

DEVELOPMENT & ANTIBACTERIAL CHARACTERIZATION OF MINT HANDWASH

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Abstract

The primary and foremost modes of transmission of bacterium are our hands. Hand hygiene is the single most important, simplest, and least expensive means of preventing nosocomial infections. Proper Hand Washing is the most important means of preventing the spread of infection. Hence it brings us to the use of antiseptic for hand washing purposes. The present research was aimed to evaluate the anti-microbial efficacy of herb and plant material such as *Mint* (*Mentha* sp.) and *Azadirachta indica* by agar-plate diffusion method of these plant extracts that were obtained by soxhlet extraction. Also the research was carried out to formulate and evaluate the herbal hand wash liquid containing the above extracts. The anti-microbial activity of the formulated hand wash gel was tested against *E. coli* and *S. aureus* by Agar plate diffusion method. Thus this work suggests and supports the incorporation and utilization of herbs and traditional plant materials in the formulation to give better anti-microbial effect. Results revealed that Mint containing soap formulation was more efficient in reducing the number of organisms from hands thus it can be used as an antiseptic soap with less or no side effects.

Keyword: *Azadirachta indica*, *Mint* (*Mentha* sp.), hand wash.

INTRODUCTION

Plants play a very important role in maintaining the health of humans as they are rich in a wide variety of secondary metabolites with different therapeutic effects. The secondary metabolites like alkaloids, tannins, terpenoids, flavonoids, glycosides and phenolic compounds have proven for their *in vitro* antimicrobial properties. Now a day's microbial resistance is emerging as an issue in hospital care outcome whereas the transfer of bacteria from the hands to food, objects, or people plays a vital role in spreading disease [1, 2].

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. To protect the skin from harmful microorganisms and to prevent spreading of many contagious diseases, hand washing is absolutely an important precaution. Food production workers and foodservice personnel must be taught to use correct hand and fingertip washing by management in preparation for work [3].

Although good & simple hygiene technique is single most important, easy and least expensive means of preventing health care-associated (nosocomial) infections and the spread of antimicrobial multidrug resistance; but, unfortunately poor hand-hygiene practices are still observed due to lack of scientific knowledge, unawareness of risks and unavailability of hand-hygiene facilities [4].

In this study, we evaluated. Mints (*Mentha* sp.) are famous aromatic and medicinal herb that are used in traditional and folk medicines in the world for the antimicrobial and antioxidant properties. *Mentha* genus contains about 25 species and some hybrids and belongs to the *Lamiaceae* family [4]. Mints contain volatile components, flavonoids, organic acids, quinones, such as for the digestive system, central nervous system, respiratory system [5].

Azadirachta indica (Neem) Neem possesses a wide spectrum antibacterial activity and has been extensively used in Ayurveda, Unani and Homoeopathic medicine. Citrus flavonoids have a large spectrum biological activity including antibacterial, antifungal, and antioxidant activity [6].

MATERIALS AND METHOD

Collection of plant material

Fresh leaves of Mints (*Mentha* sp.) plant collected from Botanical garden of Shri Ram Murti Smarak College of Engineering and Technology. The leaves were washed under running tap water to eliminate dust and other foreign particles. Dried leaves were pulverized and taken for extraction and further studies. [13]

Authentication of plants

The leaves of Mint (*Mentha* sp.) and *Azadirachta indica* (Neem) were identified and authenticated by the Department of Pharmacognosy. The Herbarium specimen (No. 172 and 176) of plant was deposited for future use.

Preparation of leaf extracts

50g of each powder Mint (*Mentha sp.*) and *Azadirachta indica* (Neem) was extracted with methanol 250 ml in Soxhlet apparatus for 24 hours. The extract was concentrated, dried on water bath. The weight of extract was taken and % yield was calculated and mentioned in Table 1.

Culture media preparation

Nutrient Agar (for bacterial cultivation, Standard antibiotic (Amoxicillin) was purchased from Hi-Media Laboratories Pvt. Ltd., Mumbai. Culture of microorganisms (overnight incubated) and alcohol were of lab grade. Pathogens selected for evaluation of anti-microbial activity of the test drug were specified in table.

Preparation of gel base

Carbapol-940 gel base was soaked in distilled water for 60 mins. The polymer was swelled and stirred using a mechanical stirrer (instrument name) to ensure the uniform dispersion. The pH of gel base was neutralizing to 7.0 by using Triethanolamine. [16] Selected concentration is mentioned in Table 4.

Preparations of the hand wash (7)

Mint (*Mentha sp.*) and *Azadirachta indica* (Neem) based extracts were incorporated with different concentrations in the gel base as per the requirement of standard procedure for preparation of hand wash (fig.1). The solution was made homogenous using homogenizer under room temperature. The formulated herbal hand wash was used initially for physical evaluation, and then for screening for antimicrobial sensitivity and checked for stability. The formula for the preparation of hand wash was shown in Table 5.



Fig. 1. Formulated mint containing hand wash

Table 1: Preparation of the hand wash.

S.NO.	INGREDIENTS	I	II	III
1	Carbapol gel base (0.5%)	18g	18g	18g
2	Sodium Lauryl sulphate (g)	0.5%	0.5%	0.5%
3	<i>Mint and Azadirachta indica</i> (Neem) extract(1:1)	1%	2%	2.5%
5	Methyl paraben sodium (ml)	1	1	1
6	Glycerine (ml)	5	5	5
7	Triethanolamine	q.s	q.s	q.s
8	Distilled water	q.s	q.s	q.s

Evaluation of formulated herbal hand wash

Physical evaluation of formulated herbal hand wash [8]

Formulated hand was evaluated for the color, odor, pH, viscosity, spreadability, homogeneity, Skin irritation test and Stability. The observations mentioned in Table 6.

pH

The pH of herbal hand wash was found to be 6.8 and pH was determined by using digital pH meter. The measurement of pH of each formulation was done in triplicate and average values were calculated.

Viscosity

50ml of herbal hand wash was taken into 100ml of beaker and the tip of viscometer was dipped into the beaker and its viscosity was measured using digital Brookfield viscometer. The viscosity range of hand wash was found to be 40–120 mP.

Determination of Spreadability

The 0.01 g of the formulated hand wash was placed between two glass plates and diameter was measured after 1 minute and the spreadability of formulated hand wash was good.

Determination of Homogeneity

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

Primary Skin irritation test

Primary skin irritation test was planned to perform on human volunteers, for each gel, five volunteers were selected. 1.0g of formulated gel 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.

Stability

The stability studies were carried out by storing the formulation at different temperatures like 25°C, 37°C & 40°C for 7 days. Stability studies carried shown no change in color and no phase separation was observed in the formulated hand wash. Stability studies of gel were done according to the International Conference on Harmonization (ICH) harmonized guidelines (ICH guidelines 8th, 2003). [17]

Table 2: Physical Evaluation Herbal hand Wash

Herbal Hand Wash	Color	pH	Viscosity (Cps)	Spredability (gm. c/sec)	Homogeneity	Skin irritation test	Stability
I	Green	6.76±0.056	95±0.57	12±0.56	Homogenous	Nil	Well
II	Green	6.66±0.057	96±0.57	13±0.58	Homogenous	Nil	Stable
III	Green	6.66±0.057	98±0.57	13±0.58	Homogenous	Nil	Stable

*Mean ± S.D. value

EVALUATION OF ANTIMICROBIAL ACTIVITY

The organisms used were *Staphylococcus aureus* (Gram +ve) and *Escherichia coli* (Gram -ve). They were obtained from the Department of Microbiology, Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly, and standard antimicrobial used was Amoxicillin.

Determination of Antimicrobial activity by Agar plate diffusion method [9]

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. In these well, all the three formulated herbal hand wash of different concentrations were individually loaded. The cavities was numbered from 1 to 4 filled with Amoxicillin, herbal hand wash having concentration 1%, 2%, 2.5% respectively. 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 Amoxicillin (10µg/ml).

Table 3: Zone Inhibition of Herbal Hand Wash with *Escherichia coli*

Zone Inhibition	Std. Amoxicillin	Hand Wash (1%)	Hand Wash (2%)	Hand Wash (2.5%)
Mean	29.25	15.25	17.66666667	25.33333333
Sem	0.692218655	0.692218655	0.666666667	0.527046277

Table 4: Zone Inhibition of Herbal Hand Wash with *Staphylococcus aureus*

Zone Inhibition	Std. Amoxicillin	Hand Wash (1%)	Hand Wash (2%)	Hand Wash (2.5%)
Mean	28.25	17.25	14.66666667	25
Sem	0.588075959	0.588075959	0.682218552	0.40824829

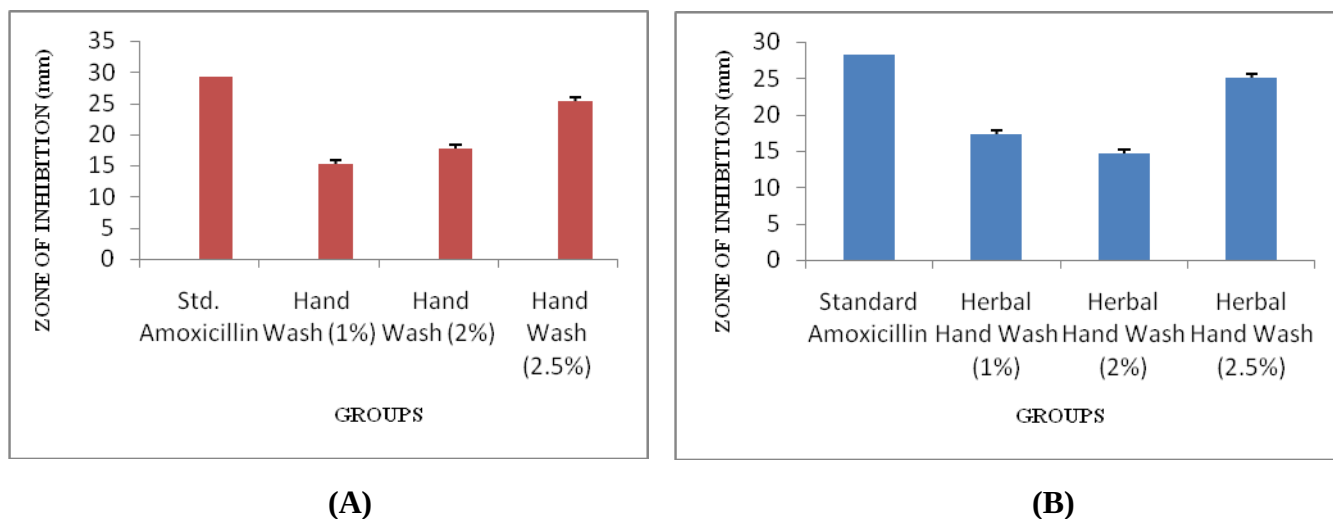


Fig 1: Graphical representation of Zone inhibition of Herbal Hand washes with (A) *Escherichia coli* (B) *Staphylococcus aureus*

Zone Inhibition	Std. Amoxicillin	Hand Wash (1%)	Hand Wash (2%)	Hand Wash (2.5%)
<i>Escherichia coli</i>	29.25	15.25	17.66666667	25.33333333
<i>Staphylococcus aureus</i>	28.25	17.25	14.66666667	25

Table 5: Combine Zone Inhibition of Herbal Hand Wash with *Escherichia coli* and *Staphylococcus aureus*

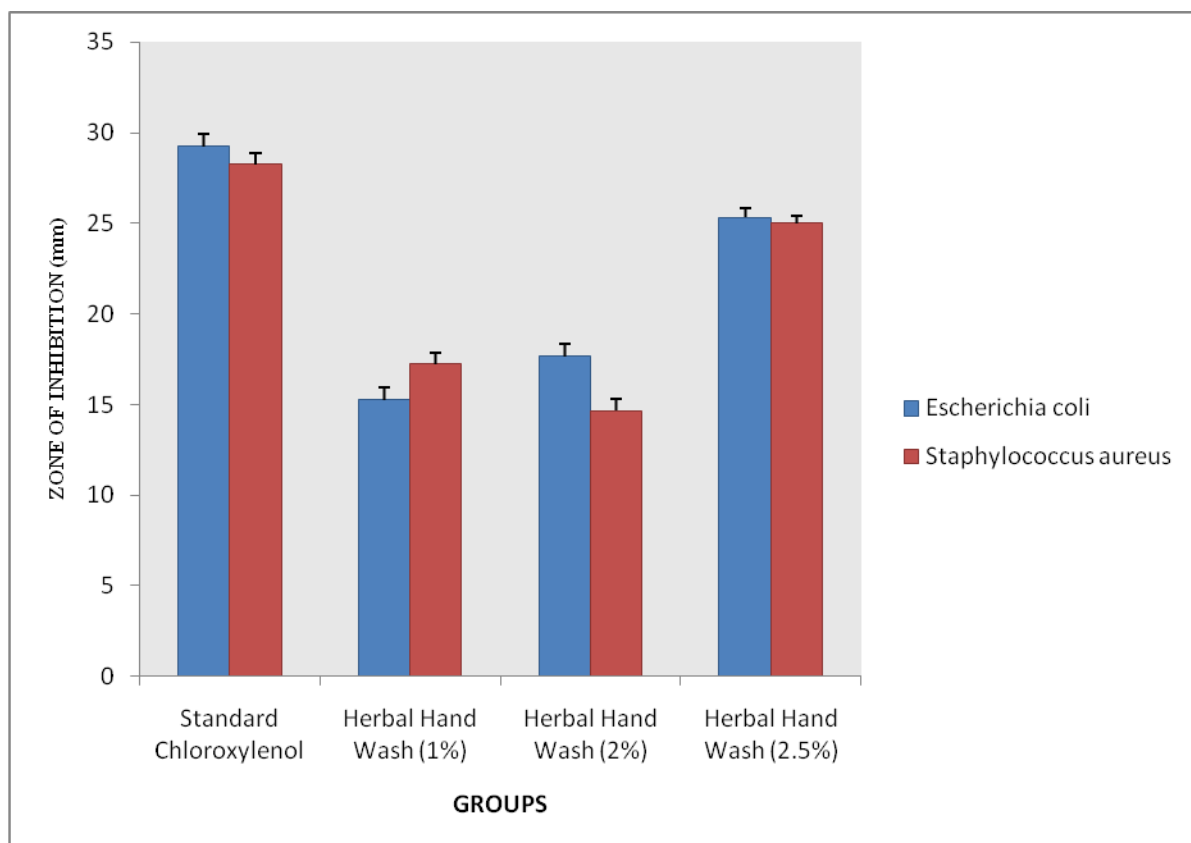


Fig 2: Graphical representation of Zone inhibition of Herbal Hand washes with combination of *Escherichia coli* and *Staphylococcus aureus*

Conclusion

Purpose of hand hygiene is sufficient prevention of microbial counts on the skin to prevent cross-transmission of microorganism. It is easier to keep the hands clean than to make them clean. The critical density of microorganisms on the hands needed for the spread of pathogens remains unknown and it may depend on the type and duration of contact, the type of microorganism, the patient's resident flora and their colonization resistance. According to recently revised hand-hygiene guidelines, the use of an herbs based hand rubbing is the preferred method of hand hygiene. Formulated mint containing herbal hand wash is safe, effective and with no adverse skin reactions to humans. The formulated hand wash has good antibacterial effect against *Staphylococcus aureus* and *Escherichia coli* on surface of hands. Extracts of mint and neem in this study demonstrated a broad-spectrum activity due to the identified alkaloids; diterpenoids, flavonoids and tannins further confirm its use as a health remedy in popular medicine. With reference to all findings done the 2.5 % concentration of the herbal hand wash shown better antimicrobial activity due to combined activity of phytoconstituents present in the extracts with no side effects on human tissue and proven for the sake of hygiene.

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