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Research Article

Development and Detection of Antimicrobial Properties of Polyherbal Handwash

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Abstract: Many of the medications marketed as traditional herbal medicines have unquestionably been used for a very long time. Hands have always been the dominant source of transmission of infection to patients. Thus, encouraging "personal hygiene" is the main objective of developing a herbal hand wash. The current study's goal was to develop a formulation of polyherbal handwash employing methanolic extracts of dried leaves of *Azadirachta indica*, *Ocimum gratissimum*, and *Coriandrum sativum*. Other ingredients entailed lemon juice, aloe vera, lavender oil, HPMC, triethanolamine, sodium lauryl sulphate (SLS), glycerine, and methylparaben. Four batches of hand wash formulations were prepared, and each batch was tested for stability, appearance, colour, grittiness, pH, viscosity, foam height, and other physical characteristics. Using the agar well diffusion method, the anti-microbial effectiveness of the prepared polyherbal hand wash was tested on a variety of bacteria, including *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeroginosa*, *Salmonella typhi, Aspergillus niger,* and *Candida albicans*. The results demonstrated that manufactured herbal handwash formulations, particularly F3, displayed a prominent zone of inhibition in comparison to standard commercial handwash, indicating that the extract of these phytoconstituents may be employed to manufacture handwash with antimicrobial properties. As a result, the research shows that the herbal handwash formulation is analogous to commercial handwash in reducing the amount of bacteria on hands and may be used as a replacement handwash made from natural sources without experiencing any undesirable effects.

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1. Introduction

Skin, the largest organ, is the most exposed organ of the human body. Due to the fact that hands are the major means by which infections spread, maintaining adequate health and hygiene, particularly with regard to hands, is quite essential (Acharya et al., 2018). Hand hygiene, the act of cleansing hands, is a very important, effective, simplest and affordable measure to prevent and decrease the transmission of harmful microbes and help fight the spread of the disease (Bagade et al., 2021). Hands', serving the maximum work for the human body, come in contact with dirt and harmful microbes acquired from the

soils, food, raw materials, equipments, etc. (Sinha et al., 2022). Thus, hand washing, the best way to maintain personal hygiene, is the most effective measure to help fight the spread of the disease and to protect the skin from harmful microbes (Singh et al., 2022).

Historically, plant species are the earliest and most good source of pharmacologically active molecules. The bioactive compounds, as well as the plant extracts, have been utilised for ages in the preparation of traditional and Ayurvedic medicines, food, natural dyes, and cosmetics, and in the treatment of different ailments (Powar et al., 2015). Herbal Plants, possessing a wide variety of bioactive compounds such as tannins, terpenoids, flavonoids, alkaloids, etc., are found to have potential *in-vitro* antimicrobial properties which are efficient against a wide spectrum of microbes (Takó et al. 2020; Vaou et al., 2021).

In the present research, herbal hand wash was formulated and prepared using different herbal plants like neem, tulsi, coriander, aloe vera, and lemon juice because of their ease of availability, less expensive, increased efficiency, and fewer side effects - benefits of using herbal handwash (Eshete and Molla, 2021). Neem (*Azadirachta indica*) belongs to the Meliaceae family and is also known as Margosa or Indian lilac. Different parts of the Neem such as leaves and barks hold therapeutic importance (Latif et al., 2020). The bioactive compounds, such as Azadirachtin, extracted possess antiviral, antifungal, antibacterial, insecticidal, and antiseptic properties (Islas et al., 2020). Also, these extracts are useful as folk medicine to control leprosy, respiratory diseases, and constipation (Ganguly et al., 2022). Nimma Tulsi, scientifically known as *Ocimum gratissimum* belonging to the Lamiaceae family, is widespread in India and South Africa. It is also grown across tropical regions on the globe (Bhavani et al., 2019). The leaves, flowers, stems, roots, seeds, fruits, and bark of this plant can all provide phytochemical constituents such as alkaloids, tannins, flavonoids, steroids, triterpenoids, and carbohydrates possessing pharmacological properties such as antimicrobial, antidiabetic, wound healing, etc. (Sharma and Upadhyaya, 2019). *Coriandrum sativum*, an annual herb belonging to the Apiaceae family, is commonly known as coriander, dhania, or cilantro. Though it is used as a flavouring agent in food preparation, it possesses both nutritional and medicinal properties (Wei et al., 2019). The most utilized part of the coriander plant is the dried ripe fruits, commonly known as coriander seeds, and leaves (Mahleyuddin et al., 2021). Major active constituents of *Coriander sativum* is fatty oil such as Linoleic acid, and oleic acid, and essential oils such as Linalool, Geraniol (Sobhani et al., 2022). Aloe vera, a cactus-like plant, also known as *Aloe barbadensis miller* belongs to the Liliaceae family (Hęś et al., 2019). It is utilized for its pharmacological properties such as anticancer, antimicrobial, antiviral, cleansing, and wound healing due to the presence of numerous bioactive compounds such as Vitamin B12, aloin, emodin, etc. (Sánchez et al., 2020). Thus, the herbal handwash prepared is in solution form which underwent several evaluation tests.

2. Material and Methods

2.1. Herbs, chemicals, and microbes collection

The herbal plants viz. *Azadirachta indica*, *Ocimum gratissimum*, *Coriandrum sativum,* and Aloe vera were collected from the herbal garden of the Brainware University Campus, Barasat, Kolkata, West Bengal, India. The lemon juice was collected from the lemon which was procured from the local market area of Barasat. All the chemicals, media, and reagents employed in the study were of analytical grade. The microbial strains (*Bacillus subtilis* ATCC 6633*, Escherichia coli* ATCC 8739*, Staphylococcus aureus* ATCC 6538*, Salmonella typhi* NCTC 786*, Pseudomonas aeruginosa* ATCC 9027*, Aspergillus niger* ATCC 16404*, Candida albicans* ATCC 10231) were procured from the Central Drug Laboratory, Kolkata, West Bengal, India.

2.2. Preparation of herbal extract

The dried leaves of *Azadirachta indica*, *Ocimum gratissimum,* and *Coriandrum sativum* were coarsely powdered. 5 grams of coarsely powdered leaves of each plant were soaked separately in 100 ml of methanol. The soaked leaves were kept for maceration for 4 days. The extract was filtered once the maceration was completed. The collected filtrate was used for manufacturing of herbal handwash (Bereksi et al., 2018; Kamalapurkar and Shendge, 2022; Rukari et al. 2022; Nurcholis et al., 2023).

2.3. Preparation of polyherbal handwash formulation

The formulation of the polyherbal handwash was prepared from the methanolic leaf extract of *Azadirachta indica*, *Ocimum gratissimum,* and *Coriandrum sativum,* with addition to lemon juice, aloe vera, Oil of lavender for perfume, using HPMC E-50 as gelling agent, Triethanolamine for adjusting the pH, Sodium Lauryl Sulphate (SLS) as foaming agent, glycerine for moisturizing and methyl paraben for preservative in various formulation batches (Table 1) (Bagade et al., 2021). The solution prepared, as per the standard procedure for the preparation of handwash, was made homogenous under room temperature and stored for further studies (Barman et al., 2020).

Table 1. Different formulations of polyherbal handwash

Composition	Formulation Batch (Quantities Taken)			
	F1	F2	F3	F4
Methanolic Extract of Neem	2 ml	2 ml	2 ml	2 ml
Methanolic Extract of Tulsi	2 ml	2 ml	2 ml	2 ml
Methanolic Extract of Coriander	2 ml	2 ml	2 ml	2 ml
Aloe vera	2 ml	2 ml	2 ml	2 ml
Lemon Juice	2 ml	2 ml	2 ml	2 ml
HPMC	0.6 gr	0.7 gr	0.8 gr	0.9 gr
Triethanolamine	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Glycerine	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Methyl Paraben	0.08 gr	0.08 gr	0.08 gr	0.08 gr
SLS	0.5 gr	0.6 gr	0.7 gr	0.8 gr
Oil of Lavender	0.2 ml	0.2 ml	0.2 ml	0.2 ml
Purified Water q.s.*	q.s to 30 ml	q.s to 30 ml	q.s to 30 ml	q.s to 30 ml

*q.s. – as much as is sufficient.

2.4. Evaluation of polyherbal handwash

2.4.1. Physical evaluation

The polyherbal handwash prepared was visually inspected where the physical parameters such as colour, odour, texture, appearance, grittiness, and homogeneity were evaluated (Patel et al., 2017; Mulani et al., 2021).

2.4.2. pH

The pH of the formulations was examined using a standardised digital pH meter at room temperature (Mali et al., 2020).

2.4.3. Foam height

In 50 ml of distilled water, 1 gram of the prepared polyherbal handwash formulation was dispersed. It was then transferred into a stoppered measuring cylinder of 500 ml capacity, making up the volume to 100 ml with water. After applying 25 strokes, it was allowed to stand until the aqueous volume reached 100 ml. The foam height was then measured above the aqueous volume (Kuril et al., 2020).

2.4.4. Foam retention

In a 200 ml graduated cylinder, 50 ml of the prepared polyherbal handwash formulation was agitated 10 times. The quantity of the foam was measured for 4 minutes at 1-minute intervals ensuring the foam retained should be stable for at least 5 minutes (Chitkara et al., 2020).

2.4.5. Skin irritation test

The skin irritancy test was performed by applying the formulations on the skin and observed for 30 minutes (Wal et al., 2021).

2.4.6. Stability

The stability study was done by storing the formulations at different temperatures such as 17 ºC, 28 ºC, and 37 ºC for a period of one month (Rajalakshmi, 2019).

2.4.7. Antimicrobial activity of polyherbal handwash

The screening of antimicrobial activity of the prepared poly-herbal handwash formulation was performed against microorganisms such as *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeroginosa, Salmonella typhi, Aspergillus niger,* and *Candida albicans* using agar well diffusion method on Muller-Hinton agar and Potato Dextrose agar respectively (Sumaiya et al., 2017; Tunio et al., 2022). The microbes were stabbed over the Muller-Hinton agar plate and Potato Dextrose agar plate using sterile cotton swabs and 10 µl of the prepared polyherbal handwash formulation of each batch was poured into the wells on the plates. After 24 hours of incubation at 37 °C, the antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition by taking triplicates (Donio et al., 2022).

3. Results

3.1. Physical evaluation

The polyherbal handwash prepared was inspected by visual observation where all formulations were found to be greenish in colour, mild lavender fragrant, sleek, pellucid, with no grittiness, and maintain homogeneous form respectively.

3.2. pH

The pH of all the formulations was determined by calibrated digital pH meter and it was found to be 6 to 7 (Table 2) which are near to the pH range of the human skin.

3.3. Foam height

After applying 25 strokes, it was allowed to stand until the aqueous volume reached 100 ml. The foam height of all the formulated herbal handwash was found to be as per the following Table 2. From the observation table, it is concluded that the F3 batch shows good foam height.

3.4. Foam retention

In a 50 ml graduated cylinder, 12.5 ml of the prepared polyherbal handwash formulation was agitated 10 times. The quantity of the foam was measured for 4 minutes at 1-minute intervals. The foam retention of all the formulated herbal handwash was found to be as per the following Table 2. From the observation table, it is concluded that the F3 batch shows good retention time than the others which was almost stable for 6 min thus it was best to be used as a herbal hand wash.

Table 2. pH, foam height, and foam retention of polyherbal handwash

Formulation batch	pH range	Foam height (cm)	Retention time (mins)
F1	6.8	8	2
F2	6.7	10	3
F3	6.9	12	6
F4	7.2	11	4

3.5. Skin irritation test

Ten final-year students voluntarily participated, and the formulation was applied to their skin for 30 minutes to test for skin irritation. Since the formulations' pH was within the acceptable range, no irritation or redness was found. This demonstrates that there was no chance for skin sensitivity.

3.6. Stability

A stability study was carried out at different temperatures such as 17 ºC, 28 ºC, and 37 ºC for a period of one month (Table 3), and found that there was not many changes in pH, physical evaluation, foam height & foam retention. From the observation table, it is concluded that F3 is a stable and more suitable batch than the others.

Table 3. Stability studies of polyherbal handwash

Formulation batch	Physical evaluation	pH	Foam height (cm)	Foam retention (mins)
F1	Slight change in color	6	5	2
F2	Slight change in color	6	7	2
F3	No change	6.7	11	6
F4	No change	6.2	9	4

3.7. Antimicrobial activity of polyherbal handwash

The antimicrobial activity of the prepared poly-herbal handwash formulation was performed against seven different microorganisms using the agar well diffusion method. The zone of inhibition indicates that the poly-herbal hand wash prepared from methanol extracts of the combined plant materials expressed significant antimicrobial activity. From the observation in Table 4, it is concluded that the F3 batch has shown good antibacterial activity against five different microorganisms.

Table 4. Antimicrobial activity of polyherbal handwash

Pathogens	Zone of Inhibition (cm)				
	Standard	F1	F2	F3	F4
Bacillus subtilis	2.3 ± 1.041	2 ± 0.194	2.3± 1.053	2± 1.064	1.8± 0.862
Escherichia coli	1.6 ± 0.764	1.5± 0.364	1.5± 0.684	1.6± 0.931	1.6± 0.485
Staphylococcus aureus	3.4 ± 0.414	2.4± 1.244	2± 1.912	2± 1.053	2± 1.154
Pseudomonas aeroginosa	2.7 ± 0.661	1.8± 0.743	1.9± 0.674	2± 1.041	1.8± 0.772
Salmonella typhi	3.2 ± 0.434	2.3± 1.037	2.1± 1.762	2.6± 2.00	2.1± 2.520
Aspergillus niger	1.8 ± 0.814	1.6 ± 0.672	1.6 ± 0.264	1.7 ± 0.392	1.7 ± 0.147
Candida albicans	1.6 ± 0.524	1.2 ± 0.533	1.4 ± 0.447	2 ± 1.113	1.8 ± 0.810

Values are expressed as Mean ± SD.

4. Discussion

The Covid-19 outbreak has raised consciousness about the significance that proper hand hygiene serves in preventing the spread of infection which many people were unaware (Natarajan et al., 2021). Bacteria, viruses, and other microbes are most commonly transmitted by hand. Thus, proper methods of hand washing can prevent the spread of infection (Ghurghure et al., 2019; Chen et al., 2022). In addition to restricting the spread of the infection, the way the world has embraced the appropriate utilisation of handwashes and hand sanitizers has also been crucial in minimizing the transmission of many other contagious diseases (Alzyood et al., 2020). In order to assess the medicinal potential of diverse herbs, a huge quantity of research has been done on the usage of traditional herbal products in south-east Asian nations (Kusarkar et al., 2022). A plethora of medicinal properties, as well as many medicinal plants, have been reported to be helpful for managing infectious diseases (Khan and Raghav, 2021). In the current scenario, natural plant-based products are widely in use for the management and prevention of different microbial infections and also for the betterment of life (Adhikari, 2021). In this era, the leading infection caused is skin infection which causes non-fatal diseases for a prolonged period of time resulting in expensive treatment (Sharma and Singh, 2020). As per different reports, herbal plants have effective pharmacological activities, especially in dermatological treatments (Rukari et al., 2022). To minimize and combat the bacterial pathogens which affect the skin, the most exposed part of the body, polyherbal handwash was formulated (Aware et al., 2022). The formulation was prepared having no or minimal side effect along with potential antimicrobial activity (Singh and Singh, 2022).

Ayurvedic, Unani, and homeopathic remedies frequently use neem, also known scientifically as *Azadirachta indica*. Neem has been reported to have anti-inflammatory, antifungal, antibacterial, and

antipyretic activities in its active components (Pallai et al., 2021; Reddy and Neelima, 2022). Native to the Indian subcontinent, Tulsi is known as the "Solution of Existence" in the Ayurvedic system. According to Sahoo et al. (2002), it has pharmacological properties including healing attributes, anticancer activity, antioxidant activity, anti-diabetic activity, anti-inflammatory activity, antibacterial activity, etc. The most popular culinary spice used globally is coriander, sometimes known as the herb of delight. The varied pharmacological effects of coriander include antibacterial, antioxidant, neuroprotective, migraine-relieving, and analgesic effects (Dhakshayani and Alias, 2022). Aloe vera, a succulent plant that resembles a cactus, is well known for its therapeutic properties and has been used for many years as a treatment for illnesses like sunburn, wounds, and skin issues. Aloe vera's pharmacological advantages are ascribed to its capacity to heal wounds as well as its immunomodulatory, anti-inflammatory, antioxidant, and antibacterial capabilities (Figueiredo et al., 2022). Lemon, a member of the Rutaceae family, has cancer-preventive, antibacterial, antifungal, and antidiabetic effects (Rukari et al., 2022). Neem, tulsi, coriander, aloe vera, and lemon juice are all common therapeutic herbs that are employed in the formulation of the polyherbal handwash in this study. The primary goal of incorporating all five herbal plants was to achieve a synergistic effect between the active components of various plants, which would aid in boosting the handwash formulation's antibacterial capability.

Four batches of polyherbal handwash were prepared for the present research. Based on their established pharmacological efficacy, inexpensive, compatibility with skin types, and ease of availability, the herbal plants used in the formulation were selected (Bhagwan et al., 2021). Every parameter, such as physical attributes, pH, foam retention, foam height, and stability, that was looked into for each batch of the polyherbal handwash was determined to be effective, but the third batch (F3) in particular showed that the formulation prepared had similar antibacterial properties to the formulation that was marketed. The handwash formulation was skin-friendly because it passed the test for skin irritation, and it can also be regarded as eco-friendly because it contained herbal plants. Microorganisms, mostly gram-positive ones, are abundant on our skin (Chindarkar, 2020). The antibacterial activity of the handwash preparations demonstrated a pronounced zone of inhibition against various pathogens. The F3 formulation produced the greatest results, demonstrating a synergistic response against the pathogens and demonstrating the presence of antibacterial properties. Hence, based on the findings and observations, it is possible to use polyherbal handwash and reduce the usage of handwash containing synthetic chemicals that might injure the skin.

The preparation and formulation of polyherbal handwash is thus a result of the growing demand for polyherbal formulations on the international market. The benefits of natural medicines are greater since they have fewer negative effects than synthetic ones (Giri et al, 2022).

Conclusion

When combined with lemon juice, aloe vera, and lavender oil, the methanolic extract of dried leaves of *Azadirachta indica*, *Ocimum gratissimum*, and *Coriandrum sativum* generate a significant zone of inhibition to fight against microorganisms similar to that of the commercial handwash employed in the study. In order to develop an environment-friendly and effective antibacterial handwash, these compounds could be isolated and incorporated to hand wash bases. The specifically formulated F3 hand wash demonstrates equivalent outcomes to the conventional hand wash in terms of physical and chemical parameters as well as close antibacterial activity against all tested microorganisms. The regular use of formulation can also assist in promoting good hygiene in both adults and children. As a result, a novel approach to combating pathogenic organisms' antibiotic resistance can be developed, allowing for the provision of safe and healthy living through the use of hands-free germs. Despite the fact that not all can be removed, a large portion may, and they can preserve their good health, which is a valuable resource for our everyday lives.

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