



Formulation and Evaluation of Alcohol Free Herbal Hand wash Containing Ocimum Sanctum

***Mali Kamlesh D, Chaudhari Himanshu P, Chaudhari Komal R and Chaudhari Dipak I**

***Assistant Professor, R.C.P. Institute of Pharmaceutical Education and Research, Shirpur, Dist-Dhule, Maharashtra-425405.**

Received: 16 Jan 2020 / Accepted: 14 March 2020 / Published online: 01 April 2020

*Corresponding Author Email: kamaleshmalipharma@gmail.com

Abstract

Hand hygiene is the single most important, simplest, and least expensive measure of preventing nosocomial infections as our hands are the primary and foremost modes of transmission of microbes. Spreading of infection prevented by Proper Hand Washing. Hence it brings us to the use of hand wash for hand washing purposes. The present research was aimed to evaluate the anti- microbial efficacy of Ocimum Sanctum (tulsi extract) by agar-plate diffusion method. Also the research was carried out to formulate and evaluate the herbal hand wash liquid containing the tulsi extracts. The anti-microbial activity of the formulated hand wash was tested against E. coli and S. aureus by Agar plate diffusion method. Thus this work suggests the incorporation and utilization of tulsi extract in the formulation to give better anti-microbial effect. Results show that tulsi extract containing herbal hand wash was more efficient in reducing the number of organisms from hands. Thus it can be used as better alternative to other hand wash.

Keywords

Ocimum Sanctum, tulsi extract, herbal hand wash, antimicrobial activities.

INTRODUCTION:

Skin is first protection line of human body it covered the inner part of body and protect them protection from the pathogens. So protect the skin from harmful microbes and to prevent spreading of many contagious diseases hand washing is important precaution. Hands are primary mode of transmission of microbes and infections. Hand hygiene is therefore the most important measure to avoid the transmission of harmful germs and prevent the health care associate infection. Hand hygiene is the simplest, and least expensive measure to prevent infection. Now a day corona virus pandemic condition (COVID-19) hand washing getting a lots of

important. Prevention is better than cure. Hand washing is the act of cleaning hands with the purpose of removing soil, dirt, pathogenic microorganisms and avoid transmitting of transient micro organism¹. Hand Washing removes visible dirt from hands and reduces the number of harmful microorganisms such as E. coli and S aureus can be carried by people, food, animal or equipment & transmitted. To protect the skin from harmful microorganism and to avoid spreading of numerous contagious diseases, hand washing is extremely important. Books on Ayurvedic medicine, written in the Vedic period (3500–1600 B.C.) describe practices, including the use of medicinal plants. In modern complementary and

alternative medical practice, plants are the primary source of therapeutics because bioactive components present in each part of the plant, including the seeds, root, stem, leaves, and fruit. The benefits associated with the use of medicinal plants are like they are cost-effectiveness and global availability. They are safe as compared to synthetic compounds. They are natural products so they are side effect free which is the most important advantage of medicinal plants². Historically, plants have provided a good source of anti-infective agents. Plant extracts have a potential as antimicrobial compounds against several pathogenic microorganisms which cause infectious diseases and resistance towards synthetic. *Ocimum sanctum*, Tulsi, or Holy Basil from the family Lamiaceae has been described as the “Queen of plants” and the “mother medicine of nature” due to its important medicinal qualities. It has been one of the most valued and holistic herbs used over years in traditional medicine in India. Traditionally, Tulsi is used in different forms; aqueous extracts from the leaves (fresh or dried as powder) are used in herbal teas or mixed with other herbs or honey to enhance the medicinal value. Traditional uses of Tulsi aqueous extracts include the treatment of different types of poisoning, stomach-ache, common colds, headaches, malaria, inflammation, and heart disease⁴. Oils extracted from the Tulsi leaves and inflorescence of Tulsi have been claimed to have useful properties, like expectorants, analgesics, anti-emetics, and antipyretics; stress reducers and inflammation relievers; and as anti-asthmatic, hypoglycemic, hepatoprotective, hypotensive, hypolipidemic, and immunomodulatory agents. Tulsi products obtained by different extraction methods, such as steam distillation, benzene extraction and petroleum extraction⁵. Tulsi extract possesses in-vitro antimicrobial properties. Considering this ultimatum and by screening literature survey for the herbs with antimicrobial properties and it has been found that *Ocimum sanctum* (Tulsi), extract holds that antimicrobial property⁶. Thus, in present work we

aimed to formulate and evaluate herbal hand wash comprising of alcoholic extracts using other suitable excipients which can be used as herbal hand wash.

MATERIALS AND METHODS:

Samples of *Tulsi leaf* were collected from medicinal garden of R.C.P. Institute of pharmaceutical education and research Shirpur, Maharashtra. Specimens were identified by a botanist for their authenticity. Leaves were separated and washed in clear water and dried. Dried leaves were powdered. Ethanol extract was prepared from the powder obtained using “cold extraction method.” A total of 100 g of finely powdered Tulsi was macerated with 100% ethanol for 2 days. The alcoholic decoction was subjected to filtration with Whatman #1 filter paper to obtain a clear filtrate. The filtrate was subjected to evaporation to obtain a solid residue of Tulsi extract.

Chemicals and Reagents

The tulsi extract, carbopol 940, sodium lauryl sulphate, Triethanolamine and glycerin were taken from Loba Chem Mumbai. All other reagents / chemicals used were analytical grade.

Formulation of Herbal Hand wash Gel⁷

Procedure

Various herbal hand wash formulations were prepared according to composition given in table 1. First prepared the gel base by using desired concentration of Carbopol-940 gelling agent with sodium lauryl sulphate, glycerin was measured accurately and dispersed in purified water with moderate stirring. Then the required quantity of methyl paraben was dissolved in remaining quantity of purified water by gentle heating. Desired quantity of Tulsi extract was added in gel base with continuous stirring. And Triethanolamine was added to adjust the pH. Of formulation. The formulated hand wash gel was filled in container and stored at cool and dry place until further evaluation. The flow chart of procedure given below see Figure 1.

Table 1: Herbal Hand Wash Formulations

Composition (%w/v)	Formulation code			
	F1	F2	F3	F4
Tulsi extract (gm)	5	5	5	5
Carbopol-940 (gm)	0.2	0.4	0.6	0.8
Glycerin (ml)	2.0	2.0	2.0	2.0
Sodium lauryl sulphate (gm)	2.0	2.0	2.0	2.0
Methyl Paraben (ml)	0.2	0.2	0.2	0.2
Triethanolamine (ml)	qs	qs	qs	qs
Purified water up to (in ml)	100	100	100	100

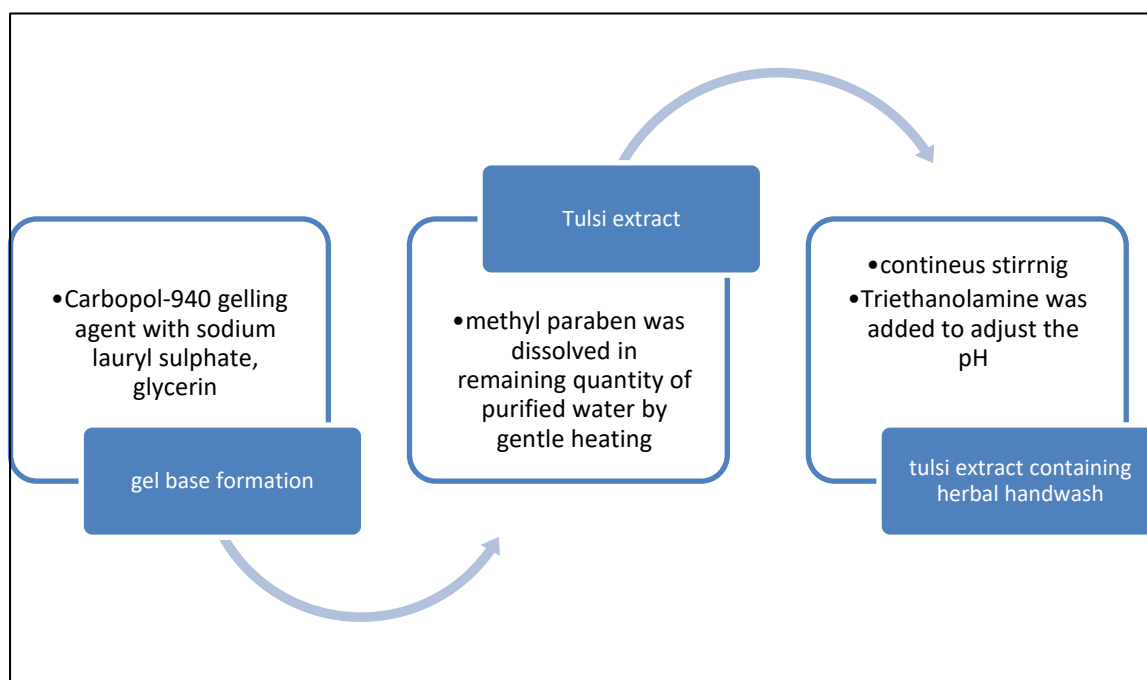


Fig 1: Flow chart of formulation process of tulsi extracts containing herbal hand wash

EVALUATION^{8,9}

Physical Evaluation Physical evaluation (color) was done by sensory and visual inspection.

pH determination One gram of sample of herbal hand wash was taken and dissolved it into 100ml distilled water. The pH of solution was measured by previously standardized digital pH meter.

Viscosity The viscosity of hand wash gel was determined by using digital Brook filed viscometer DV-II. Measured quantity of hand wash gel was taken into a beaker and the tip of viscometer was immersed into the hand wash gel and viscosity was measured.

Spread ability A sample of 0.5 g of each formula 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 spread ability. The results obtained are average of three determinations.

Determination of Homogeneity

The formulated gels were examined for their color, clarity, and homogeneity and phase Separation by visual inspection.

Skin irritation test

Skin irritation test (primary) was planned to perform on human volunteers, for each gel, five volunteers were selected. 1.0g of formulation herbal hand wash was applied on an area of 2 square inch to the back of hand, covered with cotton and secured firmly in adhesive plaster. This was allowed to remain in close

contact with the skin for over 24 hours, after which the site of application was examined for any signs of lesions or irritation.

Antibacterial efficiency of herbal hand sanitizer gel on volunteers

The antibacterial efficiency was performed by spread plate technique. Samples were collected from the five different volunteers showing no clinical signs of dermal abrasion, trauma and infection. Approximately 500 µl of herbal hand wash was applied to both hands. After washing the hands, the samples were collected from each volunteer in a separate glass beaker and the collected samples were allowed to grow on nutrient agar media for overnight at 37°C and per ml CFU were calculated.

Stability The stability studies were carried out for all the gel formulation by freeze - thaw cycling. Here, by subjecting the product to a temperature of 4° C for 3 months, then at 25°C for 3 months and then at 40°C for 3 months and studied for appearance, pH, viscosity and spread ability.

Evaluation of antimicrobial activity¹⁰

The organisms used were Staphylococcus aureus (Gram +ve) and Escherichia coli (Gram -ve). They were obtained from the Department of Microbiology, R.C.P. art commerce and science college, Shirpur. Standard antimicrobial used was Streptomycin

Determination of Antimicrobial activity by Agar plate diffusion method

The Bacterial cultures were lawn cultured on Nutrient agar media using sterile cotton swab under aseptic condition using laminar air flow. Then wells were made in each plate with the help of borer of 6 mm diameter. After solidification the microorganisms from the subculture were inoculated into the nutrient agar media and four cavities were

made in it. Then four formulated herbal hand wash were individually loaded in those well. The cavities were numbered from 1 to 4 filled with Streptomycin & herbal hand wash. Petri plates were incubated for 24 hrs at 37° C in the incubator. After incubation, the diameter of clear zone of inhibition produced around the well was measured in mm compared to the standard drug Streptomycin (0.8 µg/ml).

Table 2: Diameter of inhibition zone in mm of two bacterial strains which caused by leaves extract of Ocimum sanctum as follow

Zone Inhibition	Std. Streptomycin.	F1	F2	F3	F4
Escherichia coli	20.0±0.5	25.0 ± 0.61	26.0 ± 0.61	24.0 ± 0.45	23.0 ± 0.15
Staphylococcus aureus	23.0±0.21	16.0 ± 0.14	18.0 ± 0.51	18.0 ± 0.33	17.0 ± 0.2

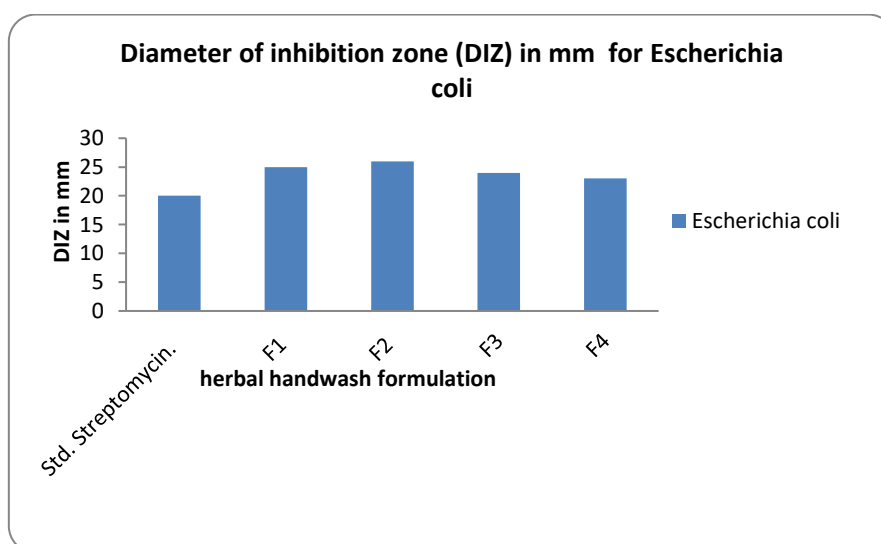


Fig 2: Graphical representation of Zone inhibition of Herbal Hand washes with Escherichia coli

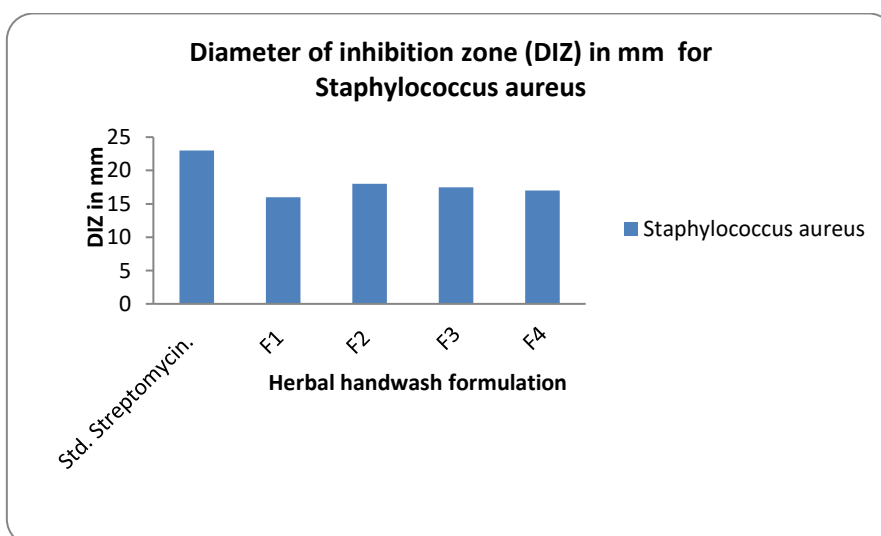


Fig 3: Graphical representation of Zone inhibition of Herbal Hand washes with Staphylococcus aureus

RESULTS AND DISCUSSIONS:

According to zone of inhibition herbal hand wash prepared with tulsi extract was equally effective against both the bacteria i.e. gram positive (*E. coli*) and gram negative (*S. aureus*). F2 Formulation show more zone of inhibition as compare the other formulation so F2 is optimize formulation. It produces wider zone of inhibition against *E. coli* 26.0 ± 0.61 & *S.aureus* 18.0 ± 0.51 mm. The inhibition of microbes due to the presence of active constituents such as Flavonoids, proline and Ascorbate Methyl – Isoleugenol in tulsi extract see table 2.

The herbal hand wash gel was greenish in colour and translucent in appearance and gave smooth on application which was maintained after tested stability study. pH of formulations also maintained throughout the study which was found 6.21 to 6.94 which are good for skin so no irritation or itching occur see table 3. The viscosities of formulations were measured using Brookfield viscometer before stability and after stability study. The viscosities of formulation were found to be in range 56 ± 0.5 to 182 ± 0.13 , it increases because of increase in concentration of gelling agent i.e carbopol 930. See

in see table 3. Spread ability was also measured and found to be in range 15 ± 0.58 to 07 ± 0.58 . Formulation F2 show good spreadability as compare to other formulation. As concentration of carbopol 930 was increase the spreadability was reduce. see table 3. The formulations were examined for homogeneity in terms of their color, clarity, and phase separation by visual inspection. After visual inspection we found that formulation had uniform color, good clarity and there was no phase separation found see table 3. The formulation didn't show skin irritation as the pH of formulations was within range and maintained throughout the study see table 3. Formulation F2 show good Antibacterial efficiency as compare to other formulation. It is due to less spreadability or more viscosities than F2. See table 3. stability test for optimize formulation was done for three months has been carried out at different temperature of 4°C, 25°C and 40°C for 3 months and then evaluation done for color, pH, viscosity and spread ability and results obtained was tabulated in table 2. After stability study there were not much variation at different temperature and humidity. So we conclude the formulation was stable see table 4.

Table 3: Evaluation parameters for herbal hand wash formulation

Formulation Code	F1	F2	F3	F4
Color	Greenish	Greenish	Greenish	Greenish
pH	6.21	6.54	6.79	6.94
Viscosity (Cps)	56 ± 0.5	92 ± 0.27	140 ± 0.54	182 ± 0.13
Spread ability (gm. c/sec)	15 ± 0.58	12 ± 0.58	09 ± 0.58	07 ± 0.58
Homogeneity	Homogenous	Homogenous	Homogenous	Homogenous
Skin irritation test	Nil	Nil	Nil	Nil
Antibacterial efficiency	+	+++	++	++
stability	+	+++	++	++

Table 4: Stability study of Formulation F2

Parameter	Observation After 3 Month	Inference
color,	Greenish	No significant change
pH,	6.48	No significant change
viscosity	92 ± 0.12	No significant change
Spread ability	12 ± 0.10	No significant change

CONCLUSION:

Prevention is better than cure. To protect the skin from harmful microorganism and to avoid spreading of numerous contagious diseases, hand washing is extremely important. herbal remedies are more acceptable in the belief as they are safer with no or less side effects than the synthetic compound. So this attempt was made to formulate the herbal hand wash containing tulsi extract. Its formulation is prepared according to delicateness of skin so that it cannot cause any type of irritation. so from this study it concluded that Formulation is good in appearance,

homogeneity. Formulation is alcohol free hand wash was as effective against pathogenic bacteria in volunteer's samples with no side effects on human skin. Hence it can be concluded that this herbal hand wash provides an effective and safe alternative to existing marketed hand wash.

REFERENCES:

1. Natarajan SB, Shah MA. Formulation Evaluation and Antibacterial Efficiency of Herbal Hand Wash Gel. International Journal of Pharmaceutical Science 23, 2014, 120-124.

2. Padalia U, Salgaonkar S. Development of Anti-Fungal Herbal Hand Wash Gel. International Journal of life science A5 2015, 86-88
3. Yamani HA, Pang EC, Mantri N and Deighton MA Antimicrobial Activity of Tulsi (*Ocimum tenuiflorum*) Essential Oil and Their Major Constituents against Three Species of Bacteria. Front. Microbiol. 7: 681.doi: 10.3389/fmicb.2016.0068
4. Wani NS, Bhalerao AK, Ranaware VP, Zanje R Formulation and Evaluation of Herbal Sanitizer International Journal of PharmTech Research 5 (1) 2016,40-43.
5. Nair R, Kalariya T, Chanda Antibacterial Activity of Some Selected Indian Medicinal Flora Turk J Biol 29 (2005) 41-47.
6. Vidhani SI, Vyas VG, Parmar HJ, Bhalani VM, MM, Ahmed Gaber and Golakiya BA Evaluation of Some Chemical Composition, Minerals Fatty Acid Profiles, antioxidant and Antimicrobial Activities of Tulsi (*Ocimum sanctum*) from India by *American Journal of Food Science and Technology*. 4(2), 2016, 52-57 doi:10.12691/ajfst-4-2-5
7. Rathi PB.et al, Development and Evaluation of Herbal Gel for Treatment of Psoriasis. Journal of Innovations in Pharmaceuticals and Biological Sciences 2(1), 2015, 772-87.
8. Zeeshan Asfisar, Salma Khanam et al. "Formulation and Evaluation of Poly herbal soap and Hand Sanitizer International Research Journal of Pharmacy 7(8), 2016, 54-57
9. Rassol Bazigha Kadhim et al, Ali Heyam Saad, "Formulation and Evaluation of Herbal Hand Wash from Matricaria Chamomilla Flowers". International Journal of Research in Ayurveda and Pharmacy 6, 2011, 11-1813.
10. Ritesh Kumar Tiwari, Devika Tripathi, Lalit Singh, vijay sharma, shashi verma Development & Antibacterial Characterization of Mint Hand wash, Journal of Research Publication 9(1) 2018,1-10