Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 12 [3] February 2023 : 167-170
©2023 Academy for Environment and Life Sciences, India
Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Development and Anti-Microbial Study of Herbal Hand Wash Using Nimba, Tulsi, Sourabhanimba, Kumari

Nameera H. A., Priyanshi Goyal, Maanya. M. Lingam, Vinaykumar R Kadibagil, Govinda Sharma.K
Sri Dharmasthala Manjunateshwara College of Ayurveda and Hospital, Hassan-573201
Correspondence Email: drvinaykadibagil@gmail.com

ABSTRACT
Hands are the main route of transmission of germs in healthcare, so hand hygiene is the most important measure to prevent the spread of harmful germs and prevent healthcare-related infections. Many infectious diseases can be transmitted from one person to another through contaminated hands. These diseases include gastrointestinal infections, such as salmonella, and respiratory infections, such as the flu. Proper hand washing can help prevent the spread of germs (such as bacteria and viruses) that cause these diseases. So an attempt is made to prepare herbal hand wash using drugs like nimba, girinimba, tulsi and kumari which are having antibacterial, antiviral, anti-fungal activities and skin hydration properties. Herbal hand wash was prepared as per standard method using herbal ingredients and swab samples streaked on Blood and Mac-conkey agar, incubated at 37°C for 24 hrs in aerobic condition for microbial load assessment study. The results suggest that hand-washing herbs can create excellent suppression zones to protect against skin pathogens. This could be the reason for using herbs in preparation for hand washing and using these compounds in the production of disinfectant lotions or soaps instead of chemicals.
Keywords: *Herbal hand wash, nimba, tulasi, saurabhanimba, antimicrobial study*

Received 02.11.2022 Revised 22.01.2023 Accepted 27.01.2023

INTRODUCTION

Food and water-borne diseases combined with nosocomial infections kill millions of people each year. A discussion of hospital acquisition and control of food-related infections and poisoning would be incomplete without including hand hygiene. They describe the means by which surgical procedures are most likely to be free from human pathogens and contact surface contaminants in food handling and consumption, without being complicated by hand-carried contaminants [1]. Hands are the main route of transmission of germs in healthcare, so hand hygiene is the most important measure to prevent the spread of harmful germs and prevent healthcare-related infections. Many infectious diseases can be transmitted from one person to another through contaminated hands. These diseases include gastrointestinal infections, such as salmonella, and respiratory infections, such as the flu. Proper hand washing can help prevent the spread of germs (such as bacteria and viruses) that cause these diseases. Some forms of gastrointestinal and respiratory infections can cause serious complications, especially in children, the elderly, or people with weakened immune systems. Therefore, simple hand washing is still an effective way to prevent infection [2].

Frequent hand washing with soap and water can dry out and crack your skin. This can be uncomfortable and increases the risk of skin infections. Frequent use of chemical handwashing causes obsessive-compulsive disorder (OCD) and dry skin, in extreme cases eczema and psoriasis. There is need of herbal hand wash which will protect ourselves from infection and also avoid the harmful effects of chemical hand wash available in market. So, an attempt is made to prepare herbal hand wash using drugs like nimba, girinimba, tulsi and kumari which are having antibacterial, antiviral, anti-fungal activities and skin hydration properties [3,4].

MATERIAL AND METHODS

Pharmaceutical study

Preparation of herbal hand wash

First nimba, tulasi and saurabhanimbaarka was prepared. Kumari swarasa was extracted from freshly collected drugs. To arka and swarasa, other ingredients i.elavana, essential oil, Methyl Paraben and Sodium Lauryl Ether Sulfate (SLES) were added one by one slowly. Mixture was stirred continuously for 30 minutes for homogenous mixing of ingredients.

Table 1 Ingredients of herbal hand wash

Sr.no.	Ingredients	Latin/English Name	Quantity
1.	NimbaArka	*Azadirachta indica*	183 ml
2.	TulasiArka	*Ocimum sanctum*	183 ml
3.	SaurabhanimbaArka	*Murraya koenigii*	183 ml
4.	Kumari swarasa (Aleo vera)	*Aloe barbadensis*	100 ml
5.	Lavana	Sodium chloride (Salt)	50 g
6.	Lemon grass essential oil		4 ml
7.	Methyl Paraben		2 g
8.	Sodium Lauryl Ether Sulfate (SLES)		300

Analytical study

Appearance:

The prepared formulation of hand wash appears as greenish brown.

1) pH:

The pH of formulations was measured by digital pH meter.

2) Viscosity:

The viscosity of hand wash was determined by using Brookfield viscometer. 50ml of herbal hand wash is taken into 100ml of beaker and the tip of viscometer was dipped into the beaker containing hand wash formulation and its viscosity was measured.

3) Foam Height:

0.5gm of sample of Herbal handwash was taken and dispersed in 25 ml distilled water. Then, transferred it into 500 ml stoppered measuring cylinder; volume was making up to 50 ml with water. 25 strokes were given & stand till aqueous volume measured up to 50ml& measured the foam height; above the aqueous volume.

4) Foam Retention:

50 ml of the Herbal handwash was taken into a 200ml graduated cylinder & shaken 10 times. The volume of foam at 1-minute intervals for 4 minutes was recorded [2].

Assessment of Antimicrobial Study

Collection of Samples for evaluation

a. Wet Swab method.

One swab will be collected before any application from both the palms from the same volunteer which will be marked as before treatment sample. Both palm will be treated with 10ml of herbal hand wash by Scholar for 30 seconds and then washed with water. One swab will be collected from each palm following exposure to herbal hand wash in sterile swab cover itself and labeled appropriately.

Preparation of Media

Culturing method: Streak culture method

Requirements:

MacConkey agar plate, blood agar plates, nichrome loop, gas burner, incubator (37^{o}C).

For culturing the bacteria in sputum sample, the culture media was needed to be prepared. Culture media was prepared by the following methods:

MacConkey Agar preparation

For preparation of 500 ml of MacConkey agar solid media: 17.5 gm of MacConkey agar was weighed and mixed with 7.5 gm of Agar. Further the powder was dissolved in 500 ml of distilled water and transferred to a 1000ml conical flask. Mouth of the conical flask was sealed tightly with cotton plug and was autoclaved at 121^{o}C for 20 minutes.

Procedure for culturing of bacteria

The zone of inoculation was marked over the outer lower surface of the sterile MacConkey and blood agar plates by using a glass marking pencil. The nichrome loop is heated red and was allowed to cool, then a loop full of bacteria was transferred aseptically to each of the previously marked zone of inoculation on the MacConkey and blood Agar plates and was streaked perpendicular to the zone of inoculation. The plates which were streaked in this manner were kept in the incubator at 37°C and observed the results after 24-48 hours. Further microscopic examination was done for the identification and characterisation if the bacteria.

Microbial Load Assessment

1ml of each dilution will be freshly pipetted onto a sterile, appropriately labeled Petri dish. Warm media will be poured over the 1 ml sample and shaken in all directions on the planar surface to ensure uniform spread and distribution of media. Vacuum shunting will be ensured to minimalize cross-contamination from external sources. After proper incubation, standardized to 24 to 36 hours in the incubation chamber,

the samples will be taken out. Distinct Colony Forming Units (CFU) will be subjected to photo documentation as well as counting by identifying unique colonies.

RESULTS ND DISCUSSION

Table 2 Summary of analytical results of herbal hand wash

Sr. no	Test name	Result
1	pH	6.24
2	Viscosity	62 CPS
3	Foam height	300 ml
4	Foam Retention	20 ml

Table 3 Aerobic culture report after 24 hrs of incubation

Sr No	Surface sampling Site	Before Hand wash	After Hand wash
1	Swab 01	40 – 45 Colonies	25 – 30 Colonies
2	Swab 02	25 – 30 Colonies	10-15 Colonies
3	Swab 03	35 – 40 Colonies	15 – 20 Colonies
4	Swab 04	45 – 50 Colonies	30-35 colonies
5	Swab 05	25 – 30 Colonies	20-25 colonies

Five swab samples were collected from health workers of SDM College of Ayurveda and Hospital. Swab samples streaked on Blood and Mac-conkey agar, incubated at 370 c for 24 hrs in aerobic condition. Results shows that number of colonies was reduced after washing with herbal hand wash.

Neem (*Azadirachta indica*) plants parts shows antimicrobial role through inhibitory effect on microbial growth/potentiality of cell wall breakdown. Azadirachtin, a complex tetranortriterpenoid limonoid present in seeds, is the key constituent responsible for both antifeedant and toxic effects in insects. 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 ßsitosterol, polyphenolic flavonoids, were purified from neem fresh leaves and were known to have antibacterial and antifungal properties and seeds hold valuable constituents including gedunin and azadirachtin[3.4,5].

Methanolic fraction and aqueous fraction of *Ocimum sanctum* showed anti-fungal activity against dermatophytic fungus i.e. T. rubrum etc. Aqueous fraction showed better anti dermatophytic activity as compared to methanolic fraction. Antibacterial activity of the aqueous, alcoholic, chloroform extract and oil obtained from leaves of *Ocimum sanctum* were studied against *E. coli, P.aeruginosa, S. typhimurium* and *S.aureus*. Extract obtained from *Ocimum sanctum* were observed equally effective against pathogenic gram positive and gram-negative bacteria [6,7].

Murraya koenigii has broad types of characteristics, such as antibacterial activity, activity of antifungal, activity of antiprotozoal. The essential oil from *M. koenigii* leaves showed antibacterial effect against *B. subtilis, Staph. aureus, C. pyogenes, P. vulgaris and Pasteurella multicida*. The pure oil was active against the first three organisms even at a dilution of 1: 50027. The acetone extract of the fresh leaves of M. koenigii on fractionation gives three bioactive carbazole alkaloids named as mahanimbine, murrayanol and mahanine, which has shown mosquitocidal, antimicrobial and topisomerase I and II inhibition activities. Acetone extract of *M. koenigii* is active against *Aspergillus niger*, benzene extract is most active against *Alternaria solani* and *Helminthosporium solani* and ethanol extract is active against *Penicillum notatum*. The leaves of this plant also are wealthy in diverse compounds inclusive of flavonoids, polyphenols, alkaloids, etc. The primary constituent is tannins. Tannins are water soluble polyphenols which can be typically located in classes hydrolysable and condensed tannins. Tannins are found in many foods. Tannins were stated to be bacteriostatic or bactericidal towards Staphylococcus aureus. They act at the membranes of the organisms.

In the current situation, the plants studied are rich in these various compounds and are therefore more effective against skin pathogens. The main ideology behind the combination of plant materials is the observation of the additive effect of the active ingredients of various plants. This combination has proven to be beneficial and is used to hand wash herbs. The results clearly prove that the herbal soap thus produced is much more active than the commercial antiseptic soap. The active ingredients in manufactured soap may be more effective at killing or eliminating organisms than the chemicals used in soaps. Thus, these compounds can be extracted and incorporated into soap bases to produce a superior antiseptic soap with little or no side effects. Thus, a new way can be found to combat antibiotic resistance

of pathogenic organisms and enable a safer and healthier life through germ-free hands. Although the elimination is not 100%, a significant number can be reduced [8,9].
Mucopolyssacarides aid in the skin's moisture retention. It was claimed that products containing Aloe vera increased skin hydration through a humectant mechanism. Several approaches have been used to demonstrate the activity of Aloe vera inner gel against Gram-positive and Gram-negative bacteria. Aloe vera gel has been shown to suppress the bacteria Streptococcus pyogenes and Streptococcus faecalis. In a monolayer culture, aloe vera gel proved bactericidal against Pseudomonas aeruginosa, whereas acemannan inhibited it from attaching to human lung epithelial cells. A processed Aloe vera gel preparation reportedly inhibited the growth of Candida albicans. Aloe extracts' antiviral properties could be attributed to indirect or direct actions. They have these effects both indirectly and directly by boosting the immune system. Various enveloped viruses, such as Herpes simplex, Varicella zoster, and Influenza, are inactivated by the anthraquinone aloin [10]. The inclusion of six antiseptic agents, namely Lupeol, salicylic acid, urea nitrogen, cinnamonic acid, phenols, and sulphur, contributes to Aloe vera's antibacterial activity. They all have antifungal, antibacterial, and antiviral properties [11,12].

CONCLUSION

The drugs used for developing herbal hand wash i.e. nimba, tulsi, saurabhanimba and kumari are having antibacterial and antifungal properties. Kumari will help in skin hydration possibly by means of a humectant mechanism. The analytical study shows that values are under normal range and suitable for hand wash. The microbial load study results suggest that hand-washing herbs reduces the microbial load to protect against skin pathogens. This could be the reason for using herbs in preparation for hand washing and using these compounds in the production of disinfectant lotions or soaps instead of chemicals.

ACKNOWLEDGEMENT

Rajiv Gandhi University of Health Sciences, Bengaluru, Karnataka. Short term research grant for UG students with project name Development and anti-microbial study of herbal hand wash and project code-UG21AYU042.

REFERENCES

1. Oranusi, S. U., Akande, V. A., & Dahunsi, S. O. (2013). Assessment of microbial quality and antibacterial activity of commonly used hand washes. *Journal of Biological and chemical research*, *30*(2), 570-580.
2. Sandeep, D. S., Charyulu, R. N., Nayak, P., Maharjan, A., & Ghalan, I. (2016). Formulations of antimicrobial polyherbal hand wash. *Research Journal of Pharmacy and Technology*, *9*(7), 864-866.
3. Islas, J. F., Acosta, E., Zuca, G., Delgado-Gallegos, J. L., Moreno-Treviño, M. G., Escalante, B., & Moreno-Cuevas, J. E. (2020). An overview of Neem (Azadirachta indica) and its potential impact on health. *Journal of Functional Foods*, *74*, 104171.
4. Maragathavalli, S., Brindha, S., Kaviyarasi, N. S., & Gangwar, S. K. (2012). Antimicrobial activity in leaf extract of neem (Azadirachta indica Linn.). *International journal of science and nature*, *3*(1), 110-113.
5. Alzohairy, M. A. (2016). Therapeutics role of Azadirachta indica (Neem) and their active constituents in diseases prevention and treatment. *Evidence-Based Complementary and Alternative Medicine*, *2016*.
6. Kulkarni, K. V., & Adavirao, B. V. (2018). A review on: Indian traditional shrub Tulsi (Ocimum sanctum): the unique medicinal plant. *Journal of Medicinal Plants Studies*, *6*(2), 106-110.
7. Patil, U. (2018). Studies on antiviral activity of tulsi (Ocimum sanctum) crude extracts on selected viruses of veterinary importance. *International Journal of Ayurveda And Pharma Research*.
8. Kumar, S. R., Loveleena, D., & Godwin, S. (2013). Medicinal property of Murraya koenigii-a review. *International Research Journal of Biological Sciences*, *2*(9), 80-83.
9. Al Harbi, H., Irfan, U. M., & Ali, S. (2016). The antibacterial effect of curry leaves (Murraya Koenigii). *European journal of pharmaceutical and medical research*,*3*, 382-387.
10. Gupta, V. K., & Malhotra, S. (2012). Pharmacological attribute of Aloe vera: Revalidation through experimental and clinical studies. *Ayu*, *33*(2), 193.
11. Lanka, S. (2018). A review on Aloe vera-The wonder medicinal plant. *Journal of Drug Delivery and Therapeutics*, *8*(5-s), 94-99.
12. Haq, A., Ali, Q., Rashid, M. S., Waheed, F., Hayat, S., & Malik, A. (2020). Antibacterial and antifungal activity of Aloe vera plant. *Life Science Journal*, *17*(7), 76-82.

CITATION OF THIS ARTICLE

Nameera H. A. Priyanshi Goyal, Maanya. M. Lingam, Vinaykumar R Kadibagil, Govinda Sharma.K. Development and Anti-Microbial Study of Herbal Hand Wash Using Nimba, Tulsi, Sourabhanimba, Kumari.Bull. Env. Pharmacol. Life Sci., Vol 12[3] Feb 2023 : 156-159