

Formulations of Herbal Hand Wash with Potential Antibacterial Activity

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Abstract- Introduction: Plants has medicinal, pharmaceuticals and cosmetic potential, using it many innovative products can be prepared useful for humans. In present study herbal hand wash was formulated using soxhlet fractions of different plant *Ocimum sanctum*, *Aloe vera* gel, *Sapindus mukorossi* (Reetha) fruit, *Azadirachta indica* (Neem), *Eucalyptus spp. (nilgiri)* and citrus fruit. These plants are traditionally known to possess different medicinal properties.

Methods: Hand wash were prepared by three different methods viz, using SDS (Sodium dodecyl sulphate) as a base and plant extracts (I), saponified Cummins seed oil and plant extracts (II) and reetha fruits extracts and plant extracts (III). The antibacterial activity of formulated herbal hand wash and individual plant extracts were examined using agar well diffusion method.

Results: Among the plant extracts used, *Sapindus mukorossi* (Reetha) and citrus fruits showed potent antimicrobial activity against skin pathogen *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Salmonella typhimurium*. All three herbal soap formulations showed antibacterial activity higher than commercially available standard soap solution dettol. Among the herbal formulations, hand wash with only extracts (III) exhibit higher activity than other formulations. It also possesses moistening properties due to the *aloe vera* extracts reducing the dryness of the skin.

Conclusion: Quality enhanced herbal hand wash with unique aroma and potential antibacterial activity was formulated.

Index Terms- Herbal Handwash; Antibacterial activity; Reetha; *Aloe vera*; *Oscimum Sanctum*..

1. INTRODUCTION

Plant extracts and products have been used for centuries in traditional medicine, functional food, natural dyes, cosmetics, as a detergent and in the treatment of diseases^{1, 2,3}. The main advantage of using natural source is that they are easily available, cheap and harmless compared to chemical products. Therefore research has been increased tremendously towards making natural products with improved quality yet less expensive and no side effect over chemical products.

The emergence of bacterial resistance to the currently available antimicrobial drugs necessitates further research in the discovery of new safe and effective antimicrobial agents. 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.

Skin, especially hands are needed to protect from bacterial pathogens as they are the most exposed part of the body. Proper hand hygiene is the single most important, simplest, and least expensive means of preventing health care associated infections. Most of the hand wash available in market are made from

synthetic chemical and has side effect like dryness of skin, rashes.

We therefore, prepared herbal hand wash using plants like *Ocimum sanctum*, *Aloe vera* *Sapindus mukorossi* (Reetha), *Azardichta indica* (Neem), *Eucalyptus spp. (nilgiri)* and citrus fruit as they possess different medicinal and chemical properties useful for formulating herbal hand wash. *Ocimum sanctum* is traditionally used in many diseases and complications and as antimicrobial activity^{4,5}, *Aloe vera* has antiseptic effect, as well as possesses properties like moisturizing for nourishing the skin⁶. *Sapindus mukorossi* (Reetha) is well-known plant in the Indian medicinal system and is a popular ingredient of ayurvedic shampoos and cleaners *also* possesses Foam properties, detergent abilities and long-term preservative efficacy⁷. Neem possesses a wide spectrum antibacterial activity and has been extensively used in Ayurveda, Unani and Homoeopathic medicine⁸. Citrus flavonoids have a large spectrum biological activity including antibacterial, antifungal, and antioxidant activity⁹. *Eucalyptus (nilgiri)* is another important ethno medicinal plant having specific aroma and volatile oils which has been previously used as expectorants¹⁰ and has antibacterial activity against pathogenic organism^{11,12}.

The antibacterial activity of herbal formulation was examined against the pathogens *Staphylococcus aureus*, *Klebsiella pneumonia*, *Salmonella typhimurium*. These pathogens are responsible for food poisoning, human lung inflammation and diarrhea, fever vomiting respectively. The herbal formulation made examined for its antibacterial activity against these bacteria, results in potential antibacterial activity.

2. METHODS AND MATERIALS

2.1 Collections of Plant Materials and Preparation of Extracts

Aloe vera, *Azadirachta indica*, *Eucalyptus* was collected from Sinhgad College campus (wadgaon), in month of September. Citrus fruit, *Sapindus mukorossi* (reetha) and *Ocimum sanctum* were purchased from local market. All the plant materials collected were authenticated from Department of Botany, Sinhgad College of Science, Pune. Collected leaves of plants *Azadirachta indica*, *Eucalyptus* and whole plant of *Ocimum sanctum* were air dried and powdered using mixer grinder. 100 g of powder was fractionated using methanol followed by distilled water using soxhlet apparatus. Finally solvents were recovered and extracts were lyophilized and stored at 4°C. Methanolic and aqueous soxhlet fractions were used to examine antibacterial activity and to formulate herbal hand wash. Fruits of *Sapindus mukorossi* (reetha) were dried, powdered and extracted using distilled water. Gel of *Aloe vera* were drained and collected, while juice of citrus fruits was used to prepare herbal formulation.

Test organisms, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Salmonella typhimurium* were used from stock culture of Department of Biotechnology, Sinhgad College of Science, Pune.

2.2 Antibacterial activity of Extracts

The antimicrobial activity of individual extracts was tested using agar well diffusion technique¹³. The plates were spread with test organism with bacterial suspension of OD was 0.6 at 600 nm. Wells of 5 mm diameter were then cut on plate. 100 ul of extract were filled into each wells and incubated at 37°C for 24 hrs. Antimicrobial activity in terms of zone of inhibition (cm) was recorded after 24 hours of incubation. The antagonistic action of each extract was tested against test organism in triplicates.

2.3 Preparations of Herbal Hand wash

2.3.1 Preparation of Herbal hand wash using SDS as detergent (I)

Sodium dodecyl sulfate (SDS) is an organic compound commonly used in molecular biology laboratory. It is an anionic surfactant having amphiphilic properties required for detergent. It used in many cleaning and hygiene products. Extracts of different plants were mixed with final concentration of 10% to which 3% SDS was added. This formulation was further used to examine its antibacterial activity at different concentration.

2.3.2 Preparations of Herbal hand wash using Cummins seed oil (II).

All soap, whether hard or liquid starts with a simple chemical reaction between oils and an alkali. With bars soaps, it's sodium hydroxide. With liquid soaps, it's potassium hydroxide. In present study Cummins seed oil was used as a source of fatty acid. A potassium hydroxide solution (5%) was added to neutralize the oil resulting in formation of thick slurry. Plant extracts at concentration of 10% was added in the liquid soap.

2.3.3 Preparation of Herbal hand wash using Reetha extracts (III)

In Indian tradition reetha is used as detergents as it possess surfactant activity. Reetha and other plant extracts mixed together at 10% final concentration. This was used as herbal liquid hand wash and used further for its antibacterial activity.

3. RESULTS

3.1 Physical properties of Herbal Hand wash

Herbal hand wash was made using three different methods. Reetha and SDS are act as detergent, while soap was also made from Cummins seed oil. All the soap showed surfactant activity in which organic molecules of the skin were dissolved easily. The colour of the hand wash was dark greenish. Odor of all the hand wash was like aroma of *Ocimum sanctum*.

3.2 Antibacterial activity of the different plant extracts and herbal hand wash

The antibacterial activity of the different plant extracts, herbal hand wash and standard soap solution is summarized in table 1 and 2. The results of well diffusion method has revealed that, citrus juice, eucalyptus leaves and *Sapindus mukorossi* extracts showed broad spectrum antibiotic activity against all the pathogen examined among which citrus juice exhibit higher activity than other extracts (Table 1). *Ocimum sanctum* inhibits the growth of *Salmonella typhimurium* while *Azadirachta indica* stop the growth of *Staphylococcus aureus* only. *Aloe vera* showed no antibacterial activity against any pathogen examined (Table 1).

The antimicrobial activity of the herbal hand wash also checked along with standard soap solution (Table 2). The one well for each 3% SDS, oil and distilled water kept as a control. The standard soap solution Dettol (10% and 1%), was kept as positive control. Results revealed that all the herbal hand wash showed antibacterial activity better than commercially available standard soap solution. Zone of inhibition of 10% dettol is lower than the other herbal soap solution. 0.1% dettol showed no zone of inhibition against any bacteria. Thus all the herbal hand wash formulation showed potential antibacterial activity (Table 2). Among the three different herbal hand wash; those prepared from only plant extracts exhibit better antibacterial activity than other herbal hand wash made from SDS and saponification of oil (Table 2).

The comparative study of the antibacterial activity of herbal hand wash and the individual plant extracts showed that combination of extracts that is herbal hand wash giving better activity than individual plant extracts (Table 1 and 2)

4. TABLES

Table 1 Zone of growth inhibition (cm) showing antibacterial activity for different plants extracts against pathogenic bacteria

Plant material	Zone of inhibition in cm		
	<i>S.aureus</i>	<i>K.pneumoniae</i>	<i>S.typhimurium</i>
<i>Ocimum sanctum</i>	--	--	1.37±0.15
<i>Aloe vera</i>	--	--	--
<i>Sapindus mukorossi</i>	1.13±0.12	1.20±0.14	1.10±0
<i>Azadirachta indica</i>	1.33±0.42	--	--
<i>Citrus lemon</i>	2.80±0.26	1.63±0.32	2.13±0.06
<i>Eucalyptus</i>	1.50±0.26	1.13±0.06	1.70±0.10

Table 2 Zone of growth inhibition (cm) showing antibacterial activity for different herbal hand wash formulations and standard liquid soap against pathogenic bacteria

Handwash	Zone of inhibition in cm		
	<i>S. aureus</i>	<i>K.pneumoniae</i>	<i>S.typhimurium</i>
Formulation I	2.07±0.06	1.2±0.15	2.53±0.06
Formulation II	1.27±0.06	--	2.03±0.06

Formulation III	2.53±0.06	1.00±0.08	2.40±0.00
10% Dettol	1.13±0.15	--	1.03±0.06

5. DISCUSSION

Many medicinal plants have been found effective in the cure of bacterial diseases, and has diverse medicinal properties³. Due to increasing antibiotic resistance in microorganisms and side effects of synthetic antibiotics; medicinal plants are now gaining popularity in the treatment of bacterial infections¹⁴. To prevent the bacterial infection it is important to protect skin especially hands from bacterial pathogens as they are the most exposed part of the body.

Therefore herbal hand wash was formulate, which has no side effect and with potential antibiotic activity. Formulating the hand wash; saponification was done by three different methods (Formulation I, II and II) explain in methodology section and plant extracts were added externally. Plants were selected on the basis of their reported biological activity and their traditional use in Indian medicine. Reetha traditionally used as a detergent¹⁵ and saponin extracted from this fruit were used to formulate hand wash III. *Aloe vera* used for its moisturizing activity in many cosmetics¹⁶. *Ocimum sanctum*^{5,17}, eucalyptus^{11,10,18}, *Azadirachta indica*^{8,19} reported to have anti bacterial activity. In addition to this *Ocimum sanctum* and eucalyptus gives the unique aroma to the herbal hand wash formulation. Plants are rich in potential secondary metabolites, such as flavonoids, tannins alkaloids polyphenols ext. These compounds have diverse biological activity including antibacterial activity³. Most of the polyphenols are extracted in the methanol²⁰, therefore methanol was used as solvents for extraction in the soxhlet apparatus.

Antibacterial activity of the each plant extracts, and three herbal soap solutions were tested against the skin pathogen *Salmonella typhimurium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*. The results suggested that herbal extracts in mixture giving higher activity than the individual extracts. The combination of the antibacterial compounds from different plant extracts may show synergistic effect enhancing their antimicrobial activity. *Sapindus mukorossi* (*Reetha*) which is used as surfactant and as a detergent in formulation III. *Ocimum sanctum* selectively inhibits the growth of *Salmonella typhimurium* (Table 1) while *Azadirachta indica* stop the growth of *Staphylococcus aureus* (Table 1). When these extracts combined to formulate the herbal hand wash antibacterial activity enhance. These results were also compared to the commercially available soap solution 'dettol'. The results suggested that

herbal soap solution has higher antibacterial activity than 10 % dettol.

Hand wash was also made using SDS as base and plants extracts were added as herbal ingredients (Formulation I). The antibacterial activity of these formulations was less than the formulation III (Table 2). This may be because the SDS may interfere the activity of the extracts. Hand washes also prepared by saponification of the fatty acid that is Cummins seeds oil with sodium hydroxide and the plant extracts were added as herbal ingredients. The antibacterial activity of these hand wash also showed lesser activity than the hand wash using only extracts (Table 2). In this case the potassium hydroxide used for the saponification of the fatty acids (Oils) may interfere in the antibacterial activity. Thus Herbal hand wash prepared with only plant extracts showed maximum antibacterial activity also because of only plant extracts used it has no side effects like skin rash and dryness.

Herbal hand wash formulations prepared by three methods has unique aroma may be due to *eucalyptus* and *Ocimum sanctum* extracts. It also has moistening property reducing the skin dryness due to *Aloe vera*. As these formulations are made from only plants it is safe for human use.

6. CONCLUSION

All the herbal hand wash formulations showed more or less antibacterial activity, among which hand wash made only from plant extracts, using reetha (III) showed higher antibacterial activity. It also has moistening property reducing the skin dryness. Thus quality enhanced herbal hand wash formulation with unique aroma and potential antibacterial activity was prepared.

7. ACKNOELEDGEMENTS

I want to acknowledge Dr. M. P. Ghatule, Principle, Sinhgad College of Science, for providing all the necessary equipments.

8. REFERENCES

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