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Formulations and evaluation of antimicrobial polyherbal hand wash containing *Azadirachta indica* and *Ocimum sanctum*

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ABSTRACT: Background: Hand hygiene is a crucial initial step in preventing the transmission of diseases because they are the main way that microorganisms enter the body. Although there are several hand washes on the market, they are all chemical-based, which might trigger allergic reactions in some people. **Aim:** The goal of the study was to formulate and evaluate hand washes that contained herbal antimicrobials such as *Azadirachta indica* (Neem) and *Ocimum sanctum* (Tulasi). **Method:** The aqueous extract of the leaves of crude pharmaceuticals was made and utilized in four distinct formulations in varying quantities and evaluated. By using the Cup and Plate method, the antibacterial activity of prepared hand wash formulations was tested against the skin pathogens *Staphylococcus aureus* and *Escherichia coli.* **Result:** The developed herbal hand wash formulation was found to be effective against *S. aureus.* The zone of inhibition against *S. aureus* of various formulations was F1 (26±0.23 mm), F2 (26±0.63 mm), F3 (28±0.22 mm), and F4 (24±0.53 mm) as well as against *E. coli* was F1 (21±0.73 mm), F2 (19±0.82 mm), F3 (27±0.33 mm), and F4 (25±0.43 mm). **Conclusion:** All four of these formulations exhibit significant antibacterial properties and have no negative side effects; F3 exhibits the maximum zone of inhibition against *S. aureus* (28±0.22 mm) and *E. coli* (27±0.33 mm), indicating that the developed formulations can be utilized without risk.

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INTRODUCTION:

The area of our bodies that is most vulnerable to infections is our skin, which demands the highest level of protection. The necessity for hospitalization due to a nosocomial infection has increased, and it occasionally results in fatalities [1]. The primary means through which various diseases are spread among healthcare professionals is through their hands [2,3]. Soaps, detergents, and other sources have all been developed as a result for us to wash our hands. There are numerous antibacterial hand washes on the market. However, they

are having negative consequences because they could contain different compounds like alcohol or chlorhexidine. The main countermeasure to the negative effects of chemical hand washes is the use of herbal products [4]. Different microorganisms, including *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*, were responsible for the skin infections. The use of herbal, or plant-based, remedies can treat those skin infections without causing any negative side effects [5].

Azadirachta indica (Neem), a plant that is commonly grown in tropical and semitropical settings, was once the primary treatment for plenty of a lot of skin problems. In addition to azadirachtin and limonoids, which are found in its fruits and seeds, neem oil also contains glycerides, various polyphenols, nimbolide, triterpenes, and beta-sitosterol. Quercetin, catechins, carotenes, and vitamin C are all present in the yellow, bitter oil, which also has a garlic-like flavor. Neem tree products have been used in Indian traditional medicine for many years. Neem serves as a natural insecticide and is a significant component of non-pesticidal management (NPM). The crop is sprayed with powdered neem seed that has been ground into powder and soaked in water. Neem does not kill insects directly but acts as a repellent and egg-lying deterrent and thus protects the crop from damage [6,7].

There are 150 different types of Tulasi, also known as holy basil or *Ocimum sanctum*, throughout the world. The queen of all herbs is Tulasi. The world's tropical and semitropical climates are home to this bushy shrub. It tastes different and has a distinctive aroma. It can reach heights of 3 to 5 feet. In Ayurveda, Tulasi is frequently consumed as a herbal tea. It also has significance in the Vaishnava school of Hinduism, where followers worship the Tulasi plant. *Ocimum sanctum* has a variety of chemical components, including oleanolic acid, rosmarinic acid, ursolic acid, eugenol, linalool, carvacrol, etc. Eugenol is one of Tulasi's main active ingredients. Additionally, it contains chemical substances like 3-carene, eugenol methyl ester, terpinene, and caryophyllene. It functions well as an antioxidant. It cleanses the blood. Gastric problems and indigestion are helped by it. It lowers stress and guards against heart disorders. It is used to treat asthma and relieve cold and cough symptoms. It is nematicidal in nature [8].

Neem and Tulasi plants have been chosen in the current situation to prepare polyherbal hand wash. The created herbal preparation's effectiveness was evaluated and it was tested against skin infections.

MATERIALS AND METHODS:

Crude drugs and chemicals:

Fresh leaves of *Ocimum sanctum* (Tulasi), and *Azadirachta indica* (Neem) were collected from the herbal garden of Raghu College of Pharmacy and Sandalwood oil was procured from Pachipulusu Venkata Sanniah & Sons, M G Road, Visakhapatnam. The chemicals like Carbapol-940, Glycerin, Sodium lauryl sulfate, Sodium benzoate, and Triethanolamine were obtained from Fisher Scientific, Mumbai. The chemicals utilized were all of the analytical grades.

Preparation of extract:

A clean, dust-free atmosphere was used to clean and dry the leaves of *Azadirachta indica* and *Ocimum sanctum*. Using a grinder, dried leaves were ground into a fine powder. The aqueous extract was created through a digestion process utilizing 100 ml of hot water and Neem and Tulasi powder. After 30 min, the mixture was set aside, and the aqueous extract was collected and utilized to create a hand wash [9].

Preparation of four herbal hand wash formulations:

Based on the compositions listed in Table 2, four different formulations were created. Neem and Tulasi extracts were extracted. Carbapol-940, sodium lauryl sulfate, glycerin, sodium benzoate, and triethanolamine were taken and diluted in distilled water to formulate four formulations, which were then placed in a beaker. The four formulations, F1, F2, F3, and F4, are prepared using this as their base. Neem and Tulasi extracts are added to the base in the amounts shown in the table, and a measuring cylinder filled with cleaned water is filled to a capacity of 100 ml. The four formulas each have sandalwood oil added for scent before being poured into the bottles.

Evaluation parameter of herbal hand wash:

A technique of evaluation was used on the herbal hand wash. Physical characteristics like color, smell, and texture were assessed using both sensory and visual methods.

***Colour*:**

It was identified visually as a bright yellow color.

***Odor*:**

A pleasant smell was identified and noted.

Appearance and homogeneity:
Visual inspection was used to check the herbal hand wash's homogeneity and phase separation.

***pH measurement*:**
A digital pH meter (Beckman, Germany) was used to measure the pH of a 1% solution of hand wash.

***Skin irritation test*:**
Four human volunteers were used to assess the skin irritancy of four distinct formulations. The back of the hand is cleaned with a tiny amount of hand soap, which is then coated with cotton and adhesive tape. This was left on for 24 h, after which the application site was checked for lesions or discomfort [10].

***Evaluation of antimicrobial activity*:**
Using the agar plant technique (cup plate method), the antimicrobial efficiency of the four distinct formulations was tested against *Staphylococcus aureus* (Gram +ve) and *Escherichia coli* (Gram -ve) [11, 12]. After 24 to 48 h, the zones of inhibition data were assessed.

Foam retention:
About 25 ml of polyherbal hand wash was taken and shaken in a 100 ml measuring cylinder ten times to prevent foam build-up. The foam volume will be monitored every minute for 4 min, and the foam retention must last at least 5 min [13-16].

Studies on stability:
For each of these 4 formulations, stability tests were conducted at 4°C for 3 months, 25°C, and 40 °C for 3 months, and changes in color, odor, appearance, and pH were observed [10].

RESULTS AND DISCUSSION:
Neem and Tulasi extracts are the two main components of the herbal hand wash. According to Ayurveda, these two herbs were more efficient against germs and fungi; a description is provided in Table 1 [17–20]. The formula listed in Table 2 was used to prepare the four different formulations. As shown in Fig 1 and graphically in Fig 2, the largest zone of inhibition for F3 against *S. aureus* and *E. coli* is 28±0.22 and 27±0.33 mm, respectively. We, therefore refer to the F3 formulation as the optimum formulation. The following formulations likewise display the zone diameter for *S. aureus* as well as *E. coli* of the remaining three formulations were F1: 26±0.23 mm, F2: 26±0.63 mm, F4: 24±0.53 mm and F1:21±0.73 mm, F2: 19±0.82 mm, F4: 25±0.43 mm respectively. Numerous Neem active ingredients, including azadirachtin, limonoids, and eugenol from Tulasi, are responsible for antibacterial activity. The four herbal hand washes were prepared and evaluated. Table 3 is a summary of the findings. After its stability experiments were finished, it was found that formulations had a faint yellowish-brown color, a light sandalwood aroma, and a translucent appearance.

Table 1. List of natural ingredients used in the preparations of herbal hand wash.

Name of the essential oil	Biological source	Purpose/ uses
Tulasi [17]	*Ocimum sanctum (OS)* is also known as Holy Basil or Tulsi, and belongs to the family of *Lamiaceae*.	Cough, asthma, diarrhea, fever, dysentery, arthritis, eye diseases, indigestion, gastric ailments
Neem [18,19]	*Azadirachta indica*, a member of the Meliaceae family, is commonly known as neem.	Anti bacterial, antifungal, hypolipemic activities in addition to hepatoprotective and hypertensive activities. It also possesses Anti-inflammatory, Antibacterial, Anti-hemorrhagic effects.
Sandal wood oil [20]	*Santalum album* L. (*Santalaceae*) commonly known as Indian Sandalwood	The heartwood constitutes the central part of the tree and is valued for its fragrance. used in perfumery, cosmetics, aromatherapy and pharmaceutical industry.

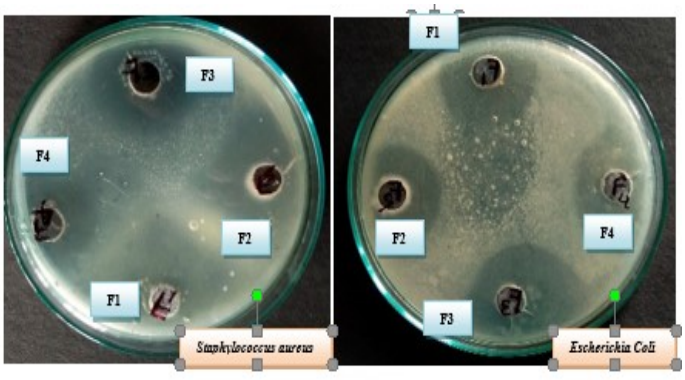

Fig 1. Antimicrobial activity of four different formulations.

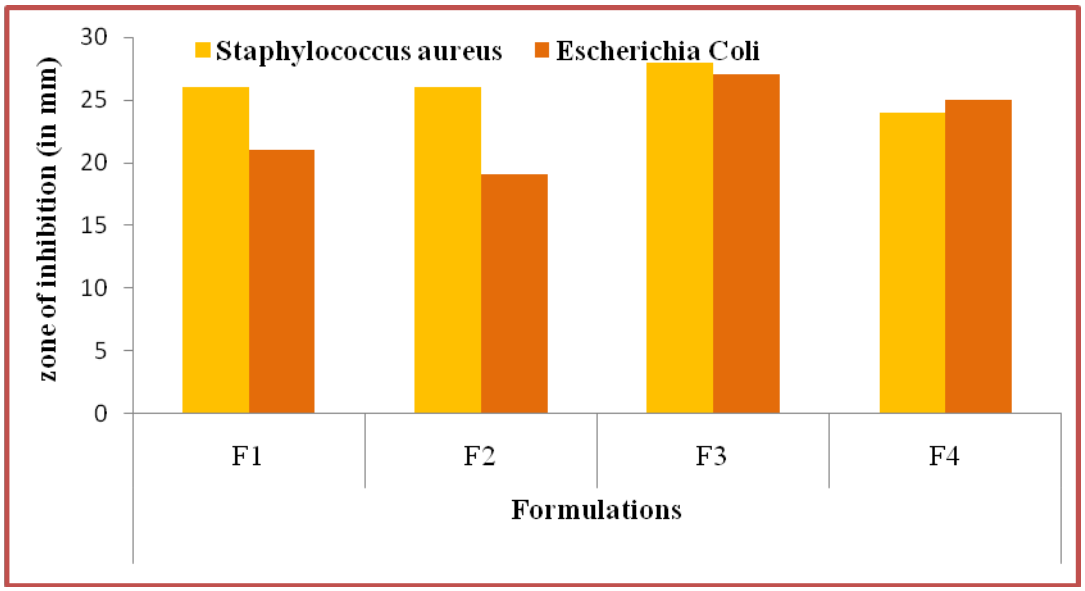

Fig 2. Antimicrobial activity of four different formulations bar graph.

Table 2. Formulations design for poly herbal hand wash.

Ingredients	F1	F2	F3	F4	Role
Tulasi extract (ml)	4	3	2	5	Antimicrobial
Neem extract (ml)	4	1	6	3	Antimicrobial
Carbapol-940 (g)	0.2	0.2	0.2	0.2	Suspension agent
Glycerin (ml)	2	2	2	2	Moisturizing agent
Sodium lauryl sulphate (g)	4	4	4	4	Surfactant
Sodium benzoate (ml)	0.2	0.2	0.2	0.2	Preservative
Triethanolamine (ml)	0.2	0.2	0.2	0.2	Emulsifiers
Sandalwood oil (ml)	0.5	0.5	0.5	0.5	Favorizing agent
Purified water (ml)	100	100	100	100	make up

Table 3. Result of evaluation parameters of Herbal hand wash.

Test	Observation of test hand wash			
	F1	F2	F3	F4
Colour	Yellowish brown	Yellowish brown	Yellowish brown	Yellowish brown
Odour	Aromatic	Aromatic	Aromatic	Aromatic
Appearance	Trans-lucent	Trans-lucent	Trans-lucent	Trans-lucent
Homogeneity	Homogenous	Homogenous	Homogenous	Homogenous
Texture	Liquid	Liquid	Liquid	Liquid
pH	6.41	6.61	6.51	6.66
Skin irritation	No any	No any	No any	No any

Table 4. Anti-bacterial activity of four different formulations.

Organism	Zone of inhibition (mm)			
	F1	F2	F3	F4
Staphylococcus aureus	26±0.23	26±0.63	28±0.22	24±0.53
Escherichia coli	21±0.73	19±0.82	27±0.33	25±0.43

Table 5. Stability studies of four different formulations of herbal hand wash.

Parameter	Observation after 3 months	Result observed
Colour	Pale yellowish brownish	No any significant changes
Odour	Agreeable and aromatic	No any significant changes
Appearance & homogeneity	No any chance in the appearance and homogeneity	No any significant changes
pH	6 approx of all formulations	No any significant changes

These characteristics remained the same. The pH varies from 6.41 to 6.66; it is considered slightly basic, suitable for skin, and doesn't cause any itching or irritation.

Table 5 of the stability study data demonstrates that no alterations were noticed following the end of the stability periods (3 months).

CONCLUSION:

In the present study, various active constituents were present in the extract of Neem, and Holy Basil showed superior inhibition against gram-positive microbial skin pathogens. Regular hand washing can prevent the entry of various microorganisms, as the proverb "prevention is better than cure" says because the hands are the primary mode of transmission of various diseases. Therefore, these compounds can be extracted and incorporated into soap bases with significant activity and little or no side effects. The formulation can also be routinely used to improve the hygiene of healthy children and adults.

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