



Assessment microbial profile of hands using herbal hand sanitizer: A case study of a stet college (Autonomous), Mannargudi

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Abstract

Human hand usually harbour microbes both as part of body normal flora as well as transient microbes acquired from the environment one common way by which transient organism of hand. Herbal hand sanitizer was prepared and formulated by using herbs like Tulsi (0.3%), Turmeric (0.2%), Ginger (0.02%), Lemon (0.01%) and mint (0.01%). A total 36 samples of hand swabs were collected from different department of STET campus. Before and after using herbal hand sanitizer isolated bacterial species were *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and fungal species such as *Candida albicans*, *Saccharomyces cerevisiae*. Number of organism was reduced after using herbal hand sanitizer. Antimicrobial studies was carried with predominant organism isolated after hand sanitizing. Using commercial herb oil such as Eucalyptus oil, Clove oil, Cinnamon oil and Peppermint oil. Among these Peppermint oil showed effective control on *Bacillus subtilis* (40 ± 2.0), *Staphylococcus aureus* (36 ± 1.4), *Pseudomonas aeruginosa* (30 ± 1.7) and fungal species such as *Candida albicans* (31 ± 1.6), *Saccharomyces cerevisiae* (25 ± 1.8) by using disc diffusion method. It is concluded that the herbal hand sanitizer has antimicrobial activity particularly against the *Bacillus subtilis*. Further study is planned to formulate the herbal hand sanitizer along with commercial herb oil.

Keywords: herbal hand sanitizer, contamination, normal flora, transient microbes, antimicrobial activity

Introduction

Hands are primary mode of transmission of microbes and infections (Padalia, 2015) [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 (Satheesh Babu Natarajan, 2014) [2]. Hand washing is the act of cleaning hands with the purpose of removing soil, dirt, pathogenic microorganisms and avoid transmitting of transient microorganism (Power, 2015) [3]. Hand Washing removes visible dirt from hands and reduces the number of harmful microorganisms such as *E. coli* and *salmonella* can be carried by people, animal or equipment & transmitted to food. To defend the skin from harmful microorganism and to avoid spreading of numerous contagious diseases, hand washing is extremely significant precaution (Suresh Choudhari, 2016) [4].

Historically, plants have provided a good source of anti-infective agents (Ali Heyam Saad, 2011) [5]. Plant extract have a potential as antimicrobial compounds against several pathogenic microorganisms which cause infections disease and resistance towards synthetic drugs. The main advantage of using natural source is that they are easily available cheap & harm less compared to chemical products (Sayant Londhe

et al., 2015) [6]. Essential oils are typically liquid, cleared unusually coloured, complex and the present compounds are volatile, characterized by a strong order and synthesized by aromatic plants during secondary metabolites (Ary Fernandes Juniores *et al.*, 2015) [7]. Hence the present study planned to formulate and evaluate the use of hand sanitizer before and after application.






The antibacterial activity, alcohol based hand sanitizers have been reported as one of the commonly recommended hand hygiene against the disease outbreaks causes by Ebola virus (Wolfe *et al.*, 2017) [8]. Eventually, people are now more interested to use hand sanitizers instead of only hand washing due to its better performance against the resident skin flora such as *Staphylococcus aureus*, *Staphylococcus epidermis* and *Enterococcus faecalis* (Pickering *et al.*, 2010) [9].

Materials and Methods

Collection of plant materials

The herbal plants such as Thulasi (*Ocimum tenuiflorum*), Lemon (*Citrus limon*), Mint (*Mentha*), Turmeric (*Curcuma longa*), Ginger (*Zingiber officinale*) were collected from various markets at Thiruvavur Dt, Tamil Nadu, India.

Table 1: Extraction (Sirsendughosh 2018) ^[10]

S.no	Ingredients	Quantity %
1	 Thulasi	0.2%
2	 Turmeric	0.2%
3	 Lemon	0.01%
4	 Ginger	0.01%
5	 Mint	0.03%
6	Distilled water	1.5%
7	Glycerol	0.05%
8	Alcohol	3%

After sun drying the materials was made in to powder with help of grinder and each plant material is weighed.Ten grams of each dry plant material (leaves) were added separately in 100ml 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, Methods of Medicinal Plant Extract Preparation, National Institute of Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria, 2000.)

Formulation of herbal hand sanitizer

Methanolicextract ofThulasi (*Ocimumtenuiflorum*), Lemon (*Citrus limon*), Mint (*Mentha*), Turmeric (*Curcuma longa*), Ginger (*Zingiber officinale*) was prepared by maceration process. Other ingredients glycerol, were added in water and stirred well using a mechanical stirrer. All the extracts were added and stirred. Then clover oil is added for good fragrance and the volume was made up using alcohol. Formulated hand sanitizer are filled in the suitable container and stored in room temperature (Nandkishoreet al., 2013) ^[11]

Evaluation parameters

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, and then at 25

°C for 3months and then at 40 °C for 3 month and studied for appearance,pH, viscosity and spreadability.

- **Colour:** It was determined visually- Light green
- **Odour:** It was determined manually- Fragrant smell
- **pH:** It was determined using pH meter -6.5

Sample collection

36 hand swab samples were collected in various departments are designated as (A-L) in STET Women's college, Mannargudi, Tiruvarur district, Tamil Nadu, India by using sterilized cotton The sterile swab was gently rotated on the hand region. Sterile condition was maintained during the sample collection. The swabs were collected in triplicated for each sample and labelled approximately (Price, 1938) ^[12].

Antimicrobial efficiency of herbal hand sanitizer on volunteers (Kolhapure. 2004) ^[13]

Beforehand sanitizing

36 healthy volunteers were selected for the study. The sterile petriplates are named properly. Nutrient agar and Rose Bengal Chloramphenicol agar was prepared and poured into the petriplates. Hand swab were directly kept in petriplates. The inoculated petriplates of nutrient agar incubated at 37°Cfor 24 to 48 hrs and Rose Bengal with

Chloramphenicol incubated at 25-28 °C for 3-4 days. After incubation pure culture was obtained and maintained by inoculating the colonies on nutrient agar and Rose Bengal with Chloramphenicol agar medium.

After hand sanitizing

One ml of pure herbal hand sanitizer was squeezed out and applied on both hands. Hands are gently cleaned. Hand swabs are directly kept in nutrient agar and Rose Bengal Chloramphenicol media. The inoculated petriplates of nutrient agar incubated at 37 °C for 24 to 48 hrs and Rose Bengal Chloramphenicol were incubated at 25-28 °C for 3-4 days. After incubation pure culture was obtained and maintained by inoculating the colonies on nutrient agar and Rose Bengal with Chloramphenicol agar medium.

Isolation and identification of bacteria by standard method (Aneja, 2001)^[14]

Morphological characteristic was done by using gram staining and motility test. Biochemical tests such as indole test, vogesproskauer test, catalase test, oxidase test and urease test are done for identifying bacteria. The isolated and identified bacteria were subjected to pure culture by specific medium as nutrient agar.

Isolation of fungi by Lactophenol cotton blue (Gillman, 1957)^[15]

Rose Bengal Chloramphenicol agar medium was used to isolate fungal pathogens from samples. The medium was prepared and sterilized in the autoclave at 121 °C for 1b pressure for 15 minutes. Then the medium was allowed to cool. The cooled medium was poured into the sterile petriplates. It was allowed to solidify. After solidification, the swab was lawned on the surface of Rose Bengal Chloramphenicol medium. Then, the plates were incubated at 25 – 28 °C for 3-4 days. After incubation pure culture was obtained and maintained by inoculating the colonies on

Rose Bengal Chloramphenicol agar medium.

Antimicrobial studies of commercial herb oil (Mashood Ahmed Shah 2014)^[16]

The screening of antimicrobial efficacy of the herbal oils was performed on various microorganisms by using dip well method as per standard procedure. Three sterile petriplates were taken for testing the antimicrobial activity of herbs oils against five different microorganism i.e. *B. subtilis*, *S. aureus*, *P. aeruginosa*, *C. albicans*, *S. cerevisiae*. The plates were filled with Macconkey and Muller Hinton agar solution and allowed for solidification. After solidification the microorganisms from the sub culture were inoculated in to the Nutrient agar media and five discs were inoculated with Eucalyptus oil, Peppermint oil, Clove oil and Cinnamon oil respectively. The plates were incubated at 37 °C for overnight. After 24 hrs of incubation, the plates were observed for the zone of inhibition. From the zone of inhibition the antimicrobial activity of formulation is estimated with the aid of ruler (mm).

Results and Discussion

The present study was carried out to isolate bacterial and fungal pathogens from the hands swabs on volunteers. The fungal and bacterial species tested with disc diffusion method using commercial oil.

Formulation of herbal hand sanitizer

Physical evaluation

Colour, Odour, pH and Stability was also done. (Table 1)

Table 1: Evaluation parameters

Parameters	Observations
Colour	Light green
Odour	Fragrant smell
p ^H	6.5
Stability	No change in colour

Table 2: Microorganisms isolated from hand swabs a from different departments on campus before using hand sanitizer

Beforehand sanitizing	
Departments	Isolated organism
A	<i>Staphylococcus spp, Streptococcus spp, Bacillus spp, Actinomycetes.</i>
B	<i>Staphylococcus spp, Streptococcus spp, Bacillus spp, Aspergillus spp.</i>
C	<i>Bacillus sp, Staphylococcus spp., Aspergillus spp, Streptococcus spp.</i>
D	<i>Bacillus subtilis, Staphylococcus spp, Neiseriaspp, Fusarium, Streptococcus sp, Actinomycetes.</i>
E	<i>Bacillus sp, Staphylococcus spp, Klebsiellaspp, Streptococcus spp, Fusariumsp Actinomycetes.</i>
F	<i>Bacillus spp, Fusarium spp, Actinomycetes, Staphylococcus spp, Aspergillus spp.</i>
G	<i>Bacillus spp, Staphylococcus spp, Streptococcus spp, Aspergillus fumigatus, Fusarium, Penicillium, Aspergillus spp.</i>
H	<i>Bacillus spp, Streptococcus spp, Aspergillus spp, Penicillium spp, Fusarium spp.</i>
I	<i>Bacillus spp, Streptococcus spp, Aspergillus spp, Penicillium spp, Fusarium spp.</i>
J	<i>Bacillus spp, Aspergillus spp, Staphylococcus spp, Actinomycetes.</i>
K	<i>Bacillus spp, Staphylococcus sp, E. coli Streptococcus spp, Penicillium spp,</i>
L	<i>Enterococci, Escherichia coli, Staphylococcus aureus, Salmonella spp., Candida spp, Aspergillus niger.</i>

Table 3: Microorganisms isolated from hand swabs a from different departments on campus after using hand sanitizer

After hand sanitizing	
Departments	Isolated organism
A	<i>Bacillus subtilis, Staphylococcus aureus</i>
B	<i>Pseudomonas aeruginosa, Candida albicans</i>
C	<i>Bacillus subtilis</i>

D	<i>Pseudomonas aeruginosa</i>
E	<i>Bacillus subtilis, candidaalbicans</i>
F	<i>Saccharomyces cerevisiae</i>
G	<i>Bacillus subtilis, Staphylococcus aureus</i>
H	<i>Staphylococcus aureus</i>
I	<i>Bacillus subtilis</i>
J	<i>Pseudomonas aeruginosa, Candida albicans</i>
K	<i>Staphylococcus aureus</i>
L	<i>Saccharomyces cerevisiae</i>

Table 4- Antimicrobial activity of commercial herb oil

Name of the oil	Pathogens				
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>S. cerevisiae</i>
Eucalyptus oil	25 ± 1.8	22 ± 1.9	18 ± 2.0	21 ± 1.0	24 ± 1.3
Peppermint oil	40 ± 2.0	36 ± 1.4	30 ± 1.7	31 ± 1.6	25 ± 1.8
Clove oil	26 ± 1.3	28 ± 1.9	20 ± 1.7	21 ± 1.5	18 ± 2.0
Cinnamon oil	16 ± 1.3	21 ± 1.2	15 ± 1.0	12 ± 2.0	14 ± 1.6

Values are triplicates, expressed as mean ± standard deviation

Effect of herb oil on isolated bacterial and fungal species

Bacterial and fungal pathogens are same in both aspects of using before and after hand sanitizing of herbal hand sanitizer. But the number of organism reduced after hand sanitizing. So over all control measured used by antimicrobial activity using different commercial herb oil such as Eucalyptus oil, Peppermint oil, Clove oil, Cinnamon oil. Using peppermint oil (*B. subtilis* [40 ± 2.0], *S. aureus* [36 ± 1.4], *Ps. aeruginosa* [30 ± 1.7]) and fungal sp (*C. albicans* [31 ± 1.6], *S. cerevisiae* [25 ± 1.8]) Among the herbal oil peppermint oil was showed better result for controlling of these pathogens.

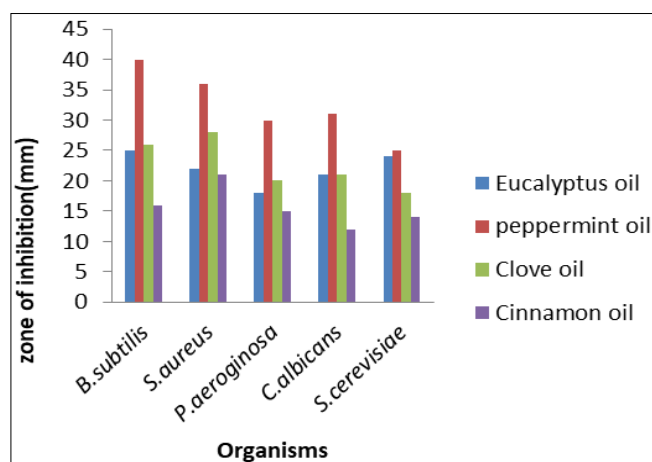


Fig 1: Antimicrobial activity of commercial herb oil

Discussion

Our study was reported that the antimicrobial activity of the formulated herbal hand sanitizer and efficiency was checked by using the hand sanitizer on volunteers and it was correlated to Mounika (2015) the antimicrobial activity of the formulated herbal hand wash gel was tested against *E. coli* and *S. aureus* by pour plate technique and the results obtained were commercial antibacterial standards. Also the efficiency was checked by using the hand wash gel on volunteers.

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 ease of use support of alcohol-based hand sanitizer during patient care. It may be concluded that herbal hand sanitizer and commercial herb oil has a significant antimicrobial effect on the isolated microorganisms. Thus, there is immense in potential in establishing the use of antimicrobial herbal products as a measure to control the multi-drug resistant microbes as well as check their spread through hands from one geographical region to another. Further study is to be planned to formulate Polyherbal hand sanitizer to provide safe and germ free hand and minimize the use of alcohol hand sanitizer.

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