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Formulation and evaluation of polyherbal hand sanitizer

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Abstract
Hand hygiene is a vital principle and exercise in the prevention, control and reduction of healthcare acquired infections. A practise of hand sanitizing is very important to eliminate the microbial contamination especially work in laboratories, hospital and even at home. Proper use of hand sanitizer reduces the transmission of infection also. Herbal hand sanitizer was prepared by using herbs like Neem (0.3%), Turmeric (0.2%), Ginger (0.2%) Lemon (0.01%), and Mint (0.01%). The surface swabs were collected from various places of STET Campus. From the sample predominant organisms were isolated such as *E.coli, P.aeroginosa, S.aureus* and fungal species such as *Candida albicans.* The isolated organisms tested with herbal hand sanitizer at different concentrations such as 25%, 50% and 100% by using agar well diffusion method. Among these 100% of herbal hand sanitizer showed the highest zone diameter was observed as (65±5.3) against *E.coli*. It is concluded that herbal hand sanitizer has a significant antimicrobial effect on isolated microorganisms. The further study is to be planned to formulate the herbal hand sanitizer in gel form also.

Keywords: hand hygiene, antimicrobial effect, contamination, herbal sanitizer

Introduction

Skin is the most exposed part of the body to the sunlight, environmental pollution and also to some protection against the pathogens. The most common skin disorders are eczema (atopic dermatitis), warts, acne, rashes, psoriasis, allergy etc. To protect the skin from harmful microorganisms and to prevent spreading of many skin infection. Hand washing is absolutely an important precaution. The aim of the present work is to prepare and physically evaluate a poly herbal hand wash from commonly available plants, instead of adopting synthetic preparation. Hand sanitizer is an antiseptic and supplement to the hand washing with soap and water. There are different preparations in hand sanitizer like gel, foam, liquid solution etc. The commonly used ingredient in hand sanitizer is alcohol and inactive ingredients include a thickening agent, humectants etc. Alcohol based hand sanitizer are very effective in killing microorganisms than compared to soaps. All hand sanitizer products require a designation called "national drug code" in the United States (Fatima Grace *et al.*, 2015) [1].

These are antiseptic products used to avoid the transmission of skin infections/pathogens. Alcoholic hand sanitizer kills 99% of the bacteria on hands for seconds after application. Drying of the skin is less and leaves more moisture. 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 diseases (Palak Vya*t al.*, 2011) [2]. 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 facilities(Larson., 1999) [3].

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, ampledisease and mortality, and excessive costs (Burke, 2003) [4]. *Escherichia coli, Pseudomonas spp., and Staphylococcus aureus* are commonly involved opportunistic microorganisms that primarily cause nosocomial infections. Generally infectious sitesare 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 immune suppressed 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 Cryptococcosis (Black, 1981) [5]. 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) (Widmer.,2000) [6]. 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 properties (Cowan, 1999) [7]. Hence the present study was aimed to formulate and evaluate the herbal hand sanitizer on surface swab collected from STET women's College, Mannargudi, Thiruvarur District, Tamil Nadu, India.

Materials and Methods

Collection of Plant Material

The herbal plants such as Neem (Azadirachta indica), Lemon (*Citrus limon*), Mint *(Mentha*), Turmericn (*Curcuma longa*), Ginger (*Zingiber officinale*) were collected from various markets at Thiruvarur Dt, Tamil Nadu, India.

Extraction (Sirsendughosh 2018) [8]

After sun drying the materials is made in to powder with help of grinder and each plant material is weighed Ten grams of each dry plant material wee added separately in 100ml of methanol solution (9 parts of methanol: 1 part of water). This mixture was heated on water bath at 60℃ for 60 min.(Okogun, Methods of Medicinal Plant Extract Preparation, National Iinstitute of Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria,2000.)

Formulation of Herbal Hand Sanitizer (Nandhikisor 2013) [9]

Methanolic extract of Neem (Azadirachta indica), 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

Table 1

S.no	Ingredients	Quantity %
1	 Neem	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%

Evaluation Parameters

Stability: The stability studies were carried out by storing at different temperature conditions like 40ºC, 25ºC and 37ºC for 4 weeks. During the stability studies no change in colour and no phase separation were observed in the formulated hand sanitizer.

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

Surface swab samples were collected by using sterilized cotton swab from per square of the table top, door handle, taps handle, toile flushers, laboratory doors and tables, books from STET Women's college, Mannargudi, Thiruvarur district, Tamil Nadu, India. The sterile swab was gently rotated on the surface. Sterile condition was maintained during the sample collection. The collected samples were immediately transferred to the laboratory for isolation and identification of bacterial and fungal species.

Isolation and Identification of Bacteria (Aneja, 2001) [10]

Morphological characteristic was done by using gram staining and motility test. Biochemical tests such us indole test, voges proskauer 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 (Gillman, 1957) [11]

Rose Bengal Chloramphenical agar medium was used to isolate fungal pathogens from samples. The medium was prepared and sterilized in the autoclave at 121℃ for lb 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 Chloramphenical medium. Then, the plates were incubated at 25 – 28 ℃ for 3-4 days. After incubation pure culture was obtained and maintained by inoculating the colonies on Rose Bengal Chloramphenical agar medium.

Agar diffusion test (well varient) to determine susceptibility of test organisms to hand sanitizer (Touhida Ishma., 2019) [12]

The susceptibility of the test organism to the hand sanitizers was investigated using the well variant of the agar diffusion method. Sterile Mueller hinton agar plates were inoculated was dipped into a tube containing the inoculums and was rotated properly to allow maximum contact. Excess inoculums were removed by pressing and rotating the swab firmly against the inside of the tube above the liquid level. The swab was then streaked over the surface of the medium three times while rotating the plates through an angle 60º after each application. The swab was also passed round the edge of the agar surface. The inoculum was left to dry for a few minutes at room temperature with the lid closed.

With the aid of a sterile 6mm Cork borer, 4 equally spaced holes were bored in the agar plate with a fifth Hole in the centre of the plate. The agar plugs were discarded using the sterile needle. Fifty microlitres (50 µL) of the hand sanitizer was then introduced into each of 4 wells while the central well was filled with an equal volume of sterile water to serve as control. This was done for all the test organisms and hand sanitizers. The plates were incubated for 24 hours at 37℃ in an upright position. They were then examined for zones of inhibition which indicate the degree of

susceptibility or resistance of the test organism to the antibacterial agent. The test was carried out in duplicates and the average of 2 readings was taken as the zone of inhibition in each case. Inhibhition zones were measured with the acid of a ruler (mm)

Results

The present study was carried out to isolate bacteria and fungal pathogens from surface swabs such as table top, door handle, tap handles, toilet flushers, laboratory doors and books. The fungal and bacterial species tested with agar diffusion test to obtain the effectiveness of herbal hand sanitizer.

Formulation of herbal hand sanitizer

Physical evaluation

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

Table 1: Evaluation parameters

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

Detection of different bacteia and fungi from the surface of various places

Pseudomonas sp, E.coli, Staphlococcus spp, Saccharomyces cerevisiae were found from the surface of Door knobs, Table top, Laboratory doors and Tap handle. Among these the highest growth rate of *E.coli* was found on the samples collected from the surface. (Table2).

Table 2: Isolation of microorganism from different location of STET campus

Sample source	*E.coli*	*Staphylococcus aureus*	*Pseudomonas aeroginosa*	*Saccharomyces cerevisiae*
Table top	+++	++	+++	+
Door handle	+++	++	++	+
Tap handle	+++	+++	+	++
Toilet flushers	++	++	+++	
Laboratory doors	++	+	++	++
Books	+	+	++	+++

+++: High growth rate, ++: Moderate growth rate, +: Low growth rate

Different concentration such as 25%, 50% & 100% of herbal hand sanitizer showed their anti-microbial activity against *E.coli, Ps.aeroginosa, Staphylcoccus aureus, Saccharromyces cerevisiae* those were isolated from surface swab. 25% of herbal hand sanitizer exhibhited 45±3. 4mm zone of diameter against *E.coli* whereas 28±2.0mm zone of diameter against *Pseudomonas aeroginosa*, 17±1.5mm zone of diameter against *Staphylococcus aureus* and 15±1.0mm zone of diameter against *Saccharromyces cerevisiae* respectively. 100% of hand sanitizer shows the highest zone diameter was observed as 65±5.3mm against *E. coli*. (Table 3)

Table 3: Inhibitory effect of herbal hand sanitizer through agar well diffusion technique.

Organism	Concentration of herbal hand sanitizer	Zone of Inhibhition
E. coli	25%	45±3.4
	50%	55±4.1
	100%	65±5.3
Pseudomonas aeroginosa	25%	28±2.0
	50%	35±1.9
	100%	40±2.5
Streptococcus aureus	25%	17±1.5
	50%	22±1.7
	100%	36±1.9
Saccharromyces cerevisiae	25%	15±1.0
	50%	25±1.2
	100%	30±1.1

Values are triplicates, expressed as Mean± Standard deviation

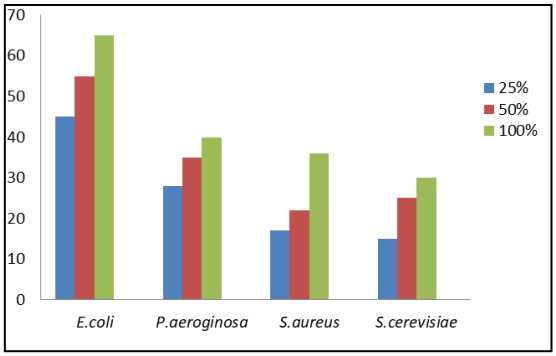

Fig 1: Inhibitory effect of herbaal hand sanitizer through agar well diffusion techniqes.

Discussion

SowmiyaK.V (2015) reported that the Poly herbal sanitizer Formulation and evaluation Mainly hand sanitizer can stop the chain of transmission of micro-organisms and other bacteria from hand to different parts of our body. Hand hygiene is important and one of the most critical steps in food production, food service as well as in homes and other day care preparations. Hand sanitizer is an antiseptic and supplement to hand washing with soap and water. The commonly used ingredient in hand sanitizer is alcohol and inactive ingredients include a thickening agent, humectants etc. Alcohol based hand sanitizer are very effective in killing micro-organisms than compared to soaps. All hand

sanitizer products require a designation called “national drug code” in the United States.

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 case of use support of alcohol-based hand sanitizers during patient care. It may be concluded that herbal hand sanitizer, has a significant antimicrobial effect on the isolated microorganisms. 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 surfaces from one geographical region to another. The further study is to be planned to formulate this herbal hand sanitizer in gel form also.

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