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Assessment of the Antimicrobial Properties of Citrus Fruit Peel Extracts & Formulation of Herbal Handwash

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Abstract: ***This study explores the antimicrobial potential of citrus fruit peels Citrus limon, Citrus macroptera, and Citrus reticulata through phytochemical screening and antimicrobial assays. The peel extracts revealed key bioactive compounds including alkaloids, flavonoids, tannins, and phenols. Microbes isolated from hand swabs (e.g., Staphylococcus sp., E. coli, Candida albicans) were tested against the extracts, with Citrus reticulata showing the highest antimicrobial activity. An herbal handwash was formulated using the extracts, and evaluated for physical parameters, pH, foam properties, stability, and antimicrobial efficacy. The results showed effective microbial inhibition, supporting the potential of citrus peel-based hand wash as a natural hygiene product.***

Keywords: Citrus limon, Citrus macroptera, Citrus reticulata, phytochemicals, antimicrobial activity, herbal handwash.

1. Introduction

Hand hygiene is one of the most effective and essential practices to prevent the transmission of infectious diseases. Since hands serve as primary carriers of pathogens, proper handwashing significantly reduces microbial load and the risk of healthcare-associated infections (Ganguly et al., 2022). Microorganisms on the skin are classified into resident flora, which are less harmful and harder to remove, and transient flora, which are more pathogenic and easily eliminated through proper hand hygiene (Kolhapure et al., 2004).

While soap and water remain effective, there is increasing interest in using natural, plant-based antimicrobial agents for hand hygiene. Medicinal plants offer numerous health benefits due to their antibacterial, antifungal, antiviral, and antioxidant properties. Among them, citrus fruits of the Rutaceae family such as *Citrus limon* (lemon), *Citrus macroptera* (Satkara), and *Citrus reticulata* (mandarin) are rich in bioactive compounds like flavonoids, terpenoids, alkaloids, and essential oils (Marta et al., 2020).

Citrus peels, often discarded as waste, contain a high concentration of phytochemicals such as polymethoxylated flavones, limonene, and pectin, which have shown significant antimicrobial and therapeutic effects. *Citrus limon* is valued for its juice and peel in traditional medicine. *Citrus macroptera* exhibits antioxidant and neuroprotective properties, while *Citrus reticulata* is traditionally used for treating gastrointestinal and respiratory conditions (Ritupriya et al., 2019; Collen et al., 2020).

Utilizing citrus peel waste for developing antimicrobial products not only supports waste reduction but also offers sustainable alternatives in the context of rising antimicrobial resistance (Shoba et al., 2023). This study investigates the antimicrobial potential of ethanolic extracts of *Citrus limon*, *Citrus macroptera*, and *Citrus reticulata* against common pathogens isolated from hand swabs. An herbal handwash was formulated using these extracts, and its efficacy was compared to a commercial handwash product using the agar well diffusion and Standard Plate Count methods.

2. Materials and Methods

Collection and Preparation of Citrus Fruit Samples

Citrus fruits (Lemon *Citrus limon*, Satkara *Citrus macroptera*, and Mandarin orange *Citrus reticulata*) were collected from Kollengode, Palakkad. The peels were separated, washed thoroughly, and shade dried for 4–6 days.

Peel Powder Preparation

Dried peels were ground into fine powder using a blender and stored in airtight containers at room temperature.

Peel Extract Preparation

10 g of peel powder was macerated in 100 ml of 95% ethanol for 72 hours with occasional stirring. The mixture was then filtered using Whatman No.1 filter paper.

Extract Concentration

Filtrate was evaporated in a water bath at 60°C. The crude extract was mixed with DMSO in 1:2 ratio and stored in the refrigerator.

Phytochemical Screening

The peel extracts were screened for the presence of Alkaloids, Terpenoids, Flavonoids, Saponins, Phenols, Tannins, Glycosides, and Reducing sugars using standard tests. Hand swabs were collected from students (Microbiology Department, SNGC) using sterile cotton swabs covering palms, fingers, and nails. Swabs were inoculated on Nutrient Agar and Sabouraud Dextrose Agar (SDA).

Identification and Characterization of isolated microorganisms was conducted using staining (Gram's-Bacteria, Germ Tube Test-Yeast &LPCB Staining-Fungi) and Biochemical Tests (IMViC, TSI, Urease, Catalase and Oxidase Tests)

Antimicrobial Assay
The antimicrobial activity of citrus peel extracts was evaluated using the Agar Well Diffusion Method against bacteria (*Staphylococcus sp., E. coli, Streptococcus sp., Pseudomonas sp.*) and fungi (*Candida albicans, Aspergillus sp., Penicillium sp.*). Media: Mueller Hinton Agar (MHA) was sterilized and poured into Petri plates.

Formulation of Herbal Handwash:
Herbal handwash was prepared by mixing 20 ml of ethanolic citrus peel extract with 5 g of SLS dissolved in 10 ml distilled water, along with glycerin, lemon juice, citric acid, and orange essential oil. The final volume was made up to 100 ml using distilled water.

Antimicrobial testing was done by agar well diffusion:
Two wells were filled with 100 µl of handwash (with and without extract) and incubated. Zones of inhibition were measured to assess antibacterial and antifungal activity

Ingredients of Herbal Handwash (with Roles):
Citrus peel extract (20 ml): Antimicrobial agent, Lemon juice (2 ml): Antiseptic agent, SLS (10 ml): Foaming agent, Glycerine (40 ml): Moisturizing agent, Citric acid (0.3 g): Preservative Orange essential oil (3 ml): Fragrance, Distilled water (up to 100 ml): Solvent/volume adjuster

Evaluation of Herbal Handwash
1) Physical Parameters: Appearance, color, odour, and homogeneity were visually inspected; pH was checked using pH paper. Foam height and retention were tested by shaking diluted hand wash and measuring foam volume and stability. Stability was assessed by storing samples at room temperature and in the refrigerator for one week
2) Antimicrobial Efficiency :Tested via agar well diffusion on MHA against *Staphylococcus sp., E. coli, Streptococcus sp., Pseudomonas sp., Candida albicans, Aspergillus sp., Penicillium sp.*

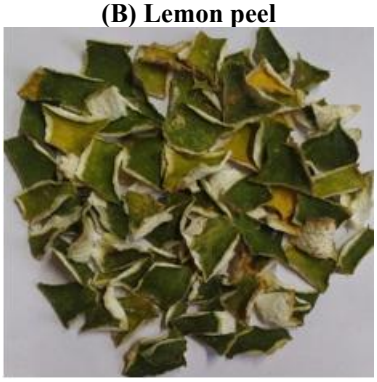
Hand Swab Test and Microbial Count
Purpose: To compare microbial load before and after using peel-extract and commercial hand wash.
Method: Hand swabs were collected before and after 20 s of washing, plated on Nutrient Agar, incubated at 37°C for 24 h, and bacterial colonies were counted to assess microbial reduction.

3. Results

Collection of Citrus Fruit Peels
Citrus fruits (Orange, Lemon and Satkara) were collected, shade dried and finely powdered for further analysis.

(a) Peel

(B) Lemon peel



(C) Large lemon peel
Figure 1: Citrus Fruit Peels

Extraction of Citrus Fruit Peel Powder
The powdered peels were subjected for ethanolic extraction by maceration method.

(A) Powdered peel mixed with ethanol

(B) After 72 hours of extraction

(C) Extract after filtration

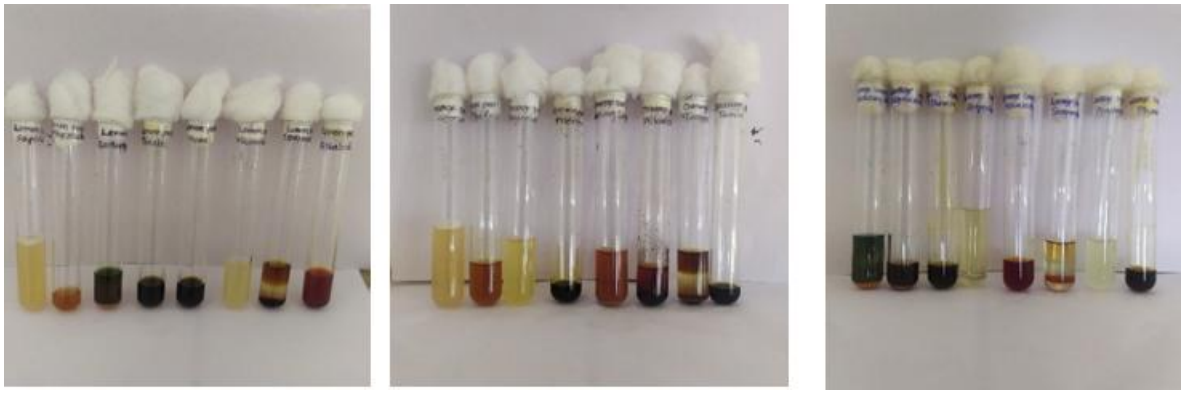
Figure 2: Extraction Process

Phytochemical Analysis

Phytochemical constituents present in Ethanolic extract of Citrus fruit peel (*Citrus limon, Citrus macroptera* and *Citrus reticulata*) and the results are shown in Table 1 and Figure 2 (A, B, C).

Table 1

S. No	Phytochemical Test	*Citrus limon*	*Citrus macroptera*	*Citrus reticulata*
1.	Alkaloids	-ve	-ve	+ve
2.	Terpenoids	+ve	+ve	+ve
3.	Flavanoids	+ve	+ve	+ve
4.	Saponin	+ve	-ve	+ve
5.	Phenol	+ve	+ve	+ve
6.	Tannin	+ve	+ve	+ve
7.	Glycosidase	+ve	+ve	+ve
8.	Reducing sugar	+ve	+ve	+ve

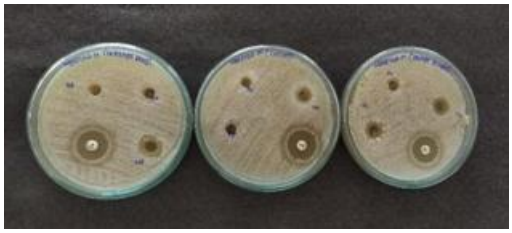


A) Lemon peel extract **B) Orange peel extract** **C) Large lemon peel extract**

Figure 3: Phytochemical Test Results of Ethanolic Peel Extract

Microscopy and Biochemical test confirmed the identity of bacterial isolates as *Staphylococcus* sp., *E. coli*, *Streptococcus* sp., and *Pseudomonas* sp. Based on LPCB staining Isolated fungi were characterized as *Penicillium* sp., (F) *Aspergillus* sp., and (G) *Candida albicans*.

Antimicrobial activity of citrus fruit peels (Citrus limon, Citrus macroptera and Citrus reticulata) extracts were determined by the Agar Well diffusion method. The Antimicrobial activity of citrus fruit peel extracts were estimated against *Staphylococcus sp, E. coli, Streptococcus sp*, *Pseudomonas sp, Candida albicans*, *Aspergillus sp* and *Penicillium sp on* MHA plates.It is shown in Figure 4 and Table 2



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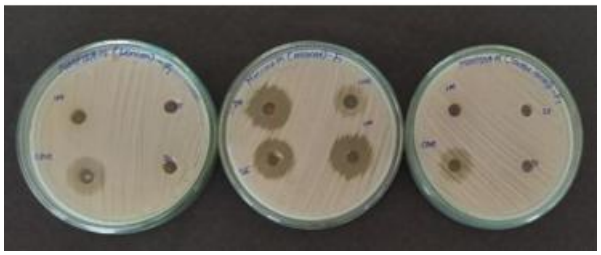

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Figure 4: A) *Staphylococcus sp* D) *Pseudomonas sp*

Table 2: Antimicrobial activity of *Citrus Fruit peel Extracts*

S. No	Test organisms	Zone of inhibition (mm)								
		25µl			50µl			100µl		
		L	O	LL	L	O	LL	L	O	LL
1	*Staphylococcus sp*	-	-	7	10	10	10	12	16	14
2	*E.coli E.coli*	11	-	-	12	10	11	14	16	14
3	*Streptococcus sp*	-	20	-	-	23	-	-	25	-
4	*Pseudomonas sp*	11	12	8	13	15	15	15	19	17
5	*Candida albicans*	-	20	-	-	21	-	8	22	-
6	*Aspergillus sp*	-	10	-	-	13	-	17	23	17
7	*Penicillium sp*	8	10	-	10	12		12	13	13

Figure 5: Citrus Fruit Peel Hand wash

Evaluation of Herbal Handwash

Physical Parameters

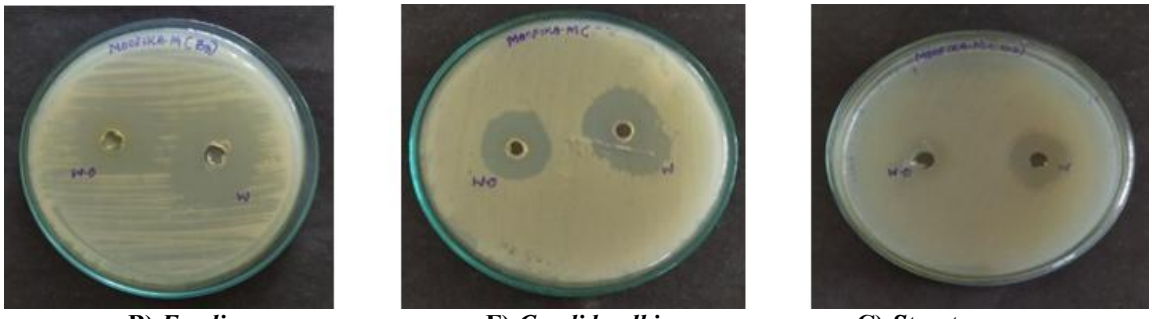
Colour, odour, Appearance and Homogenicity were evaluated. It is shown in the Table 3 given below

Table 3: Physical Evaluation of Handwash

Test	Observations
Colour	Bright orange
Odour	Aromatic
Appearance	Translucent
Homogenicity	Good
pH	6.0
Foam Height	6 cm
Foam Retention	Stable
Skin Test	No skin irritation.

Antimicrobial Efficiency

Assessed using **agar well diffusion** on MHA against microbes using hand wash with and without extract



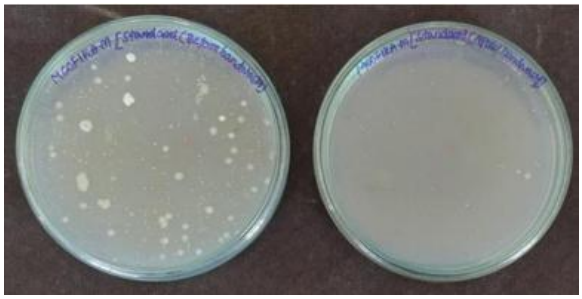
B) ***E.coli*** **F)** ***Candida albicans*** **C)** ***Streptococcus sp***

Figure 6: Antimicrobial Activity of Handwash with or Without Extract

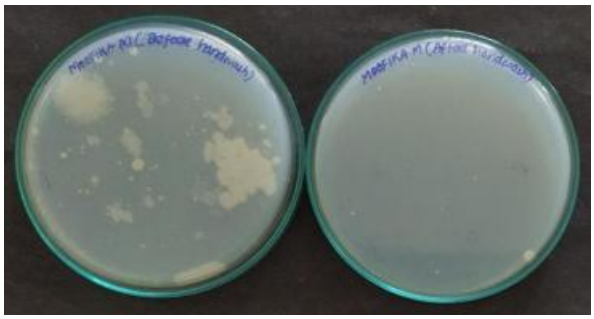
Table 4: Antimicrobial Activity of Hand Wash with or without ExtractsHand Swab Test and Microbial Count

S. No	Test organisms	Zone of inhibition (mm)	
		Without extract	With extract
1	*Staphylococcus sp*	18mm	26mm
2	*E.coli*	9mm	19mm
3	*Streptococcus sp*	23mm	32mm
4	*Pseudomonas sp*	13mm	16mm
5	*Candida albicans*	22mm	32mm
6	*Aspergillus sp*	21mm	24mm
7	*Penicillium sp*	33mm	23mm

The Hand swab tests were performed before and after handwashing with two different hand wash; one is the Sample-Citrus fruit peel extract Hand Wash and other is Standard-commercial hand wash. The figure is given below (Figure 7) and the Microbial count was tabulated in Table no 5



A) Commercial hand wash



B) Test (Citrus fruit peel) handwash

Figure 7: Before and After Using

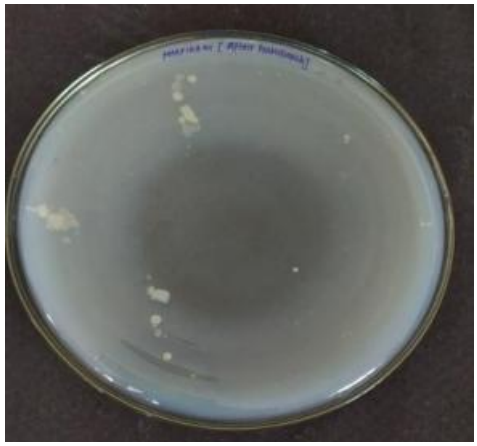
Table 5: Standard Plate Count Test Results

Average No. of colony forming unit			
S. No	Handwash	Before	After
1	Citrus fruit peel handwash	103	11
2	Commercial hand wash	TNTC	42

Handprint- Before and After Handwashing

The Handprints were captured before and after handwashing with Citrus fruit peel extract hand wash. The Handprint on Nutrient agar was shown in the figure below.

(a) Before handwashing



(b) After handwashing

Figure 8: Handprint of Before and After Handwashing with Citrus Peel Handwash

4. Discussion

This study explored the antimicrobial potential of citrus fruit peel extracts (Citrus limon, Citrus macroptera, and Citrus reticulata) and their formulation into a herbal handwash. Phytochemical screening revealed the presence of alkaloids, flavonoids, terpenoids, phenols, tannins, glycosides, and reducing sugars, supporting their antimicrobial properties (Justin et al., 2014).

Microbial isolates (Staphylococcus sp, E. coli, Streptococcus sp, Pseudomonas sp, Candida albicans, Aspergillus sp, and Penicillium sp) were identified from hand swabs. Citrus peel extracts exhibited antimicrobial activity against these pathogens using the agar well diffusion method. Citrus reticulata showed the highest inhibition against Streptococcus sp, Candida albicans, and Pseudomonas sp, while Citrus macroptera was most effective against Aspergillus spp and Staphylococcus sp. These findings align with previous studies on Citrus species (Hasan et al., 2022; Ochate et al., 2023).

The formulated herbal handwash showed good physical properties, non-irritancy, and significant antimicrobial efficacy, reducing microbial load more effectively than commercial hand wash. This supports the potential of citrus peel extracts in developing eco-friendly hygiene products.

5. Conclusion

Hand hygiene is a cornerstone of preventing most communicable diseases. Various Studies prove that hand washing with natural ingredients shows promising results in terms of antimicrobial efficacy and could be considered as an alternative to commercial handwashes. Repurposing the fruit waste as innovative ways to create a novel products with greater efficiency. The study demonstrated the potential of citrus fruit peel extracts as natural antimicrobial agents. The phytochemical composition contribute their antimicrobial property. The ethanolic extract of peel exhibited antimicrobial activity against various bacteria and fungi found in hands including *Staphylococcus sp, E. coli, Streptococcus sp, Pseudomonas sp, Candida albicans, Aspergillus sp* and *Penicillium sp*. The formulated herbal handwash using citrus fruit peel extracts shows promising results in antimicrobial efficacy by comparing it with a commercial hand wash. This means that the two liquid handwashes were not significantly different from one another and were effective in certain levels. Moreover, based on product evaluation the citrus fruit peel extract hand wash is acceptable.

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