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Research Article

Comparison and Evaluation of Antimicrobial Activity of Mimosa pudica, Azadiracta Indica and Citrus Limon in Aqueous Based Hand Wash With Marketed Herbal Products

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ABSTRACT

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The aim of this study was to prepare poyherbal handwash formulations using ethanolic extracts of *Mimosa pudica* (Touch me not), *Azadirachta indica* (Neem), and lemon extract. Two hand wash formulations were prepared, and the formulations were assessed for physical attributes such as appearance, pH, and viscosity. *Bacilus subtilus, Staphylococcus aureus, Psuedomonas aeruginosa* and *Escherichia coli*, these skin pathogens were being used to investigate the antimicrobial activity of hand wash formulations by agar diffusion method. The results revealed that prepared herbal hand wash formulations showed significant zone of inhibition compared with marketed herbal product. As a result, these plant elements can be used to make herbal hand wash on a commercial basis.

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INTRODUCTION

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 infections. Hand hygiene is the single most important, simplest, and expensive mean of preventing least nosocomial infections. Hand washing is the act of cleaning hands with the purpose of removing soil, dirt, pathogenic microorganisms and avoid transmitting of transient microorganism. Hand Washing removes visible dirt from hands and reduces number the ofharmful microorganisms E.coli such as and salmonella can be carried by people, animal or equipment & transmitted to food. To defend the skin from harmful microorganism and to avoid spreading of contagious diseases, numerous hand washing is extremely significant precaution. (1-4)

Historically, plants have provided a good source of anti-infective agents. Plant extract have a potential as antimicrobial compounds against several pathogenic microorganisms which cause infections disease and resistance towards synthetic drugs. The main advantage of using natural source is that they are easily available cheap & harm less compared to chemical products. (5-6)

Antimicrobial properties of certain Indian

medicinal plants were reported based on folklore information and only few reports are available on inhibitory activity against certain pathogenic bacteria and fungi. Use of plants as source of medicine has been inherited and is an important component of the health care system in India. In these systems of Indian medicine, most practitioners formulate and dispense their own recipes; hence this requires proper documentation and research.7

Mimosa pudica L. is a creeping annual or perennial herb. It has been identified as lajjalu in Ayurveda and has been found to have antiasthmatic, aphrodisiac, analgesic, and antidepressant properties. M. pudica is known to possess sedative, emetic, and tonic properties, and has been used traditionally in the treatment of various ailments including alopecia, diarrhea, dysentery, insomnia, tumor, and various urogenital infections. The herb has been used traditionally for ages, in the treatment of urogenital disorders, piles, dysentery, sinus, and also applied on wounds. (8-9) Mimosa pudica L. (neem) shows therapeutics role in health management due rich source of various types of ingredients. Neem plays role as free radical scavenging properties due to rich source of antioxidant. Azadirachtin and nimbolide showed concentration-dependent antiradical scavenging activity and reductive potential in the following order:

nimbolide > azadirachtin > ascorbate .10

The antimicrobial activities of Citrus plants oil and extracts were investigated, the effect of essential oils from C. aurantium, C. limon, C. paradisi and many other plant oils and extracts and found that the mini-mum inhibitory concentrations (MIC) were between 5-2% (v/v).11

Therefore, it is reasonable to expect a variety of plant compound in these extract specific as well as general antimicrobial activity and antibiotic potential. They have antimicrobial properties against E.coli, Bacillus, and Pseudomonas aeruginosa. Staphylococcus, Streptococcus and Candida etc. The cause of the antimicrobial properties believed to be disruption of the bacteria membrane.

MATERIALS AND METHODS

Plant materials and extraction

The plants Mimosa pudica and *Azadirachta indica* were collected from the garden area of SJCPS, Naharkanta, Bhubaneswar and verified in the Department of Pharmacognosy at SJCPS, Bhubaneswar.

Preparation of herbal leaf extracts¹²

About 0.5 kg of fresh plants leaves (*Mimosa pudica* and *Azadirachta indica*) were collected in bulk, washed under running tap water to remove adhering dust, dried under shade and powdered. 10gm of coarsely powdered leaves of both plants

were soaked in 200 mL of ethanol and kept for maceration for about 3-4 days. After maceration the extract is filtered and the filtrate was collected and used for making hand wash.

Preparation of Lemon Peel extract13

The plants used in this study were Citrus limon (common name: Lemon). The peels were collected from the local fruit juice shops. After collection, the peels were shade dried at room temperature (32 -35°C) to constant weight over a period of 5 days. 15g of the plant parts was coarsely powdered using a mortar and pestle and were further reduced to powder using an blender. electric The powder was transferred into closed containers. 15g of the powdered plant was soaked in 200 ml of distilled water at ambient temperature for 24 h under shaking condition at 130 rpm. The extract was then filtered using Whatman filter paper No 1 .Extract was transferred to glass vials and kept at 4 °C before use.

Preparations of Herbal Hand Wash Formulations¹⁴

Formulation 1 (S-1)

In this formulation the hand wash was prepared using 20 mL of Ethanolic extract filtrate. To this filtrate 6g of SLS, glycerin 40 ml, 0.3 g of Propyl paraben, 1.5 g of HEC, 5ml of rose merry oil is added and the volume is made up to 100ml with purified water (Table-1).

Formulation 2 (S-2)

This formulation was prepared by adding 20 ml of lemon extract to 20 ml of ethanolic extract filtrate of *Mimosa pudica*

and *Azadirachta indica* leaves. The remaining ingredients include all the same as mentioned above in formulation 1 (Table-2).

Table 1: Formulation of Poly herbal hand wash S-1

INGREDIENTS	QUANTITY	
Ethanolic extract of <i>Mimosa pudica</i> and <i>Azadirachta indica</i>	20 ml	
Lemon peel Extract	20 ml	
Sodium lauryl sulphate (SLS)	6 g	
Glycerin	40 ml	
Hydroxyethyl Cellulose	1.5 g	
Propylparaben	0.3 g	
Rose merry oil	5 ml	
Purified water q.s.	100 ml	

Table 2: Formulation of Poly herbal hand wash S-2

INGREDIENTS	QUANTITY
Ethanolic extract of <i>Mimosa pudica</i> and <i>Azadirachta indica</i>	20 ml
Sodium lauryl sulphate (SLS)	6 g
Glycerin	40 ml
Hydroxyethyl Cellulose	1.5 g
Propylparaben	0.3 g
Rose merry oil	5 ml
Purified water q.s	100 ml

Evaluation of Antimicrobial Activity¹⁵⁻¹⁶

The screening of anti-microbial efficacy of the formulated poly herbal hand wash was performed on various microorganisms by using Agar plate method as per standard procedure. Four sterile petri plates were taken for testing the anti-microbial activity against four different microorganisms *Bacillus subtillis*, *Staphylococcus aereus*, *Psuedomonas aeruginosa* and *Escherchia*

coli. The plates were filled with nutrient agar solution and allowed for solidification. After solidification the microorganisms from the subculture were inoculated into the nutrient agar media and three cavities were made in it. The first cavity is filled with standard marketed product (Patanjanli Anti-bacterial herbal hand wash/ Sri Tattva Kleanup handwash), second one with herbal hand wash without lime extract(S-1) and third cavity is filled with herbal hand wash with lime extract (S-2). It was taken care that sample should be placed at the level of cavity. The plates are placed in incubator at 37°C to test the activity. After 24 hours the plates were observed for the formation of zone of inhibition. From the zone of inhibition the anti-microbial activity of formulation is estimated.

Physical Evaluation

Physical evaluation (color, dour) was done by sensory and visual inspection and compared with the marketed hand wash gel. (Patanjanli Anti-bacterial herbal hand wash/ Sri Sri Tattva Kleanup handwash)

Grittiness

1ml of hand wash was taken on finger tips and rubbed between two fingertips then the formulation was evaluated.

рΗ

One gram of sample of poly 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 in triplicate.

RESULTS AND DISCUSSION

Physical Evaluation

The prepared two formulations of hand wash appear as greenish brown and greenish yellow colour.

Grittiness

No grittiness was found and gave smooth on application.

рΗ

The pH of formulations was measured by digital pH meter. The pH of two formulations was found to be 6.3 and 6.2.

Viscosity

The viscosity of hand wash was determined by using Brookfield viscometer. The viscosity of S-1 and S-2 was found to be 64 and 72 CPS.

Antimicrobial Activity

The Anti-microbial efficacy of the formulations of Polyherbal Hand Wash was tested on Staphylococcus aureus, Psuedomonas aeruginosa, Bacillus subtillis and Escherichia coli by agar plate technique. The results of zone of inhibition (Table 3) showed that the hand wash

prepared from ethanol extract of the combined plant materials shown significant antimicrobial activity (Fig-1). The hand wash prepared with lemon extract (S-2)

showed little higher activity than the formulation prepared without lemon extract (S-1).

Table 3: Antimicrobial Activities of Polyherbal Hand Wash Formulations

MICROORGANISM	ZONE OF INHIBITION (in cm)			
	MARKETED FORMULATION	S1	S2	
Bacilus subtilus	1.5	3.5	3.9	
Staphylococcus aureus	2.8	3.9	4.5	
Psuedomonas aeruginosa	2.5	3.7	4.4	
Escherichia coli	2.0	3.5	3.7	

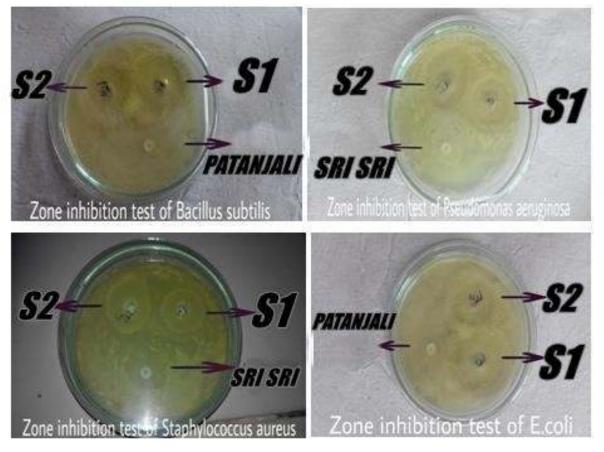


Figure 1: Plates Showing Zone of Inhibition for Different Organisms

CONCLUSION

Natural remedies are more acceptable in the belief as they are safer with fewer side effects than the synthetic ones. Herbal formulations have emergent demand in the global market. An attempt was made to formulate the herbal aqueous handwash using ethanolic extract of Azadiracta indica. Mimosa pudica and combinations with lemon with comparison of marketed herbal formulation. The results suggest that ethanolic extract of Azadiracta pudica indica. Mimosa and their combinations with lemon extract are capable of giving superior zone of inhibition to protect against the skin pathogens.

Hence it can be concluded that this herbal aqueous hand wash provide an effective and safe alternative to existing marketed synthetic hand wash.

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