

RESEARCH ARTICLE

Formulation and Evaluation of Polyherbal Antimicrobial Handwash

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Abstract: Maintaining hand hygiene and cleanliness is crucial. Instead of relying on chemical agents, we formulated a polyherbal handwash using extracts from herbs with antimicrobial properties. Extracts from neem, turmeric, reetha, lemongrass, orange peel, and glycerin were used to prepare the handwash. The aim was to develop a more hygienic and reliable handwashing routine.

The results demonstrated that the handwash prepared with methanol extracts exhibited greater antimicrobial activity compared to existing commercial handwashes. Stability studies showed no color change, phase separation, and the formulation retained its activity. The pH and viscosity were found to be 6.88 and 40-120 mPa.s, respectively. The antimicrobial efficacy of the developed polyherbal handwash was evaluated against skin pathogens such as *Staphylococcus aureus* and *E. coli* using the dip-well method. The efficacy of the handwash was validated, and the findings were compared with a commercially available handwash. The handwash formulation with lemon juice exhibited enhanced antimicrobial activity compared to the one without it. The combined action of phytoconstituents from the herbal extracts, known to be devoid of adverse effects on human tissues while promoting hygiene, rendered the desired antimicrobial effect to the polyherbal handwash formulation.

Keywords: Polyherbal; Handwash; Antimicrobial Activity; Eco-friendly; Phytoconstituents

1. Introduction

Hand hygiene is a fundamental practice in maintaining personal and public health, as well as preventing the transmission of infectious diseases. The hands serve as vectors for a myriad of pathogenic microorganisms, increasing the risk of contracting illnesses through direct contact or cross-contamination. [1-4] Numerous studies have highlighted the importance of proper handwashing practices, particularly in healthcare settings, food handling establishments, and childcare facilities. Inadequate hand hygiene has been identified as a significant contributor to the escalating prevalence of healthcare-associated infections (HAIs), which substantially impact patient outcomes, mortality rates, and healthcare expenditures. Traditionally, handwashing protocols have relied heavily on the use of antimicrobial agents, such as chlorhexidine gluconate, triclosan, and alcohol-based sanitizers. While these chemical agents have demonstrated efficacy in reducing microbial loads on hands, their prolonged and indiscriminate use has raised concerns regarding the development of antimicrobial resistance, adverse effects on the skin microbiome, and potential environmental implications. Additionally, some individuals may experience skin irritation, dryness, or allergic reactions to certain chemical agents present in commercial handwashes and sanitizers. [5, 6] In recent years, there has been a growing interest in exploring natural, plant-based alternatives that exhibit antimicrobial properties while minimizing the risk of adverse effects. Numerous medicinal plants and their derivatives have been investigated for their potential antimicrobial activities against a wide range of pathogens, including bacteria, fungi, and viruses. [7] These plant-derived compounds, often referred to as phytochemicals or secondary metabolites, encompass a diverse array of chemical classes, such as alkaloids [4], terpenoids [5], flavonoids [6], phenolics [7], and saponins [8].

Among the plant species that have garnered significant attention for their antimicrobial properties are *Azadirachta indica* (neem), *Curcuma longa* (turmeric), *Sapindus mukorossi* (reetha), *Cymbopogon citratus* (lemongrass), and *Citrus limon* (lemon). Neem, revered for its medicinal value, possesses antimicrobial, antifeedant, insect repellent, and nematicidal properties attributed to its rich phytochemical composition. [9] Turmeric, a widely used spice and coloring agent, exhibits anti-inflammatory, antiseptic, and potent antioxidant activities due to the presence of curcumin and other bioactive compounds. [10] Reetha, commonly known as the soapnut, contains saponins that contribute to its unique cleansing properties and traditional medicinal applications. Lemongrass and lemon peel extracts are valued for their refreshing aroma, cooling effects, and potential antimicrobial activities. [11] The present study aimed to formulate a polyherbal handwash by combining extracts from these plant sources, leveraging their synergistic antimicrobial effects. The formulated handwash was subjected to rigorous evaluation, including stability studies,

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physicochemical characterization, and antimicrobial efficacy assessments against relevant skin pathogens, such as *Staphylococcus aureus* and *Escherichia coli*.

2. Materials and methods

2.1. Plant Materials

The plant materials used in this study were obtained from various sources. Fresh neem (*Azadirachta indica*) leaves were collected from nearby trees in Nashik, Maharashtra, India. Turmeric (*Curcuma longa*) rhizomes, reetha (*Sapindus mukorossi*) seeds, and lemongrass (*Cymbopogon citratus*) roots were purchased from a local market. Lemon (*Citrus limon*) fruits were also procured from the local market. All plant materials were thoroughly washed with running tap water to remove any dirt, debris, or extraneous matter, followed by shade drying.

2.2. Preparation of Extracts

2.2.1. Reetha Seed Extract

Dried reetha seeds were coarsely powdered using a mechanical grinder. The powdered material (50 g) was subjected to maceration with water as the solvent in a ratio of 1:10. Solution was mixed homogeneously for 6-8 hours, ensuring complete extraction of bioactive compounds. The aqueous extract was concentrated using a hot plate and controlled temperature to obtain a semi-solid extract. [12]

2.2.2. Neem Leaf Extract

Fresh neem leaves were washed, air-dried, and then ground to a coarse powder. The powdered material (50 g) subjected to maceration with water as the solvent in a ratio of 1:10 for 3 days. The aqueous extract was filtered through the whatman no. 1 filter paper. The filtrate was evaporated to obtain a dry and aqueous extract which was then stored in a refrigerator [13]

2.2.3. Turmeric Extract

Dried turmeric rhizomes were ground into a moderately coarse powder (#22) using an electronic grinder. Two grams of the powdered material were added to an ethanol-water system (1:1 ratio). The mixture was boiled on a water bath to concentrate the extract. The hot solution was filtered through a Whatman No. 1 filter paper. The filtrate was heat-evaporated to dryness, and the resulting dry extract was stored in a refrigerator. [14]

2.2.4. Lemongrass Extract

The lemongrass material (2.3 kg) was powdered, and the powder was soaked in 70% aqueous methanol at room temperature for three days with occasional shaking. The mixture was filtered first through a muslin cloth and then through a filter paper. This procedure was repeated three times, and the combined filtrates were evaporated using a rotary evaporator. The obtained extract was stored and preserved in a refrigerator. [15]

2.2.5. Orange Peel Extract

One gram of orange peel powder was added to a solvent system consisting of 50% methanol in distilled water. The mixture was placed in a laboratory shaker for two days. After shaking, the mixture was filtered, and the filtrate was evaporated at 50°C. The dried extract obtained was stored at room temperature. [16]

2.3. Formulation of Polyherbal Handwash

The polyherbal handwash was formulated by combining the extracts obtained from the above-mentioned plant materials. The extracts were weighed in predetermined quantities and added to distilled water in a beaker. [17] The mixture was heated on a water bath at 90°C for 90 minutes with continuous stirring to facilitate the dissolution of the extracts. After cooling to room temperature, glycerin was added as a base and emollient.

The pH of the solution was adjusted to 7.0 using small quantities of triethanolamine with constant mixing. [18] The solution was then homogenized using a mechanical homogenizer at room temperature to ensure a uniform and homogeneous mixture. The final formulation was made up to the desired volume with distilled water. The composition of the polyherbal handwash formulation is presented in Table 1.

Table 1. Composition of the Polyherbal Handwash Formulation

Ingredient	Quantity	Role
Neem extract	5 g	Antimicrobial
Turmeric extract	1 g	Antiseptic, Antioxidant
Reetha extract	0.5 g	Foaming agent
Lemongrass extract	1 g	Antimicrobial, Cooling agent
Orange peel extract	0.5 g	Antioxidant, Fragrance
Glycerin	30 mL	Base, Emollient
Distilled water	q.s. 100 mL	Vehicle

2.4. Evaluation of the Polyherbal Handwash

2.4.1. Organoleptic Evaluation

The prepared polyherbal handwash was visually inspected for color, clarity, homogeneity, and phase separation. The odor of the formulation was also evaluated organoleptically. [19]

2.4.2. pH Measurement

A digital pH meter was used to measure the pH of the polyherbal handwash formulation. One gram of the handwash was dissolved in 100 mL of distilled water, and the pH of the resulting solution was measured after calibrating the pH meter with standard buffer solutions. [20]

2.4.3. Viscosity Determination

The viscosity of the polyherbal handwash was determined using a digital Brookfield viscometer. A sample of 10 mL of the handwash was transferred to a 100 mL beaker, and the viscometer spindle was immersed in the sample. The viscosity was measured in milliPascal seconds (mPa.s) at room temperature.

2.4.4. Spreadability Evaluation

The spreadability of the polyherbal handwash was evaluated by placing a known quantity (0.01 g) of the formulation between two glass plates and measuring the diameter of the spread after one minute. Good spreadability is desirable for effective application and distribution of the handwash on the skin. [21]

2.4.5. Foam Height and Retention

The foaming ability and foam retention of the polyherbal handwash were evaluated using a graduated cylinder. One gram of the handwash was added to 50 mL of distilled water in a 500 mL stoppered measuring cylinder. The mixture was shaken vigorously for 25 strokes, and the foam height was recorded immediately. The foam retention was measured by recording the foam volume at one-minute intervals for up to four minutes.

2.4.6. Stability Studies

Stability studies were conducted following the International Council for Harmonisation (ICH) guidelines. The polyherbal handwash formulation was sealed in a container and stored under different temperature and humidity conditions for three months. At predetermined time intervals, the formulation was evaluated for changes in appearance, pH, viscosity, and spreadability. [22]

2.4.7. Antimicrobial Activity Evaluation

The antimicrobial efficacy of the polyherbal handwash was evaluated against two relevant skin pathogens, *Staphylococcus aureus* and *Escherichia coli*, using the dip-well method according to standard operating procedures (SOPs). [23]

2.4.8. Bacterial Strains and Culture Conditions

Standardized strains of *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) were obtained from a certified culture collection. The bacterial strains were revived and sub-cultured on nutrient agar plates according to standard microbiological techniques. [24]

2.4.9. Dip-well Method

Nutrient agar plates were prepared and allowed to solidify. Five wells were carefully cut into the agar medium using a sterile cork borer. The wells were labeled for the test samples and controls. The bacterial cultures were swabbed onto the agar surface using a sterile cotton swab to create a lawn of bacterial growth. The polyherbal handwash, individual herbal extracts, and a commercially available handwash (as a positive control) were added to the respective wells. [25] The plates were incubated at 37°C for 24 hours.

After incubation, the plates were observed for the formation of zones of inhibition around the wells. The diameters of the zones were measured using a ruler or a digital caliper and recorded. The antimicrobial activity of the polyherbal handwash and the individual extracts was evaluated based on the size of the inhibition zones and compared with the positive control. [26]

3. Results

3.1. Organoleptic Evaluation

The prepared polyherbal handwash formulation exhibited a light yellow color with a slightly cloudy appearance. The cloudiness was attributed to the presence of plant extracts and suspended particles. The handwash had a pleasant, refreshing aroma derived from the combination of lemongrass and orange peel extracts. No visible phase separation or sedimentation was observed, indicating a stable and homogeneous formulation. [27, 28]

3.2. pH Measurement

The pH of the polyherbal handwash formulation was found to be 6.88 ± 0.12 , which is within the acceptable range for topical formulations intended for skin application. A slightly acidic pH is desirable as it mimics the natural pH of the skin, which ranges from 4.5 to 6.5. Maintaining a pH close to the skin's pH helps preserve the skin's acid mantle, which serves as a protective barrier against microbial invasion and maintains the skin's moisture balance. [29, 30]

3.3. Viscosity Determination

The viscosity of the polyherbal handwash formulation was measured to be in the range of 40-120 mPa.s at room temperature. This viscosity range is considered suitable for handwash formulations, as it ensures easy dispensing and spreading on the skin while providing a desirable thickness and body to the product. The viscosity can be attributed to the combined effects of the plant extracts, glycerin, and other ingredients used in the formulation. The results of evaluation parameters are enlisted in Table 1.

Table 1. Evaluation of polyherbal handwash

Sl. no.	Evaluation parameter	Observed values
1	Colour and odour	Green and sweet lemon
2	Homogeneity	Homogenous
3	pH	6.9
4	Viscosity Cps	40-120
5	Spreadability gm.cm/sec	11 ± 0.50
6	Foam height	150ml
7	Foam retention	25ml
8	Stability	Stable

3.4. Spreadability

The spreadability of the polyherbal handwash was evaluated to ensure efficient application and distribution on the skin during handwashing. The formulation exhibited good spreadability, with a diameter of spread ranging from 6.5 to 7.2 cm after one minute. Adequate spreadability is crucial for effective cleaning and antimicrobial action, as it allows the active ingredients to come into contact with a larger surface area of the skin. [28]

3.5. Foam Height and Retention

The foaming ability and foam retention of the handwash formulation were evaluated, as these properties contribute to the overall user experience and cleansing efficacy. The initial foam height generated upon vigorous shaking was found to be 12.5 ± 0.8 cm. The foam retention was monitored for four minutes, and the foam volume remained stable, with a gradual decrease of approximately 20% after four minutes. The presence of the reetha extract, which contains saponins, contributed to the foaming properties of the handwash, enhancing its cleansing ability. [29]

3.6. Stability Studies

The polyherbal handwash formulation was subjected to stability studies under different temperature and humidity conditions to assess its shelf-life and ensure the maintenance of its physicochemical properties and antimicrobial activity over time. After three months of storage, no significant changes in appearance, color, odor, or phase separation were observed. The pH and viscosity values remained within the acceptable ranges, indicating the formulation's stability. [30]

3.7. Antimicrobial Activity Evaluation

The antimicrobial efficacy of the polyherbal handwash was evaluated against two clinically relevant skin pathogens, *Staphylococcus aureus* and *Escherichia coli*, using the dip-well method. The results were compared with a commercially available handwash as a positive control. [31, 32]

3.7.1. *Staphylococcus aureus*

The polyherbal handwash formulation exhibited significant antimicrobial activity against *S. aureus*, with a zone of inhibition ranging from 18.5 ± 0.7 mm to 22.1 ± 0.9 mm. The individual herbal extracts also displayed varying degrees of antimicrobial activity, with the neem extract showing the largest inhibition zone (19.8 ± 0.6 mm), followed by the lemongrass extract (16.2 ± 0.5 mm) and the turmeric extract (14.7 ± 0.4 mm). The commercially available handwash, used as a positive control, demonstrated a zone of inhibition of 17.3 ± 0.6 mm.

3.7.2. *Escherichia coli*

Against *E. coli*, the polyherbal handwash formulation displayed moderate antimicrobial activity, with a zone of inhibition ranging from 14.2 ± 0.5 mm to 17.8 ± 0.7 mm. The individual herbal extracts exhibited varying levels of efficacy, with the lemongrass extract showing the highest activity (16.1 ± 0.6 mm), followed by the neem extract (14.8 ± 0.5 mm) and the turmeric extract (12.5 ± 0.4 mm). The commercially available handwash exhibited a zone of inhibition of 15.6 ± 0.5 mm against *E. coli*.

The antimicrobial activity of the polyherbal handwash can be attributed to the synergistic effects of the various phytoconstituents present in the herbal extracts. [33] Neem is known for its antimicrobial properties, which are primarily due to the presence of compounds like azadirachtin, nimbidin, and nimbolide. Turmeric contains curcumin and other curcuminoids, which have demonstrated potent antimicrobial and anti-inflammatory activities. [34] Lemongrass is rich in essential oils, including citral, geraniol, and limonene, which possess antimicrobial properties. The orange peel extract contributes antioxidant and antimicrobial activities due to the presence of flavonoids, phenolic acids, and essential oils. [35] The combined action of these phytochemicals, along with the potential synergistic interactions between them, contributed to the enhanced antimicrobial efficacy of the polyherbal handwash formulation

4. Conclusion

In conclusion, this study successfully formulated a polyherbal handwash by combining extracts from neem, turmeric, reetha, lemongrass, and orange peel. The handwash exhibited desirable physicochemical properties, stability, and significant antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. The synergistic effects of the various phytoconstituents present in the herbal extracts contributed to the enhanced antimicrobial efficacy. This formulation represents a promising natural and eco-friendly alternative to conventional chemical-based handwashes, promoting hygiene while minimizing the risk of adverse effects and antimicrobial resistance.

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