IJPSR (2022), Volume 13, Issue 10 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 13 February 2022; received in revised form, 03 April 2022; accepted, 24 April 2022; published 01 October 2022

IN-VITRO EVALUATION OF THE ANTIBACTERIAL POTENTIAL OF POLYHERBAL HAND WASH FORMULATION AGAINST SOME SKIN PATHOGENS

Preeti Chaudhary *, Rupesh Pingale, Purva Batwal, Yogita Ghode, Jayesh Lodha, Vrushabh Ajmera and Monali Phadtare

Department of Pharmacy, NCRD's Sterling Institute of Pharmacy, Nerul, Navi Mumbai - 400706, Maharashtra, India.

Keywords:

Polyherbal hand wash, *Moringa oleifera*, *Calotropis gigantea*, *Azadirachta indica*, Lemon juice, antibacterial activity

Correspondence to Author:
Preeti Chaudhary

Assistant Professor,
Department of Pharmacy, NCRD's Sterling Institute of Pharmacy, Nerul, Navi Mumbai - 400706, Maharashtra, India.

E-mail: chpreeti03@gmail.com

ABSTRACT: The aim of the present research work was the *in-vitro* evaluation of the antibacterial potential of polyherbal hand wash formulation against some skin pathogens. Herbal remedies are widely used in healthcare around the World. Herbal medicines have been widely used as effective preventative and treatment options for various ailments. There are a variety of hand washes available in the market many of them have some adverse effects such as itching, dermatitis, irritation *etc*. An attempt has been made to produce a polyherbal hand wash to prevent these adverse effects of synthetic handwash formulations. For the preparation of polyherbal hand wash the hydroalcoholic leaves extracts of *Moringa oleifera* (Drumstick tree), *Calotropis gigantea* (Giant milkweed), *Azadirachta indica* (Neem) and lemon juice were used. Two hand wash formulations were prepared, and the formulations were also tested for physical attributes such as pH, viscosity, and appearance. The antibacterial activity of formulated herbal hand wash was tested against skin pathogens such as *Escherichia coli*, *Staphylococcus aureus* by the streak-plate method. The results obtained were compared with a standard antibiotic drug (Amoxicillin). The efficiency of hand wash was also checked by using the hand wash on volunteers. The findings revealed that prepared herbal hand wash formulations showed a significant zone of inhibition compared with a standard antibiotic.

INTRODUCTION: Skin is the most exposed part of our body, and it requires protection from different skin pathogens [1]. The principal routes of transmission of these skin pathogens are through the hands of health care workers, from whom patients get infection [2]. As a result, it raises the issue of using antiseptic for hand washing purposes. The most prevalent skin pathogens are *S. aureus, E. coli*, coryne form bacteria *etc*.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(10).4187-98

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(10).4187-98

These organisms normally enter the body through a break in the skin, such as a bite from an insect, and may cause skin infections such as cellulitis, impetigo, and staphylococcal (staph) infections. Hand washing is a vital step in preventing the spread of disease. Hand washing removes visible filth and reduces the quantity of hazardous bacteria on the hands [3, 4].

A number of chemical antiseptics such as alcohol-based sanitizers, hand wash and chlorhexidine products are now available on the market. These synthetic sanitizers, hand wash, chemicals, or solutions enable to reduce of health-care-associated spread of contagious diseases and microorganisms more efficiently. Still, these synthetic sanitizers and

hand wash preparations have adverse effects and are risk factors for the skin. Their frequent usage might cause skin irritation as well as create resistance among pathogens.

Also, the companies that manufacture synthetic formulations release these harmful chemicals into the environment, potentially disrupting numerous ecosystems. So, our research provides a novel strategy to reverse antibiotic resistance in pathogenic organisms while also providing a safe and healthy living environment through germ-free hands.

As Herbal Hand wash is made up of natural substances. So, it is able to overcome pathogenic resistance and also cause no harm to the environment if left in the environment. Plants are the earliest source of pharmacologically active molecules and they have offered many medicinally beneficial compounds to humans for centuries. Herbal medicines have long been used as effective treatments for the prevention and treatment of various illnesses [5].

Only a few reports on inhibitory action against specific pathogenic bacteria and fungi are available, and the antibacterial properties of several Indian medicinal herbs have been described based on folklore information. We used three medicinal plants in our research work to check the antibacterial potential.

1. *Moringa oleifera:* *Moringa oleifera* (MO) is a plant that belongs to the Moringacea family **Fig. 1**. Moringa is a plant native to Africa and Asia that is widely cultivated in Northwestern India. It's also called a 'drumstick tree' or a 'horseradish tree'. Moringa is commonly farmed around the world because it can resist both severe drought and moderate winter [6].

Plant Profile:

Kingdom: Plantae

Class: Tracheophytes

Order: Brassicales

Family: Moringaceae

Genus: *Moringa*

Species: *M. oleifera* [7]

FIG. 1: *MORINGA OLEIFERA* LEAVES

Chemical Constituents: Extracts from leaves contain various alkaloids, glycosides, flavonoids, saponins, hydrocarbons, phytosterols, fatty acids, alcohols, esters, and phenols.9-Octadecenoic acid, L-(+) - Ascorbic acid- 2, 6- dihexadecanoate, 14 – methyl -8- Hexadecenal, 4- hydroxyl-4-methyl2-pentanone, 3-ethyl-2, 4-dimethylpentaneand phytol are the major chemical constituents [8].

Uses: The plant has traditionally been used as an antispasmodic, stimulant, expectorant, and diuretic. The root is bitter and vesicant when fresh (has the taste of horse-radish). It's utilized as a stimulant, diuretic, and antilithic on the inside. Gum is a mucilaginous, bland substance. The seeds are astringent and stimulating. Bark has emmenagogue and even abortifacient properties, as well as antifungal and antibacterial properties. Flowers are cholagogues, stimulants, tonics, and diuretics that can help enhance bile flow. The plant is also an antibacterial and a heart circulatory tonic [9].

2. *Calotropis gigantea*: *Calotropis gigantea* is a weed that thrives in Africa and Asia's wastelands. "Crown flower," "giant milkweed, and "shallow wort" are some of the common names for it [10]. It is called “aak,” “akauwa,” or “arka” in India. This plant may be characterized by its thick oblong leaves and purplish blossoms, which are odourless **Fig. 2.**

Plant Profile:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Gentianales

Family: Apocynaceae

Genus: *Calotropis*

Species: *Calotropis gigantea* [15]

FIG. 2: *CALOTROPIS GIGANTEA* LEAVES

Chemical Constituents: Several researchers have reported the presence of metabolites such as flavonoids, tannins, terpenoids, saponins, alkaloids, steroids, and cardiac glycosides in various parts of the plant [11, 12, 13]. The major phytochemical groups reported in the leaf extracts of *C. procera* are fatty acid ethyl esters, palmitic acid esters, linoleic acids, and amino acids [14].

Uses: *Calotropis gigantea* is used to treat asthma, colds, coughs, diarrhea, fever, indigestion, leprosy, leukoderma, and rheumatism in Ayurveda Chinese and homeopathic medicines [15].

3. *Azadirachta indica*: *Azadirachta indica* A. Juss, (Neem tree), from the Meliaceae family (Figure 3), also known as Margosa or Indian lilac. Various parts of the Neem tree have been used in traditional Ayurvedic medicine in India. Neem oil, the bark, and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders, and constipation and also as a general health promoter. Neem leaves possess a wide spectrum of antibacterial action against gram-negative and gram-positive microorganisms[5].

Plant Profile:

Kingdom: Plantae

Division: Tracheophyta

Class: Magnoliopsida

Order: Rutales

Family: Meliaceae

Genus: *Azadirachta*

Species: *Azadirachta indica* [16]

FIG. 3: *AZADIRACHTA INDICA* LEAVES

Chemical Constituents: The most important active constituent is azadirachtin, and the others are nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin, and quercetin. Leaves contain ingredients such as nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol and amino acid, 7-desacetyl-7- benzoylazadiradione, 7-desacetyl - 7 - benzoylgedunin, 17-hydroxyazadiradione and nimbiol. Quercetin and ß-sitosterol, polyphenolic flavonoids, were also isolated from neem fresh leaves [16].

Uses: Various parts of the Neem tree have been used as traditional Ayurvedic medicine in India. Neem oil, the bark, and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders, and constipation and also as a general health promoter. Neem leaves possess a wide spectrum of antibacterial action against gram-negative and gram-positive microorganisms [5].

Lemon juice which is obtained from fruits of *Citrus limon* L. belonging to the family *Rutaceae* is traditionally used for the purpose of cleaning due to its disinfectant properties. Lemon juice is also used as a short-term preservative in some food preparations.

Lemon juice is used in Indian medicinal systems because of its anti-microbial properties of lemon. It is also used to add taste to many food preparations [17].

MATERIALS AND METHODS:

Chemicals: Standard antibiotic amoxicillin was obtained from the local medical store (Almox-250, Alkem Laboratories Ltd, Solan, H.P, India). Sodium lauryl sulphate (SLS), Methylparaben (methyl – p - hydroxybenzoate), Glycerine, Peppermint oil, Calcium carbonate, Sodium chloride were purchased from Research-Lab Fine Chem Industries, Mumbai. Agar powder, peptone and beef extract were purchased from HiMedia Laboratories Pvt. Ltd. 23, Vadhani Ind. Est., LBS Marg, Mumbai-400086, India. The solvents and other chemicals used were analytical grade and were obtained from local dealers.

Plants Collection and Authentication: The leaves of *Calotropis gigantea* and *Azadirachta indica* were collected from the garden of Ekta Vihar, Belapur, Navi Mumbai, and leaves of *Moringa oleifera* were collected from the roadside of Patvin Engineering Pvt. Ltd. W-193, TTC Industrial Area, Khairane MIDC, Behind Reliance Silicon, Thane, Belapur Road, Navi Mumbai in the month of December and then authenticated by Dr. Rajendra D. Shinde, Principal & Head, Department of Botany & Director, Blatter Herbarium, St. Xavier's College, Mumbai: 400001vide specimen no. (NI 1718 of N.A. Irani, NI 4891 of N.A. Irani).

Preparation of Leaf Extracts: The leaves of collected plants *Moringa oleifera*, *Calotropis gigantea* and *Azadirachta indica* are taken and coarsely powdered. 50 grams of coarsely powdered leaves of all three plants were soaked in 400 ml of 70% (V/V) ethanol and water mixture and kept for maceration for about 1 week. After maceration, the extract is filtered, and the filtrate was collected and used for making hand wash [18].

Phytochemical Screening: The hydroalcoholic extract of plants was screened for the presence of various phytoconstituents by adopting standard procedures **Fig. 5** [19].

Preparation of Herbal Hand Wash Formulations: Two formulations of herbal hand wash: Formulation 1 (F-1) & Formulation 2 (F-2) were prepared.

Formulation 1 (F-1): In this formulation, the hand wash was prepared using 21 ml of hydroalcoholic extract filtrate. To this filtrate 6 gms of SLS, glycerin 50 ml, 0.3 gms of methylparaben, 1 ml of peppermint oil is added and the volume is made up to 100 ml with purified water **Table 1**.[17]

TABLE 1: FORMULATION 1 (F-1)

S. no.	Ingredients	Quantity
1	Hydroalcoholic extract of *Moringa oleifera*, *Calotropis gigantea* and *Azadirachta indica*.	21 ml
2	Sodium Lauryl Sulphate (SLS)	6 gms
3	Glycerin	50 ml
4	Methyl Paraben	0.3 gms
5	Peppermint Oil	1 ml
6	Purified Water q. s	100 ml

Formulation 2 (F-2): This formulation was prepared by adding 20 ml of lemon juice to 21 ml of hydroalcoholic extract filtrate of *Moringa oleifera*, *Calotropis gigantea*, and *Azadirachta indica* leaves. The remaining ingredients include all the same as mentioned above in formulation-1 **Table 2** [17].

TABLE 2: FORMULATION 2 (F-2)

S. no.	Ingredients	Quantity
1	Hydroalcoholic extract of *Moringa oleifera*, *Calotropis gigantea*, and *Azadirachta indica*.	21 ml
2	Lemon Water	20 ml
3	Sodium Lauryl Sulphate (SLS)	6 gms
4	Glycerin	50 ml
5	Methyl Paraben	0.3 gms
6	Peppermint Oil	1 ml
7	Purified Water q. s	100 ml

Evaluation Parameters:

1. **Physical Evaluation:** Physical evaluation (color, odour) was done by sensory and visual inspection and compared with the marketed hand wash (Dettol hand wash).
2. **Grittiness:** 1ml of hand wash was taken on fingertips and rubbed between two fingertips, then the formulation was evaluated.
3. **pH:** For the pH evaluation, the end of the pH strip was dipped into the hand wash formulations. After a couple of seconds, remove the paper and compare the color of the pH strip to the color chart provided with the pH paper kit **Fig. 7.**
4. **Viscosity:** The viscosity of hand wash gel was determined by using a digital Brook filed viscometer DV-II. Measured quantity of hand

wash gel was taken into a beaker, and the tip of the viscometer was immersed into the hand wash gel, and viscosity was measured in triplicate.

5. **Spreadability:** A sample of 0.5 g of both formulations was pressed between two slides and left for about 5 minutes where no more spreading was expected. Diameters of spreaded circles were measured in cm and were taken as comparative values for spreadability. The results obtained were an average of three determinations.
6. **Foam Height:** One gram of sample of hand wash was taken and dispersed in 50 ml distilled water. The dispersion was transferred to 500 ml measuring cylinder. Volume was made up to 100 ml with water. 25 strokes were given and kept aside. The foam height above the aqueous volume was noted.
7. **Foam Retention:** 25 ml of the 1% hand wash gel was taken into a 100 ml graduated cylinder. The cylinder was covered with a hand and shaken 10 times. The volume of foam at 1 min interval was recorded for 4 min.
8. **Skin Irritation:** This evaluation was performed on healthy human volunteers, including both male and female candidates. About 0.5 g of polyherbal hand wash was applied on area of 6cm^2 of skin. At the end of the exposure period of 1 hour, the skin was checked for any irritation or redness [20].

Antibacterial Activity:

Selection of Bacteria: Different strains of bacteria were used for antimicrobial screening, namely *Staphylococcus aureus* (gram-positive) & *Escherichia coli* (gram-negative).

Bacterial Sample: Gram-positive and Gram-negative bacteria, *i.e., Staphylococcus aureus*, and Escherichia coli, were collected from a soil sample.

Volunteers Sample: Swabs from hand palm skin of volunteers were also included in this study.

Preparation of Soil Extract: Take 5 gm of garden soil and add 50 ml of tap water, mix it properly and leave for 20-30 min. Supernatant separated and autoclave at 21 lb for 30 min.

Identification of Bacteria: Gram staining consists of four steps **Fig. 4**:

1. Fix the slide by heat.
2. **Primary Stain:** Stain the slide smear with gentian violet or crystal violet. It will penetrate the cell membrane.
3. **Mordant:** wash the violet stain and flood the smear with the Iodine solution. This acts as mordant and forms complex with Crystal violet.
4. **Decolorizer:** Wash off the smear and flood it with alcohol (95 %) or Acetone-alcohol mixture.

a) This will remove the outer cell membrane in gram-negative bacteria, where the complex will also be washed off.

b) While in Gram-positive bacteria cell membrane remain intact, and the stain will not be washed off after alcohol treatment.

5. **Secondary Stain:** Counterstain the smear with safranin O, which is a red dye.

a) In gram, positive bacteria counterstain cannot enter, so the bacteria are purple.

b) While in gram-negative bacteria, safranin can enter and give pink color [21].

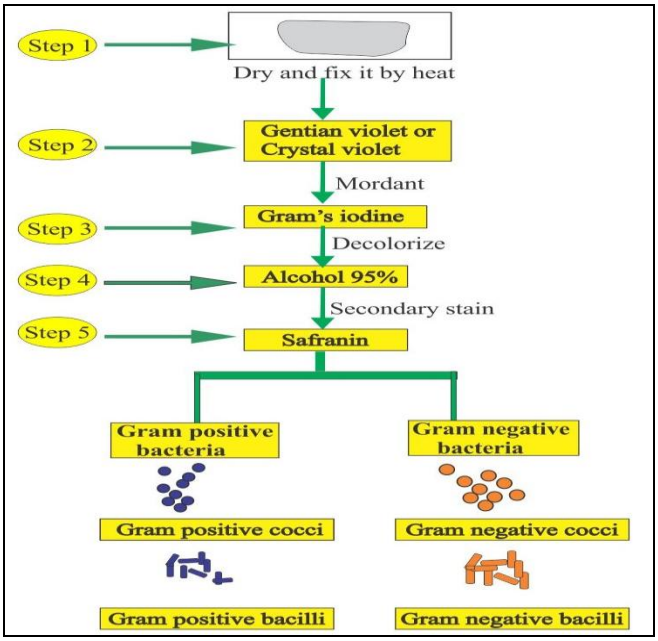

FIG. 4: GRAM STAINING

Media: Nutrient agar.

Preparation of Nutrient Agar: Nutrient agar or culture medium allows a liquid or gel to provide all the nutrients needed for bacteria to grow successfully **Table 3.**

TABLE 3: COMPOSITION OF NUTRIENT AGAR

S. no.	Ingredients	Quantity
1	Beef Extract	1 gm
2	Peptone	1 gm
3	Sodium Chloride	0.5 gm
4	Agar	2 gm
5	Distilled Water	q.s. 100 ml

Procedure: Each ingredient except agar is dissolved in the appropriate volume of distilled water. The pH of the fluid medium is determined with a pH meter and adjusted by using 1N HCI or 1N NaOH. Add agar powder and the medium is heated to dissolve the agar to form a clear liquid. The medium is dispensed into the flask. Plug the flasks containing medium by using nonabsorbent cotton. Sterilize the media at 121°C 15 lbs pressure for 15 min in an autoclave. Allow the flasks to cool up to 50 °C [22].

Preparation of Agar Plates: In each sterile Petri plate 25 ml of agar medium was poured in an aseptic condition and kept at room temperature for solidification at least for 20 min.

Streak Plate Method: The screening of antibacterial activity of the formulated poly herbal hand wash formulations was performed on various skin pathogens by using the streak-plate method as per the standard procedure. Two sterile petri plates were taken to test the antibacterial activity against *Escherichia coli* & *Staphylococcus aureus*. Take a small quantity of inoculum on the wire loop and streak the agar plates using the streak plate method under aseptic conditions. Streaked plates are incubated at 37°C for 24 h. After that, three cavities were made in it. The first cavity is filled with standard antibiotic amoxicillin second one with herbal hand wash without lime water (F-1), and the third cavity is filled with herbal hand wash with lime water (F-2). It was taken care that the sample should be placed at the cavity level. The plates are placed in an incubator at 37°C to test the activity. After 24 h the plates were observed to form a zone of inhibition.

From the zone of inhibition, the antibacterial activity of both the formulations was estimated [23].

RESULTS & DISCUSSION: The preliminary phytochemical screening confirmed the presence of alkaloids, glycosides, saponins, flavonoids, phenols, and proteins in the hydroalcoholic leaves extract of *Moringa oleifera*. After the phytochemical screening of the hydroalcoholic extract of *Calotropis gigantea*, it was found that the extract contains alkaloids, glycosides, saponins, phytosterols, flavonoids, and the hydroalcoholic extract of *Azadirachta indica* showed the presence of alkaloids, carbohydrates, glycosides, saponins, flavonoids, phenols and proteins **Table 4.**

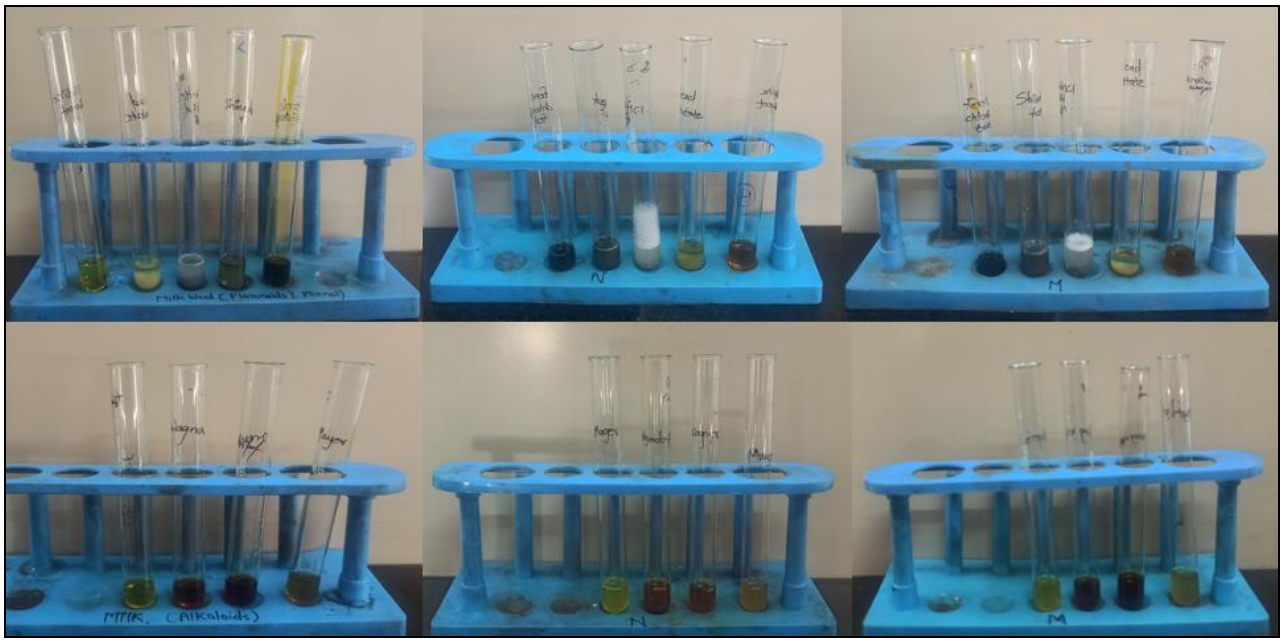

FIG. 5: PHYTOCHEMICAL SCREENING OF HYDROALCOHOLIC EXTRACTS

TABLE 4: PHYTOCONSTITUENTS PRESENT IN HYDROALCOHOLIC EXTRACTS OF *MORINGA OLEIFERA*, *CALOTROPIS GIGANTEA* AND *AZADIRACHTA INDICA*

S. no.	Chemical Constituents	Tests	*Moringa oleifera*	*Calotropis gigantea*	*Azadirachta indica*
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	+	+	+
3.	Glycosides	Modified Borntrager's	+	+	+
		Legal test	+	+	-
4.	Saponins	Foam test	+	+	+
		Froth test	+	+	+
5.	Phytosterols	Salkowski's test	+	+	+
		Libermann Burchard test	+	+	+
6.	Resins	Acetone-Water test	-	+	-
7.	Fixed oils	Filter Paper	+	+	+
8.	Phenols	Ferric Chloride test	+	+	+
9.	Tannins	Gelatin test	+	-	+
10.	Flavonoids	Alkaline Reagent test	+	+	+
		Lead acetate	+	+	+
		Zn-HCl acid reduction	+	+	+
		Shinoda test	+	+	+
11.	Proteins	Xanthoproteic test	-	-	-
		Ninhydrin test	+	+	+
		Biuret test	+	+	+

Note: '+' sign indicates presence and '—' sign indicates absence

Preparation of Herbal Hand Wash Formulations: Two formulations of herbal hand wash: Formulation 1 (F-1) & Formulation 2 (F-2) were prepared **Fig. 6.**

FIG. 6: POLYHERBAL HANDWASH FORMULATIONS

Evaluation Parameters: The prepared formulations (F-1 & F-2) of polyherbal hand wash were subjected to physical evaluation and other evaluation parameters.

1. Physical Evaluation: The prepared two formulations of hand wash appear fluorescent green and greenish-yellow colour.

Both the formulations were found to be translucent in nature with a pleasant odour, and smooth and cooling feel on topical application. The results are shown in **Table 5.**

TABLE 5: PHYSICAL EVALUATION

S. no.	Formulation	Color	Odour	Feel on Application
1	F-1	Fluorescent green	Pleasant	Smooth and cooling feel
2	F-2	Greenish-yellow	Pleasant	Smooth and cooling feel

2. Grittiness: Both formulations were evaluated for the presence of any appreciable particulate matter. Both hand wash formulations were taken on fingertips and rubbed between two fingertips, no grittiness was found.

3. pH: The pH of formulations was measured by pH paper. The pH of both formulations was in the range of the pH of the skin *i.e.,* 4 to 5.9 which are in range and it is shown in **Table 6.**

TABLE 6: PH OF POLYHERBAL HANDWASH FORMULATIONS

S. no.	Formulation	pH
1	F-1	5.8
2	F-2	4.0

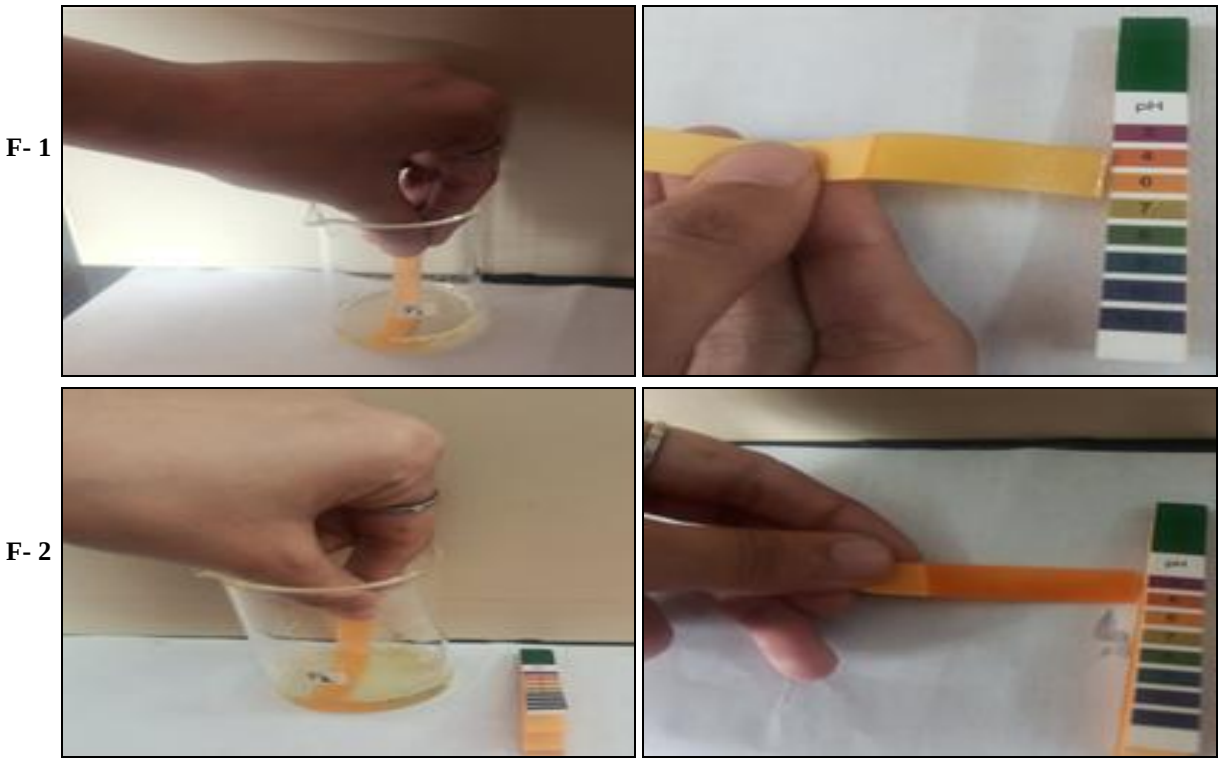

FIG. 7: PH OF HANDWASH FORMULATIONS. The pH of F-1 & F-2 formulations was found to be 5.8 and 4.0 respectively.

4. Viscosity: The viscosity of both hand wash formulations was determined by using a Brookfield viscometer.

50 ml of polyherbal hand wash is taken into 100 ml of beaker and the tip of the viscometer was dipped into the beaker containing hand wash formulation and its viscosity was measured. The viscosity of F-1 and F-2 was found to be 61 and 58 CPS.

5. Spreadability: Diameters of spreaded circles of F-1 & F-2 formulations was found to be 7.3 cm and 7.7 cm, respectively **Fig. 8.**

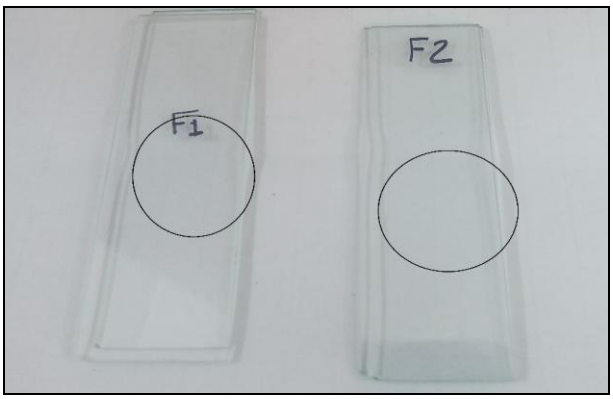

FIG. 8: SPREADABILITY OF HANDWASH FORMULATIONS

6. Foam Height: The foam height of formulation 1 (F-1) and formulation 2 (F-2) was found to be 120 ml and 110 ml respectively **Fig. 9.**

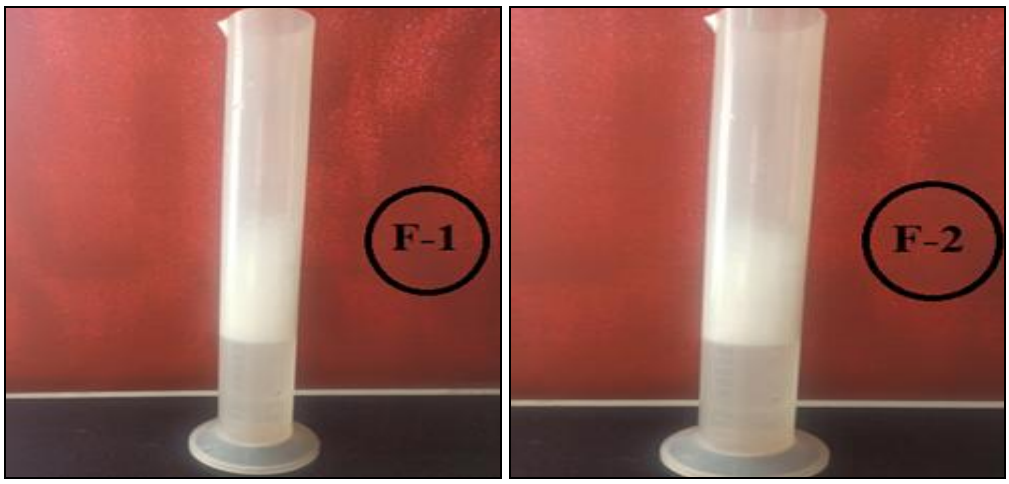

FIG. 9: FOAM HEIGHT OF HANDWASH FORMULATIONS

7. Foam Retention: The volume of foam of formulation 1 (F-1) and formulation 2 (F-2) at 1 min interval was found to be 25 ml & 21 ml, respectively **Fig. 10.**

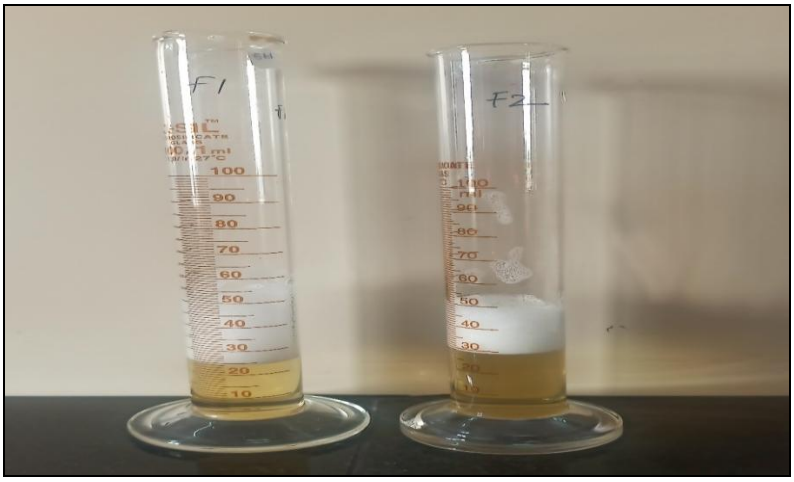

FIG. 10: FOAM RETENTION OF HANDWASH FORMULATIONS

8. Skin Irritation: Both formulations (F-1 & F-2) were subjected to a skin irritation study on voluntary individuals. No formulation showed any sign of skin irritation or redness. This implies that the formulations are not allergic to the skin **Fig. 11.**

FIG. 11: SKIN IRRITATION TEST

Evaluation of Antimicrobial Activity: The antimicrobial efficacy of the formulations of polyherbal hand wash was tested on *Staphylococcus aureus* and *Escherichia coli* by streak-plate technique. Two sterile petri plates were taken to test the antibacterial activity. The plates were filled with nutrient agar solution and allowed for solidification **Fig. 12** & **13.**

FIG. 12: PREPARATION OF AGAR MEDIA AND PETRI PLATES

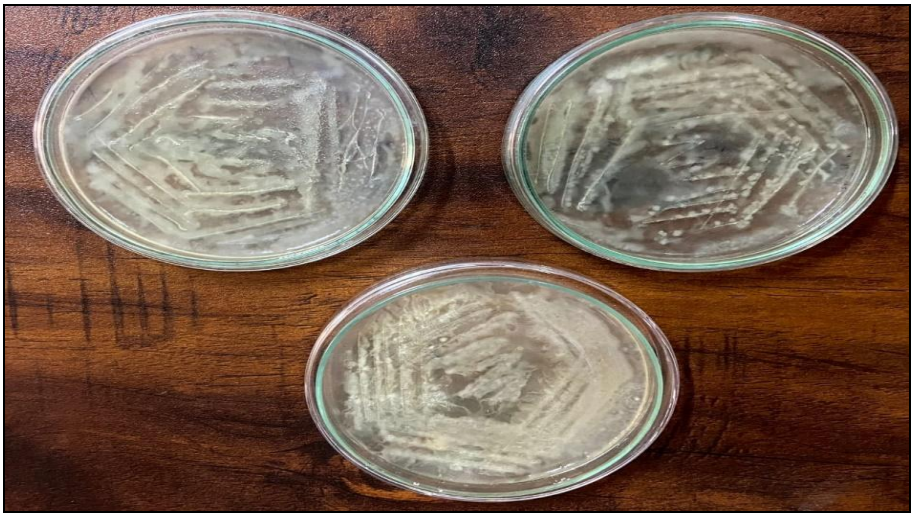

FIG. 13: STREAK PLATES

Bacterias were identified by the Gram staining method. Both *Staphylococcus aureus* and *Escherichia coli* bacterias were present in the soil extract and skin swab **Fig. 14.**

The results of zone of inhibition showed that the herbal hand wash formulations (F-1 & F-2) prepared from hydroalcoholic extract of the combined plant materials showed significant antimicrobial activity than standard drug amoxicillin. The hand wash prepared with lemon juice (F-2) showed little higher activity than the formulation prepared without lemon juice (F-1).

The data of the zone of inhibition of formulations are shown in **Table 7**. The zone of inhibition for different organisms is shown below in **Fig. 15.**

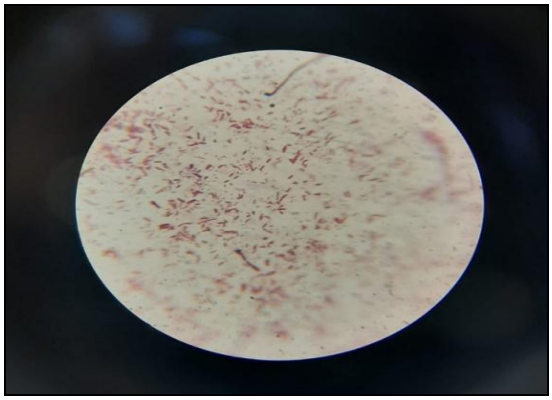

FIG. 14: GRAM STAINING FOR BACTERIAS

TABLE 7: ZONE OF INHIBITION OF FORMULATIONS (F-1 & F-2)

S. no.	Sample	*Staphylococcus aureus*	*Escherichia coli*
1	Standard Amoxicillin	23mm	24mm
2	Formulation-1 (F-1)	19mm	17mm
3	Formulation-2 (F-2)	22mm	25mm

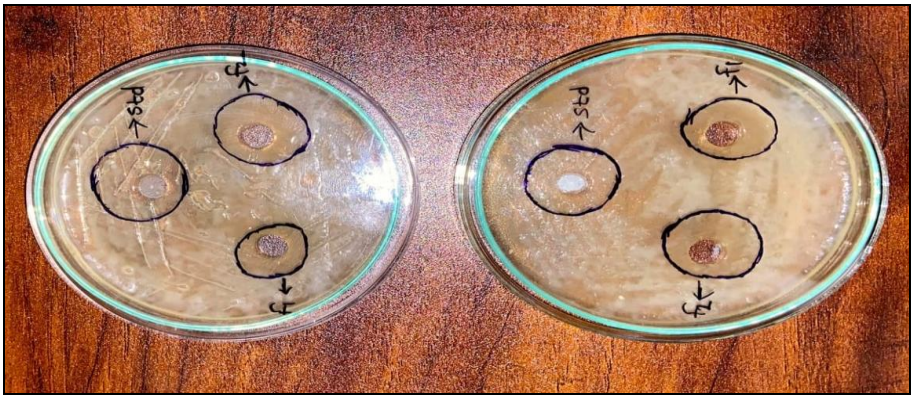

FIG. 15: PLATES SHOWING ZONE OF INHIBITION FOR *STAPHYLOCOCCUS AUREUS* & *ESCHERICHIA COLI*

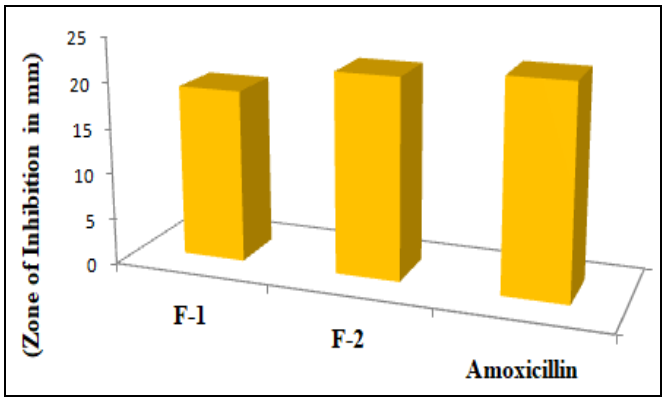

FIG. 16: ANTIBACTERIAL ACTIVITY OF FORMULATIONS ON *STAPHYLOCOCCUS AUREUS*

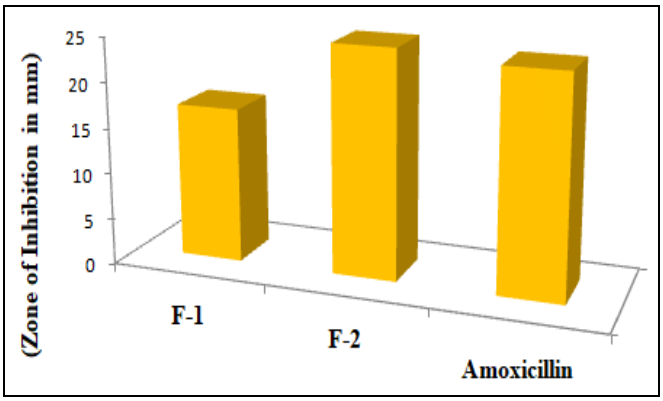

FIG. 17: ANTIBACTERIAL ACTIVITY OF FORMULATIONS ON *ESCHERICHIA COLI*

CONCLUSION: The results suggest that hydroalcoholic extract of *Moringa oleifera*, *Calotropis gigantea*, *Azadirachta indica*, and their combinations with lemon water are capable of giving a superior zone of inhibition to protect against the skin pathogens compared to standard amoxicillin **Fig. 16** & **17.** Thus, polyherbal hand wash prepared with different plant extracts shows maximum activity, and it has no side effects like skin rash and dryness.

ACKNOWLEDGEMENT: The authors are very much thankful to NCRD's Sterling Institute of Pharmacy, Nerul, Navi Mumbai, India, for

providing the necessary support to complete this work successfully.

CONFLICTS OF INTEREST: All authors declared no conflicts of interest.

REFERENCE:

1. Surwase VB, Savale MM, Murgude MM, Mohite SK and Magdum CS: Antimicrobial polyherbal hand wash formulation. International Journal of Creative Research Thoughts 2021; 9(6): 21-24.
2. Padalia U and Salgaonkar S: Development of Anti-Fungal Herbal Hand Wash Gel. International Journal of Life Science A5 2015; 1: 86-88
3. Aiello AE and Elaine BL: Antibacterial cleaning and hygiene products as an emerging risk factor for antibiotic resistance in the community. The Lancet Infectious Diseases 2003; 3(8): 501–506.
4. Snyder OP and Paul S: Safe hand washing. Hospitality Institute of Technology and Management 1988; 1: 1-3.
5. Powar PV, Bhandari NR, Arya Ashwini and Sharma PH: Formulation and evaluation of poly herbal anti-bacterial gel based hand wash. International J of Pharmaceutical Sciences Review and Research 2015; 33(1): 79-82.
6. Lakshmipriya G, Kruthi D and Devarai SK: *Moringa oleifera:* A review on nutritive importance and its medicinal application. Food Science and Human Wellness 2016; 5: 49-56.
7. Mishra G, Singh P, Verma R, Kumar S, Srivastav S, Jha KK and Khosa RL: Traditional uses, phytochemistry and pharmacological properties of *Moringa oleifera* plant: An overview. Der Pharmacia Lettre 2011; 3(2): 141-164.
8. Aja P.M,Nwachukwu N,Ibiam1 U.A,Igwenyi I.O,Offor C.E andOrji U.O:Chemical Constituents of Moringa oleifera Leaves and Seeds from Abakaliki, Nigeria. American Journal of Phytomedicine and Clinical Therapeutics 2014; 2(3): 310-321.
9. Marcela VJ, Manal MA and Maria LF: Bioactive Components in *Moringa oleifera* Leaves Protect against Chronic Disease. Antioxidants 2017; 6(91): 1-13. doi:10.3390/antiox6040091
10. Tanuj K and Alok A: *Calotropis gigantea*. Wilderness & Environmental Medicine 2015; 1-2.
11. Mossa JS, Tariq M, Mohsin A, Ageel AM, Al-Yahya MA and Al-Said MS: Pharmacological studies on aerial parts of *Calotropis procera*. Am J Chinese Med 1991; 19: 223–231. doi: 10.1142/S0192415X91000302
12. Moustafa AMY, Ahmed SH, Nabil ZI, Hussein AA and Omran MA: Extraction and phytochemical investigation of *Calotropis procera*: effect of plant extracts on the activity of diverse muscles. Pharm. Biol 2010; 48: 1080–1190. doi: 10.3109/13880200903490513
13. Al-Rowaily SL, Abd-ElGawad AM, Assaeed AM, Elgamal AM, El Gendy AENG and Mohamed TA: Essential oil of *Calotropis procera*: comparative chemical profiles, antimicrobial activity and allelopathic potential on weeds. Molecules 2020; 25: 5203. doi: 10.3390/molecules25215203
14. Pattnaik PK, Kar D, Chhatoi H, Shahbazi S, Ghosh G and Kuanar A: Chemometric profile & antimicrobial activities of leaf extract of *Calotropis procera* and *Calotropis gigantea*. Nat Prod Res 2017; 31: 1954–1957. doi: 10.1080/14786419.2016.1266349
15. Kanchan T and Atreya A: *Calotropis gigantea*. Wilderness & Environmental Medicine 2015; 1: 1-2.
16. Mohammad AA: Therapeutics role of *azadirachta indica* (neem) and their active constituents in diseases prevention and treatment. Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine 2016; 1: 1-11. http://dx.doi.org/10.1155/2016/7382506
17. Sandeep DS, Narayana Charyulu R, Prashant N, Aliss M and Indira G: Formulations of antimicrobial polyherbal hand wash. Research J Pharm and Tech 2016; 9(7): 1-3.
18. Azwanida NN: A review on the extraction methods use in medicinal plants, principle, strength and limitation. Medicinal & Aromatic Plants 2015; 4(3): 1-6. doi: 10.4172/2167-0412.1000196
19. Kokate CK: Practical pharmacognosy, Vallabh Prakashan, New Delhi 1999; 107-121.
20. Yadav R, Singh A, Saxena J and Mittal L: Formulation and Evaluation of Antibacterial Hydrogel. Turkish Journal of Physiotherapy and Rehabilitation 2021; 32(3): 31822-31843.
21. Kokare CR: Pharmaceutical microbiology experiments and techniques. Career Publication Nashik Maharashtra 2008; 43.
22. Jadhav P, Sonne M, Kadam A, Patil S, Dahigaonkar K and Oberoi JK: Formulation of cost effective alternative bacterial culture media using fruit and vegetables waste. International Journal of Current Research and Review 2018; 10(2): 6-15. doi: 10.7324/IJCRR.2018.1022
23. Elizabeth E: Pouring agar plates and streaking or spreading to isolate individual colonies. Methods in Enzymology 2013; 533: 3-14. doi: https://doi.org/10.1016/B978-0-12-420067-8.00001-5
24. Powar PV, Bhandari NR, Arya A and Sharma PH: Formulation and Evaluation of Poly Herbal Anti-Bacterial Gel Based Hand Wash. Int J Pharm Sci Rev Res 2015; 33(1): 79-82.
25. Katakam RS, Pedarla B, Vasimalla A, Shaik A, Mogudumpuram H and Sudhakarbabu: Formulation of poly herbal hand wash with antimicrobial activity. Indo American Journal of Pharmaceutical Research 2017; 7(3): 7869-7872.
26. Mounika A, Vijayanand P and Jyothi V: Formulation and evaluation of poly herbal hand wash gel containing essential oils. Int J of Pharmacy and Analytical Research 2017; 6(4): 645-653.
27. Terkar N, Sharma A, Tekawade J, Momin T and Sayyad E: Formulation and Evaluation of Polyherbal Hand Wash (Gel). International Journal of Science and Research IJSR 2021; 10(8): 1213-1219.

How to cite this article:
Chaudhary P, Pingale R, Batwal P, Ghode Y, Lodha J, Ajmera V and Phadtare M: *In-vitro* evaluation of the antibacterial potential of polyherbal hand wash formulation against some skin pathogens. Int J Pharm Sci & Res 2022; 13(10): 4187-98. doi: 10.13040/IJPSR.0975-8232.13(10). 4187-98.

All © 2022 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **Android OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)