Original Article

Alum-based Herbal Handwash: Formulation and Evaluation Study

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ABSTRACT

Hand washing is one of the most important strategies for preventing the spread of surface-borne diseases. A variety of hand washes are currently available on the market, the majority of which are alcohol-based and synthetic. Since synthetic substances can cause various allergic reactions among people, as well as roughness of the skin, society's interest has shifted towards the use of herbal hand washes. However, in addition to the herbal extracts, we investigated alum as one of the most ancient and effective antimicrobial ingredients in hand wash formulations. In this study, F-1 to F-5 formulation batches of hand washes were prepared using a trial and error method by varying the quantity of ingredients used. In these formulations, hydro-alcoholic extracts of lemon peels and reetha, as well as lemon grass oil as a volatile oil and alum as a mineral, were used as the main constituents, which were analysed by phytochemical screening. These formulations were then evaluated using a variety of methods, such as pH, foam height, foam retention, skin irritation, stability study, and an antimicrobial assay. According to our findings, the alum-based F-3 formulation performed significantly better than the other formulations. With the success of this study, we can conclude that, in the future, as herbal drug technology advances, various hygienic and life-saving products will firmly hold their place in human life.

Key words: herbal extracts, alum, soap base, physical evaluation, antimicrobial

Hand hygiene, or washing your hands, is a crucial, practical, easy, and reasonable way to stop the spread of disease [1]. In the past, the earliest and best sources of pharmacologically active compounds were plant species. For centuries, bioactive compounds and plant extracts have been employed in the preparation of traditional and Ayurvedic medicines, foods, natural dyes, and cosmetics for the treatment of various illnesses. It has been discovered that herbal drugs, which contain a diverse range of bioactive compounds like flavonoids, terpenoids, volatile oils, tannins, and alkaloids, have the potential to exhibit effective antimicrobial properties *in vitro* against a broad range of microorganisms with relative safety [2-5].

Nowadays, people are very much aware of the adverse effects of the synthetic materials used in various health products. As a result, the demand for herbal products worldwide is on the rise. Although on the market, various hand wash formulations are available, most of them contain synthetic antimicrobials [6-7].

So, in this study, the formulation and evaluation of herbal hand wash is proposed using various herbal drug extracts, volatile oils, and minerals such as lemon peel, reetha, lemon grass oil, and potassium alum, as they are easily available, less expensive, and more efficient with fewer side effects.

Access this article online

Quick Response Code

Received – 21st June 2024
Initial Review – 04th October 2024
Accepted – 08th October 2024

Plants and mineral profile

Lemon peel (*Limonis cortex*) is obtained from the fruit of *Citrus limon* (L.) Burm. belonging to the family Rutaceae. It's a small tree, 3–5 m high, cultivated in the countries bordering the Mediterranean countries. Dried lemon peel is official in the BP and EP. The major chemical constituents of dried lemon peels are 2.5% volatile oil, vitamin C, hesperidin, flavanone glycosides, and mucilage. In this work, the lemon peel extract was used as an antimicrobial agent and as a perfuming agent [8].

Reetha consists of the fruits of *Sapindus mukorossi* Gaertn., also known as soapnut or washnut, a member of the Sapindaceae family. The plant is a deciduous tree that grows in tropical and subtropical regions of Asia, including India, China, Japan, and Pakistan. Saponins, sugars, sesquiterpene oligoglycosides, and mucilage are among the most important chemical constituents of reetha. It has been used since antiquity as a foaming and cleansing agent. Several studies have demonstrated its potential for antibacterial, anticancer, and hepatoprotective activity. In this work, the reetha extract was used as an antimicrobial, foaming, and cleansing agent [9].

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Lemongrass oil is distilled from the *Cymbopogon flexuosus* plant, which belongs to the Gramineae family. Lemongrass oil is either reddish yellow or brown. It has an odor similar to lemon oil. Lemongrass oil primarily contains citral and citronellal (75–85%). Other terpenes found include geraniol, nerol, linalool, methyl heptenol, and limonene. It is primarily used as a flavoring and perfume agent in soaps and cosmetics. In this work, lemongrass oil was used as a perfuming and antimicrobial agent [10].

Potassium alum, also known as alum, was first mentioned in ancient Indian texts such as the Charaka Samhita and Sushruta Samhita. In Ayurveda, alum in the form of bhasma, known as 'Sphatika Bhasma', is used to treat whooping cough. It is widely used in the purification of water, leather tanning, aftershave lotion, and deodorant. Various studies have demonstrated that alum can be effectively used in various formulations to kill and control the growth of microorganisms. So, in this work, the alum was used as an antimicrobial agent [11–12].

MATERIALS AND METHODS

Plant materials, chemicals, and microbes

For this formulation, lemon and reetha fruits were purchased from the local market of Talegaon, District-Wardha (India). Lemon grass was obtained from the farm of Talegaon. All these plant materials were thoroughly cleaned with distilled water after collection and dried in the shed. In the case of lemons, after removing their juice, the peels were sundried and used. Further, these plant materials were subjected to pulverization and extraction to obtain extract and volatile oil. Potassium alum (aluminium potassium sulfate purified dodecahydrate), hydroxyl propyl methyl cellulose (HPMC), and sodium lauryl sulfate (SLS) were purchased from CDH Fine Chemical, India. Ethanol (purity ≥ 99%) was purchased from Alsucrose Corporation, India. A soap base was prepared in the laboratory. Throughout the experiment, the double-distilled water prepared in the laboratory was used. For the evaluation of antimicrobial activity, the soil sample was used as a source of microbes.

Preparation of extracts and isolation of volatile oils

The plant materials, namely lemon peels and reetha, were pulverized to a coarse powder after drying. It was then extracted with solvent (ethanol: water 50:50) using a Soxhlet apparatus. After the extraction, the solvent recovery was carried out using a distillation apparatus. Further, the extracts were concentrated and dried in a water bath, and the yield was noted down. To obtain the lemon grass oil, the Clevenger apparatus was used, and the yield was calculated.

Preparation of soap base

Take 6 g of cooking fat into an Erlenmeyer flask. Add the 12.5% NaOH solution prepared in 40 ml of distilled water and ethanol (1:1) to the oil and mix thoroughly. Heat the solution in a boiling water bath for 45 minutes. After 45 minutes, remove the mixture and place the flask in ice-cold water, followed by pouring into the 16.5% NaCl solution for several minutes. Filter the precipitated soap and wash twice with ice-cold water [13].

Phytochemical screening

The prepared extracts were further subjected to phytochemical screening for the presence of carbohydrates, proteins, alkaloids, saponin glycosides, steroids, flavonoids, terpenoids, tannins, and amino acids by various standard procedures [14-16].

Tests for carbohydrates

Molisch's test (general test) To 2-3 ml of aqueous extract, add a few drops of alpha-napthol solution in alcohol, shake, and add conc. H_2SO_4 from the sides of the test tube. A violet ring is formed at the junction of two liquids.

Fehling's test (for reducing sugars): Mix 1 ml of Fehling's A and 1 ml of Fehling's B solution; boil for one minute. Add an equal volume of test solution. Heat in a boiling water bath for 5-10 minutes. First yellow, then brick red precipitate is observed.

Barfoed's test (for monosaccharides): Mix equal volumes of Barfoed's reagent and test solution in the test tube. Heat in boiling water bath for 5 minutes. The solution appears green, yellow, or red depending on the amount of reducing sugar present in the test solution.

Bial's orcinol test (for pentose sugars): To boil Bial's reagent, add a few drops of test solution. Green or purple coloration appears.

Selwinoff's test (for hexose sugars): Heat 3 ml of Selwinoff's reagent and 1 ml of test solution in a water bath for 1-2 minutes. A red color is formed.

Tollen's phloroglucinol test (for hexose sugars): Mix 2.5 ml conc. HCl and 4 ml 0.5% phloroglucinol. Add 1-2 ml of test solution. Heat the mixture. Yellow to red color appears.

Tests for proteins

Biuret test (general test): To the 3 ml test solution, add 4% NaOH and a few drops of 1% $CuSO_4$ solution. Violet or pink color appears.

Million's test: Mix 3 ml of test solution with 5 ml of Million's reagent. White precipitate obtained. Warm precipitate, turn brick red, or the precipitate dissolves, giving a red-colored solution.

Test for sulfur-containing proteins: Mix 5 ml test solution with 2 ml of 40% NaOH solution and 2-3 drops of 10% lead acetate solution. Boil. Solution turns black or brownish due to lead sulfide formation.

Tests for alkaloids

For the alkaloidal test, 2 ml of dilute HCl was added to 1 g of dry extracts, shaken well, filtered, and used for the following tests.

Mayer's Test: To 3 ml of the filtrates, add 1 ml of Mayer's reagent (potassium mercuric iodide). The creamy precipitate indicates the presence of alkaloids.

Wagner's Test: To 3 ml of the filtrates, add 1 ml of Wagner's reagent (iodine in potassium iodide). The reddish brown precipitate indicates the presence of alkaloids.

Hager's Test: To 3 ml of the filtrates, add 1 ml of Hager's reagent (saturated picric acid solution). The yellow precipitate indicates the presence of alkaloids.

Dragendroff's Test: To 3 ml of the filtrates, add 1 ml of Dragendroff's reagent (potassium bismuth iodide). The appearance of orange-brown precipitate indicates the presence of alkaloids.

Tests for saponin glycosides

Foam test Shake a little quantity of extract with water. The persistent foam for 10 minutes confirms the presence of saponins.

Haemolysis test: Mix a small amount of extract with blood on the slide. The hemolytic zone represents the saponin glycosides.

Tests for steroids

Salkowski's test: To the 2 ml test solution, add 2 ml chloroform and 2 ml conc. H_2SO_4. Shake well. The chloroform layer appears red, and the acid layer shows greenish-yellow fluorescence.

Legal's test (for cardenolides): To the 1 ml of test solution, add 1 ml of pyridine and 1 ml of sodium nitroprusside solution. Pink to red color appears.

Tests for flavonoids

Shinoda tests Dissolve the extract in 5 ml of 95% v/v ethanol and add a few drops of conc. HCl and 0.5 g of magnesium turnings. The pink, crimson, or magenta color represents flavonoids.

Tests for terpenoids

Salkowaski's test: To the extract, add 2 ml of chloroform and 2 ml of conc. sulfuric acid from the side of the test tube. Shake it for few minutes. Red color forms.

Liebermann-Burchard's test: Dissolve the extract in chloroform, add a few ml of acetic anhydride, and heat it. Cool it and add a few drops of conc. sulphuric acid from the side of the test tube. The blue colour forms.

Tests for tannins

Ferric chloride test: With the 5% ferric chloride solution, the extract gives a dark green or deep blue color.

Lead acetate test: Add a 10% w/v solution of basic lead acetate in distilled water to extract. Precipitate is obtained.

Potassium dichromate test: With the extract, potassium dichromate solution produces a dark precipitate.

Gelatin test: Add a 1% w/v solution of gelatin in water containing 10% sodium chloride. White precipitate indicates presence of tannins.

Tests for amino acids

Ninhydrin test: Heat 3 ml of test solution and 3 drops of 5% ninhydrin solution in a boiling water bath for 10 minutes. Purple or bluish color appears.

Handwash formulation procedure

The formulation of alum-based herbal handwash was carried out by trial and error method. In brief, a total five formulation batches with varying amounts of ingredients were used (Table 1.1), and the best formulation was finally selected based on the various evaluation parameters to produce the bulk quantity.

Table1.1: Formulation of handwash batches by trial and error method

S. No.	Ingredients used	Formulation batches /quantity used (mg)				
		F-1	F-2	F-3	F-4	F-5
1.	Potassium alum	400	450	500	550	600
2.	Lemon peel extract	500	650	750	800	900
3.	Reetha extract	500	650	750	800	900
4.	Lemon grass oil	500	600	400	300	250
5.	Soap based	1500	2000	2700	3000	3500
6.	HPMC	200	400	700	800	900
7.	SLS	50	200	350	500	600
8.	Distilled water	q.s. to 100 ml				

Figure 1.1: alum-based handwash formulation (Batch F-3)

In this process, the desired quantity of the soap base in the form of powder was allowed to dissolve in the warm water, followed by HPMC. The prepared extracts were further dissolved in the distilled water and added to the gel prepared in the previous step, followed by SLS, potassium alum, and lemon grass oil, and the quantity was adjusted with distilled water. The formulations were made homogenous at room temperature and stored for further studies (Figure 1.1).

Evaluation of prepared handwash

Physical evaluation

The prepared handwash formulations were first evaluated for various physical parameters like colour, odour, appearance, texture, homogeneity, and grittiness by visual inspection.

pH

The digital pH meter was used for the determination of the pH of the various prepared formulations. For this process, a 1% handwash solution was used, and readings were recorded at room temperature.

Foam height

For the determination of foam height, 1 g of handwash formulation was diluted with 50 ml of distilled water in a 500 ml stoppered measuring cylinder, followed by water up to 100 ml. Further, the stopper measuring cylinder was shaken vigorously for 25 strokes and kept aside for some time, and the height of the foam was measured.

Foam retention

To determine foam retention, 1 g of handwash formulation was diluted with distilled water in a 500-ml stoppered measuring cylinder up to 100 ml. The preparation was then shaken vigorously ten times, and the quantity of foam produced was measured for four minutes at one-minute intervals.

Skin irritation test

To determine whether any skin irritation or redness was caused by the prepared handwash formulation, 15 students were voluntarily selected. The formulations were applied to their skin for 30 minutes, then washed off. During this time, any skin irritation or redness that occurred was noted down.

Stability study

The stability of handwash was tested for one month at 5 ± 3°C, 30 ± 2°C, and 40 ± 2°C, following ICH guidelines. After one month, the tested formulation was evaluated for physical changes as well as changes in other parameters [17].

Selection of microbes and antimicrobial assay

For the evaluation of the antimicrobial potential of the prepared handwash, a soil sample was used as a source of microbes. In this process, a soil sample was prepared by the serial dilution method and used. For the antimicrobial assay, the agar well diffusion method was used. In brief, the sterilized nutrient agar medium plates were spread with the diluted soil sample prepared in sterile water using a sterile glass spreader. Further, the holes were punched using a sterile cork borer, and a volume of 50 µl (0.1%) of prepared handwash formulation, marketed handwash formulation, and sterile water was filled in each bore. Here, sterile water was used as a control and marketed handwash as a standard. The plates were incubated at 35-37 °C for 24 hours. After 24 hours, the diameter of the inhibition zone in mm was measured [18].

RESULT

After being extracted using ethanol and water, the extractive values of the crude drugs, lemon peel, and reetha were found to be 3.7% and 4.3%, respectively, upon complete drying on the water bath. While the yield of volatile oil from the lemon grass using the Clevenger apparatus was found to be 3.4%. In order to analyse the classes of chemical constituents present in these extracts, various phytochemical tests were carried out. The details of the phytochemical screening are tabulated below (Table 1.2).

The phytochemical screening indicated the presence of all the phytoconstituents for which the phytochemical screening has been conducted except amino acids.

Table 1.2: Phytochemical screening of extracts

Phytoconstituents	Tests	Lemon peel extract	Reetha extract
Carbohydrates	Molisch's	+	+
	Fehling's	+	+
	Barfoed's	+	+
	Bial's Orcinol	+	+
	Selwinoff's	+	+
	Tollen's phloroglucinol	+	+
Proteins	Biuret	+	+
	Million's	+	+
	Sulphur containing	+	+
Alkaloids	Mayer's	+	+
	Wagner's	+	+
	Hager's	+	+
	Dragendroff's	+	+
Saponin glycosides	Foam	+	+
	Haemolysis	+	+
Steroids	Salkowski's	+	+
	Legal's	+	+
Flavonoids	Shinoda	+	+
Terpenoids	Salkowski's	+	+
	Liebermann-Burchard's	+	+
Tannins	Ferric chloride	+	+
	Lead acetate	+	+
	Potassium dichromate	+	+
	Gelatin	+	+
Amino acids	Ninhydrin	-	-

+ Present ; - Absent

The evaluation results of the different parameters for the prepared formulation batches are as follows:

Physical evaluation

Each of the five formulations had a light buff colour and a pleasing scent of lemon peel extract and lemon grass. The texture was glossy, non-gritty, and had an opaque appearance. The preparations were uniform and simple to clean.

pH

The pH of all the tested formulations was found to be in the range of 6.7–7.4, which is within the permissible limit (Table 1.3).

Table 1.3: Evaluation of prepared formulation batches

Formulation batches	pH	Foam height (cm)	Foam retention (Min.)
F-1	6.9	24	8
F-2	6.9	19	3
F-3	7.1	32	12
F-4	6.8	19	5
F-5	7.4	22	5

Foam height

The foam height of all the tested formulations of herbal handwash was given in the table (Table 1.3). From the observation table, it was found that the F-3 formulation has good foam height as compared to other formulations.

Foam retention

According to the observation table (Table 1.3), the F-3 formulation has a better retention time than the others, which was almost stable for 12 minutes. So, the F-3 formulation can be considered good on this parameter as well.

Skin irritation test

No irritation or redness to the skin was reported on the evaluation of all formulations of handwash.

Stability

On testing prepared handwash formulations for one month at various conditions, the F-3 formulation was found to be more stable as compared to others. Other than the F-3 formulation, slight changes in the colour as well as the odour have been observed.

Table 1.4: Antimicrobial assay

S. No.	Diameter of inhibition zone in (mm)		
	Formulation batches 50µl (0.1%)		Standard formulation 50µl (0.1%)
1.	F-1	11	42
2.	F-2	06	
3.	F-3	24	
4.	F-4	15	
5.	F-5	16	

Antimicrobial assay

The antimicrobial assay revealed that the F-3 handwash formulation is effective at inhibiting the growth of microorganisms, similar to the marketed product (Table 1.4). As

a result, we can conclude that the F-3 formulation is suitable for bulk preparation and subsequent application.

DISCUSSION

The herbal handwash formulations in this study were made with reetha and lemon peel hydro-alcoholic extracts. Lemon grass and alum were also utilized as primary ingredients. All of the extracts had a similar chemical composition, with the exception of the presence of amino acids, as discovered during the phytochemical screening process. These phytochemical data suggested that the strong antimicrobial activity observed in the antimicrobial assay may be attributed to the presence of a few key constituents, primarily tannins, saponin glycosides, and alkaloids. Additionally, the quality parameters for the aforementioned herbal handwash were found to be satisfactory.

CONCLUSION

With COVID-19, people are becoming more aware of the various diseases that spread through skin surfaces, such as hands. Hand washing is one of the simplest ways to protect ourselves from infectious diseases. Hand wash liquids are commonly used in society to provide protection. Various handwash formulations with a variety of ingredients are available on the market, the majority of which are synthetic or alcohol-based. Despite the fact that these formulations have passed various quality tests, people prefer formulations containing natural ingredients such as herbs, minerals, and volatile oils due to their relative non-toxic effects and to control the continuous buildup of microbe resistance. So, in this study, while keeping this fact in mind, alum-based herbal extracts and a volatile oil-containing herbal handwash were prepared. The prepared handwash formulation batches were tested for a variety of parameters, including antimicrobial activity. Among them, we discovered that formulation batch F-3 performed exceptionally well in all aspects of the hand washing requirement. The antimicrobial assay revealed that F-3 at 0.1% concentration effectively inhibited the growth of soil bacteria. So, to summarize the study, the alum-based herbal handwash was successfully developed and tested.

ACKNOWLEDGEMENT

We are grateful to the college management for providing laboratory and library facilities during our course of work.

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How to cite this article: Mohit J. Umarkar, Pawan V. Jibhkate, Payal G. Thakare, Nilesh B. Banarase, Koshish B. Gabhane, Vikrant L. Salode. Alum-based Herbal Handwash-Formulation and Evaluation Study. Indian J Pharm Drug Studies. 2024; Online First.

Funding: None; Conflicts of Interest: None Stated