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Evaluating the Synergistic Efficacy of Homemade Penta-Herbal Hand Sanitizer against Bioburden

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Abstract

Increased resistance against antimicrobial products threatens our lives and demands the use of natural compounds for assessing good hand hygiene. Herbs can be considered as the best choice for hand disinfection. In herbal sciences, synergy is a new approach concerning the phytomedicinal research. The current study was carried out to evaluate the potential of synergistic herbs against the pathogenic organisms. For this purpose, the sanitizer was prepared by using the combination of Penta-herbs along with apple cider vinegar and essential oils. Antimicrobial activity was determined by agar disk diffusion method and agar well diffusion method; good sensitivity was observed against tested microbes owing to sanitizer action. Afterwards, sampling was done following hand disinfection according to the European Standard (EN 1500) and the impression of 62 personals were taken on nutrient agar before and after the application of sanitizer. The results demonstrated that after the application of homemade herbal sanitizer, the bacterial load on hands gets reduced by 37% to 83%. Synergistic use of herbs might be considered as a preferable choice as organisms are adapting more and more resistivity against alcohol-based rubs.

Keywords: Hand hygiene, antimicrobial products, synergy, herbs, and alcohol-based rubs.



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INTRODUCTION

The skin is the human body's largest organ being colonized by a diversified microflora, most of which are harmless or even beneficial to their host (Grice and Segre, 2011). The number of microorganisms within an individual can vary from $100-10^6/cm^2$ on intact areas of the skin (Faergemann and Fredriksson, 1981; Noble and Somerville, 1974). The microbial population of the skin is divided into resident flora and transient flora (Sanford and Gallo, 2013). The resident flora are associated with the deeper layers of the skin and these organisms are inaccessible to hand hygiene preparations and rarely cause infection unless the skin is breached by a device such as a central venous catheter. The transient flora colonize the superficial layers of the skin and are less adherent so they are easily removed by hand washing practices and may be passed on by direct hand contact between human skin and the inanimate environment such as work surfaces or food and are frequently associated with nosocomial infections (World Health Organization, 2009).

Hand hygiene is the most effective measure for interrupting the transmission of microorganisms within the community and also in the healthcare setting. It has been estimated that hand washing with soap could save a million lives per year (World Health Organization, 2009; McLaws, 2015; Curtis, 2003). According to experts at the Food and Drug Administration (FDA), consumers use antibacterial soaps and body washes at home, office, school and in other public settings daily; FDA believes that this ought to be beneficial for balancing any potential risks. Though the risks associated with regular and long-term use of antibacterial soaps may perhaps outweigh the benefits, as there are indications that certain ingredients in these soaps, may contribute to bacterial resistance to antibiotics and may have unanticipated hormonal effects that are of serious concern (Food and Drug Administration, 2013).

A hand sanitizer is a supplement or alternative to hand washing with soap and water. The Centers for Disease Control recommends the use of hand sanitizer with children to promote good hygiene, especially when traveling, in

order to avoid the transmission of disease through dirty hands (Center for Disease Control and Prevention, 2017). The active ingredient in commercial hand Sanitizers may be isopropyl alcohol (isopropanol), ethanol, n-propanol, or povidone-iodine (Reynolds *et al.*, 2006). Furthermore, antibacterial soap and other related products contain chemical compounds, such as triclosan (TCS) and triclocarban, which might carry hazards and their benefits are unproven (Lenz *et al.*, 2017). It has been reported that the continual dispose of TCS into the sewage system is creating a major environmental and public health menace, due to its chemical properties of bioaccumulation and resistance to degradation (Halden, 2014). This problem might be solved by the synergy of herbs, an essential characteristic of herbal medicines considering that every plant is an orchestra of chemicals and combinations of herbs used in all herbal traditions e.g. TCM, Tibetan, Ayurveda and Western herbal medicine. These herbs contain several active compounds and as a result, they may serve as antimicrobial agents that may be directed against the surveillance of hand contaminants. The combination of two or more herbs (synergy) makes an herbal medicine more efficient than the individual ones. Therefore, herbal synergy (multi-targeting) increases efficacy and reduces resistance (Che *et al.*, 2013).

In the current study considering the antimicrobial activities of herbs, herbal sanitizer was prepared using a combination of synergistically active herbs. *In-vitro* effectiveness of the sanitizer was observed against the microbial population, we encountered in our daily life. The hand disinfection sampling was done after educating the samplers about hand disinfection procedures according to EN1500 as shown in figure 1 (Babeluk *et al.*, 2014). EN1500 (European Standard) specified test method simulating practical conditions for establishing whether a product for hygienic hand rub reduces the release of transient microbial flora on the hands when rubbed into the artificially contaminated hands of volunteers (Fichtner *et al.*, 2013).



Fig.1. Hand disinfection procedure steps according to EN1500. (Babeluk *et al.*, 2014)

MATERIALS AND METHODS

Culture

The cultures of microorganisms (*E. coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella* specie, *Bacillus subtilis*, *Acinetobacter* specie, *Lactobacillus* specie, *Bifidobacterium* specie, *Staphylococcus saprophyticus*, *Candida albicans* and *Aspergillus niger*) were obtained from the Culture Bank of the Department of Microbiology, Jinnah University for Women.

Preparation of Sanitizer

Penta-herbal sanitizer was prepared by taking a combination of herbs including dried *Lavandula angustifolia* (lavender), *Mentha piperita* (peppermint), *Rosemarinus officinalis* (rosemary), *Artemisia absinthium* (wormwood) and *S. officinalis* (sage), these were then ground up in a mortar and pestle. These herbal powders were added in apple cider vinegar along with essential oils of cinnamon and jasmine. The prepared herbal mixture was placed under the sunlight for two weeks. Subsequently, the apple cider vinegar solution was strained, and the extract was filtered and was then poured into the spray bottles for usage.

In-vitro Sensitivity Testing

The susceptibility of organisms against the Penta-Herbal Sanitizers was determined by agar well diffusion and agar disk diffusion methods. For Agar disk diffusion method, 5mm sterilized discs were impregnated aseptically into sanitizer and were placed in the Mueller-Hinton agar (MHA) plates inoculated with test organisms. Plates were then incubated at 37°C for 18 hours. For Agar well diffusion method, two-fold dilutions of the extract were prepared i.e. 1:2, 1:4, 1:8 and 1:16 for determining the MIC against the targeted organism. Inoculated MHA plates were bored and 100µl of each concentration of herbal sanitizer, crude and sterile saline was poured into its respective wells. Plates were then incubated at 37°C for 18 hours. The antimicrobial activity was determined by measuring the zone of inhibition against the tested organism.

In-vivo Sensitivity Testing

For ensuring the fact that the sanitizer is safe for application on human skin, it was applied to the restricted area of hand and no hypersensitivity reactions were observed. For assessing the efficacy of Sanitizers in reducing the viable counts of contaminants on the hands of subjects, sixty-two individuals were selected at random and verbal enlightened consent was obtained from all participating subjects beforehand of conducting the experiment. The hands of 62 of the randomly selected subjects were examined for microbial count reduction with

sanitizer. Firstly, hand impressions were taken from 62 personnel on the respective nutrient agar plate before applying sanitizer. The samplers were then educated about hand disinfection procedures according to EN1500 as shown in figure 1. Sanitizer was then massaged by the samplers on unwashed hands and allowed to absorb for a few seconds, afterwards hand impressions were taken on the plates. All the plates were then incubated at 37°C for 18 hours. Then the colonies of microorganisms were counted and percentage reduction in the cfu/ml after the application of sanitizer was calculated by following formula;

$$\text{Percent reduction} = \frac{(A-B) \times 100}{A}$$

Where,

A is the no of viable organisms before sanitizer application.

B is the no of viable organisms after sanitizer application.

RESULTS AND DISCUSSION

The present era demands the use of some natural antimicrobial products instead of synthetic ones. *In-vitro* synergistic effect of Penta-herbal hand sanitizer was evaluated by the two methods. In the Disk Diffusion Method; halos were appeared in MHA plate against all the tested organisms except for *Candida*, *Enterococcus* and *Shigella* which showed resistivity (Table 1). This might be because the amount was so little to be effective against them as these are the more resistant against the active compounds of penta-herbal sanitizer. As shown in table 2, we observed susceptibility results against all the tested organisms in agar well diffusion method around the well of crude extract; it indicates that this penta-herbal compound has great efficacy against a number of microorganisms. On the other hand, against the diluted amount of sanitizer only some organisms gave zones of susceptibility and at the dilution of 1:8 and 1:16 no organism showed susceptibility. The reason might be same in accordance with the results of the disk diffusion method that the diluted amount of the sanitizer was not sufficient to show its bacteriostatic activity. Several studies suggested that herbal compounds contain key active ingredients having the ability to inhibit the growth of bacteria, yeast and mold. As, herbs and their essential oils are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, linalool, p-Cymene, phenolic acids, cinnamaldehyde and flavonoids, which have been found *in-vitro* to have antimicrobial properties (Food and Drug Administration, 2013). The antimicrobial activity of most of the terpenoids is related to their functional groups, and the hydroxyl group of the phenolic terpenoids and the presence of delocalized electrons are important elements of their antimicrobial action (Nazzaro *et al.*, 2013). Rosemary essential oil is also used as an

antibacterial, antifungal (Oluwatuyi *et al.*, 2004) and anticancer agent (Leal *et al.*, 2003). Rosemary plants are effective against both Gram-positive and Gram-negative bacteria as these plants are rich in phenolic compounds with high antimicrobial activity (Moreno *et al.*, 2006). Furthermore, terpenoid thujone acts as GABAA receptor chloride channel blocker, much like the plant convulsant picrotoxin, and related synthetic analogs (Johnston and Beart, 2004). P-Cymene (precursor of carvacrol) is a monoterpene with a benzene ring without any functional groups on its side chains. Moreover, p-cymene has strong antimicrobial activity when it is used alone, and it can also enhance the antimicrobial activity of other compounds, such as its derivative carvacrol (Marchese *et al.*, 2017). Flavonoids in sage functions as an antioxidant (free radical scavenger), could modulate enzymatic activities and also inhibits the process of cell proliferation (Jing *et al.*, 2015). Cinnamon's essential oil has strong antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus thuringiensis*, *Streptomyces microflavus*,

Diplococcus catarrhalis and Salmonella (Chouhan *et al.*, 2017), and also exhibited inhibitory activity against plant pathogenic fungi and dermatophytes (Wei *et al.*, 2006). Considering the in-vitro antimicrobial activities of herbs, we preferred to work with the herbal sanitizer to perceive in-vivo effectiveness of Penta-herbal sanitizer against the microbial population, we encountered in our daily life (hand contaminants); as shown in figures 2 and 3, there was a prominent reduction in the number of colonies. In some of the hand contaminants samples, too numerous to count colonies became countable after the application of sanitizer, but in the rest of the samples number of colonies were reduced by 37% to 83%. The surviving organisms after the application of sanitizer were might be the resident flora of hand like *Staphylococcus*. This suggests that a synergistic combination of herbs in a form of Penta-herbal sanitizer possess strong antibacterial activity.

Table 1. Susceptibility Pattern of Microorganisms against Penta-Herbal Sanitizer (Agar disk diffusion method).

<u>Name of Organisms</u>	<u>Zone of Inhibition</u> (in mm)	<u>Interpretation</u>
<i>Candida</i>	No zone	Resistant
<i>E. coli</i>	12mm	Sensitive
<i>Staphylococcus</i>	8mm	Sensitive
<i>Streptococci</i>	16mm	Sensitive
<i>Klebsiella</i>	16mm	Sensitive
<i>Pseudomonas</i>	18mm	Sensitive
<i>Enterococcus</i>	No zone	Resistant
<i>Acinetobacter</i>	7mm	Sensitive
<i>Shigella</i>	No zone	Resistant
<i>Salmonella</i>	10mm	Sensitive

Table 2. Susceptibility Pattern of Microorganisms against Penta-Herbal Sanitizer (Agar well diffusion method).

<u>Organisms</u>	Zone of inhibition (in mm)					
	1:2	1:4	1:6	1:8	Crude	Control
<i>Bacillus</i>	8mm	-	-	-	16mm	-
<i>Salmonella</i>	-	-	-	-	30mm	-
<i>Pseudomonas</i>	-	-	-	-	25mm	-
<i>Candida</i>	-	-	-	-	14mm	-
<i>Proteus</i>	-	-	-	-	14mm	-
<i>Klebsiella</i>	22mm	-	-	-	28mm	-
<i>Streptococcus</i>	32mm	18mm	-	-	40mm	-
<i>Lactobacillus</i>	19mm	-	-	-	34mm	-
<i>Bifidobacterium</i>	14mm	-	-	-	28mm	-
<i>S. saprophyticus</i>	-	14mm	-	-	36mm	-
<i>Aspergillus</i>	-	-	-	-	16mm	-

- : No zone

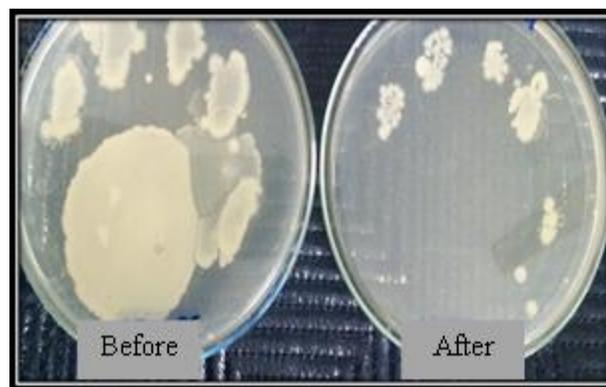


Fig. 2. TNTC to countable colonies

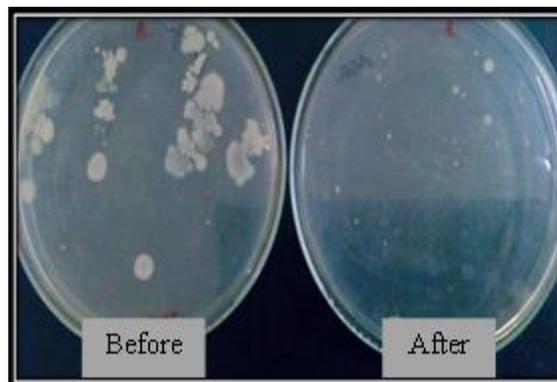


Fig. 3. 63 to 20 colonies

CONCLUSION

In herbal synergy, antimicrobial activity of herb enhances with the activity of other and they should be considered worthy not only because they are actively inhibiting the potential pathogens but also because they are not harmful for us. Good hand sanitization practices are a big contribution in order to be safer from the pathogens in daily life and serve as a key source in lessening the spread of infections and diseases. Hence, a sanitizer containing combination of multiple herbs may provide preventions against different infections by lessening the bacterial load from personnel hands and may control the rising menace of resistant microbes in our society.

CONFLICT OF INTEREST

The authors declare that no competing interests exist.

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