

Research Article



Formulation, Evaluation, and Antibacterial Activity of Polyherbal Hand Wash

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Received: 18-03-2023; Revised: 26-05-2023; Accepted: 03-06-2023; Published on: 15-06-2023.

ABSTRACT

The objective of the current experiment was to evaluate *in vitro* the antibacterial activity of a polyherbal hand wash formulation against various skin infections. The use of herbal treatments in healthcare is widespread around the world. Numerous ailments can be effectively treated and prevented with the use of herbal medications. There are many synthetic hand washes available in the market and many of them have side effects including dermatitis, itching, and irritation. To counteract these negative impacts of synthetic handwash formulations, an effort has been undertaken to create a polyherbal hand wash. The hydroalcoholic extracts of *Tridax procumbens*, *Azadirachta indica*, *Glycyrrhiza glabra*, and *Hibiscus rosa-sinensis* were utilized to make the polyherbal hand wash. Two hand wash formulations were developed, and the preparation's physical characteristics, including pH, viscosity, and appearance, were investigated. The agar plate method was used for antibacterial effectiveness against skin infections including *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. The outcomes were contrasted with those of common antibacterial medication (Amoxicillin). The efficacy of hand cleaning was also assessed by applying the hand wash to individuals. The results demonstrated that when compared to a conventional antibiotic, produced herbal hand wash formulations showed a considerable zone of inhibition.

Keywords: Polyherbal hand wash, *Tridax procumbens*, *Azadirachta indica*, *Glycyrrhiza glabra*, *Hibiscus rosa-sinensis*, Antibacterial activity

QUICK RESPONSE CODE →

DOI:

10.47583/ijpsrr.2023.v80i02.011



DOI link: <http://dx.doi.org/10.47583/ijpsrr.2023.v80i02.011>

INTRODUCTION

The area of our bodies that is exposed the most is our skin, which must be shielded from various skin diseases¹. Healthcare workers (HCWs) hands are the major aspects of infection and multidrug-resistant pathogens spreading to patients. As a result, it raises the issue of using antiseptic for hand hygiene. Many chemical antiseptics are nowadays offered in the market as chlorhexidine products, alcohol-based sanitizers, etc. These soaps or solutions make it easier to lessen the spread of infectious illnesses in healthcare, but they also have certain drawbacks or undesirable side effects. Their repeated usage can cause infections to become resistant as well as irritate the skin. Skin pathogens include organisms including *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*². These microorganisms often cause skin infections including cellulitis, impetigo, and staphylococcal infections, and enter the body through an abrasion in the skin, like those caused by insect bites. Hand washing is crucial for preventing the spread of illness and reducing the number of harmful germs on hands^{3,4}. Most practitioners in these systems of Indian medicine create

and administer their own medications, therefore adequate documentation and research are necessary⁵. *Tridax procumbens*, an Asian Ayurvedic plant with a long history of usage, is a member of the Asteraceae family. *T. procumbens* has been used in traditional medicine for centuries to heal wounds, dermatitis, and blood clotting. It has anti-leishmanial, anti-coagulant, antioxidant, anticancer, immunomodulatory, insecticidal, anthelmintic, cardio-vascular, antiseptic, antimicrobial, and insecticidal actions⁶. *Azadirachta indica* also known as neem, margosa tree, and Indian lilac components have been used in traditional Ayurvedic medicine all throughout the world. Neem leaves include terpenoids, alkaloids, tannins, saponins, flavonoids, and amino acids. Both gram-positive and gram-negative bacteria are susceptible to its broad-spectrum antibacterial action. Additionally, it is also used to treat conditions affecting the skin, blood detoxification, control pests, and illnesses, lowering fever, dental treatments, cough, asthma, ulcers, and intestinal worms⁷. *Glycyrrhiza glabra* L. (Fabaceae), a native of Iran and southwest Asia, is a member of the Fabaceae family. Liquorice, also known as *Glycyrrhiza glabra* L., is a highly sweet, moist, calming herb with anti-inflammatory and expectorant characteristics, which also coughs and has hormonal effects. Additionally, it detoxifies and protects the liver. It is administered internally for the treatment of arthritis, gastric ulcers, Addison's illness, bronchitis, asthma, and coughs. Previous studies have investigated the antimicrobial properties of roots and rhizomes, but there have only been a few reports on the antimicrobial properties of liquorice leaves⁸. *Hibiscus rosa-sinensis* is a



member of the Malvaceae family. In ancient times, flowers have been utilized for prophylaxis of asthma. Many chemical components have been identified from this plant, including cyanidin, quercetin, hentriacontane, calcium oxalate, thiamine, riboflavin, niacin, and ascorbic acids. Lemon water, which is made from the fruits of *Citrus limon* L. and is a member of the Rutaceae family, has traditionally been used for cleaning because of its disinfecting properties. Lemon water is used in Indian medicine because of its antibacterial properties, but it is also used as a preservative in several culinary preparations. Moreover, it is employed to flavour several food preparations^{9,10}. The goal of the current investigation is to identify the function of Polyherbal extract in vitro antibacterial activity against human pathogens, specifically *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*.

MATERIALS AND METHODS

Plants Collection and Authentication

The plant's powder *Tridax procumbens*, *Azadirachta indica*, *Glycyrrhiza glabra*, and *Hibiscus rosa-sinensis* were purchased and authenticated from Sanchomee Herboveda Pvt. Ltd. Padwal Lane, Chinchwadgaon, Pune 411033

Preparation of Extracts

50 grams of coarsely powdered *Tridax procumbens*, *Azadirachta indica*, *Glycyrrhiza glabra*, and *Hibiscus rosa-sinensis* were soaked in 400 ml of 70% (V/V) methanol and water mixture and kept for maceration for about 1 week. After maceration, the extract is filtered, and the filtrate was collected and stored for preparation of hand wash¹¹.

Preparation of Herbal Hand Wash Formulations

Formulation 1 (F-1):

The hand wash was prepared using 20 ml of hydroalcoholic extract filtrate in this formulation. To this filtrate, 6 gms of Sodium Lauryl Sulphate, glycerin 40 ml, 0.3 gms of methylparaben, and 5 ml of Strawberry oil is added, and the volume is made up to 100 ml with purified water².

Table 1: Formulation 1 (F-1)

Sr. No.	Ingredients	Quantity
1	Hydroalcoholic extract of <i>Tridax procumbens</i> , <i>Azadirachta indica</i> , <i>Glycyrrhiza glabra</i> , and <i>Hibiscus rosa-sinensis</i>	20 ml
2	Sodium Lauryl Sulphate (SLS)	6 gms
3	Glycerin	40 ml
4	Methyl Paraben	0.3 ml
5	Strawberry oil	5 ml
6	Purified Water q. s	100 ml

Formulation 2 (F-2):

This formulation was prepared by adding 20 ml of lemon water to 20 ml of hydroalcoholic extract filtrate of *Tridax procumbens*, *Azadirachta indica*, *Glycyrrhiza glabra* and *Hibiscus rosa-sinensis*. The remaining ingredients are all the same as in formulation 1².

Table 2: Formulation 2 (F-2)

Sr. No.	Ingredients	Quantity
1	Hydroalcoholic extract of <i>Tridax procumbens</i> , <i>Azadirachta indica</i> , <i>Glycyrrhiza glabra</i> , and <i>Hibiscus rosa-sinensis</i>	20 ml
2	Lemon Water	20 ml
3	Sodium Lauryl Sulphate (SLS)	6 gms
4	Glycerin	40 ml
5	Methyl Paraben	0.3 ml
6	Strawberry oil	5 ml
7	Purified Water q. s	100 ml

Evaluation Parameters

1. Physical Evaluation:

Through sensory and visual examination, a physical assessment (colour, odour) was evaluated and compared to the hand wash currently being sold (Dettol hand wash)¹².

2. Grittiness:

1ml of hand wash was taken on the fingertips and rubbed between two fingertips, the formulation was evaluated to check the grittiness of handwash¹³.

3. pH:

1 g of herbal hand wash was dissolved in 100 ml of purified water. Using a previously established digital pH meter, the pH of the handwash was determined¹².

4. Viscosity:

Using a digital Brookfield viscometer model DV-II, the viscosity of the hand wash was calculated. A predetermined amount of hand soap was placed in a beaker, the viscometer's tip was inserted, and the viscosity was measured three times¹⁴.

5. Foam Height:

A hand wash sample weighing one gram was diluted in 50 ml of distilled water. A measuring cylinder with a capacity of 500 ml received the dispersion. Water was added to the volume to make it 100 ml. There were awarded and set away 25 strokes. The height of the foam above the aqueous volume was observed¹⁴.

6. Foam Retention:

A 100 ml graduated cylinder was filled with 25 ml of the hand wash gel with 1% alcohol. A hand was placed over the cylinder and shaken ten times. For four minutes, the amount of foam was measured at 1-minute intervals¹⁴.

7. Skin Irritation:

The skin (Hand) was treated with the formulation, and it was kept on for 30 minutes and evaluated for any irritation that persist¹⁵.

Evaluation of antimicrobial activity

The prepared polyherbal hand wash was tested for antimicrobial effectiveness on a variety of microorganisms using the agar plate method, in accordance with normal practice. To evaluate the antimicrobial activity against three distinct bacteria *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* three sterile petri plates were used. Agar solution containing nutrients and cultured bacteria was poured into the plates, and once they solidified, three cavities were created. Amoxicillin, a common antibiotic, is placed in the first cavity. Herbal hand wash without lime water (F-1) is placed in the second cavity. Herbal hand wash with lime water is placed in the third cavity (F-2). It was made sure that the sample was positioned level with the cavity. To assess the activity, the plates are put in an incubator set at 37°C. The plates were checked for the development of an inhibitory zone after 24 hours. The antimicrobial activity of the formulation is assessed from the zone of inhibition².

RESULTS AND DISCUSSION**Preliminary Phytochemical Screening**

Preliminary phytochemical analysis of the hydroalcoholic extract of *Tridax procumbens*, *Azadirachta indica*, *Glycyrrhiza glabra*, and *Hibiscus rosa-sinensis* revealed the presence of alkaloids, carbohydrates, glycosides, saponins, phytosterols, fixed oils, flavonoids, phenols, tannins, and proteins^[16].

Table 3: Phytoconstituents Present in Hydroalcoholic Extracts of *Tridax Procumbens*, *Azadirachta Indica*, *Glycyrrhiza Glabra*, And *Hibiscus Rosa-Sinensis*

Sr. No.	Phytochemical	Tests	Result
1	Alkaloids	Mayer's test	+
		Dragendorff's test	+
		Wagner's test	+
		Hager's test	+
2	Carbohydrates	Molisch's test	+
		Benedict's test	+
		Fehling's test	+
		Benedict's Test	-
3	Anthraquinone Glycosides	Borntrager's Test	+
		Modified	+
		Borntrager's Test	

4	Cardiac Glycosides	Baljet's Test	+
		Legal test	+
5	Saponins	Foam test	+
		Froth test	+
		Haemolysis Test	+
6	Phytosterols	Salkowski's test	+
		Libermann	+
		Burchard test	
7	Fixed oils	Filter Paper	+
8	Flavonoids	Shinoda Test	+
		Lead acetate	+
		Zn-HCl acid reduction	+
9	Phenols	Ferric Chloride test	+
10	Tannins	Gelatin test	-
		Vanillin-HCL Test	+
		Matchstick Test	+
11	Proteins	Xanthoproteic test	-
		Ninhydrin test	-
		Biuret test	+
		Million's Test	

Note: Present '+', Absent '-'.

Evaluation Parameters of Polyherbal Handwash:

Physical assessment and other evaluation criteria were used for the polyherbal hand wash formulations (F-1 and F-2) that had been developed.

Table 4: Evaluation Parameters

Sr No.	Parameter	Formulation	Observation
1	Colour	F1	Dark Brown
		F2	Light Orange
2	Odour	F1	Strawberry Like
		F2	Pleasant
3	Grittiness	F1	Non – Gritty
		F2	Non - Gritty
4	pH	F1	7
		F2	3.6
5	Viscosity	F1	27.6 cP
		F2	28.8 cP
6	Foam Height	F1	210 ml
		F2	250 ml
7	Foam Retention	F1	70 ml
		F2	60 ml
8	Skin Irritation	F1	No Irritation
		F2	No Irritation

Evaluation of Antimicrobial Activity

By using the agar plate technique, the anti-microbial effectiveness of the Polyherbal Hand Wash formulations was evaluated against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. The zone of inhibition test findings demonstrated that the combined hydroalcoholic extract of the plant components used to make the hand wash had high antibacterial activity. The formulation without lemon water (F-1) had somewhat lower activity than the hand wash made with lemon water. The table below displays the data on formulations' zones of inhibition.

Table 5: Zone of Inhibition of Formulations (F-1 & F-2)

Sr No.	Sample	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>
1	Standard Amoxicillin	26mm	24mm	20mm
2	Formulation -1 (F-1)	17mm	16mm	17mm
3	Formulation -2 (F-2)	16mm	14mm	18mm

The zone of inhibition for different organisms is shown in the below figure



Figure 1: Anti-bacterial activity of hand wash was tested using *Staphylococcus aureus*



Figure 2: Anti-bacterial activity of hand wash was tested using *Bacillus subtilis*



Figure 3: Anti-bacterial activity of hand wash was tested using *Escherichia coli*

CONCLUSION

According to the research, hydroalcoholic extracts of *Tridax procumbens*, *Azadirachta indica*, *Glycyrrhiza glabra*, and *Hibiscus rosa-sinensis*, when combined with lemon water, can provide a superior zone of inhibition to defend against skin infections when compared to regular Amoxicillin. Hence, polyherbal hand wash made from many plant extracts exhibits maximal action and has no negative side effects like skin irritation and dryness.

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Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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