

Innovations

Polyherbal Hand Hygiene Solutions: Formulation Optimization and Broad-Spectrum Antimicrobial Activity

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Abstract: *In recent pandemic condition, the personal hygiene is considerable as vital factor more over it was recommended by WHO to protect us from life threatening Covid 19. Many companies formulated sanitizer using alcohol and other chemicals. With or without our knowledge we used them in excess to protect ourselves from dreadful condition. Most of the sanitizer were found to unwanted effect on the skin, allergic, causes environmental pollution and cost effective. So, we decided to formulate the sanitizer purely based on herbs without any chemical or artificial colour and perfumes. An attempt was made to formulate and evaluate herbal hand sanitizer and herbal hand wash based available on earlier claims of our indigenous plants. The formulations were evaluated for PH, stability test, organoleptic test and preliminary chemical test. The evaluation results were found to be within the standard limit. The presence of poly phenolic compounds, steroids, alkaloids and tannins were found by chemical test. Both the formulations were tested for antimicrobial activity with bacterial species and fungal species. The selected bacterial species are bacillus subtiles (MTCC 6133), Escherichia coli (MTCC 1687) & pseudomonas aeruginosa (MTCC 7925) and fungal species. Hand sanitizer showed the very good suitability effect on the both fungal species and bacterial species. Hand wash shows the good sustainability effect on the bacterial species whereas the poor effect on the fungal species. Comparatively hand sanitizer shows the good antibacterial activity as well as antifungal activity against tested organism by agar well diffusion method. It shows that out of two formulations, hand sanitiser formula was found as good antimicrobial agent. The results of study was very encouraging to us.*

Keywords: *Herbal Hand Sanitizer, Herbal Hand wash, Evaluation, Antimicrobial activity*

Introduction

Hands are the primary mode of trans mission of microbes and infections. Contaminated hands can give out vectors for the transmission of microorganisms. Thus Hand Hygiene is the most important measure to

evade the transmission of microbes and infections.^[1] Hand hygiene is simplest and least expensive means of nosocomial infections. Nosocomial infections are those which are acquired or originated in hospital or healthcare association and are results of high prevalence of pathogens. Hand cleanliness is now considered one of the most critical aspects of infection prevention. In response to the rising burden of health-care-associated infections (HCAIs), the increasing severity of sickness and complexity of treatment, and the emergence of multi-drug-resistant (MDR) pathogen infections, health-care practitioners (HCPs) are reverting to the basics of infection prevention, such as hand cleanliness. This is because there is substantial scientific data to suggest that, when correctly implemented, hand cleanliness alone can greatly lower the risk of infection cross-transmission in healthcare facilities (HCFs).^[2] Hand sanitizer use has grown popular among the people recently. Several hand sanitizer solutions are available on the market, and they typically contain a high alcohol content, which can cause skin irritation. As a result, using an alcohol-based antiseptic on a regular basis is inconvenient. As a result, natural antiseptics that can be used multiple times are being created.^[3] Microbes living on hands are traditionally categorised into resident and transient floras. *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Enterococcus faecalis* are common resident floras that colonise the deeper skin layers and are resistant to mechanical removal. *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa* are among the transitory floras, which invade the skin's surface layers in a short period of time. ^[4]As a result, we chose some of these organisms and also some fungal species to investigate their susceptibility to the herbal hand sanitizer and herbal hand wash used in this investigation.

Materials and Methods

Materials

Based on the literature review and our own idea we have selected the herbs for the formulation of poly herbal hand sanitizer and hand wash. Rose oil and rose water was purchased in the local shop. Turmeric rhizome, vasambu, myrobalan fruits, soapnuts were purchased from commercial shop. Neem leaves are collected from neem tree. The bacterial cultures for antimicrobial activity were procured from the Microbial Type Culture Collection & Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh, India. The fungal cultures Mueller Hinton agar, Sabourou's Dextrose Agar were procured from Research and Development, Marina Labs, Chennai.

Formulation of herbal hand sanitizer

Aqueous extraction of the neem leaves, turmeric rhizome, vasambu rhizome and myrobalan fruits were prepared by hot decoction method. 1ml of concentrated extract of each were mixed and added with 20 ml of pure rose

essence in a glass beaker The extracts and rose water were mixed well in porcelain mortar with continuous stirring with pestle. The content was poured into the labeled container, thus the poly herbal hand sanitizer was formulated and stored at normal temperature for evaluation.

Formulation of herbal hand wash

Aqueous extraction of the neem leaves, turmeric rhizome, vasambu rhizome and myrobalan fruits were prepared by hot decoction method. The ratio selected for the formulation of herbal hand wash is 20:5:5:5:5 %v/v (soapnut: myrobalan: sweet flag: neem: turmeric). 20 ml of aqueous extract of soap nuts was added to a porcelain mortar. 5ml of aqueous extract of neem was added to the soapnut extract in porcelain mortar with continuous stirring with pestle. 5ml of aqueous extract of myrobalan was added to the mixed extracts of soapnut and neem extract in porcelain mortar with continuous stirring with pestle. 5ml of aqueous extract of turmeric was added to the mixed extracts in porcelain mortar with continuous stirring with pestle. 5ml of aqueous extract of sweet flag was added to the mixed extracts in porcelain mortar with continuous stirring with pestle. To the mixed extracts add 2 drops of clove oil was added for aroma and also as preservative with continuous stirring using the pestle. The content was poured into the labeled container, thus the poly herbal hand wash was formulated and it was stored at normal temperature for further evaluations.

Evaluation

Physiochemical Characterization and evaluation

Organoleptic test

The formulations were inspected visually to check the texture, odour, and colour

pH test^[5]

A pH measurements were carried out using pH Universal indicator paper by placing the 1 ml of formulated herbal hand sanitizer and hand wash on the pH indicator paper, after which it was observed and the colour-matched with a pH indicator paper comparison.

Stability test^[6]

Both the formulations were tested for stability as per standard protocol at room temperature for three months

Anti Microbial Activity:

Bacterial Cultures^[7,8]

The Microorganisms *Bacillus subtilis* (MTCC 6133), *Escherichia coli* (MTCC 1687) and *Pseudomonas aeruginosa* (MTCC 7925) were procured from Microbial Type Culture Collection & Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh, India. Fresh bacterial cultures were prepared by sub-culturing

stock bacterial cultures into freshly prepared nutrient agar and incubating at 37°C for 24 hours.

Fungal Cultures

The fungal cultures of *Aspergillus niger*, *Aspergillus flavus* and *Paecilomyces variotii* were procured from Research and Development, Marina Labs, Chennai. The fungal cultures were maintained in Potato Dextrose Agar for further usage.

Mueller Hinton Agar ^[9]

Mueller–Hinton agar (MHA) is a microbiological growth medium that is commonly used for antibiotic susceptibility testing, specifically well diffusion methods. It is composed of casein acid hydrolysate (17.5 g/L), Meat extract (2 g/L), Starch (1.5 g/L) and Agar (17 g/L). To prepare Mueller Hinton Agar, Suspend 38 gm of the powder in 1 liter of sterile distilled water. Heat with frequent agitation and boil for one minute to completely dissolve the medium. Autoclave at 121°C for 15 minutes. Cool to room temperature and pour into Petri dishes.

Sabouraud's Dextrose Agar ^[10]

Sabouraud's Dextrose Agar (Sda) Is a Selective medium prominently used for the isolation of fungi. It is composed of Mycological peptone enzymatic digest of casein and animal tissues (10 g/L), Dextrose (40 g/L) and Agar(15g/L). To prepare SDA, suspend 65 gm of the medium in one liter of sterile distilled water. Heat to boil for one minute to completely dissolve the medium. Autoclave at 15 lbs. pressure (121° C) for 15 minutes. Cool to room temperature and pour into Petri dishes.

Agar well diffusion assay for Antibacterial activity^[11]

The agar well diffusion method was adopted to test the antimicrobial activity of the HAW and HS. The HAW and HS were tested using Mueller Hinton II agar plates. The surface of the agar plate was streaked with the respective cultures (bacterial strains). Then 5 mm diameter wells were cut into the agar medium using a sterile cork borer. The plates are allowed to dry to remove excess moisture for 20 min. The extracts of HAW and HS of 15 µl, 20 µl and 25 µl were dispensed into each well respectively. The plates were incubated at 37°C. The tests were conducted in triplicates. After 24 hours of incubation, each plate was examined for zones of inhibition. The zone of inhibition was recorded as the diameter of inhibition zone.

Well diffusion as say for Antifungal activity^[12]

The agar well diffusion method was adopted to test the antimicrobial activity of the Herbal hand wash and Herbal Santizer. The Herbal Handwash and Hand Santizer were tested using Sabouraud;s Dextrose Agar plates. The surface of the agar plate was streaked with the respective cultures (fungal cultures). Then 5

mm diameter wells were cut into the agar medium using a sterile cork borer. The plates are allowed to dry to remove excess moisture for 20 min. The extracts of HAW and HS of 15 μ l, 20 μ l and 25 μ l were dispensed into each well respectively. The plates were incubated at 37°C. The tests were conducted in Triplicates. After 24 hours of incubation, each plate was examined for zones of inhibition. The zone of inhibition was recorded as the diameter of inhibition zone.

Results

Organoleptic parameters were shown table -1 in the prepared formulations. The comparative evaluation of the physical characteristics shows that both the herbal hand sanitizer and hand wash are liquid formulations, but they differ noticeably in texture, appearance, and viscosity. The hand sanitizer is slightly viscous, pale green, translucent, and has a watery, volatile texture, indicating the presence of alcohol or other fast evaporating components that allow rapid drying after application. In contrast, the herbal hand wash is highly viscous, yellowish brown, opalescent, and exhibits a thicker, non-volatile texture, meaning it does not evaporate quickly and is designed to remain on the skin longer during washing to facilitate foam formation and effective cleansing. Both products possess a pleasant odour, enhancing user acceptability, but their physical differences reflect their intended functions quick-drying sanitization versus prolonged contact cleansing.

Table 1: Organoleptic parameters observed in the formulations

Characteristics	Hand Sanitizer	Hand Wash
State	Liquid	Liquid
Nature	Slightly viscous	Highly viscous
Colour	Pale green	Yellowish brown
Odour	Pleasant odour	Pleasant odour
Appearance	translucent	Opalescent
Texture	Watery volatile	Slightly viscous Non- volatile

The antifungal evaluation of the herbal handwash and herbal hand sanitizer showed in table -2 notable differences in their inhibitory activity against the tested fungal species. The herbal hand sanitizer demonstrated strong and consistent antifungal effects across all volumes, especially against *Aspergillus niger*, which showed a maximum inhibition zone of 40 mm at all tested concentrations. *Aspergillus flavus* exhibited no sensitivity to the herbal handwash, whereas the sanitizer produced increasing inhibition zones from 29 \pm

8.98 mm to 40 mm with rising volume. In contrast, *Paecilomyces variotii* showed moderate sensitivity to the herbal handwash with inhibition zones increasing from 9 mm to 15 ± 1.63 mm, while the sanitizer produced higher and more uniform inhibition ranging from 35 ± 0.57 mm to 40 mm. Overall, the results indicate that the herbal hand sanitizer possesses significantly superior antifungal activity compared to the herbal handwash, demonstrating broad-spectrum and dose-dependent efficacy.

Table 2: Results of antifungal activity at the end of 24h for herbal hand sanitizer and hand wash against fungal species

SL. NO	Fungal Species	Herbal Hand wash			Herbal Hand Sanitizer		
		15 ul	20ul	25 ul	15 ul	20 ul	25 ul
1	<i>Aspergillus niger</i>	0	0	11 ± 1.63	40 ± 0.00	40 ± 0.00	40 ± 0.00
2	<i>Aspergillus flavus</i>	0	0	0	29 ± 8.98	38 ± 1.63	40 ± 0.00
3	<i>Paecilomyces variotii</i>	9 ± 0.00	11 ± 0.00	15 ± 1.63	35 ± 0.57	38 ± 0.00	40 ± 0.00

Values are means of triplicate and represent in mean \pm SD: zone of inhibition (in mm)

The antibacterial assessment of the herbal handwash and herbal hand sanitizer revealed effective inhibition against all tested bacterial strains, with varying degrees of activity across different concentrations. *Bacillus subtilis* (MTCC 6133) showed a progressive increase in susceptibility to both formulations, with the herbal handwash producing inhibition zones from 23 ± 1.63 mm to 28 ± 2.58 mm, while the sanitizer showed comparable activity ranging from 24 ± 2.08 mm to 28 ± 0.57 mm. *Escherichia coli* (MTCC 1687) demonstrated equal sensitivity toward both products, exhibiting identical inhibition patterns of 20 ± 1.29 mm at 15 μ L, increasing to 24 ± 1.29 mm at 25 μ L. For *Pseudomonas aeruginosa* (MTCC 7925), the herbal handwash displayed stronger antibacterial action, with inhibition zones increasing up to 33 ± 0.81 mm at 25 μ L, whereas the sanitizer showed moderate inhibition ranging between 18 ± 1.03 mm and 26 ± 0.57 mm. Overall, the herbal handwash exhibited comparatively higher antibacterial potency against *P. aeruginosa*, while both formulations demonstrated similar and dose-dependent activity against *B. subtilis* and *E. coli*.

Table 3: Results of antibacterial activity at the end of 24h for herbal hand sanitizer and hand wash against Bacterial species

SL NO	Bacterial Species	Herbal Hand Wash			Herbal Hand Sanitizer		
		15 ul	20ul	25 ul	15 ul	20 ul	25 ul
1	Bacillus subtilis (MTCC 6133)	23±1.63	27±1.63	28±2.58	24±2.08	26±0.57	28±0.57
2	Escherichia coli (MTCC 1687)	20±1.29	22±1.29	24±1.29	20±1.29	22±1.29	24±1.29
3	Pseudomonas aeruginosa (MTCC 7925)	26±0.57	24±0.81	33±0.81	18±1.03	24±0.00	26±0.57

Values are means of triplicate and represent in mean±SD: zone of inhibition (in mm)

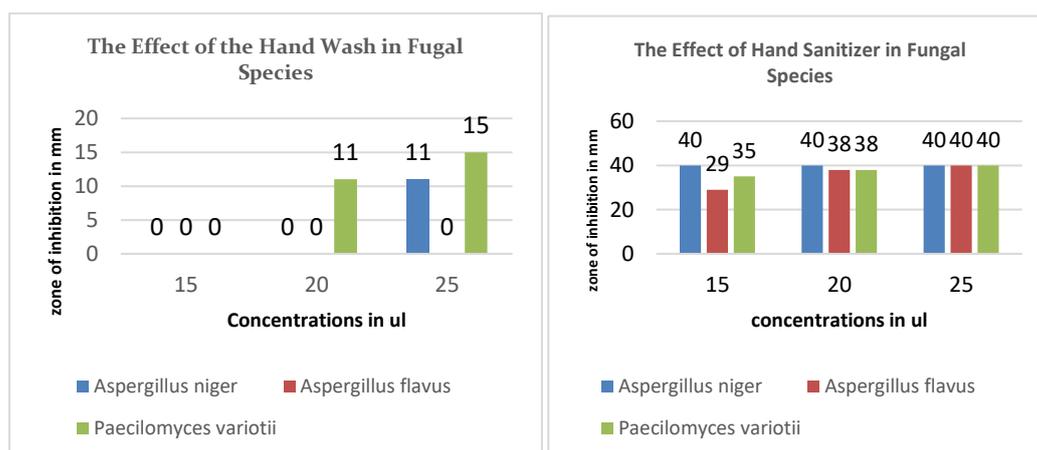


Fig 1(a): Effect of hand wash in fungal species 1(b): Effect of hand sanitizer in fungal species

The graphical comparison clearly highlights the difference in antifungal efficacy between the herbal handwash and the herbal hand sanitizer across all three fungal species. The herbal handwash shows minimal to moderate activity, with *Aspergillus niger* and *Aspergillus flavus* exhibiting no inhibition at 15 µL and 20 µL, and only *A. niger* showing a small zone (11 mm) at 25 µL. In contrast, *Paecilomyces variotii* responds better to the handwash, with inhibition zones increasing from 11 mm at 20 µL to 15 mm at 25 µL. On the other hand, the herbal hand sanitizer demonstrates strong and consistent antifungal activity across all concentrations, producing 40 mm zones of inhibition against *Aspergillus niger* at every level tested. *Aspergillus flavus* shows increasing inhibition from 29 mm at 15 µL to 40 mm at 25 µL, while *Paecilomyces variotii* also exhibits robust inhibition ranging from 35 mm to 40 mm. Overall, the charts confirm that the

herbal hand sanitizer is significantly more effective than the herbal handwash against all tested fungal species, showing both broader spectrum and higher magnitude of antifungal activity.

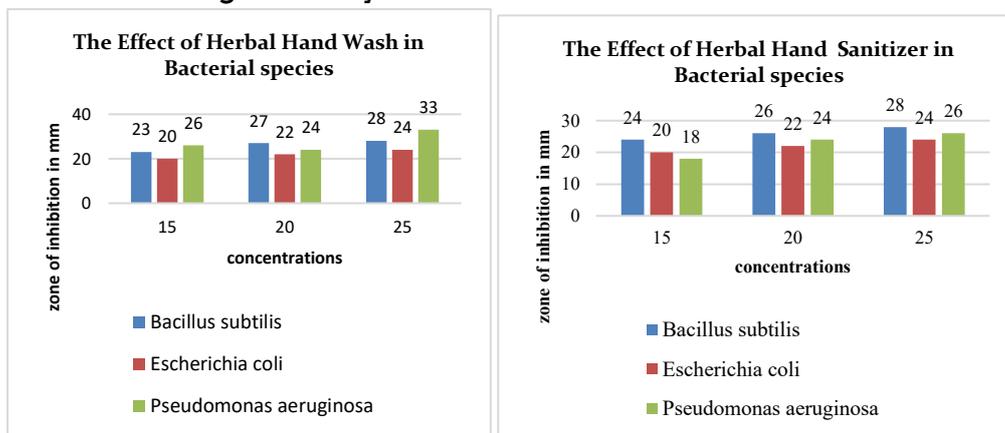


Fig 2(a): Effect of hand wash in bacterial species 2(b): Effect of hand sanitizer in bacterial species

The comparative analysis of the graphs shows that both the herbal handwash and herbal hand sanitizer exhibit measurable antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, with variations in potency across concentrations. The herbal handwash demonstrates a steady increase in inhibition zones with rising volumes, showing the highest activity at 25 μ L, particularly against *Pseudomonas aeruginosa* (33 mm), followed by *Bacillus subtilis* (28 mm) and *E. coli* (24 mm). In contrast, the herbal hand sanitizer also shows dose dependent antibacterial action but with a comparatively more balanced effect across species. At 25 μ L, it produces inhibition zones of 28 mm for *Bacillus subtilis*, 24 mm for *E. coli*, and 26 mm for *P. aeruginosa*. Overall, while both formulations are effective, the herbal handwash displays stronger activity against *P. aeruginosa*, whereas the sanitizer maintains consistent antibacterial performance across all three species, highlighting its broad-spectrum efficacy.

Discussion

In recent pandemic condition, the personal hygiene is considerable as vital factor, more over it was recommended by WHO to protect us from life threatening Covid 19 [13] . Many companies formulated sanitizer using alcohol and other chemicals. With or without our knowledge we used them in excess to protect ourselves from deadful condition. Most of the sanitiser were found to unwanted effect on the skin, allergic and environmental pollutants and more cost. To overcome all the above is suedecided to formulate the herbal sanitizer purely based on herbs without any chemical or artificial colour and perfumes. So an attempt to formulate both herbal hand sanitiser and herbal hand wash based on easily available, indigenous plantsmotive of formulation was to create a

chemical free, and alcohol-free poly herbal hand sanitiser and poly herbal hand wash which aids in preventing from microbial infections..

The organoleptic test of poly herbal hand sanitizer and herbal hand wash was conducted to evaluate the physical appearance of the prepared formulations. Following the visual quality inspection of prepared poly herbal hand sanitizer and herbal hand wash, the results indicated good characteristics observed for the prepared formulations. The poly herbal sanitiser and herbal hand wash were homogenous, clear with the EO's distinctive odour, no syneresis occurred, were easy to apply, light to spread, and had a consistent flow. There was a bubble-like appearance that was formed upon overnight storage, but disappeared after slight shaking. The herbal hand sanitiser and herbal hand was exhibited no coarse particles upon spreading on a transparent glass, owing to the homogeneity of the prepared formulations.

The formulated poly herbal hand wash and herbal hand sanitiser were inspected visually to check the texture, odour, and colour of the poly herbal sanitiser and hand wash. The pH values of the formulated poly hand sanitizer and hand wash were measured using a pH paper. The study was conducted to check the neutralization of different prepared formulations. The ideal standards for a pH value of a topical dosage form should be within the broad pH range of the skin, i.e., 4.0 to 7.0, in order to avoid skin inflammation and irritation. The pH measurement showed that all prepared formulations were neutral, with pH values around 7. The formulations were evaluated for PH, stability test, organoleptic test and preliminary chemical test. The evaluation results were found to be within the standard limit. The presence of poly phenolic compounds, steroids, alkaloids and tannins were found by chemical test^[14]. Both the formulations were tested for antimicrobial activity with bacterial species and fungal species. The selected bacterial species are bacillus subtilis (MTCC 6133), Escherichia coli (MTCC 1687) & pseudomonas aeruginosa (MTCC 7925) and fungal species are aspergillusniger, aspergillusflavus and paecilomycesvariotii hand sanitizer shows the very good sustability effect on the both fungal species and bacterial species. Hand wash shows the good sustainability effect on the bacterial species whereas the poor effect on the fungal species. Comparatively hand sanitizer shows the good antibacterial activity as well as antifungal activity against tested organism by algae well diffusion method^[15]. It shows that out of two formulations, hand sanitiser formula was found as good antimicrobial agent. In future planned to investigate in preclinical studies and clinical examination and commercialization of our product.

Conclusion

In recent pandemic condition, the personal hygiene is considerable as vital factor, more over it was recommended by WHO to protect us from life threatening Covid 19. So the hand sanitizer and hand wash plays a vital role in this pandemic condition. Therefore we made an attempt to formulate a

completely alcohol free and chemical free hand sanitizer and hand wash and the formulations were evaluated for antimicrobial activity. It shows that our formulations have good sustainability effect on the both bacterial and fungal organisms.

Conflict Of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this article.

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