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Research Article

Formulation and Evaluation of Polyherbal Handwash

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ABSTRACT

Since hands are a major source of microbiological contamination, the main goals of creating essential oil-based herbal hand wash are to encourage personal cleanliness and stop the spread of microbes. The oils from the eucalyptus, lavender, and neem trees were used to create the polyherbal handwash. When these oils are combined, they have synergistic effects. These oils served as the primary component, or API, during formulation preparation. Additional excipients, such as carbapol, HPMC, TEA, glycerin, rosewater, SLS, and others, were added to each batch in the necessary amounts. Nine batches were processed, eight of which were rejected because they did not meet the standard specifications provided. The ninth batch on the other hand, was chosen and taken into consideration since it satisfied all the requirements and had a decent look and smell. The examination took into account both chemical and physical criteria, such as pH, viscosity, foam height, foam retention, irritancy test, and antibacterial activity. Microbial activity against Staphylococcus aureus is assessed using the Kirby-Bauer disc diffusion technique. Additionally, a large zone of inhibition was seen when compared to typical antimicrobial medications, and all data were confirmed to be within normal limits with little to no adverse effects. These plant resources have high microbiological activity, which makes them suitable for commercial usage in the production of herbal hand wash. Additionally, customers nowadays are always looking for natural cosmetics to prevent allergic reactions and other adverse effects brought on by the chemicals included in everyday cosmetics.

INTRODUCTION

Hygiene is defined as maintenance of cleanliness practices which carries utmost importance in maintenance of health. Keeping bodily hygiene and usage of cleansers are requisites of healthy living. These concepts highlight the need of maintaining hygiene in prevention of diseases. Spread of infections (bacterial and viral) can be prevented by following hygiene practices.[1] Hands are primary mode of transmission of microbes and

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infections. Hand hygiene is therefore the most important measure to avoid the transmission of harmful germs and prevent the infections. Hand hygiene is the single most important, simplest, and least expensive means of preventing nosocomial infections. Contaminated hands can serve as vectors for the transmission of microorganisms.[2] The recent global spread of COVID-19has fostered diverse initiatives such as the Safe Hands‟ challenge led by the World Health Organization. Individuals are encouraged to regularly wash their hands for 40–60s under running water with soap. This call for Safe Hands‟comes at a time when water in security and limited access to hand washing facilities in Africa is heightened. The underlying rationale is clear: washing hands regularly and thoroughly physically degrades and removes viral particles, therefore, it lowers the likelihood of infection transmission. While millions of people living in developing countries lack access to potable water, this affect the implementation of handwashing activities, despite at this stage, hand washing being one of the most effective ways to contain the fast spreading of disease.[3]The concept of cleansing hands with an antiseptic agent probably emerged in the early 19th century. As early as 1822,a French pharmacist demonstrated that solutions containing chlorides of lime or soda could eradicate the foul odors associated with human corpses and that such solutions could be used as disinfectants and antiseptics. In a paper published in 1825, this pharmacist stated that physicians and other persons attending patients with contagious diseases would benefit from moistening their hands with a liquid chloride solution.[2] Skin is one of the most exposed parts of the body requires protection from the pathogens. To protect the skin from harmful micro-organisms and to prevent spreading of many contagious diseases hand washing is absolutely an important precaution.[1] To protect the skin from harmful microorganisms and to prevent spreading of many contagious diseases, hand washing is absolutely an important precaution. Hand washing removes visible dirt from hands and reduce the number harmful microorganisms such as, E. coli and Salmonella can be carried by people, animals or equipment and transmitted to food.[2]The WHO standard requires people to wash their hands with no antibacterial soap and water. The time duration ranged on average as short as 15 to 30 seconds, including rubbing the backs of hands, wrists, between fingers, and under fingernails.[4] The most vulnerable part of body, skin epidermis, needs to be protected from being exposed to pathogenic bacteria. Nosocomial infection has become a crucial problem in the outcome of hospital treatment, leading to prolonged hospitalization with the risk of mortality. The Health Care Workers‟ (HCWs) hands are the main routes of exposure of drug-resistant pathogens and severe infections. Many of the chemicals antiseptic are commercially available as sanitizers consisting alcohol, chlorhexidine and soon. These hand-washes help to control contagious disease transmission associated with health care more effectively but they produce diverse effects on prolonged use. Their repeated application can lead to dermal irritation and also pathogen resistance. Some researchers have shown that growing resistance in microbes towards chemical antiseptics has led to severe disease outbreaks.[5] *Staphylococcus aureus* is a frequent cause of infections in both the community and hospital. Worldwide, the increasing resistance of this pathogen to various antibiotics complicates treatment of *Saureus* infections. Effective measures to prevent *S aureus* infections are therefore urgently needed.[6]A teaspoonful of dirt may contain more than 1billion bacterial cells.[9] Because of high incidence, morbidity, and antimicrobial resistance, *Staphylococcus aureus* infections are a growing concern for family

physicians.*S. aureus* is a common pathogen in skin, soft-tissue, catheter- related, bone joint, pulmonary, and central nervous system infections. *S.aureus* bacteremias are particularly problematic because of the high incidence of associated complicated infections, including infective endocarditis. Adherence to precautions recommended by the Centers for Disease Control and Prevention, especially hand washing, is suboptimal.[7]Approximately 30% of the human population is colonized with *S. aureus*. Simultaneously, it is a leading cause of bacteremia and infective endocarditis (IE) as well as osteo articular, skin and soft tissue, pleura pulmonary, and device-related infections.[8]

About Plants:

Eucalyptus oil is obtained from the leaves of eucalyptus globose (*Eucalyptus globules* Labill), which belongs to the *Myrtaceae* family.[9] The genus eucalyptus contains about 700 species; among them, more than 300 contains volatile oil in their leaves. Essential oils of various eucalyptus species are used in the pharmaceutical, toiletries, cosmetics and food industries. Eucalyptus globulus commonly known as blue gum was introduced into India as a fuel tree in 1843.[11]It is called the fever tree based on its strong antimicrobial ability.[12] Eucalypti are evergreen trees or shrubs that reach a height of up to 100 m. Most species drop their barks every year. Leaves of *E. globulus*, from which oil is obtained, should be greyish-green, up to 25 cm long and up to 5 cm wide, thick, and sickle-shaped. The flowers are usually white, but in some species yellow, pink, or red. Eucalyptus EO has antimicrobial activity against viruses, bacteria, yeasts, and filamentous fungi. Eucalyptus EO is more active against Gram-negative than Gram-positive bacteria, which is attributed to the presence of components such as 1.8-cineole,*p*-cymene,*cis*-geraniol, and terpinolene, which can increase the permeability of the cytoplasmic membrane through functional impairment.[9] The essential oil consisted mainly of oxygenated monoterpenes, monoterpenes and oxygenated sesquiterpenes. Of these, 1, 8-eucalyptus (72.71%) α- terpined (2.54%), terpiene-4-ol (0.34%), and linalool (0.24%) were the main oxygenated monoterpenes, while α-eudesmol (0.39%), (-)- globulol (2.77%), and epilobulol (0.44%) were the main sesquiterpene. Several significant compounds were αterpineol acetate (3.1%), geranyl acetate (0.71%), Lpinocarveol (0.36%), β-sabinene (0.25%), and terpinolene (0.19%). A portion (0.26%) of the total constituents remains unidentified.[10]

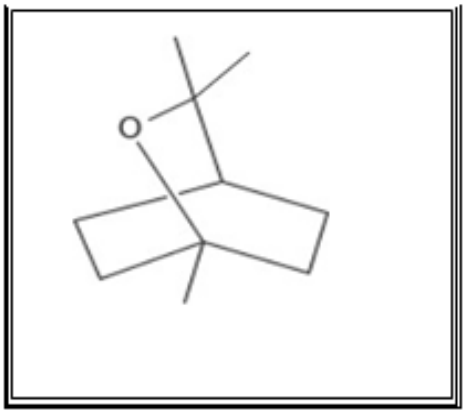


Figure 1: Eucalyptus.

Eucalyptus E Otoxicity studies performed in vitro and in animals have shown that it is low in toxicity. In addition, studies conducted with the participation of human volunteers indicated its low allergenicity.[13]High concentrations of several polyphenolic compounds including flavonols, hydroxybenzoic acids, and hydrolyzable tannins have been found in the extract of eucalyptus. These compounds are the reason for the high antimicrobial and antioxidant activity of eucalyptus. Recent studies have revealed the strong antibacterial ability of eucalyptus against S. aureus, Listeria mono cyto genes, Bacillus, Klebsiella pneumoniae, Enterococcus faecalis, Pseudomonas aeruginosa, Salmonella Enteritidis, and Escherichia coli. These results suggest that eucalyptus has high antimicrobial and antioxidant abilities.[14] In present study we formulate herbal

hand wash using different plant extracts with potential antibacterial activity and thereby establishing them as a potent antimicrobial agent in the formulation of herbal hand wash.[1] Lavender is one of the most commonly cultivated plants in the world on account of its EO properties. Lavender belongs to the Lamiaceae family, formally called Labiatea. The genus Lavandula includes about 40 different species and hundreds of varieties and its hybrids.The three species most commonly grown type sare: L.angustifolia Mill. (narrow-leaved lavender, usually medical), L. stoechas(French lavender),L. latifolia.[13]The plant flowers and essential oils are principally used in the toiletry and perfumery industries. [10]In India, it was introduced in the Kashmir Valley in 1983, where its commercial cultivation was found to be successful. This plant is cultivated primarily for its aromatic inflorescence from which the essential oil is isolated.[13] Lavender EO is a colorless or pale yellow liquid with a characteristic odor. Lavender EO is obtained after distillation with water vapor of fresh or dried tips of blooming plants. The main components are Renantiomers of linalool (20–45%) and linalyl acetate (25 to 46%). The high content of these ingredients determines the quality of the oil. The content of other ingredients should be in the following ranges: limonene (>1.0%), eucalyptol (<2.5%), camphor (>1.2%), terpin-4-ol (0.1–6.0) %), lawandulol (<0.1%), lavandulyl acetate (<0.2%), and α-terpineol (>2.0%).[9]The pleasant aroma of this plant is mainly due to the occurrence of low molecular weight terpenoids synthesized and accumulated in aerial parts, especially in inflorescences.[33]

Figure 2: Eucalyptus Tree[10]

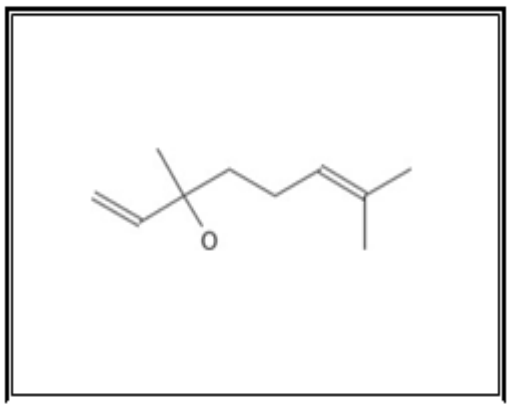


Figure 3:Linalool

It is known medicinally for its powerful antibacterial, anti-inflammatory, and analgesic properties.[14]It has shown antibacterial activity to a much greater extent. It is worth noting that the EO of *L. angustifolia* Mill. has a strong antiseptic effect against antibiotic-resistant strains, e.g., *Staphylococcus aureus* resistant to methicillin or vancomycin-resistant strains of *Entero coccus*sp. (VRE). Lavender EO is also active against the strain of *E.coli* resistant to piperacillin.

Figure 4: Lavender Tree[15]

Azadirachta indica(A. Juss), commonly known as the neem tree, is a tropical evergreen tree that is native to the Indian subcontinent.[16] *Neem*tree is also named as "village dispensary" as every part including the seeds, leaves and barkhas amedicinal value.[17]Neem seed is a part of Neem tree which has high concentration of oil.[18]Since time immemorial it has been used by Indian people for treatment of various diseases due to its medicinal properties. It possesses anti- bacterial, anti-cariogenic, anti-helminthic, anti-diabetic, anti-oxidant, astringent, anti- viral, cytotoxic, and anti-inflammatory activity. Nimbidin, Azadirachtin and nimbinin are active compounds present in *Neem*which are responsible for antibacterial activity.[20]The phytochemical constituents present in *neem*are nimbidin, nimbin, nimbolide, Azadirachtin, gallic acid, epicatechin, catechin, and margolone. All these exhibit potent antibacterial activity. The chief active constituent of *neem*is azadirachtin, which is an effective antimicrobial agent.[19]Extracts from Neem leaves, fruits and seeds, containing approximately 0.13% oil, have been used from ancient times in pharmaceutical preparations. Nimbin, accounting for much of the biological activities of Neem oil, shows anti-inflammatory, antipyretic, fungicidal, antihistamine and antiseptic properties. More than 140 compounds have been isolated from all parts of the Neemtree (flowers, leaves, seeds, fruits, roots, bark),to which interferon inducing activity (bark),immune modulatory, anti pyretic and anti-inflammatory, antiulcer, anti malarial, antifungal, antibacterial, antiviral against skin ailments activity (leaves), as well as antioxidant and anti mutagenic properties were recognized. The antimicrobial activity of Neem oil was evaluated as a synergistic interaction with other essential oils, which combine terpenoid compounds, demonstrating that the presence of Neem oil confers a broad spectrum of antibacterial activity.[20]The oil possesses a wide spectrum of antibacterial action against various microorganisms, including M. tuberculosis and streptomycin resistant strains. The Neem oil showed considerably activity against bacterial Gram-positive bacteria: example, Staphylococcus species and the Gram-negative bacteria: example Escherichia coli [21]

Figure 5: Neem Tree [19]

In present study we formulate herbal hand wash using different plant extracts with potential antibacterial activity and thereby establishing them as a potent antimicrobial agent in the formulation of herbal hand wash.[1]

MATERIALS AND METHODOLOGY:

Table 1: List of Materials

Sr. No.	Materials	Used As
1.	Neem oil	Anti-microbial agent
2.	Eucalyptus oil	Anti-Microbial and cooling Agent and analgesic
3.	Lavender oil	Anti-micobial agent and fragrance
4.	Carbopol934	Gelling agent
5.	Ethanol	Disinfectant
6.	Hydroxy Propyl Methyl Cellulose	Polymer
7.	Tween 80	Surfactant
8.	Sodiyum Lauryl Sulphate	Surfactant
9.	Glycerine	Moisturising agent

Table 2: List of Equipment's

Sr. No.	Equipments	Make/Model No.
1.	Weighing balance	WENSAR Class II (Serialno.91499)
2.	pH meter	SYSTRONICS Type 335 (Serialno.13976)
3.	Viscometer	Fungilab Type Visco Lead ADV (Serialno.ADVL252087)
4.	Mechanical stirrer	NSAW™

Table 3: Optimized Formulation Batches.

Contents	F1	F2	F3	F4	F5	F6	F7	F8	F9*
Neem oil (ml)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	**0.25**
Eucalyptus oil (ml)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	**0.25**
Lavender oil (ml)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	**0.25**
Ethanol (ml)	4	4	4	4	2	2	2	2	**2**
Carbopol 934 (gm)	2	2	2	2	2	2	5	5	**2**
HPMC (gm)	-	-	-	2	2	2	2	2	**2**
Tween80 (ml)	4	-	4	4	-	4	8	8	**4**
SLS (gm)	6	6	-	-	6	4	3	3	**3**
TEA (ml)	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	**Q.S.**
Glycerine (ml)	40	40	40	40	40	40	40	40	**40**
Rosewater (ml)	5	5	5	5	10	10	10	10	**10**
Turmeric (gm)	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	**Q.S.**
Distilled water(ml)	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	**Q.S.**

***Indicates the selected formulation batch passing all the required parameters.**

The following procedures applicable for all poly herbal hand wash batches only the quantity of excipient varied.

METHODOLOGY:

Essential Oils Selection: Neem oil, Eucalyptus oil, and Lavender oil are chosen for their known antimicrobial properties, which can effectively cleanse and protect the hands from harmful pathogens.

Solvent Choice: Ethanol is selected as the solvent for dissolving the essential oils due to its abilityto effectively dissolve a wide range of compounds and its rapid evaporation, leaving no residue.

Polymer Selection for Base: Carbopol 934 and HPMC are chosen aspolymers for the base formulation due to their thickening and suspending properties. They help create a gel-like consistency that is easy to apply and provides a smooth texture.

Surfactant Addition: Tween 80 is added to the base formulation as a surfactant to improve the dispersibility of the essential oils and ensure even distribution throughout the formulation. This helps enhance the efficacy of the handwash.

Mixing Method: Continuous stirring is employed during the formulation process to ensure thorough mixing of the essential oils with the base. This helps achieve uniform distribution of the active ingredients and ensures consistency in product quality.

Colorants addition: Turmeric added as a colorant for the better appearance.

Neutralizer addition: Adjustment of the PH done by adding sufficient quantity of Triethanolamine which act as a neutralizer.

Quality Control: Throughout the formulation process, it's important to monitor parameters such as pH, colour, Irritancy, Appearance, viscosity, Foam retention at 5 min, Homogenicity and clarity

to ensure the final product meets quality standards and delivers the desired performance as a polyherbal handwash.

Procedure: *

Take each 0.25ml of essential oil (Neem oil, Eucalyptus oil, Lavander oil Which can effectively cleanse and protect the hands from harmful pathogens. Dissolve the essential oils in 2ml of ethanol due to its ability to effectively dissolve a wide range of compounds and its rapid evaporation, leaving no residue. Mix each 2gm of Carbopol 934 and HPMC along with 4ml of Tween 80 by continuous stirring and make a smooth homogenus base. Add 3gm of SLS to the base formed. Now, mix the ethanol along with the essential oil to the base. Add 10 ml of rose water for the fragrance and 40 ml of glycerine as a moisturizing agent in the above mixture .Make up the volume up to 100ml by using distilled water. Adjustment of the PH done by adding sufficient quantity of Triethanolamine which act as a neutralizer. Add sufficient quantity of turmeric as a colourant in above mixture for better appearance. Pump fillers are versatile and can handle a wide range of liquid viscosities, making them suitable for filling herbal liquid hand wash into containers. Appropriate filling machinery used to fill the handwash into the prepared packaging containers. Ensure proper sealing to maintain product integrity.

Pharmaceutical Evaluation Of Polyherbal Handwash:*

The herbal liquid soap was evaluated for various physiochemical parameters such as physical appearance (colour, odour), pH, Viscosity.

Colour examination:

Colour of the hand wash was examined by visual inspection.

Odour examination:

The odour of the herbal hand wash was evaluated manually

Visual Appearance:

The formulation was determined homogenous in appearance by visual assessment and no phase separation was observed.

Determination of pH:

The pH of the hand wash was evaluated by using digital pH meter.

Determination of viscosity:

Using a digital Brookfield viscometer, the viscosity of the hand wash was calculated.

Foam retention:

The formulation is stirred by mechanical stirrer for about 15-30 minutes and allowed to stand. After the time to retain the foam was recorded.

Skin irritation:

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

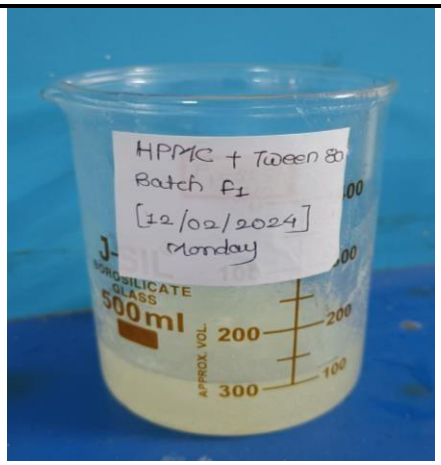
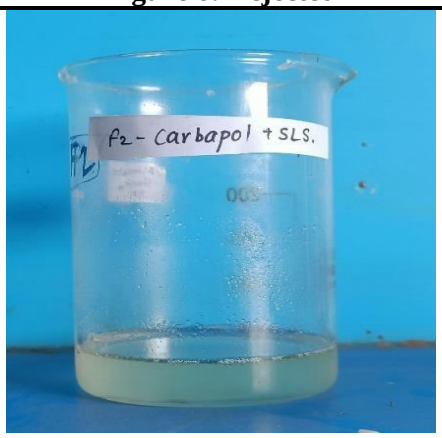
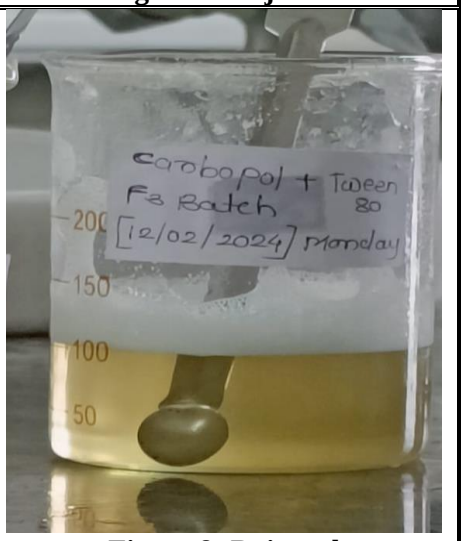
Evaluation of anti-microbial activity:

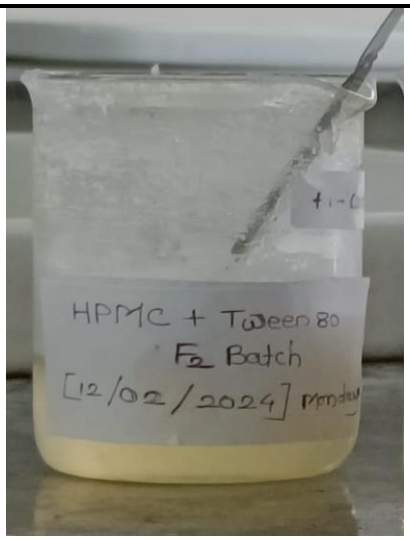
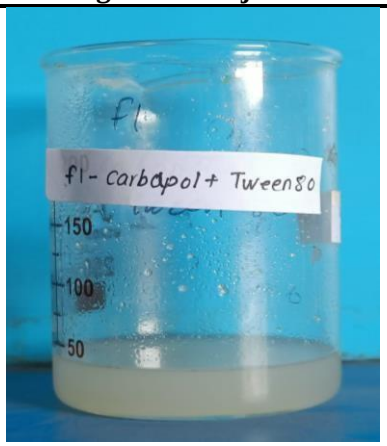
By using the agar plate technique, the anti-microbial effectiveness of the Polyherbal Hand Wash formulation was evaluated against Staphylococcus aureus.

RESULTS & DISCUSSIONS:

Table 4: Trials With Observations

Sr.no.	Formulations	Observation	Appearance

1)	F1	This batch of formulation is rejected due to low homogenicity and cloudy appearance.	 **Figure 6: Rejected**
2)	F2	This batch of formulation is rejected due to low viscosity.	 **Figure 7: Rejected**
3)	F3	This batch of formulation is rejected due to high foam retention.	 **Figure 8: Rejected**

4)	F4	This batch of formulation is rejected due to high viscosity.	 **Figure 9: Rejected**
5)	F5	This batch of formulation is rejected due to lump formation.	 **Figure 10: Rejected**
6)	F6	This batch of formulation is rejected due to less foam formation.	 **Figure 11: Rejected**

***Indicates the selected formulation batch passing all the required parameters.**

All the observation data for physical and chemical valuations of F9 batch of polyherbal handwash is presented as follows:*

Table 5: Comparison of evaluation parameters for prepared formulation and marketed formulation.

Sr. No.	Evaluation Parameters	Prepared Formulation	Specification [22]
1.	Colour	Yellow	White
2.	Odour	Pleasant	Floral
3.	Appearance	Opaque	Opaque
4.	Texture	Smooth	Smooth
5.	PH of solution	7.1	8.5
6.	Viscosity	360cp	435cp
7.	Irritancy	No	No
8.	Foam retention	12.5 ml	17.2 ml
9.	Spreadability	4 cm	3.7 cm
10.	Stability	Stable	stable

In-Vitro Antimicrobial Activity by using agar plate method:

In this evaluation test, the antimicrobial activity of herbal hand wash was found to be good, and the gel-based herbal hand wash created possesses antimicrobial activity towards species of bacteria like S. aureus. The preparation had a good zone of inhibition (30 mm), as shown in the table below:

•Control: Ciprofloxacin

•Interpretation–Sensitive: Zone of inhibition in more than12mm

•Moderately Sensitive: Zone of inhibition between12-8mm

•Resistant: Zone of inhibition less than 8mm

Interpretive Criterion:

1. Incubation on Standard Culture media for 24 hrs at 37^0C for interpretation
2. Zone of inhibition was measured in Millimeter(mm)
3. Samples are tested against Standard ATCC culture stains

Table 6: Result of Zone of inhibition for Formulation F9.

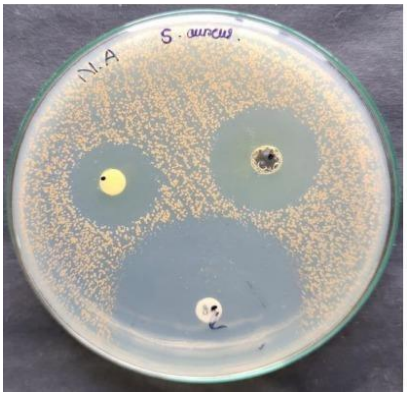
Micro-organism	Zone of inhibition	Visual appearance
Staphylococcus aureus	**30 mm**	

Figure 15: Zone of inhibition

CONCLUSION:

Skin, respiratory, gastrointestinal, and other diseases are primarily spread through the hands. Due to numerous illnesses and bacteria, the bar soap becomes contaminated, which could cause germs to spread. Soaps are typically used to clean and remove dirt and microorganisms from the skin's surface. Each person has a different preference for soap, but the soap must not irritate skin that is already sensitive and must be effective

in removing skin-infecting germs. Compared to available commercially manufactured hand washes, this formulated hand wash is more efficient. There are several liquid formulations that are offered in the pharmaceutical sector, such as poly herbal hand washes. We discovered that the majority of them used chemical pretreatment. In summary, the aforementioned results demonstrate that each of the three oils used in the poly herbal hand wash is effective against microorganisms. When compared to ciprofloxacin, these oils together may provide a higher zone of inhibition to protect against skin infections. The results unequivocally showed that the herbal hand wash gel's formulation is efficient and has no negative effects on human tissue. We provide several kinds of assessment tests, ranging from after-feel to colour, odour and pH. It is the Brooke Field Viscosity Test, which measures viscosity in the centre and is non-irritating, spreadable, washable, etc. The outcome is satisfactory and fit for human use.

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