



Formulation, Evaluation and Antibacterial Efficiency of Herbal Hand Wash Gel

Mashood Ahmed Shah*, Satheesh Babu Natarajan, Mohd.Gousuddin

¹Lincoln University College, Faculty of Pharmacy, Department of Microbiology, Mayang Plaza, Block A, No 1 Jalan SS 26/2, Taman Mayang Jaya, 47301, Petaling Jaya, Selangor, Darul Ehsan, Malaysia.

*Corresponding author's E-mail: mashood@lincoln.edu.my

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ABSTRACT

Hands are primary mode of transmission of microbes and infections. Hand-washing is critical in food production, food service and also important in healthcare setting, homes and day care preparations. The present research was aimed to evaluate the antibacterial efficacy of various herbal oils such as Cinnamon oil, Eucalyptus oil, menthol oil and lavender oil and found that cinnamon oil showed better antibacterial activity. Also the research was carried out to formulate and evaluate the poly herbal Hand wash gel containing Cinnamon oil. The anti-microbial activity of the formulated herbal hand wash gel was tested against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* by spread 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. The results from the present work suggest and support the incorporation and utilization of herbs in the formulations to give better effect.

Keywords: Herbal hand wash gel, Antimicrobial activity and Cinnamon oil.

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 least expensive means of preventing nosocomial infections.¹ Contaminated hands can serve as vectors for the transmission of microorganisms. Pathogenic microorganisms responsible for outbreaks are spread from the hands of the food handler to others when the food handler contaminates his/her hands and then passes these microorganisms to consumers via hand contact with food or drinks. The consumer is exposed following the ingestion of these microorganisms, which may cause gastrointestinal illness. Hand contact with ready-to-eat foods represents a very important mechanism by which pathogens may enter the food supply. Food handlers whose work involves touching unwrapped foods to be consumed raw or without further cooking or other forms of treatment have been identified as a particular risk group².

To protect the skin from harmful micro organisms 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 work³. Any health-care worker, caregiver or person involved in direct or indirect patient care needs to be concerned about hand hygiene and should be able to perform it correctly and at the right time⁴. Black et al. (1981) reported a study that demonstrated a decline in diarrheal illnesses (due to *Shigella*, *Giardia* and rotavirus) in day care centers when employees were taught to use good hand washing

procedures⁵. Hand washing removes visible dirt from hands and reduce the number of harmful microorganisms such as, *E. coli* and *Salmonella* can be carried by people, animals or equipment and transmitted to food⁶. By far the most common mode of pathogen transmission to food by the infected food handler is via faecally contaminated hands. Poor hand hygiene is the contributing factor². WHO has recommended all people should wash hands before during and after preparing food, before eating food, before and after caring for someone who is sick, before and after treating a cut or wound, after using the toilet and changing diapers or cleaning up a child who has used the toilet. After blowing your nose, coughing, or sneezing, after touching an animal or animal waste, after handling pet food or pet treats and after touching garbage⁷. For generations, hand washing with soap and water has been considered a measure of personal hygiene.

The concept of cleansing hands with an antiseptic agent probably emerged in the early 19th century. As early as 1822, a French pharmacist demonstrated that solutions containing chlorides of lime or soda could eradicate the foul odors associated with human corpses and that such solutions could be used as disinfectants and antiseptics. In a paper published in 1825, this pharmacist stated that physicians and other persons attending patients with contagious diseases would benefit from moistening their hands with a liquid chloride solution⁸.

MATERIALS AND METHODS

The study was conducted between January 2013 and June 2013. The study was carried out in the Department of Pharmacology and Microbiology, Lincoln university college Malaysia.



Chemicals and Regents

The Menthol oil, Cinnamon oil, Lavender oil, Eucalyptus oil and Nutmeg oil and methyl paraben and Hydroxy propyl methyl cellulose were collected from Alpha chemicals, India., Sodium lauryl sulphate, and glycerin were purchased from Scharlau, Spain. All other reagents/chemicals were used as analytical grade.

Samples

- A. Volunteers Sample:** Swabs from hand skin of volunteers without any clinical signs of infection were included in his study.
- B. Bacterial samples:** Gram positive and Gram negative bacteria i.e. *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* were collected from department of Microbiology, Lincoln University College Malaysia. Bacterial suspension of concentration 10CFU/ml was used.

Media

- Nutrient broth and agar
- MacConkey agar
- Mueller Hinton Agar

Methods

Standardization of inoculum

The inocula prepared from the stock cultures, were maintained on nutrient agar at 4°C and sub cultured onto Nutrient broth using a sterile wire loop.

Antimicrobial Studies of Commercial Herbal Oils

The screening of anti-microbial efficacy of the herbal oils was performed on various micro organisms by using dip well method as per standard procedure. Three sterile Petri plates were taken for testing the antimicrobial activity of herbal oils against three different microorganisms i.e. *Escherichia coli*, *Staphylococcus aureus* and *Salmonella*. The plates were filled with MacConkey and Muller Hinton agar solution and allowed for solidification. After solidification the microorganisms from the subculture were inoculated into the nutrient agar media and five discs were inoculated with menthol oil, cinnamon oil, lavender oil, eucalyptus oil and nutmeg oil respectively. The plates were incubated at 37°C for overnight. After 24 hours of incubation, the plates were observed for the zone of inhibition. From the zone of inhibition the anti microbial activity of formulation is estimated as shown in the tables below. (Fig 1, 2, 3 and 4) and table (2).

Determination of Minimum Inhibitory Concentration (MIC) of the Cinnamon Oil

The MIC is defined as the lowest concentration that completely inhibits the growth of microorganisms for 24 hrs incubation. Determination of minimum inhibitory concentration of Cinnamon oil was determined by preparing different concentrations of oil. 1:20, 1:40, 1:60,

1:80 and 1:100 were added respectively to the nutrient broth (Fig 5). A 50µl volume of each dilution was added aseptically into the wells of Mueller Hinton agar plates that were already 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 hours. The lowest concentration of oil showing a clear zone of inhibition was considered as the MIC.

Formulation of Herbal Hand Wash Gel

Various herbal hand wash gel formulation batches were prepared. (table.1) The desired concentration of gelling agent, foaming agent, emollient were measured accurately and dispersed in purified water with moderate stirrer speed. The required quantity of methyl paraben was dissolved in remaining quantity of purified water by gentle heating. Desired quantity of herbal oil, flavorent, colorant was added to the above formulation. Triethanolamine was added to adjust the pH. The formulated hand wash gel was filled in suitable containers and stored at cool and dry place.

Table 1: Formulation of herbal hand wash gel

Composition (%W/V)	Formulation code					
	H ₁	H ₂	H ₃	H ₄	H ₅	H ₆
Cinnamon oil	0.1	0.1	0.1	0.1	0.1	0.1
HPMC	1	2	3	4	4	4
SLS	1	1	1	1	2	2
Glycerin	1	1	1	1	1	2
TEA	*q.s	q.s	q.s	q.s	q.s	q.s
Perfume	q.s	q.s	q.s	q.s	q.s	q.s
Colorant	q.s	q.s	q.s	q.s	q.s	q.s
Methyl paraben	q.s	q.s	q.s	q.s	q.s	q.s
Purified water q.s to	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml

*q.s= quantity sufficient

Characterization of herbal hand washes gel

pH

The pH was determined by using digital pH meter and the pH of herbal hand wash was found 6.5±0.1.

Viscosity

The viscosity of hand wash was determined by using digital Brookfield viscometer. Measured quantity of herbal hand wash was taken into a beaker and the tip of viscometer was immersed into the hand wash gel and the viscosity was measured in triplicate. The viscosity was found 50c Pascals.

Antimicrobial studies of herbal hand wash gel

The screening of anti-microbial efficacy of the formulated poly herbal hand wash gel was aseptically performed on *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* by using Dip well Agar Diffusion Technique described by Bauer et al., 1966⁹ and demonstrated by Cakir et al.,

2004¹⁰ was employed for antibacterial bioassay. A well was prepared in the plates (containing 15ml of Muller–Hinton agar medium) with the help of a cork-borer (0.85cm). 100µl of the test compound (herbal hand wash gel) was introduced into the well. The standard antibiotic discs like erythromycin, penicillin, streptomycin and Ampicillin were used as a standard. The plates were incubated overnight at 37°C. Efficiency of hand wash gel was determined by measuring the diameter of zone of inhibition. (Fig 6)

Antibacterial efficiency of herbal hand wash gel on volunteers

The antibacterial efficiency was performed by spread plate technique. Samples were collected from the six different volunteers showing no clinical signs of dermal abrasion, trauma and infection. Approximately 500 µl of herbal hand wash gel was applied to both hands. After washing the hands, the samples were collected from each volunteer in a separate glass beaker and the collected samples were allowed to grow on nutrient agar media for overnight at 37°C and per ml CFU were calculated. (Fig 7)

Stability

The stability studies were carried out by storing at different temperature conditions like 40°C, 25°C & 37°C for 1 week. During the stability studies no change in color and no phase separation were observed in the formulated hand wash. Also the formulations withstand its activity.

RESULTS AND DISCUSSION

According to the zone of inhibition formed resulting from the herbal hand wash gel against different bacterial isolates, showed that the hand wash prepared with cinnamon oil had great activity. Statistical analysis findings in fig: 6 showed that herbal hand wash gel is the broad spectrum antibacterial agent with different response for different bacterial kinds tested. From the investigation it was clear that Cinnamon oil was equally effective against both the groups of bacteria. It produced the widest zone of inhibition against *S. aureus* with diameter of 4.0 cm, *E.coli* 3.5 cm followed by Salmonella 3.0 cm. The inhibition by cinnamon oil could be due to the presence of active constituents such as cinnamaldehyde and cinnamic acid. These are terpenoids in nature. Their activity is a function of the lipophilic properties of the constituent terpenes, the potency of their functional groups and their aqueous¹¹.

Table 2 Antibacterial sensitivity of different herbal oils

Name of the oil	Plate 1:	Plate 2:	Plate 3:
	<i>E. coli</i>	<i>S. aureus</i>	<i>Salmonella</i>
Zone of inhibition (cm)			
Eucalyptus oil	0.5	0.5	No zone
Cinnamon oil	3.5	4.0	3.0
Nutmeg oil	1.5	1.2	1.0
Peppermint oil	1.0	0.5	No zone

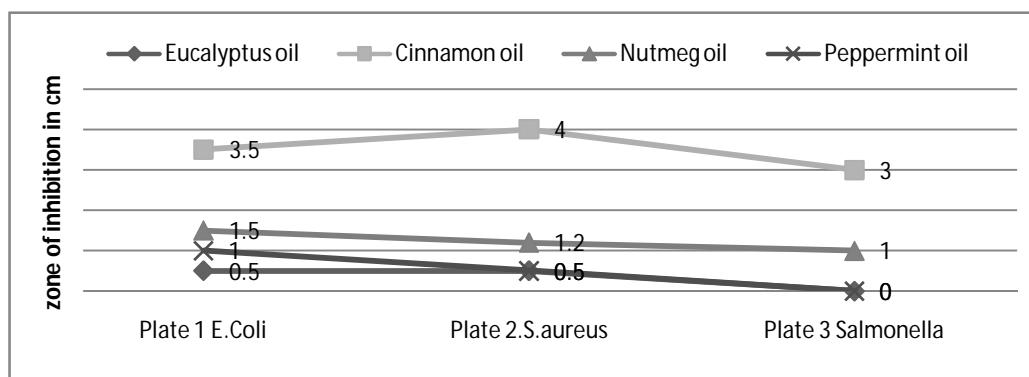
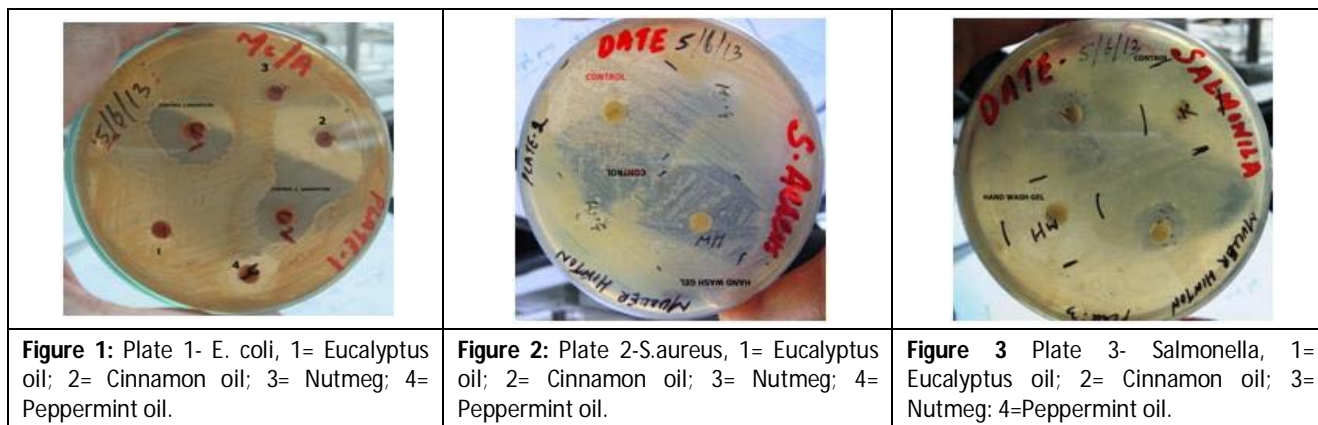


Figure 4: Antibacterial sensitivity of different herbal oils

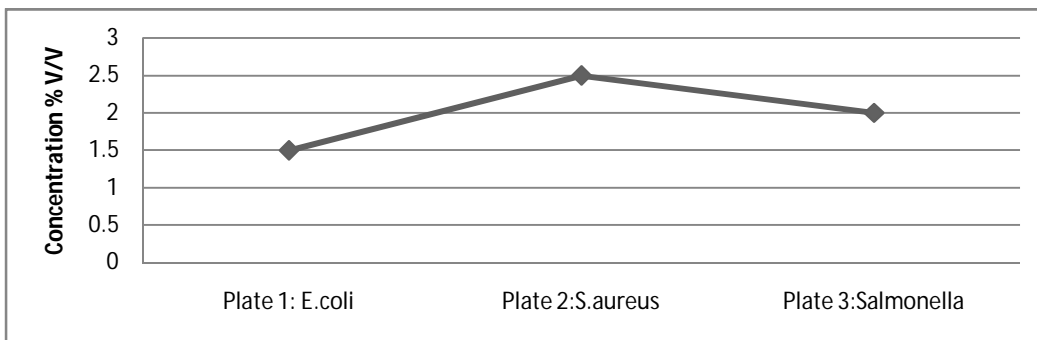


Figure 5: MIC values of Cinnamon herbal oil on selected bacteria

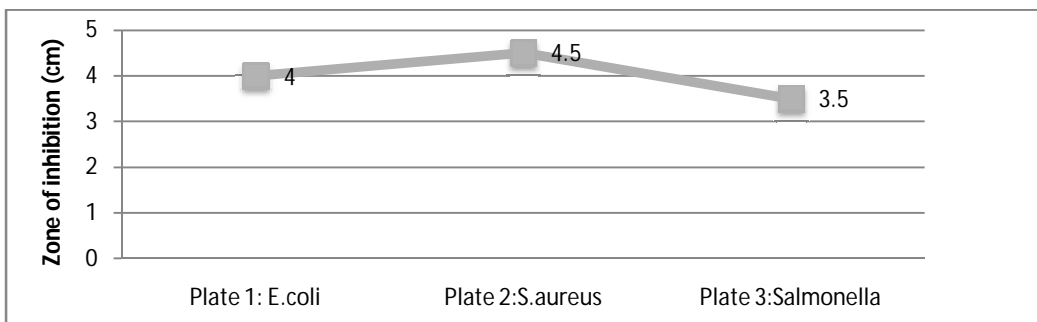


Figure 6: Zone of inhibition on Muller Hinton Agar Plate

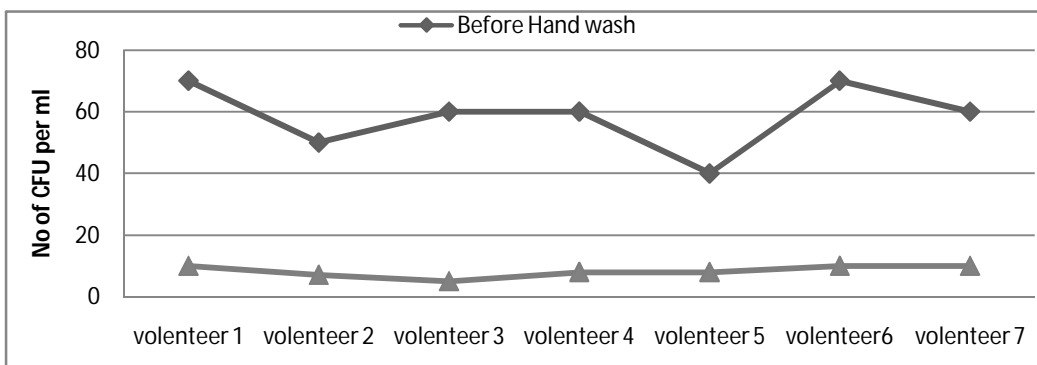


Figure 7: Effect of Herbal hand wash gel on volunteers

CONCLUSION

In conclusion, based on the above findings it is clear that cinnamon oil is active against the microorganisms. The results clearly revealed that the formulated herbal hand wash gel is effective with no side effects on human tissue. Hence a new way can be found to come back antibiotic resistant of pathogenic organism and provide safe and healthy living through germ free hand, all though the removal is not 100% but a major number can be reduced.

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