

Formulation And Evaluation Of Herbal Sanitizer

Nandkishor S. Wani^{1*}, Ashish K. Bhalerao¹,
Vikram P. Ranaware¹, Rahul Zanje¹.

¹Sinhgad Institute of Pharmaceutical Sciences, Lonavala- 410401, Dist-Pune,
Maharashtra, India.

*Corros.author : nandkishore.wani@gmail.com
Phone No: 02114 304322 Mobile: 91 9881190690.

Abstract: Hand hygiene is a vital principle and exercise in the prevention, control, and reduction of healthcare-acquired infections. Right hand washing and drying methods stop the chain of transmission of deadly pathogens (from the contaminated surface/site) from hands to other parts of the body. Hand sanitization is the preeminent aid in preventing nosocomial infections caused by different opportunistic microorganisms and to get this, the use of hand sanitizer becomes must in recent circumstances. The purpose of present study was to prepare herbal hand sanitizer incorporating the leaves extracts of *Ocimum sanctum* Linn.(Tulsi) and *Eucalyptus globulus* (Nilgiri), the well-known herbal combination with multidimensional activities; and to evaluate their respective antimicrobial efficacy and safety of hands. The formulation was evaluated against the specified microorganism (Bacteria- *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and Fungi- *Saccharomyces cerevisiae*, *Candida albicans*) by culture sensitivity test. The significance was found to be more in comparison to the standard reference.

Keywords: Hand hygiene, antimicrobial activity, nosocomial infections, herbal sanitizer.

INTRODUCTION:

Hygiene is defined as maintenance of cleanliness practices which carries utmost importance in maintenance of health. Keeping bodily hygiene and usage of cleansers are requisites of healthy living. These concepts highlight the need of maintaining hygiene in prevention of diseases¹. 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 facilities². Nosocomial infections are those which acquired or originated in a hospital or health care setting and are result of high prevalence of pathogens, high prevalence of compromised hosts, efficient mechanisms of transmission from patient to

patient. Thus occurrence of nosocomial infections is alarmingly increasing and has emerged as a serious concern in hospital care outcome; resulting in prolonged hospitalization, ample disease and mortality, and excessive costs³. *Escherichia coli*, *Pseudomonas* spp., and *Staphylococcus aureus* are commonly involved opportunistic microorganisms that primarily cause nosocomial infections. Generally infectious sites are urinary tract, surgical wounds, respiratory tract, skin, blood, gastrointestinal tract, and central nervous system. These pathogens also tend to become incorporated into the normal flora of health care workers. *Pseudomonas aeruginosa* is the most commonly detected microorganism in hospitalized patients and immunosuppressed people. Opportunistic fungal infections have become very important especially in HIV patients and the highest frequencies of opportunistic fungal infections documented are

candidiasis, aspergillosis and cryptococcosis⁴. Usually, microbes residing on the hands are divided into resident and transient flora. Resident flora (e.g. *Corynebacterium diphtheriae*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus viridans*) colonizing deeper skin layers are more resistant to mechanical removal has lower pathogenic potential. Transient flora (e.g. *Staphylococcus aureus*, Gram-negative bacilli, *Candida* species) colonizes the superficial skin layers for short periods, is usually acquired by contact with a patient or contaminated environment and these microorganisms are easily removed by mechanical means such as hand washing and are responsible for most health care-associated infections and the spread of antimicrobial resistance. In the current scenario of mechanized life style; a consumer will always prefer ready-made formulation of alcohol hand rub rather than hand washing (application of a non-antimicrobial or antimicrobial soap; and mechanical friction is generated by rubbing the hands together for 1 minute, followed by rinsing with water, and then drying thoroughly with a disposable towel)⁵. Traditional healers have long used plants to prevent or cure infectious conditions. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties⁶.

Considering this ultimatum; an attempt has been made to screen classical literature for the herbs with antimicrobial properties and found that, *Ocimum sanctum* (Tulsi leaves), *Eucalyptus globulus* (Nilgiri leaves) and *Azadirachta indica* (Neem leaves) has those antimicrobial activities. To formulate and evaluate herbal sanitizer comprise of combination of alcoholic extracts of *Ocimum sanctum* (Tulsi), *Azadirachta indica* (Neem) and *Eucalyptus globulus* (Nilgiri) using suitable excipients; which can be used as a ready-made herbal hand sanitizer.

EXPERIMENTAL:

DRUGS AND CHEMICALS:

Leaves of Tulsi (*Ocimum sanctum*) and Nilgiri (*Eucalyptus globulus*) was collected from local area of Lonavala region and authenticated by Department of Pharmacognosy, Sinhgad Institute of Pharmaceutical Sciences, Kusgaon (Bk.), Lonavala, Dist- Pune, Maharashtra, India. Nutrient Agar (For bacterial cultivation), SDA (Sabouraud's Dextrose Agar) for fungal cultivation, Standard antibiotics (Ampicillin, Amphotericin-B) were purchased from Hi-Media. Culture of microorganisms (overnight incubated), Alcohol were of lab grade.

Pathogens selected for evaluation of anti-microbial activity of the test drug were specified below.

BACTERIAL STRAINS:

1. *Escherichia coli* (gram-ve) ATCC 10531
2. *Pseudomonas aeruginosa* (gram-ve) ATCC 25619
3. *Staphylococcus aureus* (gram +ve) ATCC 6538
4. *Bacillus subtilis* (gram +ve) ATCC 6633

FUNGI STRAINS:

1. *Saccharomyces cerevisiae* (yeast) ATCC 2601
2. *Candida albicans* (yeast) ATCC 10231

EXTRACTION OF PLANT MATERIAL:

Ten grams of each dry plant material were added separately in 100 ml of methanol solution (9 parts of methanol: 1 part of water). This mixture was heated on water bath at 60 °C for 60 min. (Okogun JI, Methods of Medicinal Plant Extract Preparation, National Institute of Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria, 2000.)

FORMULATION OF HERBAL HAND SANITIZER:

Carbopol was added to deionized water with constant stirring. After uniform mixing, Tri Ethanol Amine (TEA) was added with slow stirring to avoid formation of possible air bubbles in the product and kept aside for 24 hrs. All the extracts were added to denatured alcohol along with glycerin, polysorbate 20 were mixed with aqueous phase. Finally, 0.25% each of Methyl & Propyl Paraben was added as preservative and 0.5% of perfume and mixed with slow stirring to obtain uniform product. Prepared product was stored in air tight HDPE containers. (Table 1)

Table 1: Formulation composition of Herbal Hand Sanitizer

Sr. No.	Ingredients and Excipients	Quantity %
1.	Deionized water	31.00
2.	Alcohol Denatured	62.00
3.	Tulsi leaves extract	01.00
4.	Nilgiri leaves extract	01.00
5.	Carbopol 940	00.50
6.	Tri Ethanol Amine	00.70
7.	Glycerin	02.30
8.	Polysorbate 20	00.50
9.	Perfume	00.50
10.	Presevative	00.50

IN-VITRO ANTIMICROBIAL ACTIVITY BY AGAR PLATE DIFFUSION METHOD (CUP PLATE METHOD):

In present study the antimicrobial activity of trial drug was carried out by the agar plate diffusion method. Different concentrations were incorporated into an agar medium in a petridish. Replicator device was used to inoculate multiple specimens on to a series of plates with varying concentration of antibiotics. Responses of organisms to the trial drugs were measured and compared with the response of the standard reference drug. Ampicillin was the standard reference for antibacterial study, whereas Amphoterecin - B for antifungal activity.

RESULTS:

Total four bacterial and two fungal species were selected in the present study to evaluate the antimicrobial activity of the test drugs. Different concentrations of the products were incubated and observed for the zone of inhibition. (Table 2)The extracts were mixed in a fixed ratio of 1:1 and antimicrobial activity is carried out by counting the zone of inhibition. Gel at the concentrations 400 µg/ml was observed in three bacterial species (*E. coli*, *B. subtilis* and *S. aureus*) and fungal species (*C. albicans*), found to be greater in comparison to reference standard antibacterial & antifungal drug i.e. Ampicillin & Amphotericin respectively. The other one bacterial (*Ps. aeruginosa*) and fungal species (*S. cerevisiae*) didn't significant to the maximum concentration (800 mcg/ml) of the gel in current study. This indicates that the hand Sanitizer has anti-bacterial activity particularly against *E. coli*,

B. pumillus and *S. aureus* at minimum concentration of 400 mcg/ml; (Fig.1). The resistant organism may respond to the test drugs in further concentrations.

DISCUSSION:

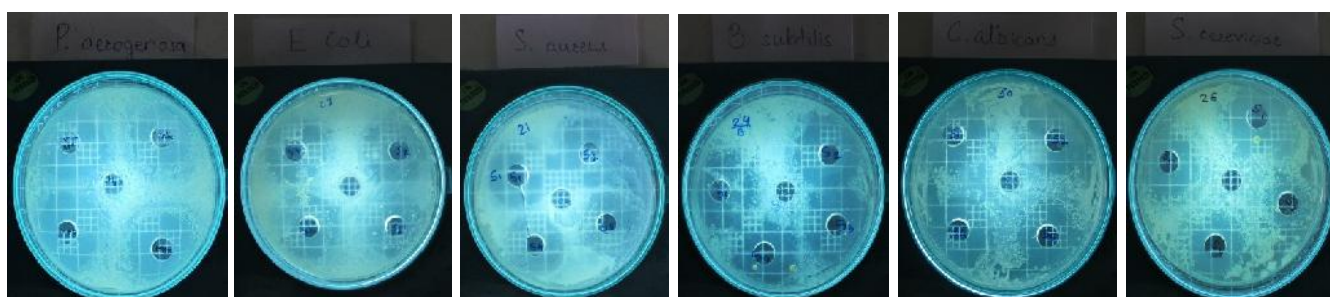
The prepared formulation of herbal hand sanitizer showed significant results at concentrations starting from 400 µg/ml against two bacterial species. The significance was found to be more in comparison to the standard reference. The composition (*Ocimum sanctum* and *Eucalyptus globulus*) has been attributed with properties like free radical scavenging, anthelmintic, antimicrobial, anti-inflammatory and analgesic etc. More concentrations may be needed to get a broad spectrum activity of the test drug.

The alcohols have excellent, rapid (within seconds) germicidal activity against vegetative bacteria, fungi, and many viruses and antimicrobial activity is based on protein denaturation of microorganisms. Alcohol rubs/sanitizers are highly effective against mycobacteria (the bacteria most resistant to the disinfection process) and multidrug-resistant pathogens. Alcohol rubs are approximately 100 times more effective against viruses than any form of hand washing^{8,9}. Sanitizers offer numerous advantages over non-alcoholic hand disinfectants, rubbing sanitizers onto both hands & until it completely evaporate, usually requires only 15 to 30 seconds. Whereas vigorous friction, rinsing with water, and drying with a towel are not needed like hand disinfectants or soaps¹⁰.

Table 2: Result of antimicrobial study of Hand-sanitizer (mean + SD) (n=3)

Conc ⁿ of Drug (µg/ml)/ Organisms	Zone of inhibition (diameter mm)				
	800	400	200	Control	STD
<i>E. coli</i>	36 ± 2.0	32 ± 1.2	29 ± 1.5	0	32 ± 1.1
<i>Ps. aeruginosa</i>	30 ± 1.7	25 ± 1.5	20 ± 1.7	0	37 ± 1.2
<i>B. subtilis</i>	32 ± 1.4	29 ± 1.6	26 ± 1.3	0	30 ± 1.2
<i>S. aureus</i>	32 ± 1.8	30 ± 2.0	28 ± 1.9	0	31 ± 1.1
<i>C. albicans</i>	31 ± 1.6	28 ± 1.4	21 ± 1.5	0	28 ± 1.4
<i>S. cerevisiae</i>	25 ± 1.8	22 ± 1.9	18 ± 2.0	0	28 ± 1.2

Fig. 1: Result of antimicrobial study of Hand-sanitizer against various pathogens.



CONCLUSION:

Hands are the most common mode of transmission of pathogens to patients and proper hand hygiene can prevent health care-associated infections and the spread of antimicrobial resistance. Scientific evidence and ease of use support of alcohol-based hand sanitizers during patient care. It may be concluded that Herbal Hand Sanitizer has a

significant anti-microbial effect on the specified microorganisms except *Ps. aeruginosa* and *S. cerevisiae*. Thus, there is immense potential in establishing the use of antimicrobial herbal products as a measure to control the multidrug resistant microbes as well as check their spread through hands from one geographical region to another.

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