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FORMULATION AND EVALUATION OF A POLYHEBAL HAND WASH

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ABSTRACT

Plants have enormous medicinal, pharmaceutical and cosmetic potential and can be explored and exploited in the form of innovative products for human use. Hand hygiene is of prime importance in order to avoid infections. ***Objective:*** *The commercially available chemical hand washes pose different side effects like dryness and skin irritation upon long term use. In the present study, different poly herbal hand washes were formulated using herbal drug extracts.* ***Methods:*** *methanolic neem (Azadirachta indica) leaf extract, tulsi (Ocimum sanctum) leaf extract, orange (citrus sinensis) peel extract and eucalyptus oil (Eucalyptus obliqua (nilgiri) were obtained using soxhlet apparatus. Carbopol 934 was used as gel base. The antibacterial activity of formulated herbal hand washes was evaluated using agar well diffusion method against common skin pathogens Staphylococcus aureus (s.aureus), Klebsiella pneumonia, Escherichia coli & Bacillus subtilis.* ***Results:*** *Though all four herbal hand wash formulations showed significant antibacterial activity, formulation I was more effective against Klebsiella pneumonia and formulation IV was more effective against Staphylococcus aureus in comparison to commercial hand wash formulation.* ***Conclusion:*** *These herbal hand wash formulations can subside the use of chemical hand washes. In addition these formulations possess natural aroma therefore assumed to be more consumer attractive products.*

KEYWORDS*: Azadiracta indica, Ocimum sanctum, Citrus sinensis, Eucalyptus obliqua, anti microbial activity.*

INTRODUCTION

Hand hygiene is the prime criterion to subside infectious diseases. This is achieved by use of antiseptic agents [1]. The chemical antiseptic hand washes available in the market are often alcohol based and are mainly chlorohexidine products. Though highly potent, these cause skin irritation, dryness and also result in development of resistant pathogens [2]. The traditional methods of curing diseases include utilization of plant extracts [3]. Secondary metabolites that are produced in plants like alkaloids, terpenoids, tannins, phenols, flavanoids [4] etc through different mechanisms show antimicrobial activity [5].Research is now being focused on to herbal extracts with potential antimicrobial activity.

Ocimum sanctum is traditionally used for its antimicrobial properties [6, 7]. *Azadirachta indica* has a broad spectrum of antimicrobial activity [8]. The active constituents of citrus peel showed extensive antimicrobial activity apart from flavoring properties [9]. *Eucalyptus* is an important medicinal plant in having unique aroma with a significant anti microbial activity against common human pathogenic microorganisms [10]. Despite of wide spread references for antibacterial activity of these plant extracts, there is no report on formulation of herbal hand wash using them. This study aims to prepare different herbal hand wash formulations involving these herbal extracts and subsequent evaluation of prepared formulations for antimicrobial activity by comparison with commercially available synthetic hand wash. The success of herbal formulation will mitigate the side effects of synthetic hand washes.

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MATERIALS AND METHODS

Collection of plant materials:

Fresh and disease free leaves of tulasi, neem and Eucalyptus were collected from local area around Dundigal village, outskirts of Hyderabad, Telangana state in the month of November. Citrus fruits were purchased from local market and peeled. The plant materials collected were authenticated by the Dept. of Pharmacognosy. The plant materials were washed with distilled water and shade dried.

The chemicals used for extraction, phytochemical screening, hand wash formulation & antimicrobial evaluation were procured from SD fine chemicals, Hyderabad.

Preparation of plant extracts:

The dried plant parts were finely powdered individually by using a blender. 50 g of each powder was extracted with 250 ml of methanol at 65^{0}C using Soxhlet apparatus. The obtained extracts were concentrated under vacuum on a rotary evaporator until a viscous mass was obtained. The extracts were stored at 4 °C in the refrigerator until they were used. Further, all the plant extracts were screened for phytochemical constituents and different formulations were developed.

Preliminary phytochemical screening:

Phytochemical screening of the plant extract was carried out to know the presence or absence of various phytochemical constituents by established procedures [11].

Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates

Ferric chloride test: Each extract (50 mg) was dissolved in 5 ml of distilled water and few drops of 5% ferric chloride were added. Bluish black colour indicated the presence of tannins.

Potassium dichromate test: To the extract add 5% potassium dichromate solution. Positive result for phenol was confirmed by a formation of brown precipitate.

Alkaline reagent test: Few drops of sodium hydroxide were added into the extracts to give intense yellow colour. The disappearance of colour after addition of dilute hydrochloride acid showed the presence of flavonoid.

Salkowski's test: The extract (0.5 mg) was added with few ml of chloroform followed by concentrated sulphuric acid to form a layer. Reddish brown colour at the interface indicated the presence of terpenoids.

Leibermann's Reaction: 3 ml. of extract was mixed with 3 ml. of acetic anhydride. The test solution was then heated and cooled. A few drops of conc. Sulfuric acid were added to the test solution. Appearance of blue colour shows the presence of sterols.

Froth test: Each extract (50 mg) was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. The development of two cm layers of foam indicated the presence of saponins.

Wagner's test: About 50 mg of extracts was stirred with few ml of dilute hydrochloric acid and filtered. Then, few drops of Wagner's reagent were added at the side of the test tube. The formation of reddish-brown precipitate showed the presence of alkaloids.

Keller-Kiliani'stest: A small amount of extract (50 mg) was treated with 2 ml of glacial acetic acid containing one drop of 5% ferric chloride, followed by addition of 1 ml of concentrated sulphuric acid. A brown ring at interface is characteristic of cardenolide deoxysugar. Appearance of the violet ring below the brown ring and greenish ring in acetic acid layer indicated the presence of cardiac glycosides.

Biuret test: Each extract (50 mg) was diluted with distilled water and treated with Biuret reagent. The appearance of pink colour indicated the presence of protein. The results of phytochemical screening are tabulated in table 1

Preparation of hand wash:

To prepare the hand wash formulation of proper consistency a gel base is used for better pourability and easy to use.

Preparation of gel base:

Carbopol-940 gel base was soaked in distilled water for 1 hr. The polymer was swelled and stirred using a homogenizer to ensure the uniform dispersion. The pH of gel base was neutralized to 7.0 by using Triethanolamine[12, 13] prior to incorporating the methanolic extracts in the base .The extracts of *Ocimum sanctum* (tulasi), *Azadirachta indica (Neem), Eucalyptus spp. (nilgiri) and citrus sinensis* (orage peel) in different concentrations were incorporated in gel base along with other excipients for preparation of hand wash. Each hand wash formulation prepared was made homogenous with a homogenizer for further evaluation studies. The formulae for the preparation of hand wash were shown in Table 2.The final volume of each formulation was adjusted to 100 ml with distilled water.

Table No. 2: Preparations of hand wash formulations

Formulation	Gel base	Neem	Tulsi	Citrus	Eucalyptus	Propyl paraben	SLS
I	30%	0.5gm	0.5gm	0.5gm	≤ 1%	≤ 0.1%	2%
II	30%	1gm	1gm	1gm	≤ 1%	≤ 0.1%	2%
III	30%	1.5gm	1.5gm	1.5gm	≤ 1%	≤ 0.1%	2%
IV	30%	2gm	2gm	2gm	≤ 1 %	≤ 0.1%	2%

The formulated herbal hand wash was subjected to stability studies, physical evaluation and antimicrobial evaluaion.10% hand wash solution was used for evaluation of antimicrobial activity using normal saline.

Microorganisms Tested:

In the present study, two gram positive bacteria viz., *Staphylococcus aureus, Bacillus subtilis and* two gram negative bacteria *E. coli, Klebsiella pneumonia* were used for antmicrobial evaluation. The test microbes were collected from Microbiology Lab, Osmania University. The microbial cultures were maintained in nutrient broth at 37 °C and on agar plates at 4°C.The test microorganisms were sub cultured regularly (every 30 days) and refrigerated as per the standard method [14].

Evaluation of formulated herbal hand wash:

Physical evaluation of formulated herbal hand wash: The physical evaluation was carried out on five healthy volunteer groups (n=5) [15]. Volunteers with damaged or wounded skin and those on antimicrobial therapy were excluded. The hand wash formulations prepared were evaluated for colour, fragrance, pH, viscosity, Spreadability, skin irritation test and stability. The results obtained were tabulated.

Colour & Fragrance: Colour and fragrance were tested by organoleptic evaluation by the volunteer groups and the results were tabulated in table 3.

pH:The measurement of pH of each formulation was done by using digital pH meter in triplicate and average values were calculated.

Viscosity: 50ml of herbal hand wash was taken in a 100ml of beaker and the tip of viscometer was dipped in the solution and its viscosity was measured using digital Brookfield viscometer. The viscosity range of hand wash was found to be 60–100 m Pascals.

Determination of Spreadability: The extent of spreading of the formulations was evaluated using standard procedure [16]. 0.01 g of the formulation was placed between two glass plates (16 x 16) and the spread of gel was measured after 1 minute.

Skin irritation test: About 1.0 gm of formulated hand wash was applied on an area of 2 square inch on the back of hand. It was covered with cotton and secured firmly with 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 herbal hand wash formulations were exposed to different temperature conditions of 4^0C, 25^0C and 37^0C for a period of four weeks as per ICH guidelines [17]. Every week small quantity was aseptically removed and observed for stability. All the formulations were stable in all climatic conditions which were indicated by absence of phase out of liquid. The results were tabulated in table 4.

Evaluation of antimicrobial activity:

The antimicrobial activity of the prepared formulations was evaluated by standard agar well diffusion method [15].The nutrient agar medium was sterilized and around 25 ml was poured in to petri dishes of 90 mm diameter to give a uniform depth of 4 mm. The agar medium was allowed to solidify at room temperature. Then about 0.1ml of test inoculums was evenly spread using sterile spreader at 35^0C on the surface of agar. Utmost care was taken so that agar was not disturbed. Four wells of 8 mm diameter and 3 mm depth were made on agar plate using a sterile borer. Then 0.1 ml of 10% hand wash formulation was filled in to each well. The plates were incubated at 32°C. The antibacterial activity was determined by measuring the diameters of zones of inhibition. The test was performed in triplicate with Methanol and 10% standard marketed hand wash controls as Negative and Positive controls respectively. The results were tabulated in table 5.

RESULTS AND DISCUSSION

In the present investigation leaf extracts of neem (*Azadirachta indica)*, tulsi (*Osmium sanctum*), orange (*citrus sinensis*) peel extract and eucalyptus oil (*Eucalyptus obliqua*) were used to formulate different herbal hand wash preparations. All the herbal extracts used in the hand wash formulation were observed to contain a wide range of phyto constituents. Phytochemical examination was carried out for all the extracts as per standard methods.

Organoleptic evaluation (colour, odour) was done by sensory and visual inspection and compared to the marketed hand wash.

Fragrance test: It was based on individual observation for its acceptability. 5 volunteer groups were asked for acceptability of fragrance and their opinion was taken. And fragrance was evaluated based on the below-described criteria;

Table No. 2: Phytochemical screening of Methanolic extracts

S.No	Chemical test	Neem	Tulasi	Eucalyptus	Citrus
1.	Alkaloids	+	+	-	+
2.	Flavanoids	-	-	+	+
3.	Phenols &tannins	+	+	+	+
4.	Steroids&sterols	+	+	+	+
5.	Carbohydrates	+	+	+	+
6.	Saponins	+	+	+	_
7.	Glycosides	+	+	-	+
8.	Volatile oils	+	+	+	+
9.	Terpenoids	+	+	+	+
10.	Proteins&amino acids	-	-	+	+

Table No. 3: Organoleptic evaluation of hand wash formulations

S.No	Formulation	colour	odour
1.	Standard	Light blue	pleasant
2.	I	Light green	slight
3.	II	Green	Pleasant
4.	III	Dark green	Pleasant
5.	IV	Brownish green	pleasant

I. Fragrance was good and comparable to the reference hand wash
II. Fragrance was good, as good as the fragrance of reference hand wash.
III. Fragrance was poor than the reference hand wash.
IV. Fragrance was better than all the formulations and as good as the reference hand wash

Table No. 4: Physical evaluation of the developed formulations

S. No.	Formulation	pH	Viscosity	Homogeneity	Spreadability	Skin irritation	Stability
1	standard	7.0±0.1	70 mP	Homogenous	semifluid gel	No	stable
2	I	7.0	65 mP	Homogenous	semifluid gel	No	stable
3	II	7.0	66mP	Homogenous	semifluid gel	No	stable
4	III	7.0	70 mP	Homogenous	semifluid gel	No	stable
5	IV	7.0	72mp	Homogenous	semifluid gel	No	stable

The consistency of all formulations was found to be semi fluid gel (55mm-70mm) and are homogenous in nature.The developed formulations did not exhibit any skin irritation. The viscosity of the formulations is comparable with that of the standard. The formulations were found to be stable over wide temperature ranges of 4^0C, 25^0C and 37^0C.All the formulations showed neutral pH. The physical parameters of all the formulations are as good as the standard marketed formulation.

Antimicrobial evaluation:

Table No. 5: Zone of inhibition of bacteria using different hand wash formulations

Formulation	Zones of Inhibition(cm) (Diluted Sample)			
	S.aureus	E. coli	B.subtilis	K.pneumoniae
Standard	3.5 ± 0.01	1.2 ± 0.01	_	2 ± 0.01
I	3 ± 0.05	1.6 ± 0.04	_	4 ± 0.05
II	2.4 ± 0.11	1.6 ± 0.10	_	2.6 ± 0.02
III	2.6 ± 0.12	1.4 ± 0.12	_	3 ± 0.11
IV	3.6 ± 0.11	1.4 ± 0.12	_	2 ± 0.20

The data represent mean of three replicate ± S.D

10% concentration of the developed formulations and standard were evaluated for antimicrobial activity. From table 5 and fig.1 it is clear that the formulation IV is showing more inhibition of 3.6 cm against *staphylococcus aureus* which is more than the standard

marketed formulation. All the formulations are inhibiting *E.coli* more effectively than the standard. The developed formulation I is showing the highest zone of inhibition of 4 cm against *Klebsiella pneumoniae.* The bacterial strain *Bacillus subtilis* was not inhibited by any of the hand wash formulations. It is clear that the *Bacillus subtilis* strain used has developed resistance.

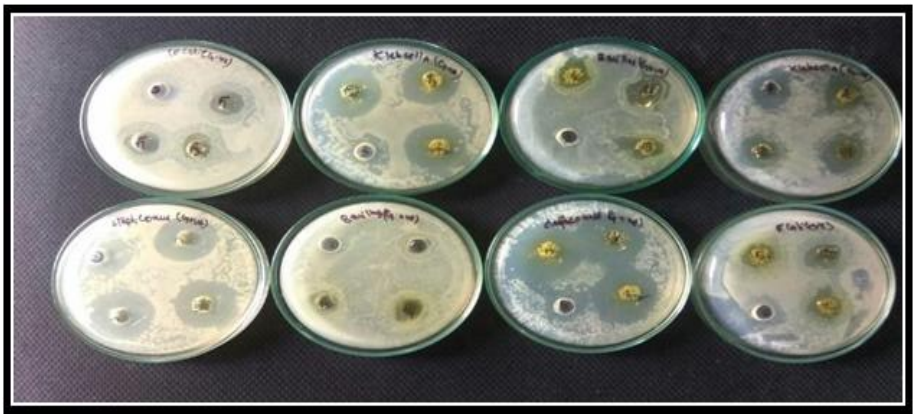

Fig. 1: Inhibition in the Growth of a) *Staphylococcus aureus,* b) *Klebsiella pneumonia,* c) *Escherichia coli* and d) *Bacillus subtilis with different* hand wash formulations.

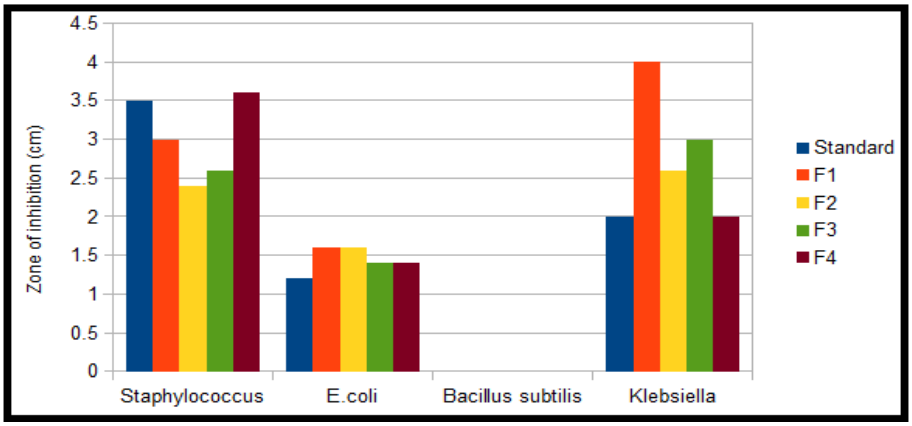

Fig. 2: Inhibition of growth of bacteria using different hand wash formulations and standard hand wash

CONCLUSION

Herbal hand wash formulations developed in the study are effective against *Staphylococcus aureus, Klebsiella pneumonia & Escherichia coli.* These formulations were not found to be effective against *Bacillus subtilis*. The *Bacillus subtilis* strain selected was found to be resistant even to reference chemical formulation .Further work can be carried out to develop a herbal formulation that can show broad spectrum of activity.

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