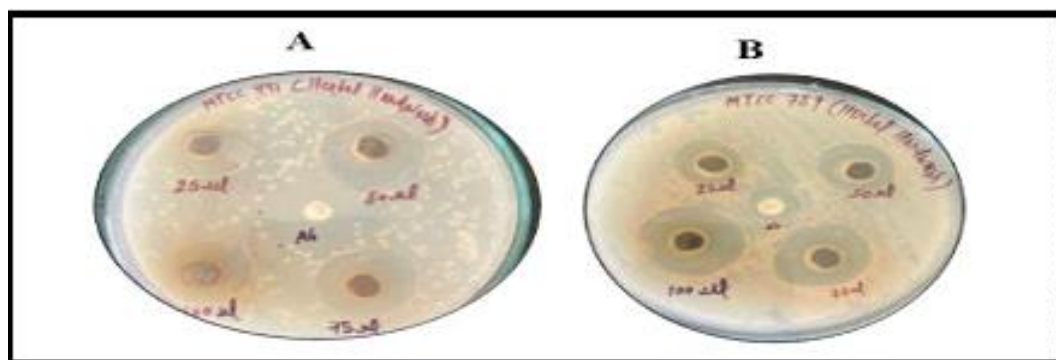


Fig. 3: Showing the zone of inhibition of Hand wash Formulation 2 containing the methanolic extract of *O. tenuiflorum* against *B.subtilis* (MTCC441) *E. coli* (MTCC 739).

Table 5: Showing the zone of inhibition of Hand wash Formulation 2 containing the methanolic extract of *O. tenuiflorum* against *B. subtilis* (MTCC 441).

Concentration of plant extract	Whole zone of inhibition (mm)	Zone of well (mm)	Zone of inhibition
25µg/ml	22	8	14mm
50µg/ml	23	8	15mm
75µg/ml	25	8	17mm
100µg/ml	27	8	19mm
Antibiotic(10mg)	20	8	12mm

Table 6: Showing the zone of inhibition of Hand wash Formulation 2 containing the methanolic extract of *O. tenuiflorum* against *E.coli* (MTCC 739).

Concentration of plant extract	Whole zone of inhibition (mm)	Zone of well (mm)	Zone of inhibition
25µg/ml	18	8	10mm
50µg/ml	20	8	12mm
75µg/ml	25	8	17mm
100µg/ml	25	8	17mm
Antibiotic(10mg)	15	8	7mm

The two bacterial strains, *B. subtilis* (MTCC 441) and *E. coli* (MTCC739) were used to check the antimicrobial activity of methanolic extract of *O. tenuiflorum* leaves. All the strains will be obtained from the repository of Division of Microbiology Lab of CPU Hamirpur, H.P.

CONCLUSION

The main aim of this study was to check the antibacterial potential of methanolic extract of *O. tenuiflorum* against gram +ve and –ve bacteria. We observed that hand wash formulation 2 showed more antibacterial activity against gram +ve as compared to gram –ve bacteria. This may be use of aqueous extract of reetha (used as foaming agent) and lemon juice (used as a preservative) instead of SLES (Sodium Lauryl Ether Sulphate) and Methylene paraben. So we conclude that plant based hand wash are much safer as compared to synthetic hand wash.

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