



A Study on Evaluation and Effectiveness of Herbal Hand Sanitizer and its Anti Bacterial Activity

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ABSTRACT

An ever increasing number of customers might want Good hygiene is a minister to health, comfort, and social interactions. Current society is well aware of hygiene. Washing hands is one of the simplest, most effective ways to get rid of germs and avoid infection. Transient microorganisms can be of any type, from any source may contact the body. As hands carry out the majority of functions of the human's body and are exposed to a variety of substances, hands hygiene plays a chief conscientiousness. Herbal plants are the unique resources of every country with potent medicinal properties pave ways towards sustainable health. In this present study, the combination of *Trachyspermum copticum*, *Coleus aromaticus*, *Acorus calamus*, *Mentha piperita*, *Piper nigrum*, *Elatteria cardamomum* and *Aloe barbadensis* were used to formulate herbal hand sanitizer. The hand wash samples were subjected to microbial analysis. The isolated strains (*Yersinia*, *Proteus*, *Escherichia coli*, *Klebsiella*, *Salmonella*, and *Pseudomonas*) were characterized by their cultural characterization and confirmed using selective media. Herbal sanitizer possesses vitamin C (.4%), vitamin A (0.062) and considerable microbial load. The antioxidant activity was found to be 49% and the susceptibility test showed more activity found in *Yersinia* sp. > *Proteus* sp > *Salmonella* sp. > *Klebseilla* sp. > *Pseudomonas* sp. > *E.coli*.

Keywords: Hygiene, Hand sanitizer, Anti microbial and Anti oxidant

1. INTRODUCTION

The Hands are the chief mode of transmission of microbial infections and diseases [1]. Hygiene of hands is hence the most vital thing to avoid the transmission of harmful germs and prevent the infections. Hand hygiene is the simplest and least expensive means of preventing nosocomial infections [2]. Hand-washing is significant in food production, food service and also chief in healthcare setting, homes and day care preparations. Though good & simple hygiene technique is single most important, easy and least expensive means of preventing health care-associated 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[3].

Plant extracts and products have been used for centuries in traditional medicine, functional food, natural dyes, cosmetics, and in the treatment of diseases [4,5,6]. The lead advantage of using natural source is that they are easily available, cheap and harmless without side effects compared to chemical products. Plants have been used for long time by traditional healers to prevent or cure infectious conditions. As plants are rich in wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids etc., they have been found to possess antimicrobial properties. Thus research has been increased enormously focusing on the production of natural products with improved quality. The emergence of bacterial resistance to the presently

available antimicrobial drugs necessitates additional research in the discovery of new safe and effective antimicrobial agents.

Hands are needed to protect from bacterial pathogens as they are the most exposed part of the body. Proper hand hygiene is the most important measure of preventing health care associated infections especially children and old aged persons with less immunity. In present study we formulate herbal hand wash using different alcoholic plant extracts holds that antimicrobial potency and thereby formulating ready to use herbal hand sanitizer.

2. MATERIALS AND METHODS

2.1 Plant Sample

Trachyspermum copticum (Omum seed)

Coleus aromaticus (Karpooravalli)

Acorus calamus (Sweet flag; Vasambu)

Mentha piperita (Peppermint)

Piper nigrum (Milagu)

Elatteria cardamomum (Ellam)

Aloe barbadensis (Aloe)

2.2 Preparation of Herbal Hand Sanitizer:

- The dried plant extracts (*T. copticum*, *A. calamus*, *P. nigrum*, *E. Cardamomum*, *C. aromaticus* and *M. piperita*) were prepared using methanol.
- Plant extracts *T. copticum* (3%), *A. calamus* (1%), *P. nigrum* (5%), *E. cardamomum* (2%) *C. aromaticus* (3%) and *M. piperita* (5%) were added in to the 100 ml beaker.
- 15% of *Aloe barbadensis* was added in to the above extracts then 30% of methanol and 10% Almond aqueous were added then the solution was mixed by using stirrer.
- Finally citric acid (preservative) was added in to the sanitizer solution and make up in to 100 ml using double distilled water.

2.3 In Vitro Evaluation of Transient Bacterial on human palm flora:

Collection of palm washes samples by Wash/rinse Procedure [7] (Hand washing).

2.4 Experimental Design:

A palm washes were taken from five volunteers in whole study period for the evaluation of transient bacterial flora from palms.

Volunteers list

S. No.	Name of the volunteers	Volunteers native	Age of the volunteers
S1	College student	Punavasal	25
S2	Software working lady	Nanjikottai	23
S3	Home maker	Pattukkottai	26
S4	Servant maid	Kanni thoppu	60
S5	Slum boy	Mariyamman koil	5

The samples were streaked onto nutrient agar.

The inoculated plates were incubated aerobically in an inverted position at 37 °C for 24 hours. The plates were then observed for the presence of isolated colonies were again sub-cultured on nutrient agar to isolate pure culture. After isolating pure cultures, bacterial isolates were further identified and characterized by Gram staining, and biochemical tests [8]. and identified based on the morphological, physiological and the biochemical characteristics presented in Bergey's Manual of Determination.

2.5 Screening of anti bacterial activity by agar well diffusion assay

The agar well diffusion method of Perez *et al.*, 1990 [9] was employed. Mueller Hinton Agar medium was inoculated with the microorganism suspended in sterile water. Once the agar was solidified, it was punched with a six millimeters diameter wells and filled with 25 µL of the sample, positive control and negative control. The test was carried out by triplicate. The plaques were incubated at 35 ± 2 °C for 24 h. The antimicrobial activity was calculated by applying the expression in mm.

2.6 In vivo efficacy of Herbal hand sanitizer

Total of five volunteers with different age groups were involved to evaluate *in vivo* efficacy of the sample. Total eight palm washes were taken from the volunteers. Control palm washes were taken by use of sterile water only.

2.6 a. Bacteriological study [10]

Point inoculation method was used for viable count of different bacterial flora. Aseptically 10 µl palm wash samples were transferred to 90 µl sterile saline (85%) solution. Appropriate serial dilutions were made and aseptically 5 µl droplets were point inoculated on selective media plates followed by incubation at 37°C for 6 hrs. Typical colonies showing characteristic coloration were counted and representative colonies were picked up randomly and counter using colony counter.

2.7 Antioxidant Activity by Power Reducing Assay [11]

1 ml of Plant extract was mixed with phosphate buffer (2.5 ml 0.2 M, pH 6.6) and potassium ferric cyanide (2.5 ml). The mixture was incubated at 50°C for 20 minutes. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and Ferric chloride (0.5 ml, 0.1%) and absorbance measured at 700 nm. Increased absorbance of the reaction mixture indicates stronger reducing power. The activity was compared with ascorbic acid as standard.

Calculation:

Percentage scavenging activity = $A_{\text{control}}/A_{\text{test}}$

×100

A_{control}

Where A_{control} is the absorbance of the control. A_{test} is the absorbance in the presence of the sample.

3.RESULTS AND DISCUSSION

The herbal hand sanitizer was prepared and it was used for evaluate the antimicrobial and antioxidant activity.

3.1 Identification of Human bacterial flora

The bacterial flora like *Yersinia* sp. *Proteus* sp. *Escherichia coli*, *Klebsiella* sp. *Salmonella* sp. and *Pseudomonas* sp. were isolated from human palm and identified based on Bergy's manual. In this study, prevalence of gram positive and gram negative bacteria organisms were 66.7% and 33.3% respectively (fig: 1). Among Gram negative

organisms, *Escherichia coli*, *Klebsiella* sp. *Salmonella* sp. were found to be the most predominant pathogen. Our skin contains large numbers of microorganisms, mainly Gram-positive. Most of the gram positive bacteria are natural flora, which are commonly found on the hands, face and in deep layers of the skin [12, 13]. The goal of hygiene is a sufficient reduction of microbial counts on the skin to prevent cross-transmission of pathogens among humans.

3.2 Antibacterial Susceptibility Testing

The antimicrobial activity of herbal hand sanitizer against different pathogens was determined by Agar Well diffusion method. The sample hand sanitizer possessed more antibacterial effective against isolated microorganisms with the inhibition zone ranging from 15–30.8 mm. More activity was found in *Yersinia* sp.> *Proteus* sp.> *Salmonella* sp.> *Klebsiella* sp.> *Pseudomonas* sp.> *E.coli*. Aloe vera used for its moisturizing activity in many cosmetics [6]. Plants are rich in potential secondary metabolites, such as flavonoids, tannins alkaloids polyphenols ext. These compounds have diverse biological activity including antibacterial activity [14]. Most of the polyphenols are extracted in the methanol [15]. The results suggested that herbal extracts in mixture giving higher activity than the individual extracts. The combination of the antibacterial compounds from different plant extracts may show synergistic effect enhancing their antimicrobial activity.

Mithun A. Thombare [16] *et al.* 2015 observed that the alcohol based herbal hand sanitizer (at concentration 400 µg/ml) showed greater inhibition zones against bacterial species *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* in comparison to commercially available hand sanitizer.

Microbial Load

Microbial load was analysed before applying sanitizer and after applying of herbal based sanitizer. The result showed that microbial load was considerably low after applying herbal hand sanitizer. Highest reduction was observed in college student and slum boy with 75 and 50% respectively after the application of herbal hand sanitizer. Many studies have been done earlier in view of evaluation of total transient microflora of hands. *Staphylococci* especially coagulase –negative *S.aureus* were found to be the dominant part of

transient hand microflora [17]. Colonization of health care worker hands with *S.aureus* has been described to range between 10 to 76.3% and transient flora varies considerably from person to person [18]. It was concluded that the herbal based sanitizer had potential to control and prevent the over population of bacterial flora in the palm.

Kavatheker *et. al.* (2004)[19], reported that pure herbal hand sanitizers were found to be more effective in reducing the total microbial load. Alcohol rubs are approximately 100 times more effective against viruses than any form of hand washing [20]. Sanitizers offer numerous advantages over non-alcoholic hand disinfectants as, 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 soaps [21].

In vitro Antioxidant activity of Herbal hand sanitizer

A concentration dependent scavenging activity was observed in herbal hand sanitizer with maximum activity of 49.4% achieved in the highest tested concentration at 10mg/ml (fig 3). The sample had relatively higher reducing power due to the presence of high bioactive compounds which was present in selected medicinal ingredients and it has been attributed with properties like free radical scavenging, anthelmintic, antimicrobial, antiinflammatory and analgesic etc. which are widely used in cosmetic, and pharmaceutical industries, natural antioxidants [22,23,24]. Hence, it could have promising skin care properties, can able to protect the human body from free radicals.

SELECTIVE PLATING

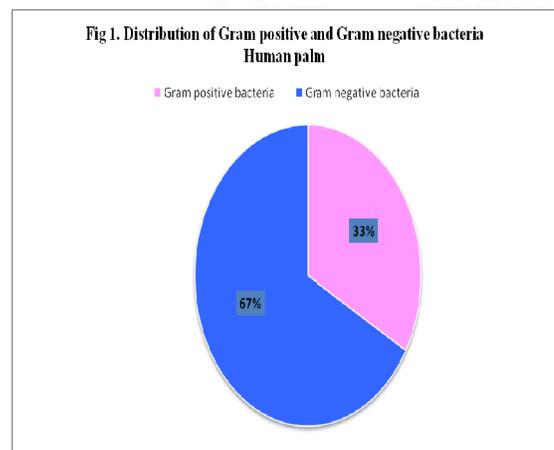
The test organisms were confirmed by plating them in selective medium and the results were observed and showed in table.2

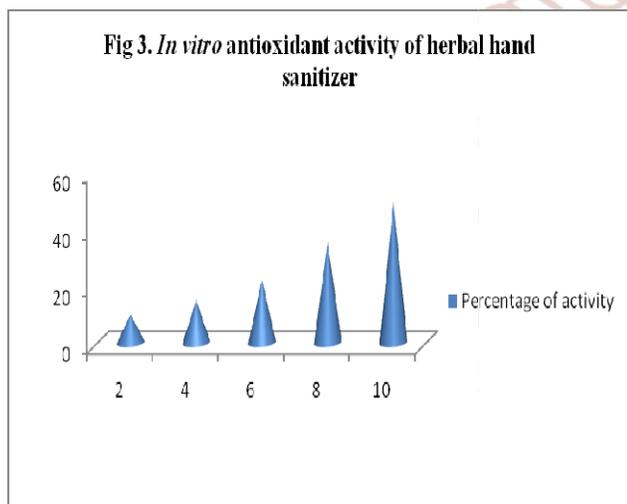
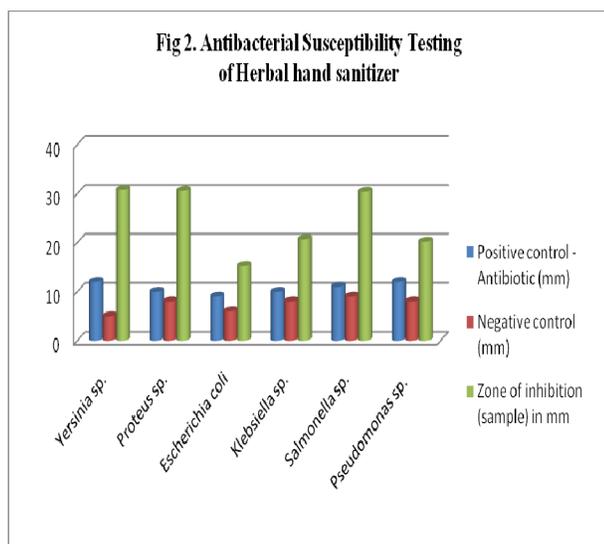
Table 2: List of selective medium and test organisms

S. No.	Test Organism	Medium used
1	<i>Yersinia sp.</i>	Yersenia agar
2	<i>Proteus sp.</i>	Macconkey agar
3	<i>Escherichia coli</i>	Brilliant green agar
4	<i>Klebsiella sp.</i>	EMB agar
5	<i>Salmonella sp.</i>	SS agar
6	<i>Pseudomonas sp.</i>	Macconkey agar

Table: 3. Microbial load before and after applying herbal hand sanitizer:

Volunteers	Load before applying sanitizer	Load after applying sanitizer	Percentage of reduction
College student	4×10 ³	3×10 ³	75
Software working lady	6×10 ³	2×10 ³	33
Home maker	5×10 ³	2×10 ³	40
Servant women	7×10 ³	3×10 ³	43
Slum boy	8×10 ³	4×10 ³	50





4. CONCLUSION

Hand hygiene is a vital principle exercise in the prevention, control, and reduction of healthcare acquired infections. Proper hand washing and drying methods prevent the chain of transmission of deadly pathogens from the contaminated site to other parts of the body. Hand sanitization is the paramount aid in preventing nosocomial infections caused by different opportunistic microorganisms. In this present study, results showed that the herbal sanitizer has antimicrobial property, antioxidant activity, and considerable microbial load. When compare to alcohol based hand sanitizers, herbal hand sanitizer is more effective against microbes, non toxic, formulate hand soft and makes the hand hygienic. The further study will be conducted for evaluation of bioactive compounds in herbal hand sanitizer responsible for medicinal properties and its shelf life time. 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 to check

their spread through hands from one geographical region to another.

5. Acknowledgment

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