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## Formulation and evaluation of poly herbal hand wash gel containing essential oils

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### ABSTRACT

The present research was aimed to evaluate the antibacterial efficacy of various herbal oils such as Cinnamon, eucalyptus, orange, peppermint; clove, geranium and rosemary oils found that cinnamon, geranium and peppermint showed better antibacterial, antimicrobial activities. The research was carried out to formulate and evaluate the poly herbal gels. Hand wash gel containing Cinnamon and geranium oil. The anti-microbial activity of the formulated herbal hand wash gel was tested against *Escherichia coli* and *Staphylococcus aureus* by pour plate techniques and the results obtained were compared with commercial antibacterial standards. Also the efficiency was checked by using the hand wash gel on volunteers.

**Keywords:** Herbal hands wash gel, Antimicrobial and Antibacterial activities, Cinnamon and geranium oils poly herbal formulations.

### INTRODUCTION

Hands are primary mode of transmission of microbes and infections [1]. 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 mean of preventing nosocomial infections [2]. Hand washing is the act of cleaning hands with the purpose of removing soil, dirt, pathogenic microorganisms and avoid transmitting of transient micro organism [4]. Hand Washing removes visible

dirt from hands and reduces the number of harmful micro organisms such as *E.coli* and salmonella can be carried by people, animal or equipment & transmitted to food<sup>2</sup>. To defend the skin from harmful microorganism and to avoid spreading of numerous contagious diseases, hand washing is extremely significant precaution [3].

Historically, plants have provided a good source of anti infective agents [5]. Plant extract have a potential as antimicrobial compounds against several pathogenic microorganisms which cause infections disease and resistance towards synthetic

drugs<sup>6</sup>. The main advantage of using natural source is that they are easily available cheap & harm less compared to chemical products [7].

Essential oils are typically liquid, cleared unusually colored, complex and the present compounds are volatile, characterized by a strong order and synthesized by aromatic plants during secondary metabolites [8]. Essential oils have been shown to possess antibacterial, antifungal, antiviral insecticidal and anti oxidant properties. There has been an increased interest in looking at antimicrobial properties of extracts from aromatic plants, particularly essential oils. Therefore, it is reasonable to expect a variety of plant compound in these oils with specific as well as general antimicrobial activity and antibiotic potential.

Essential oils also (also called volatile oils) are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation or extraction. But the method of steam distillation is most commonly used for commercial production. An estimated 3000 essential oils are known, of which 300 are commercially important in fragrance market. Essential oils are complex mixers containing many single compound [9]. 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. In this research work, hand wash gel formulated using essential oils and evaluated.

## MATERIALS AND METHODS

### Chemicals and Reagents

The eucalyptus oil, cinnamon oil, geranium oil, peppermint oil, rosemary oil, clove oil and orange oil were collected from Allin exporter Mumbai and Ooty. Hydroxy propyl methylcellulose, carbopol940 is collected from Himedia laboratories Pvt. Ltd. Secunderabad, sodium lauryl sulphate and glycerin were taken from SDFCL Laboratories, Mumbai. All other reagents / chemicals used were analytical grade. [10]

### Bacterial Sample

Gram positive and Gram negative bacteria i.e. staphylococcus aureus, Escherichia coli were collected from department of microbiology, Osmania University, Hyderabad.

### Media

Nutrient agar

## METHODS

### Standardization of Inoculums

The inocula prepared from the stock culture, were maintained on nutrient agar at 4°C and subcultured on to nutrient broth using sterile wire loop.

### Preparation of Agar plates

In each Petri plate 25ml of agar medium was poured in an aseptic condition and kept at room temperature for solidification at least for 20minutes. [11]

### Selection of Bacteria

Two strains of bacteria as were used for antimicrobial screening namely Staphylococcus aureus (gram positive) E.coli (gram negative) [11]

### Preparation of DMSO solution

Ten milliliters of DMSO was taken along with 0.5% v/v tween80. Volume was made up to using distilled water.

### Determination of minimum inhibitory concentration (MIC)

The MIC is defined as the lowest concentration that completely inhibits the growth of microorganisms for 24hrs incubation. Determination of minimum inhibitory concentration of cinnamon oil, eucalyptus Oil, geranium Oil, rosemary Oil, clove oil, orange oil and peppermint oil was determined by preparing different concentration of oil 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5%, 2.5% and 1.25% . A 10µl volume of each dilution was added aseptically in to the well of Nutrient agar plate that was seeded with the standardized inoculums of the test bacteria. All experiments were performed in triplicate. The agar plates were incubated at 37°C for 24 hrs. The lower concentration of oil showing clear zone of inhibition was considered as the MIC.

[12] (table no.3). Among above oils, only three oils showed high zone of inhibition similarly, to test the synergistic effect, MIC of combination of oils (50:50) tested (table no.4)

### Formulation of Herbal Hand wash Gel

Form literature review, HPMC E-50 and Carbopol 940 were found to be excellent gelling agent for the preparation of hand wash gels.

### Procedure

Various hand wash gel formulations were prepared (table no:1 & 2) using HPMCE-50 and

carbopol940 as gelling agents. The desired concentration of gelling agent, sodium lauryl sulphate, glycerin were measured accurately and dispersed in purified water with moderate stirring. The required quantity of methyl paraben was dissolved in remaining quantity of purified water by gentle heating. Desire quantity of herbal oils added. Triethanolamine was used to adjust the pH. The formulated hand wash gel was filled in collapsible tubes and stored at cool and dry place until further evaluation.

**Table No: 1 Formulation of herbal hand wash gel with HPMCE-50 as gelling agent**

Composition (%w/v)	Formulation code							
	F1	F2	F3	F4	F5	F6	F4	F8
Cinnamon & Geranium Oil (V/V%)	1.25	1.25	1.25	1.25	1.25	1.25	2.5	3.75
HPMC E-50	2	2	3	3	4	4	4	4
Glycerin	1	1	1	1	1	1	1	1
Sodium lauryl sulphate	1	1.5	1	1.5	1	1.5	1.5	1.5
Methyl Paraben	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Purified water up to(In ml)	100	100	100	100	100	100	100	100

**Table No: 2 Formulation of herbal hand wash gel with carbopol940 as gelling agent**

Composition (%w/v)	Formulation code									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Cinnamon & Geranium Oil (V/V%)	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	2.5	3.75
Carbopol-940	0.1	0.1	0.2	0.2	0.3	0.3	0.4	0.4	0.4	0.4
Glycerin	1	1	1	1	1	1	1	1	1	1
Sodium lauryl sulphate	1.5	2	1.5	1.5	2	1.5	1.5	2	2	2
Methyl Paraben	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Purified water up to(in ml)	100	100	100	100	100	100	100	100	100	100

## EVALUATION

### Physical Evaluation

Physical evaluation (color, dour) was done by sensory and visual inspection and compared with the marketed hand wash gel. [13] (Dettol hand wash)

### Grittiness

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

### pH

One gram of sample of poly herbal hand wash gel was taken and dissolved it into 100ml distilled

water. The pH of solution was measured by previously standardized digital pH meter. [14, 15]

### 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. [16]

### Spread ability

A sample of 0.5 g of each formula was pressed between two slides and left for about 5 minutes where no more spreading was expected Diameters of spreaded circles were measured in cm and were taken as comparative values for spreadability. The

results obtained are average of three determinations. [17]

### Foam Height

One gram of sample of hand wash gel was taken and dispersed in 50ml distilled water. Dispersion was transferred to 500ml measuring cylinder. Volume was made up to 100ml with water. 25 strokes were given and kept it aside. The foam height above the aqueous volume was noted. [18]

### Foam Retention

25ml of the 1% hand wash gel was taken into 100ml graduated cylinder. The cylinder was covered with hand and shaken 10 times. The volume of foam at 1 minute interval was recorded for 4 minutes. [18]

### Antibacterial efficiency of herbal hand wash gel on volunteers

The antibacterial efficiency was performed by pour plate method. [19]

### Before hand wash

Seven healthy volunteers groups (n=3) without any clinical signs of dermal abrasion, trauma and

infection were selected for the study. Sterile Petri dishes were serially numbered. Nutrient agar was prepared and pour in to the Petri dishes. Hands are directly kept in agar media. The Petri dishes were incubated in an incubator at 37°C for 24 to 48 hours. [20]

### After hand wash

A One ml of pure herbal hand wash gel was squeezed out and applied on both hands. Hands are gently cleaned using running water. Hands are directly kept in agar media. The Petri dishes were incubated in an incubator at 37°C for 24 to 48 h. [20]

### Stability

The stability studies were carried out for all the gel formulation by freeze - thaw cycling. Here, by subjecting the product to a temperature of 4° C for 3 month, then at 25°C for 3 month and then at 40°C for 3 month and studied for appearance, pH, viscosity and spreadability. [21]

## RESULTS AND DISCUSSIONS

**Table No: 3 Anti bacterial activity of different herbal oil**

Name of the Oil	MIC (%)		Zone of Inhibition (mm)	
	E. coli	S.A	E. coli	S.A.
Orange Oil	20	30	6.5	10
Eucalyptus Oil	40	20	8	6.5
Geranium Oil	2.5	1.25	5.5	7.5
Peppermint Oil	2.5	2.5	8	7.5
Rosemary Oil	10	20	5.5	7
Clove Oil	10	5	6	8
Cinnamon Oil	1.25	1.25	7	6.5

**Table No: 4 Anti bacterial activity of combination of herbal oils**

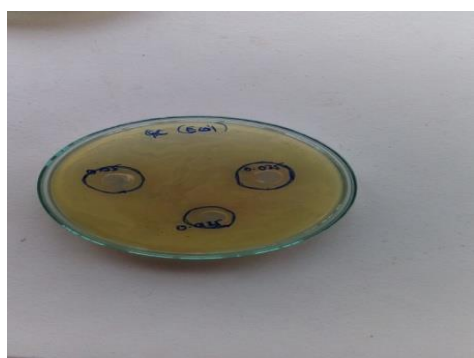
Combination Oils	MIC (%)		Zone of Inhibition (mm)	
	E. coli	S.A	E. coli	S.A.
Geranium and Cinnamon Oil	1.25	1.25	8	7.5
Cinnamon and Peppermint Oil	2.5	2.5	8	6
Geranium and Peppermint Oil	1.25	1.25	8	7



**Figure no: 1** Plate 1-E.Coli (Cinnamon and Peppermint)  
1=0.05%, 2=0.25%, 3=0.0125%



Plate 2-S.A (Cinnamon and Peppermint)  
1=0.05%, 2=0.25% 3=0.0125%.



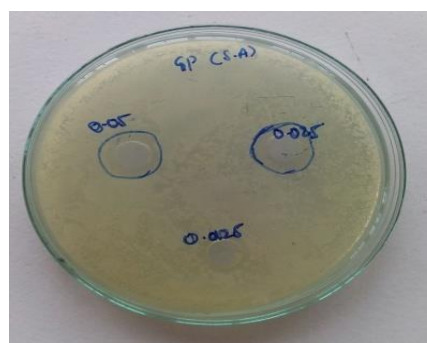
**Figure no: 2** Plate 1-E.Coli (Geranium and Cinnamon)  
1=0.05% 2=0.25%, 3=0.0125%



Plate: 2 S.A (Geranium and Cinnamon)  
1=0.05%, 2=0.25% , 3=0.0125%.



**Figure no: 3** Plate 1 -E.coli (Geranium and Peppermint) plate : 2 S.A (Geranium and Peppermint)  
1=0.05% , 2=0.25% , 3=0.0125%


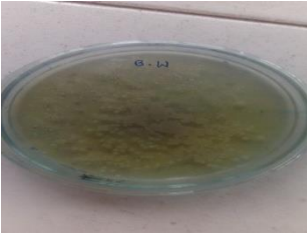


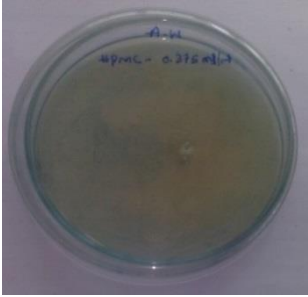
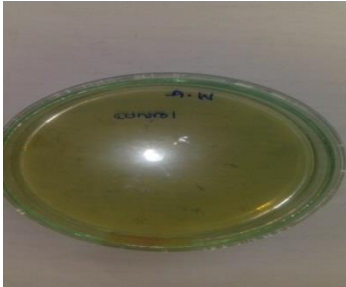


1=0.05% , 2=0.25% , 3=0.0125%

### Anti bacterial activity of different herbal oils

E.coli= (Escheria coli)

S.A= (staphylococcus aureus)

Before and after Hand Wash		
		
Carbopol F10 Formulation	HPMC F8 formulation	
		
		Marketed product(Dettol)

### Microbial assay on human volunteers

Table No: 5 Evaluation parameters for HpmcE-50 gelling agent hand wash gel

Formulation Code	Appearance and homogeneity	Grittiness	Color	pH	Foam Height (in ml)	Foam Retention (in ml)	Spread ability (cm)	Viscosity		
								50 (rpm)	60 (rpm)	100 (rpm)
F1	Translucent	Non gritty	Light yellow	6.81	140	13	12	43.2	52.3	156
F2	Translucent	Non gritty	Light yellow	6.82	160	23	10.5	45.1	55.1	161
F3	Translucent	Non gritty	Light yellow	6.82	120	11	10.1	208	216	234
F4	Translucent	Non gritty	Light yellow	6.83	160	12	10	209	219	242
F5	Translucent	Non gritty	Light yellow	6.90	150	18	9.5	210	225	253
F6	Translucent	Non gritty	Light yellow	6.92	200	25	8.73	305	342	361
F7	Translucent	Non gritty	Light yellow	6.92	200	25	8.73	305	342	361
F8	Translucent	Non gritty	Light yellow	6.92	200	25	8.73	305	342	361
Marketed formulation	Translucent	Non gritty	Light yellow	6.92	180	24	8.32	220	262	275

From above table F8 formulation was optimized and subjected for stability studies for 3 months

Table No.6 Evaluation parameters for Carbopol-940 as gelling agent hand wash gel

Formulation Code	Appearance and homogeneity	Grittiness	Color	pH	Foam Retention (in ml)	Foam Height (in ml)	Spread ability (cm)	Viscosity		
								50 (rpm)	60 (rpm)	100 (rpm)
F1	Translucent	Non gritty	Light yellow	6.90	10	50	16	30	60	70
F2	Translucent	Non gritty	Light yellow	6.45	13	60	15	58	75	80
F3	Translucent	Non gritty	Light yellow	6.46	12	80	14.2	62	90	140
F4	Translucent	Non gritty	Light yellow	6.79	15	100	14	65	93	151.1
F5	Translucent	Non gritty	Light yellow	6.78	20	120	13.5	72	92	160
F6	Translucent	Non gritty	Light yellow	6.45	23	150	13.2	75	98	170
F7	Translucent	Non gritty	Light yellow	6.11	24	150	10.5	150	193	220
F8	Translucent	Non gritty	Light yellow	6.81	27	160	10	193	268	275
F9	Translucent	Non gritty	Light yellow	6.81	27	160	10	193	268	275
F10	Translucent	Non gritty	Light yellow	6.81	27	160	10	193	268	275
Marketed formulation	Translucent	Non gritty	Light yellow	6.92	24	180	8.32	220	262	275

From above table F10 formulation was optimized and subjected for stability studies for 3 months.

### Evaluation of stability studies

Table No: 7 Evaluation parameters for HPMCE-50 gelling agent hand wash gel

Months	Formulation Code	Appearance and homogeneity	Grittiness	Color	pH	Foam Retention (in ml)	Foam Height (in ml)	Spread ability (cm)	Viscosity		
									50 (rpm)	60 (rpm)	100 (rpm)
1 <sup>st</sup> month	F8	Translucent	Non gritty	Light yellow	6.75	24	155	87	300	332	352
2 <sup>nd</sup> month	F8	Translucent	Non gritty	Light yellow	6.73	23	153	8.65	299	322	341
3 <sup>rd</sup> month	F8	Translucent	Non gritty	Light yellow	6.70	23	152	8.21	250	301	325

**Table No.8 Evaluation parameters for Carbopol-940 as gelling agent hand wash gel**

Months	Formulation Code	Appearance and homogeneity	Grittiness	Color	pH	Foam Retention (in ml)	Foam Height (in ml)	Spread ability (cm)	Viscosity		
									50 (rpm)	60 (rpm)	100 (rpm)
1 <sup>st</sup> month	F10	Translucent	Non gritty	Light yellow	6.79	25	160	9.8	190	262	270
2 <sup>nd</sup> month	F10	Translucent	Non gritty	Light yellow	6.75	26	160	9.6	185	260	285
3 <sup>rd</sup> month	F10	Translucent	Non gritty	Light yellow	6.73	24	160	9.2	180	255	280

## RESULTS AND DISCUSSIONS

According to zone of inhibition hand wash gel prepared with the combination of cinnamon and geranium had great statistical analysis finding in fig no:2.cinnamon and geranium oil was equally effective against both the bacteria i.e. gram positive(E.coli) and gram negative(S.aureus).It produces wider zone of inhibition against S.aureus 7.5mm E.coli 8 mm. The inhibition of bacteria cinnamon and geranium oil could be due to the presence of active constituents such as cinnamaldehyde, geranial acetate and geraniol. The hand wash gel was light yellow in colour and translucent in appearance and gave smooth on application which was maintained after tested stability study (Table 5, 6,7 &8). pH also maintained throughout the study which was found 6.11 to 6.92 Spreadability was also measured and found to be less variation with the initially prepared gel after performs the stability study (Table no: 5,6, 7 &8). The initial viscosities of developed gels were measured using Brookfield viscometer with spindle. Further stability test for three months has

been carried out and results revealed gel in (table no:7HF8 and table no:8C10) showed better stability than the other formulations at 100 rpm HF8 361 cps and C10 275 cps respectively and after stability study there were not much variation at different temperature and humidity. The gel was non-irritant upon application on to the skin.

## 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 gel using cinnamon and geranium containing various concentrations. Formulated gels were transparent light yellow color in appearance. Results on human volunteers showed considerable reduction in growth of microbial colonies after hand wash. Hence it can be concluded that this herbal gel hand wash provide an effective and safe alternative to existing marketed hand wash gels.

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