

# Formulation and Evaluation of the antimicrobial potential of a Herbal handwash Tablet

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**ABSTRACT:** The Herbal Handwash Tablet is a compact and travel-friendly hygiene product made with orange peel extract, which is recognized for its antimicrobial and antifungal effects. This formulation successfully removes microbes while offering a convenient and environmentally friendly option compared to traditional liquid handwashes. By combining natural plant extracts with specific synthetic ingredients, the formulation improves skin protection while remaining gentle on human skin. The scent of orange provides a refreshing sensory experience, making it an attractive option for those looking for both hygiene and skincare advantages. This study examines the therapeutic benefits of orange peel extract in handwash formulations, showcasing its effectiveness as a safe and reliable alternative to standard antiseptic products.

**KEYWORDS:** Herbal Handwash, Tablets, Orange peel extract, antimicrobial.

## I. INTRODUCTION

Escherichia coli is the leading cause of urinary tract infections as well as infections of the gastrointestinal tract. Staphylococcus aureus is often associated with skin and soft tissue infections but can also cause serious infections like pneumonia. Both pathogens are abundantly available on various surfaces, in soil, water, on the human body as well and get readily transferred through contact.

A Handwash Tablet can be designed to evaluate its antimicrobial activity, ensuring effective protection against microbes while maintaining skin barrier function. Orange peel extract is rich in bioactive compounds such as flavonoids, limonene, and polyphenols, which contribute to its antimicrobial, antifungal, and antioxidant activities. Additionally, orange peel is a natural, eco-friendly ingredient that aligns with sustainable practices by utilizing fruit waste [1, 2].

A tablet dosage form is easy to carry and use, ideal for on-the-go hygiene, ensures consistent antimicrobial activity, minimizes risk of spillage, contamination, and product wastage [3].

D-Limonene, is reported to be the major active component of orange peel extract and it can effectively combat the prevalent human-hand pathogens Staphylococcus aureus and Escherichia coli. Thus, it might be utilized as a reliable and secure substitute for synthetic Handwashing agents [4].

The current study aims to formulate a herbal handwash tablet that will be optimized for proper disintegration, ease of use, and effective release of active ingredients. Additionally, the antimicrobial efficacy will be evaluated against Escherichia coli and Staphylococcus aureus, followed by stability studies to ensure long-term effectiveness.

## II. MATERIALS & METHODS

### Materials

Orange peel extract was purchased from Research lab fine chem industries. Kaolin, sodium laurel sulfate, sodium benzoate, polyvinylpyrrolidone, citric acid, sodium starch glycolate, sodium bicarbonate, sodium chloride, barium chloride were purchased from S. D. Fine Chemicals. Agar Agar and Nutrient Agar were purchased from Hi-Media Laboratories Pvt. Ltd.

### Methodology

#### a. Preformulation Studies

Phytochemical screening was done for the orange peel extract in order to detect the phytochemical constituents present [5].

#### b. Formulation development [6]

Accurately weighed active pharmaceutical ingredient (API) and excipients were placed in a clean and dry mortar for granulation process.

Kaolin was added to the mortar, followed by the gradual addition of orange peel extract. The mixture was blended thoroughly to ensure uniform adsorption of the extract onto the kaolin. All pre-granulating excipients were added to the mixture and mixed uniformly. A polyvinylpyrrolidone (PVP) solution prepared in isopropyl alcohol (IPA) was added gradually to the dry powder mixture to form a cohesive dough. The dough was passed through sieve no. 14 to obtain uniformly sized

granules. The granules were dried to the desired moisture content. The dried granules were blended with the post-granulating agents to ensure uniform distribution. The final blend was compressed into tablets using an appropriate tablet punching machine with the die of 12mm. The tablets were individually packed in paper wrappers, and then ten packets were stored in a plastic container under appropriate conditions.



Figure 1a: Handwash Tablets



Figure 1b: Packaged Handwash Tablets

### c. Evaluation of the herbal handwash tablets

**Organoleptic properties:**The organoleptic properties such as appearance, colour, odour, shape, size, texture were determined by physical examination[7].

**pH determination:**One tablet was dissolved in 5 ml of water and pH was measured using pH meter. Comparison was also done with marketed handwash flakes and a marketed liquid handwash[8].

**Foam height:**The foam height was assessed by dissolving one tablet in 50 ml water by shaking it for 30 secs and observing the volume of foam formation. Comparison was also done with marketed handwash flakes and a marketed liquid handwash[9].

**Foam retention:**The foam retention was evaluated by measuring the time taken for the foam to collapse, indicating the tablet's ability to maintain a stable lather over time. Comparison was also done with marketed handwash flakes and a marketed liquid handwash[10].

**Disintegration test:**The disintegration test was performed to assess the time required for the herbal handwash tablets to break down into smaller particles. Six tablets from the same batch were selected, ensuring they were intact and free from defects. Each tablet was placed in one of the six tubes of the basket-rack assembly in the disintegration apparatus. Distilled water was added

to the tubes, ensuring the liquid level remained consistent throughout the test. The apparatus was operated for the specified time at the specified temperature, and the disintegration time was recorded as minutes/seconds, indicating the tablet's ability to disintegrate effectively. Comparison was also done with a Disprin tablet, as it is also an effervescent tablet [11].

**Thickness:**The thickness of the herbal handwash tablets was measured using a Vernier calliper. The reading was repeated for multiple tablets to obtain an average value [12].

**Hardness:**The hardness of the herbal handwash tablets was measured using a Monsanto hardness tester. Each tablet was placed on the tester's platform, and the probe was carefully adjusted to align directly over the tablet's center. Pressure was gradually applied by pushing the probe down until the tablet fractured, and the force required to break the tablet was recorded in kilograms or Newtons. This process was repeated for multiple tablets to determine the average hardness [13].

**Weight variation:**The weight variation test for handwash tablets is performed to ensure uniformity in tablet weight and consistency in ingredients. In this test, 6 tablets were randomly selected and weighed individually. The average weight was calculated, and the percentage deviation of each tablet from the average was determined [14].

**Pre-microbial test:**The pre-microbial test was conducted to observe the initial microbial growth before using the herbal handwash tablet. A sample from palm was collected and inoculated onto an appropriate growth medium under sterile conditions. The inoculated plates were incubated at a specified temperature for a predetermined time to allow microbial growth. After incubation, the plates were examined for the presence of microbial growth[15].

**Post-microbial test:**The post-microbial test was conducted to observe the microbial growth after using the herbal handwash tablet. Following the usage of the tablet, a sample from the palm was collected and inoculated onto an appropriate growth medium under sterile conditions. The inoculated plates were incubated at a specified temperature for a predetermined time to allow microbial growth. After incubation, the plates were examined for the presence or reduction of microbial growth compared to the pre-microbial test, indicating the tablet's antimicrobial effectiveness [16].

**Drug content determination:**A tablet of the drug formulation was placed in a 100 mL volumetric flask containing distilled water. As the solution remained turbid, 1 mL of the solution was pipetted out and diluted to 100 mL with distilled water to obtain a clear solution, ensuring accurate absorbance measurement. The absorbance of the solution was measured at a wavelength of 208 nm using a UV spectrophotometer [17].

**Microbial assay:**The microbiological assay was conducted to evaluate the antimicrobial effectiveness of the herbal handwash tablet. Slant was prepared using nutrient agar and agar agar (1.5%) by inoculating *Escherichia coli* and *Staphylococcus aureus* culture on each slant. The inoculum density was adjusted to achieve the desired concentration (0.5 McFarland standard) using freshly prepared saline. Equal volume of *Escherichia coli* and *Staphylococcus aureus* culture was mixed in a test tube. The surface of sterile agar plates was streaked with the mixed culture using a

sterile cotton swab. Wells were created in the agar using a sterile cork borer, and the handwash tablet solution (1 tablet dissolved in 5 ml of water) was added to the wells using a sterile pipette. One well of the same plate was filled with distilled water, and in the other well, an antibiotic like Kanamycin was poured to observe the negative and positive control, respectively. The agar plates were incubated overnight at temperature (37°C) for microbial growth[18].

**CAM assay:** A Chorioallantoic membrane (CAM) assay was performed to test the irritancy of the gel on a 8-day-old chicken embryo. The air sac was marked and the eggshell over the air sac and the inner shell membrane were removed carefully. Tablet solution was prepared (1 tablet dissolved in 10 ml distilled water) and 0.3 ml or 3 to 4 drops of this solution was added onto CAM and the egg was observed for 5 mins or 300 secs to check for haemorrhage, lysis, coagulation. The time taken for haemorrhage, lysis, and coagulation was noted and with the help of the following formula the Irritancy Score (IS) was calculated [19].

$$IS = [(301 - H) / 300 \times 5] + [(301 - L) / 300 \times 7] + [(301 - C) / 300 \times 9]$$

Where, H = Haemorrhage time

L = Lysis time

C = Coagulation time

**Stability studies:** The herbal handwash tablet was stored for three months at 40°C and 75% RH (relative humidity) in a humidity chamber as part of an accelerated stability test and at 25°C and 60% RH for real-time stability studies. All the tests mentioned above were performed on the tablets stored in the chamber after 3 months [20,21,22].

### III. RESULTS & DISCUSSION

**Phytochemical screening:** The orange peel extract was found to contain terpenes, steroids and flavonoids.

**Formulation development:** The composition of various formulations is summarized in **Table 1**.

Ingredients	F 1	F 2	F 3	F 4
Orange Peel Extract (gm)	0.15	0.175	0.180	0.200
Kaolin (gm)	0.4	0.445	0.425	0.475
SLS (gm)	0.06	0.075	0.080	0.085
Citric Acid (gm)	0.04	0.05	0.055	0.060
Sodium Starch Glycolate (gm)	0.07	0.06	0.055	0.065

Sodium Bicarbonate (gm)	0.09	0.1	0.095	0.1
(PVP in IPA)	q.s.	q.s.	q.s.	q.s.
<b>Post-granulating Agent:</b>				
SLS (gm)	0.006	0.0075	0.008	0.009
Sodium Starch Glycolate (gm)	0.006	0.0075	0.008	0.009
Sodium Benzoate (gm)	0.006	0.0075	0.008	0.009

**Table 1: Composition of various handwash tablet formulations**

All the formulations were found to have buff colour, round shape, smooth texture and a mild citrus odour.

The results of the **foam height, foam retention, disintegration time, thickness, hardness and weight variation** have been summarized in **Table 2**.

**pH determination:** The pH of all the formulations was found to be 6.5 which falls in a skin-friendly range.

**Table 2: Foam height of various formulations**

Formulation No.	Foam height (ml)	Foam retention (seconds)	Disintegration time (mins)	Thickness (cm)	Hardness (kg/cm <sup>2</sup> )	Weight variation (gm)
Formulation F1	49.3 ± 1.15	300 ± 20 seconds	11.7 ± 0.5	1.07 ± 0.3	1	0.7 ± 0.02
Formulation F2	66.1 ± 1.0	240 ± 45 seconds	12.5 ± 0.5	1.03 ± 0.5	1	0.69 ± 0.05
Formulation F3	38.2 ± 0.95	300 ± 00 seconds	12 ± 0.9	1.1 ± 0.7	1	0.66 ± 0.08
Formulation F4	65.5 ± 0.99	240 ± 30 seconds	11.9 ± 1.0	1.12 ± 0.5	1	0.72 ± 0.05
Handwash flakes	31.7 ± 1.52	120 ± 10 seconds	---	---	---	---
Liquid Handwash	42.3 ± 2.08	300 ± 55 seconds	---	---	---	---
Disprin Tablets	---	---	2.25 ± 1.44			

**Pre and Post Microbial test:** There was visible reduction in the microbial colonies after washing hands with Formulation F2 of the herbal handwash tablets when compared with other formulations.

The results are seen in **Figure 2**. Hence, this formulation was evaluated further for its drug content, safety and efficacy against *S. aureus* and *E. coli*.



**Figure 2: Pre and Post Microbial test of F2**

**Drug content:** The drug content of F2 was found to be  $98.75\% \pm 0.7$ .

**Microbiological assay:** Table 3 represents a summary of the zones of inhibition of the various formulations. F2 exhibited a greater inhibition on

*E. coli* and *S. aureus* when compared to positive control (Kanamycin). Figure 3 exhibits the zone of inhibition of the herbal handwash tablet F2 along with negative control and the positive control.

**Table 3: Zones of inhibition of various formulations**

	Zone of Inhibition (cm)
Negative Control	0.0
Positive Control	$3.5 \pm 0.12$
Formulation F2	$4.1 \pm 0.2$



**Figure 3: Zones of Inhibition**

**CAM assay:** The summary of lysis, haemorrhage and coagulation time for control saline, irritant 0.1N sodium hydroxide and F2 of herbal handwash

tablets is compiled in Table 4 and Figure 4. The developed herbal handwash tablets were found to be non-irritant.

**Table 4: Observation time and Irritancy score calculation of CAM assay**

Test Substance	Events			Irritation score	Category
	Hemorrhage	Lysis	Coagulation		
Negative control (Saline)	> 300 sec	> 300 sec	> 300 sec	0.069	Non - irritant
Positive control (0.1N NaOH)	20 sec	15sec	20sec	19.78	Severe irritant
Herbal Handwash Tablet	> 300 sec	> 300 sec	> 300 sec	0.069	Non - irritant

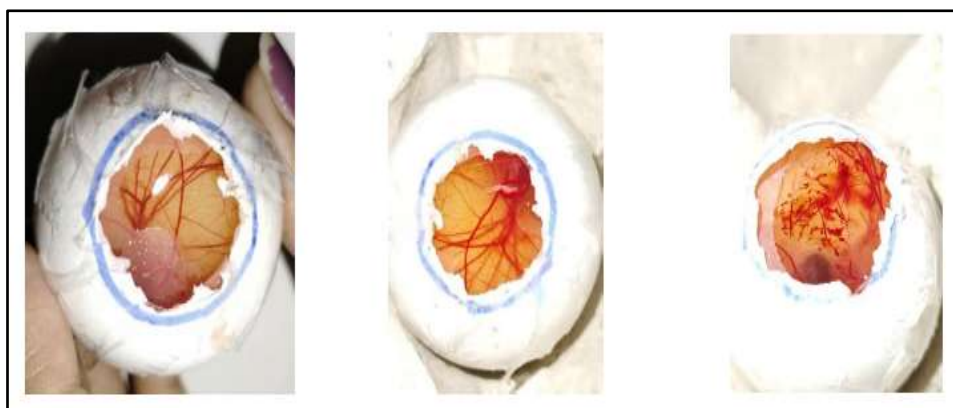


Figure 4a: Herbal handwash tablet Figure 4b: Control – Saline Figure. 4c: Irritant – 0.1N NaOH

Table 5: Irritancy score table [19]

HET-CAM score range	Irritation category
0-0.9	Nonirritant or practically none
1-4.9	Weak or slight irritation
5-8.9 or 5-9.9	Moderate irritation
9-21 or 10-21	Strong or severe irritation

HET-CAM=Hen's egg test chorioallantoic membrane,  
 IS= Irritation score

**Stability studies:** Table 6 shows the stability of the formulation at 25 °C and 65% RH after 3 months with respect to all the evaluation parameters performed previously. At accelerated stability conditions, the tablets were found to dissolve in

their package and hence could not be evaluated further. Hence, it can be concluded that the formulation needs to be stored at 25 °C in a dry place.

Table 6: Stability study at 25 °C and 60% RH

Formula tion F2	Foam height (ml)	Foam retention (seconds)	Disintegration time (mins)	Thickness (cm)	Hardness (kg/cm <sup>2</sup> )	Weight variation (gm)	Drug content (%)	Zone of Inhibition (cm)
0 month	66.1 ± 1.0	240 ± 45	12.5 ± 0.5	1.03 ± 0.5	1	0.69 ± 0.05	98.75 ± 0.7	4.1 ± 0.2
3 months	60.8 ± 1.0	200 ± 32	13.1 ± 0.9	1.03 ± 0.7	1	0.66 ± 0.08	95.17 ± 0.34	3.9 ± 0.09

#### IV. CONCLUSION

The developed herbal handwash tablet containing orange peel extract showed promising antimicrobial effectiveness against Escherichia coli and Staphylococcus aureus, affirming its potential as a natural and environmentally friendly hand hygiene product. The tablets displayed favourable physical characteristics, such as consistent size, smooth surface, and a pleasant citrus aroma right after being produced. Essential evaluation metrics, including pH, foam height and retention,

disintegration time, hardness, and thickness were optimized to ensure user-friendliness and effective microbial protection. Microbial assessments demonstrated a noteworthy decrease in microbial presence following application, and the CAM assay indicated non-irritation, making the formulation appropriate for regular use. Stability studies revealed that if stored in a cool and dry place, the formulation would retain its antimicrobial properties over time. In summary, the herbal handwash tablet offers a convenient, sustainable,

and skin-friendly alternative to traditional liquid handwashes, merging antimicrobial protection with environmental awareness.

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