IN VITRO ASSESMENT OF INDIGENOUS HERBAL AND COMMERCIAL ANTISEPTIC SOAPS FOR THEIR ANTIMICROBIAL ACTIVITY

A
Dissertation
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Of the award of degree of
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In
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Dedicated to my parents

CERTIFICATE

This is to certify that the dissertation entitled "*In vitro* assessment of indigenous herbal and commercial antiseptic soaps for their antimicrobial activity" submitted by Miss. Vineeta Chauhan, Roll no. 3040028, in partial fulfillment of the requirements for the award of the degree of Master of Science in Biotechnology, to Thapar Institute of Engineering and Technology (Deemed University), Patiala, is a record of student's own work carried out by her under my supervision and guidance. The report has not been submitted for the award of any other degree or certificate in this or any other University or Institute.

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EXECUTIVE SUMMARY

Hand hygiene is a fundamental principle and practice in the prevention, control, and reduction of healthcare-acquired infection. Good and simple hygienic practice i.e. correct hand washing and drying techniques will stop the chain of transmission of deadly pathogens (from the contaminated surface/site) form hands to other parts of the body.

In the present study i*n vivo* efficacy of four different soaps (2 commercial and 1 indigenous herbal and 1 control soap) was done. Indigenous herbal soap was found to be most effective in reducing the total bacterial flora to 80% and *S.aureus* flora to 83%. Pure isolated *S.aureus* and *E.coli* strains from palm washes were found to be resistant to antimicrobial agents. 81% of all *S.aureus* strains were detected as multi drug resistant.

The study highlights the need of antiseptics, which have less, or no resistant pattern towards the transient microflora of the skin and could check the spread of multidrug resistant microbes. The study provides evidence that can be used in formulating herbal antiseptic agents to elucidate the role of herbals in the dissemination of antibiotic resistance to human populations

ABBREVIATIONS

MRSA	Methicillin resistant *Staphylococcus aureus*
VRSA	Vancomycin resistant *Staphylococcus aureus*
CDC	Center For Disease Control
HCW's	Health care worker's
WHO	World Health Organisation
CCOH	Canada's National Occupational Health & Safety Resourse
CDR	Central Disease Review
μl	Micro litre
Cfu/ml	Colony forming unit per milli litre

CONTENTS

INTRODUCTION

Hygiene is defined as maintenance of healthy practices. Modern society refers hygiene as cleanliness. However with the development of the germ theory of disease, hygiene refers to any practice, which reduces the harmful bacteria to sub-pathogenic levels so that it does not cause any disease. Good hygiene is an aid to health, beauty, comfort, and social interactions. It aids in disease prevention and isolation. The human skin covers the external surface of the body and varies according to its function like thermoregulation, sensation; secretion of substances and serves as matrix for harboring a variety of microbes. These in turn metabolize these secretions and produce specific odorous compounds responsible for characteristic skin odor.

Microbes present on the skin can be broadly classified into two distinct categories: resident and transient. Resident microbes are those considered as permanent inhabitants of the epidermis (superficial skin surface). 10 to 20% total resident microflora has been found to be localized in the skin crevices, where skin oils and hardened skin make their removal difficult and complete sterilization of skin impossible. Bacteria representing skin resident flora include CONS (Coagulase negative Staphylococci), members of *Corynebacterium*, *Propionibacterium* and *Acinetobacter* species. *Staphylococcus aureus* is the only true pathogenic organism included in the resident as well as transient microflora of skin. About 35% of normal adults carry *S. aureus* in the anterior nostrils of the nose and are particularly susceptible to infection when the normal protective skin barrier is broken. Transient microbes are those, which are not autochthonous but found on and within the epidermal layer of skin, as well as other areas of the body, where they do not normally reside. Almost all disease-producing microorganisms belong to this category. Transient microorganisms can be of any type (bacteria, yeast, molds, viruses, and parasites), from any source with which the body has had contact, and are found on the palms of hands, fingertips, and under fingernails (Noble & Pitcher, 1978). The basic practice of hygiene is washing with water.

When this is used for cleaning the whole body is referred to as bathing and when specifically used for cleaning hands is referred to as hand washing.

Hands perform the majority of functions of the human's body and are exposed to a variety of substances which include soil during farming, food during cooking, touching raw and contaminated food material, during personal hygiene.

Clean hands stop the spread of germs; therefore hand washing is often emphasized as the single most important measure in any infection control programme for preventing cross transmission of microorganisms between patients. Hand washing is the act of cleaning the hands with or other liquid with or with out the use of soap or other detergent to remove dirt or loose transient flora thus preventing cross-infection.

Today microbes are getting refractory to antimicrobial drugs and these drug resistant microbes are responsible for occurrence of chronic diseases. Methicillin resistant *S. au*reus (MRSA) is one of the predominant microbes reported to be responsible for hospital acquired (nosocomial) or community-acquired infections. A parallel increase has also been registered in the numbers of Vancomycin resistant *Enterococci* (VRE), Vancomycin intermediate *S. aureus* (VISA) and Vancomycin resistant *S. aureus* (VRSA). MRSA has been isolated from nosocomial environment as well as from the community. Transmission of MRSA from patient to patient after direct contact with asymptomatic healthcare providers or diseased individuals in hospitals as well as community has been traced and documented (Gastmeier *et.al.*, 2002; Chambers, 2001). Hands of health care workers (HCW's) are the primary mode of transmission of MRSA and other pathogens to new admitted patients as well as in community. Viable microorganisms are easily shed from the patient, or health worker or reservoir's skin, bed linen, bed furniture and other objects in the patient's immediate environment can easily become contaminated with patient flora (Burke, 2003).

Hand washing is considered as the simplest and most effective measure that can be followed by people to reduce the risk of spread of infectious diseases. Hand washing is the act of cleaning the hands with or with out the use of soap or other detergent to remove dirt or loose transient flora thus preventing cross-infection. Hand washing was traditionally been identified as the most important infection control intervention by Dr. Ignaz Phillip Semmelweis (1818-1865) during patient examination to prevent disease transmission and is recommended before and after contact with patients, body fluids and dirty material; between dirty and clean procedures on the same patient; before and after performing invasive procedures (Pettit & Boyce, 2001). Hand hygiene is a general term that applies to hand washing, antiseptic hand wash, antiseptic hand rub, or surgical hand antisepsis (CDC, 1995). Hand hygiene is the first line of defense against the spread of pathogenic microbes and in conjunction with stringent sanitation, can greatly reduce the risks of nosocomial and community acquired diseases.

Soaps are esterified sodium or potassium salts of fatty acids. These can exist in different forms like the bar soap, tissue, leaflet and liquid preparations. The cleaning action of the soap is attributed to its detergent properties as a result of which dirt, soil and various organic compounds are removed from the hands. Plain soaps have minimal or no antimicrobial activity and removes the transient flora loosely. In majority of cases the plain soap failed to remove the pathogens from the hands of healthcare personnel. Moreover plain soaps could serve as the substrate for colonization of transient microflora. Use of antimicrobial agents in soap can effectively reduce the bacterial counts of transient microflora in hands. Triclosan (5-chloro-2-(2,4-dichlorophenoxy) phenol) is a phenolic compound which is soluble in ethanol, diethyl ether, and strong basic solution like 1 M sodium hydroxide which is used as a basic material in preparation of the soap. Triclosan is a broad spectrum antibacterial as well as antifungal agent. Its bactericidal action is due inhibition of fatty acid synthesis by binding to bacterial enoyl-acyl carrier protein reductase enzyme (ENR) (McMurry *et.al*., 1993). Triclosan was recommended for MRSA in community as well as hospitals (Zafar *et.al*., 1995). However excessive use of Triclosan as a biocide in variety of hygiene products like soaps and mouthwashes, thereafter incorporation in toys, towels and chopping boards has led to reduced susceptibility of MRSA and other pathogens (Suller & Russel, 2000). Aiello *et.al*. (2005) have recently found that antimicrobial soaps having 0.2% triclosan were not beneficial in reducing the bacterial counts in hands of household members and patients. Chlorhexidine is a chemical antiseptic which combats with both gram positive and gram negative microbes and is both bacteriostatic and bactericidal and used for general skin cleansing, a surgical scrub and a pre-operative skin preparation. It has also been reported that Chlorhexidine based hand washes have limited effectiveness towards MRSA. Mycobacteria are generally highly resistant to chlorhexidine (Russell, 1996).

Quaternary ammonium salts like Cetrimide also possess antibacterial activity. Cetrimide is also known as Alkyltrimethylammonium bromide or Hexadecyltrimethylammonium bromide and generally used as a topical agent against bacteria and fungi. It is also an anionic detergent abbreviated as CTAB (Cetyl trimethyl ammonium bromide) for isolation and purification of biological macromolecules like DNA. Cetrimide has been found to have an effect on the Proton motive force (PMF) in *S. aureus* at bacteriostatic concentration, by the discharge of the pH component of the PMF and by production of a compound having 260-nm-absorbing material. There are reports of Cetrimide resistance in human pathogenic bacteria. ZPT (Zinc Pyrithione) has more antimicrobial efficacy than that of iodine, chlorhexidine and triclosan (Paulson, 1999). ZPT basically acts by inhibition of the bacterial ATP; synthesis however ZPT is considered a better antifungal than antibacterial agent.

There is an immense need of antiseptics, which have less, or no resistant pattern towards the transient microflora of the skin, should not have irritating and drying properties and could check the spread of multidrug resistant microbes. Plant extracts consist of a variety of bioactive compounds, which possess antibacterial activities. Standardized extracts of garlic, onion and green pepper have been reported to inhibit the growth of *Escherichia coli*, *Salmonella typhi*, *Shigella dysenterae* and *S. aureus* (Sofowora, 1983). The bactericidal effect of garlic extract was apparent in *Escherichia coli* within 1 hour of incubation and 93% reduction in CFU was observed in *Salmonella typhi* and *Staphylococcus epidermidis*. Neem oil/extract has largely been used in manufacture of soap due to its antimicrobial properties. Essential oils of lavender have been traditionally used in soaps for fragrance as well as for antimicrobial action. Historically, many plant oils and extracts, such as tea tree, myrrh and clove, have been used as topical antiseptics, or have been reported to have antimicrobial properties. Various publications have documented the antimicrobial activity of essential oils and plant extracts including rosemary, peppermint, bay, basil, tea tree, celery seed and bottle brush (Saxena & Gomber 2006). Thus, there is immense potential in establishing the use of antimicrobial herbal soaps as a measure to control the multidrug resistant microbes as well as check their spread through hands from one geographical region to another.

REVIEW
OF
LITERATURE

2.1 Historical perspectives

Hand washing is a fundamental principle and practice in the prevention, control, and reduction of healthcare-acquired infection. For generations, handwashing with soap and water has been considered a measure of personal hygiene. The concept of cleansing hands with an antiseptic agent emerged in the early 19th century. As early as 1822, it has been demonstrated that solutions containing chlorides of lime or soda could eradicate the foul odors associated with human corpses and that such solutions could be used as disinfectants and antiseptics (Labarraque, 1989). Advocated by Semmelweiss (Louis & Mosby, 1985) from the 1800s to resolve an obstetric morbidity and mortality occurrence, the simple act of hand cleansing portrays the intuitive benefits to basic hygiene, health continuum, and, most important, disease prevention. The term hand washing is replaced by the new term hand hygiene, which includes hand cleansing, hand disinfecting, and surgical hand scrub.

Cleansing heavily contaminated hands with an antiseptic agent between patient contacts may reduce health-care–associated transmission of contagious diseases more effectively than hand washing with plain soap and water. In 1843, Oliver Wendell Holmes concluded the spread of puerperal fever through the hands of health personnel. Hand washing with soap and water, followed by drying with paper towels, reduces the risk of transient skin carriage of salmonellas (Pether & Gilbert*, 1971*). The primary goal of hand washing by food workers is the removal of surface soil (oil and debris) on hands and hence, the removal of transient pathogenic microorganisms (Hutchinson, 1987). This can be accomplished by washing hands with soap or detergent and water. By increasing the friction during hand washing by rubbing the hands together, or by using a nailbrush, ordinary soaps and detergents can reduce a high level of transient bacteria, as well as a minor portion of resident bacteria.

Use of non-antimicrobial soap between patient contacts and washing with antimicrobial soap before and after performing invasive procedures or caring for patients at high risk has been recommended (Steere *et. al.*1975; Garner *et. al.*1986). Either antimicrobial soap or a waterless antiseptic agent can be used for cleaning hands upon leaving the rooms of patients with multidrug-resistant pathogens (e.g., methicillin-resistant *Staphylococcus aureus* [MRSA]) (Garner, 1996). More than 150 years after Semmelweis epidemiological observations, this intervention represents the first evidence indicating that cleansing heavily contaminated hands with an antiseptic agent between patient contacts may reduce health-care–associated transmission of contagious diseases more effectively than hand washing with plain soap and water

2.2 Antimicrobial Resistance

Multidrug resistance is a continually evolving and dangerous problem that requires immediate attention as well as future planning to impede a global health crisis. Widespread use of antibiotics has spurred evolutionary changes in bacteria allowing them to flourish in the presence of powerful drugs. Penicillin resistance emerged in 1950s, cephalosporin resistance in 1970s and resistance to third generation cephalosporins in 1980s. Today virtually all-major human pathogens have acquired antimicrobial resistance genes. These include *Salmonella*, *Shigella*, *Neisseria gonorrhoeae*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *E.coli*, *Vibrio cholera*, *Enterobacter* sp., *Mycobacterium tuberculosis* and most recently *Staphylococcus aureus*. Hospital acquired bacterial infections due to multidrug resistant strains pose a formidable challenge for healthcare practitioners as patients often need to be treated empirically and a delay in the appropriate initial therapy is known to increase mortality rates significantly. Prompt treatment with an appropriate antimicrobial agent that is active against both gram-positive and gram-negative infections is prudent for such infections. This situation is worsened in under- developed countries due to high cost of such treatments. Thus the development of novel, effective and low-cost chemotherapeutic alternatives is an urgent need today.

2.3 *Staphylococcus aureus*: Biology of the Organism and Overview of Resistance

Staphylococcus aureus are gram positive, non-motile, non-spore forming, facultative anaerobic cocci, which are catalase positive and oxidase negative. Under the microscope they usually appear as grape-like clusters (Fig. 1) (Lowy, 1998; Lowy, 2003). Asymptomatic colonization of *S. aureus* occurs on human skin, anterior nares, nails, hair, axillae, perineum and vagina. *S. aureus* can cause disease, particularly if there is an opportunity for the bacteria to enter the body. Nasal carriage perhaps is a major risk factor for *S.aureus* infections (Kluytmans *et al.* 1997). The success of *S. aureus* as a pathogen and its ability to cause such a wide range of infections (Table 1) are the result of its extensive virulence factors (Archer, 1998).

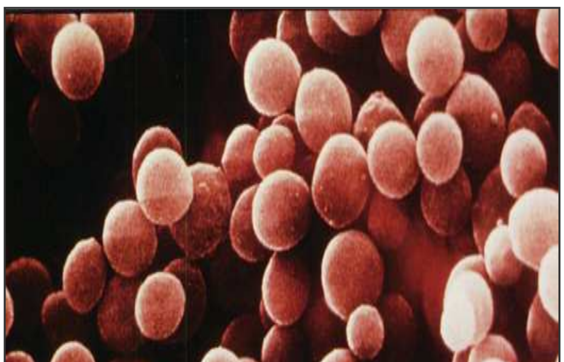


Fig 1: *S. aureus*: Electron micrograph

Table 1. Infections caused by *S.aureus*
Furuncle or carbuncle
Impetigo bullosa
Cellulitis
Surgical wound infection
Pyomyositis
Botryomycosis
Hosptial-acquired bacteremia
Acute or right-sided endocarditis
Hematogenous osteomyelitis
Septic arthritis
Epidural abscess
Brain abscess
Empyema
Toxic shock syndrome
Scalded skin syndrome
Food-borne gastroenteritis

The mortality of patients with *S. aureus* bacteremia in the pre-antibiotic era exceeded 80%, and over 70% developed metastatic infections (Skinner *et.al.,* 1941).However, as early as 1942, penicillin-resistant staphylococci were recognized, first in hospitals and subsequently in the community (Rammelkamp *et.al*., 1942). By the late 1960s, more than 80% of both community- and hospital-acquired staphylococcal isolates were resistant to penicillin. Methicillin (a penicillinase- stable β- lactam) was introduced in 1960. However the success of methicillin was short lived as cases methicillin resistant *Staphylococcus aureus* infections [due to production of a penicillin-binding protein (PBP2a)] were detected in hospitals in Britain in 1961 (Solsby, 2005). Today MRSA is one of the leading causes of bacterial infections representing 60% of the nosocomial *S. aureus* isolates detected in hospital intensive care units (Chambers, 2001). These infections are difficult to treat as MRSA are usually resistant other antibacterial agents including quinolones, sulfonamides, macrolides, tetracyclines, chloranphenicol, and cephalosporins (Baquero, 1997). Vancomycin- a glycopeptide antibiotic remains the last choice of treatment for MRSA infections. However in 1996, the first clinical isolate of *S. aureus* with reduced susceptibility to vancomycin was reported from Japan (Hiramatsu *et al*, 1997). Infections with reduced susceptibility to vancomycin continue to be reported, including 2 cases caused by *S.aureus* isolates with full resistance to vancomycin. In the United States, there have now been 9 reported clinical cases of infection with *S.aureus* with intermediate resistance to vancomycin, as well as 2 known clinical cases of infection with *S.aureus* isolates that are fully resistant to vancomycin. The emergence of VRSA has created a fear of the emergence of a pre- antibiotic era, thus necessitating the discovery of newer therapeutics from various biological matrices viz. plants and microbes.

2.4 Transmission of MRSA and VRSA

Diseases are caused by germs, which are transmitted from one person to another through the air, urine and feces, blood, saliva, skin etc. In hospital approximately 5-10% of all patients acquire a nosocomial infection (Ayliffe, 1996). Transmission of infection in a hospital requires at least three elements: a source of infecting microorganisms, a susceptible host and a means of transmission for bacteria and virus (Pyrek, 2006). Patients undergoing surgical treatment or physiotherapy showed a threefold increase in the risk of being colonized or infected with MRSA. Surgical wound infection is the most frequent among MRSA-induced infections (28%). The main transmission path of MRSA inside a hospital is bacterial spread from one patient to another through contact with the hands of nursing staff (Llacsahuanga *et. al*., 1998). Asymptomatic colonization of MRSA and VRSA occurs in healthy, non-hospitalized persons without contact with healthcare personnel or other colonized patients (Herold *et. al*., 1998). It has been strongly suspected for many years that MRSA environmental contamination is clinically significant and provides a reservoir for indirect transmission of MRSA between patients (Dancer, 1999; Hota, 2004). Healthcare workers hands may become contaminated by contact with patients, or surfaces in the workplace, and medical devices that are contaminated with body fluids containing MRSA (CCOH, 2005). Thus, cross-infection in health care occurs by transfer on the hands of staff, primarily due to a lack of handwashing or poor practice in the process of handwashing and drying. Methicillin-resistant *S. aure*us (MRSA) have been transmitted on the hands of staff and caused infections in others (Boyle, 2001; Coia, 1999). Patients also contribute to increased infection rates with their own hand hygiene practices. Overall hand hygiene compliance increased from 40% to 53% before patient contact and 39% to 59% after patient contact. Patients' hands can contribute to infection and cross-infection (Sanderson & Weissler, 1992).

Poor hand hygiene in the general population is also indicated as a contributory factor in the increasing incidence of food borne illnesses and in cross-infection in areas such as nurseries, prisons and farm visitor centres (CDR 1999). A report of MRSA infections leading to four deaths in previously healthy children demonstrated that MRSA infections can be community-acquired in persons with no exposure to the hospital system (CDC, 1997). This raises serious concerns about the possibility of transmission of MRSA outside the healthcare system.

2.5 Hand microflora

The skin is the largest and most accessible organ of the human body. The skin provides protection by serving as an impenetrable barrier between bacteria-free tissues of the body and an environment that is contaminated with all types of microorganisms. Normal human skin is colonized with a variety of bacteria. Different areas of the body have varied total aerobic bacterial counts (e.g., 1-106 colony forming units (cfus)/$cm^{2,}$ on the scalp, 5-105 cfus/cm^2 in the axilla, 4-104 cfus/cm^2on the abdomen, and 1-104 cfus/cm^2 on the forearm) (Selwyn, 1980). Total bacterial counts on the hands of medical personnel have ranged from 3.9 -104 to 4.6- 106(Price, 1938; Larson *et. al.,* 1998).

There are significant quantitative differences in the composition and density of microflora in different areas of the hands (Ginley *et. al.*, 1988). The subungual spaces have an average log_{10} cfu of 5.39, compared with a range from 2.55 to 3.53 for other hand sites. Coagulase-negative Staphylococci are the dominant organisms, with *S. epidermidis, S. haemolyticus* and *S. hominis* being the most frequently isolated species. Other bacteria recovered from subungual spaces included gram-negative bacilli in 42.3% of subjects, with *Pseudomonas* species composing 31.3% of this group, and coryneforms in 42.3% of subjects.

2.5 Efficacy of various antiseptic agents

Various hand hygiene techniques have been recommended by sanitarians. Investigators use different methods to study the *In vivo* efficacy of handwashing, antiseptic handwash and surgical hand antisepsis protocols. These protocols differ mainly weather hands are purposely contaminated with bacteria before use of test agents, the volume of hand hygiene product applied to the hands, the method use to contaminate fingers or hands and the method of expressing the efficacy of products. Despite these differences, the majority of studies can be placed into two major categories, studies focusing on products to remove transient flora and studies involving products that are used to remove resident flora from the hands. In the United States, antiseptic had wash products intended for use by HCW's is regulated by FDA's division of over-the counter drug products (OTC). Requirements for in vitro and In vivo testing of HCW's hand wash products and surgical hand scrubs are outlined in the FDA tentative final monograph for healthcare antiseptic drug products. Ayliffe *et. al*. (1978) standardized the test procedure in which fingertips are inoculated with broth cultures of organisms (*S. aureus, S. saprophyticus, E. coli and Pseudomonas aeruginosa*). 70% alcohol, with or without chlorhexidine was found to be most effective preparation among all the antiseptic agents.

Table 2 Antimicrobial agents in soaps: (Pittet & Boyce, 2001)

Group	Gram-positive bacteria	Gram-negative bacteria	Speed of action	Comments
Alcohols	+++	+++	Fast	Optimum concentration 60–90%; non-persistent activity
Chlorhexidine	+++	++	Intermediate	Persistent activity; (2% and 4% aqueous) rare allergic reactions
Iodine compounds	+++	+++	Intermediate	Causes skin burns usually too irritating for hand hygiene
Iodophors	+++	+++	Intermediate	Less irritating than iodine; acceptance varies
Phenol derivatives	+++	+++	Intermediate	Activity neutralised by non-ionic surfactants
Triclosan	+++	++	Intermediate	Acceptability on hands varies
Quaternary ammonium	+++	++	Slow	Used only in combination with compounds alcohols; ecologic concerns

Activity: +++ excellent; ++ good, does not include the entire bacterial spectrum

The efficacy of the antimicrobial effect of 0.5% chlorhexidine (Hibistat) has been found to be more as compared to 70% alcohol on hands contaminated with Serratia marcescens (P<0.01) (Aly & Maibach ,1980). Hand washing will probably prevent at least some of the diarrhea in day-care centers (Black *et. al.,* 1981). Hand washing practices may be adversely influenced by the detrimental effect of hand washing on skin. Chorhexidine and povidone-iodine containing antiseptic agents can adversely damage the stratum corneum (Larson *et. al*., 1986).

Larson *et. al.* (1988) studied the influence of two hand washing frequencies on the reduction in colonizing flora with three hand washing products used by health care personnel. 2% chlorhexidine gluconate resulted in greater reductions as compared to 0.6% parachlorometaxylenol, 0.3% triclosan, or a non-antimicrobial control. In 2005, Monique Courtenay worked on the efficacy of various hand hygiene regimes on removal and/or destruction of *Escherchia coli* on hands. The efficacy of alcohol based hand sanitizers to replace hand washing was also evaluated. Servsafe, warm water rinse and cool water rinse reduced *E.coli* cells on hands by 98.0, 64.4, 42.8 %log cfu/ml, resulting in <1,1.4 and 2,1 log cfu/ml *E.coli* on hands, respectively, from 3.6log cfu/ml on unwashed hands. Hand hygiene has indicated that the efficacy of alcohol is superior to the many other active agents such as chlorhexidiene or povidone iodine, also on the residual hand flora (CDC, 2002). Gunter *et. al.* (2005) also proved this by evaluating the efficacy of two distinct ethanol - based hand rubs for surgical hand disinfections.

Goktas *et. al.* (1992) tested the efficacy of tap water, unmedicated bar soap, benzalkonium chloride 1% (Zefiran), Na-hypochloride 1% and 5%, alcohol 70%, hexachlorophene 3%, hexachlorophene 3% liquid soap (Solu-heks), chlorhexidine 1.5% liquid soap (Savlon), chlorhexidine 4% liquid soap (Hibiscrub), povidone-iodine 10% solution (Betadine and polyod), povidone-iodine 7.5% liquid soap (Betadine and Polyod) by compairing on the gloved hands contaminated with *E.coli* and *Pseudomonas aeruginosa* Brain Heart Infusion broth cultures. It was found that washing time of 30 seconds with chlorhexidine 4% (Hibiscrub) liquid soap or povidone-iodine 7.5% liquid soap (Betadine and Polyod) was required to eradicate all the organisms inoculated from both glove surfaces.

Povidone-iodine and chlorhexidine were more effective washing agents than the other disinfectants.

Chlorhexidine gluconate and povidone iodine can be used for the disinfection of skin and scrubbing hands prior to surgery, bladder irrigation & catheterization. 2% glutaraldehyde is cost effective disinfectant and can be used for the disinfection of medical equipment like cystoscopes, bronchoscopes and decontamination in laboratories. Lysol should be preferred over phenol for cleaning floors, and walls. Savlon is not recommended for the disinfection of skin, as it is liable to become contaminated with *Pseudomonas aeruginosa.* Further studies are required to evaluate these disinfectants when applied to the real life situations (Karamat Ahmed Karamat *et. al.*, 1997).

Ekizoglu *et. al.*, (2003) studied the effect of widely used antiseptics and disinfectants on some hospital isolates of gram-negative bacteria by the quantitative suspension test. Chlorhexidine gluconate (4%), savlon (1:100), and 5.25% sodium hypochlorite were tested. Savlon and chlorhexidine gluconate were effective at in-use concentrations and sodium hypochlorite was effective at 1:50 dilution.

It has been reported that alcohols (70%) alone or with savlon reduced the virus titer by greater than 99%, whereas the reductions by proviodine, dettol, and hibisol ranged from 95 to 97%, using finger pad method. Aqueous solutions of chlorhexidine gluconate are significantly less effective for virus removal or inactivation than 70% alcohol solutions. Furthermore, savlon in water (1:200) is found to be much less effective in eliminating the virus (80.6%) than the bacterium (98.9%). The tap water alone and the soap reduced the virus titers by 83.6 and 72.5% and the bacterial titers by 90 and 68.7%, respectively (Ansari, 1989). Hand-rubbing with aqueous alcoholic solution, preceded by a 1-minute non-antiseptic hand wash before each surgeon's first procedure of the day and before any other procedure if the hands are soiled, is as effective as traditional hand-scrubbing with antiseptic soap in preventing surgical site infections (Jean Jacques parienti, 2002). Dettol-H and alpilon (having benzalkonium chloride (40%) + disodium edetate (1.5%) both have been found to be effective against a number of pathogenic bacterial organisms (Kapoor *et. al.*, 1998).

2.6 Resistance against antiseptic agents

Many bacterial strains including *S.aureus* have been found to be resistant to antiseptic agents, which are being used as ingredients in commercial antiseptic soaps**.** *S. aureus* strains with low-level resistance to triclosan have emerged (Suller & Russel, 2000). Some gram-negative bacteria are found to be resistant to chlorhexidine (Russell & Path, 1986). *Pseudomonas aeruginosa* is highly resistant to triclosan, whereas methicillin-resistant *S. aureus* strains are inhibited over a range of 0.1–2 mg/L. Enterococci are much less susceptible than S*taphylococci* (Russell, 2004).

2.7 Plant extracts as antimicrobials

The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. Ethnopharmacologists, botanists, microbiologists, and natural-products chemists are combing the Earth for phytochemicals and "leads" which could be developed for treatment of infectious diseases. 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).

Historically, plants have provided a good source of antiinfective agents. Emetine, quinine and berberine remain highly effective against microbial infections. Plant based antimicrobials represent a vast untapped source for medicines. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials.

Plants containing protoberberines and related alkaloids, picralima-type indole alkaloids and garcinia biflavonones used in traditional African system of medicine, have been found to be active against a wide variety of micro-organisms. The profile of known drugs like *Hydrastis canadensis* (goldenseal), *Garcinia* kola (bitter kola), *Polygonum s*p., *Aframomum melegueta* (grains of paradise) will be used to illustrate the enormous potential of antiinfective agents from higher plants (Maurice, 1999). Kazmi *et. al.* (1994) described an anthraquinone from *Cassia italica*, which was bacteriostatic for *Bacillus anthracis*, *Corynebacterium pseudodiphthericum*, and *Pseudomonas aeruginosa* and bactericidal for *Pseudomonas pseudomalliae* The ethanol-soluble fraction of purple prairie clover yields a terpenoid called petalostemumol, which showed excellent activity against *Bacillus subtilis* and *S. aureus* and lesser activity against gram-negative bacteria as well as *Candida albicans* . Kadota *et. al.* (1997) found that trichorabdal A; a diterpene could directly inhibit *H. pylori*. The crude khat callus extracts shows high antibacterial activity against gram positive and myocbacteria and broad cytotoxic activity against several cell-line systems (Jaber & Mossa, 1999).

2.8 Plant derived antistaphylococcal agents

Many of the plant derived antimicrobialagents are found to be effective against *S,aureus* .The ethanol-soluble fraction of purple prairie clover yields a terpenoid called petalostemumol, which showed excellent activity against *S. aureus* . Two diterpenes isolated by Batista *et. al.* (1994) have been found to be more democratic, they worked well against *S. aureus*. Extract from the leaves of *Eremophila alternifolia* (Myoporaceae) shows activity against MRSA; extracts from the leaves of *Amyema quandong* (Loranthaceae) and *Eremophila duttonii* (Myoporaceae) reported active against both VRSA and MRSA extract from the stem base of *Lepidosperma viscidum* (Cyperaceae) are active against MRSA(Palombo *et. al.*,2002). From the plant extracts screened for antibacterial activity, *Myrtus communis L.* (leaves) has antimicrobial activity, inhibiting the growth of the *S.aureus* isolates (Mansouri, 1999).

2.9 Techniques used for evaluation of transient hand microflora

Various techniques are in use for the evaluation of transient hand bacterial flora. In finger impression method, finger impressions are taken on the separate media plates for both left and right fingers and incubating the plates for 18-24 hr at 37^0C followed by colony counting (Mona *et. al.,* 2005). In rinse wash method, hand wash samples are collected in the sterile beakers/bags under sterile conditions. Temperature of sterile water should be near to 40^0C and 75 ml water is used for a single hand wash. Further enumeration of bacterial flora is carried out by serially diluting and plating on media plates and incubating them at 37^0C for 18-24 hr (Courtenary *et.al*., 2005)

3.0 Antimicrobial susceptibility testing

Kirby Bauer disc diffusion method is a quantitative assay whereby disc of paper are impregnated with a single conc. of different antibiotics. The disc diffusion method is performed in accordance to NCCLS (National committee for clinical laboratory standards) method gives reliable results. And hence predicts clinical efficacy of antibiotic tested. This is one of the more commonly used methods of antimicrobial susceptibility testing. In this test, small filter paper disks (6 mm) impregnated with a standard amount of antibiotic are placed onto an agar plate to which bacteria have been swabbed. The plates are incubated overnight, and the zone of inhibition of bacterial growth is used as a measure of susceptibility. Large zones of inhibition indicate that the organism is susceptible, while small or no zone of inhibition indicates resistance. An interpretation of intermediate is given for zones, which fall between the accepted cutoffs for the other interpretations European Antimicrobial resistance surveillance system (EARSS, 2005) defines the susceptibility of *S.aureus*. Arthur L. Barry (1974) evaluated two standardized disk methods for testing antimicrobial susceptibility of *Pseudomonas aeruginosa* and of the *Enterobacteriaceae.* In a study made by Onanuga *et. al.* (2005**)**.on *S.aureus* isolates collected from urine samples of pregnant women's werefound to be highly resistant to ampicillin (91.7%), clindamycin (78.3%), cephalexin (75%), methicillin (71.7%) and vancomycin (68.3%) but had very low resistance to gentamicin (3.3%), ciprofloxacin (3.3%).

3.1 Soaps used in present study

Two commercial antiseptic soaps (Savlon & medimix), an indigenous herbal soap and a bland soap (soap lacking herbal extract i.e. control) were evaluated in the study.

Savlon is an antiseptic soap containing 0.3g-chlorhexidine gluconate and 3.0g cetrimide as active ingredients per 100 ml and 2.84% m/v n-propyl alcohol and 0,056% m/v benzyl benzoate as preservatives. This soap has detergent and antiseptic cleansing properties with mild skin reactions therefore should not be applied repeatedly to the skin and wet dressings should not be left in contact as sensitivity may occur. Ototoxicity has been reported after direct instillation into the middle ear. Medimix is a poly herbal antiseptic soap having fewer side effects then other commercial antiseptic soaps.

AIM OF THE STUDY

The aim of the current study is to evaluate the efficacy of an indigenous herbal soap in reducing the total microflora on palms and its comparison with commercially available antimicrobial soaps viz. Savlon and Medimix.

To achieve the aim, the objectives of the dissertation were:

1. Evaluation of total transient bacterial flora on palms.

2. *In vivo* efficacy of commercial antiseptic and indigenous herbal soaps.

3. Evaluation of the antimicrobial susceptibility of the isolated strains.

MATERIAL

AND

MATHODOLOGY

4.1 *In vitro* evaluation of transient bacterial palm flora

Collection of palm washes samples by Wash/ rinse Procedure (palm washing).

4.1.1 Experimental design

A total of ninety palm washes were taken from three volunteers (30 from each) in whole study period for the evaluation of transient bacterial flora from palms. In a day, two palm washes were taken at two different timings (morning & evening) involving three consecutive palm washes for a single volunteer at a single time.

4.1.2 Rinse / wash method (Courtenary et. al., 2005)

Rinse method was used for collection of palm wash samples. Palm washes samples were collected by rinsing palms (slightly rubbing) with 75 ml sterile water (40^0C) in sterile beakers under sterile conditions. Each volunteer repeated the palm wash treatment for three times consecutively and palm wash samples were further used for enumeration of total bacterial, *S.aureus* and *E.coli* flora.

4.1.3 Culture media

Nutrient agar, Mannitol salt phenol red agar and EMB agar (Eosin Methylene- blue Lactose sucrose agar) were used respectively for the isolation of total bacterial flora, S. *aureus* and *E.coli* from palm region.

Fig. 2: Change in colour indicates the presence of *S.aureus*

4.1.4 Bacteriological study

Aseptically 10 μl palm wash samples were transferred to 90 μl sterile saline (0.85%) solution. Appropriate dilutions of the samples were plated out on one day old nutrient agar plates, mannitol salt agar salt plates and EMB Agar plates followed by incubation at 37^0C for 18-24 hours. Typical colonies showing characteristic coloration were counted and representative colonies were picked up randomly and transferred to respective broths in test tubes and incubated at 37^0c for 24-48 hrs for microscopic examination. The above isolates were purified by repeated streaking on the selective medium to obtain well-isolated colonies and preserved on specific slants.

4.1.5 Preliminary tests for the identification of *S.aureus* and *E.coli*

Microscopic examination

The isolates were examined for shape and size as well as gram's reaction.

4.2 *In vivo* efficacy of commercial soaps (herbal/ non- herbal), control soap and indigenous herbal soap

4.2.1 Experimental design

Total of four volunteers were involved for *in vivo* efficacy of four different soaps. Total 40 palm washes were taken for a single volunteer for a single soap. Control palm washes were taken by use of sterile water only.

4.2.2 Procedure

Rinse palm for 10seconds with small amount of tap water. Palm is completely lathered with the desired soap for a specified time (1 min). Volunteers then rinse palms under 40ºC tap water for 30 seconds and the samples are obtained aseptically.

4.2.3 Bacteriological study

Point inoculation method (Miles & Mishra, 1938) 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 37^0C for 6 hrs. Typical colonies showing characteristic coloration were counted and representative colonies were picked up randomly and transferred to respective broths in test tubes and incubated at 37^0c for 24-48 hrs for microscopic examination. The above isolates were purified by repeated streaking on the selective medium to obtain well-isolated colonies and preserved on specific slants.

4.2.4 *Microscopic examination for the identification of S.aureus and E.coli.*

The isolates were examined for shape and size as well as gram's reaction.

4.2.5 Percentage reductions in bacterial flora will be calculated using the formula (Courtenary et. al., 2005)

$$\textbf{\% Reduction} = \frac{\text{Control (cfu/ml) - Test (cfu/ ml)}}{\text{Control (cfu/ml)}} \times 100$$

4.3 Antimicrobial Susceptibility Testing (Bauer & Kirby, 1966)

4.3.1 Preparation of 0.5 McFarland standard

Add 0.5 ml of 0.048M $BaCl_2$ to 99.5 ml of 0.18 M H_2So_4 with constant stirring. The O.D. of the solution at should be in the range of 0.08-0.1 at 625 nm. Store the standard in amber colored bottle (to prevent it from the damaging effect of light) at room temperature. Vigoursly vortex the standary prior to use (NCCLS, 1997). Distribute in to the tubes of the same size as those of for the broth cultures.

4.3.2 Susceptibility Testing

The Kirby Bauer disk assay was used for testing 33 antibacterial drugs against test microorganisms for profiling their resistance pattern according to NCCLS, 2002. The turbidity of the activated cultures was visually adjusted with saline to that of 0.5 Mc Farland standard. Within 15 min of adjustment of the test cultures, Muller Hinton Agar plates (mean depth ± 4.00mm) were inoculated with the test culture by swabbing each plate 3 times for carrying out the antibacterial resistance profiles respectively. Sterile antibiotic disks (Hi Media) were applied on to the surface of agar plates using sterile forceps. Plates were incubated at 37^0C for 18-24 hrs. Susceptibility was evaluated by measuring the diameter of the clear zone of inhibition across the antibiotic disk. Plates were examined visually for isolated colonies within the inhibition zone that may have represented resistance.

Based on the results the cultures were classified as resistant (R), intermediate (I) and susceptible (S).

RESULTS AND DISCUSSION

5.1 Evaluation of total microflora

Colonies were counted and isolated colonies were examined microscopically for morphological identification. On nutrient agar plates, different shaped colonies were observed. On MS phenol red agar plates *S.aureus* colonies were found to be gram's positive, surrounded by yellow zone and appeared grape like in clusters. Change in colour from red to yellow indicates the presence of *S.aureus*. *E.coli* putative identification was also done by microscopic examination and gram's staining. They were found to be greenish, metallic sheen in reflected light and gram's negative rods. Table 6, fig. 3 and 4 represent the average general (morning and evening) microbial flora for three volunteers. Total average microbial flora on palms of first volunteer was found to be 129 x 10^3 cfu/ml for morning session and 95 x 10^3 cfu/ml for evening session. Reduction was observed in total microbial load on palms from first to third palm washes except in second palm washes during morning session. Average microflora counted on palms of first volunteer after first, second and third wash for morning session were found to be 132 x 10^3, 136 x 10^3 and 95 x 10^3 (Table6) respectively. It was found to be 118 x 10^3, 108 x 10^3 and, 66 x 10^3 cfu/ml for second volunteer for evening session respectively three palm washes (Table 6).

Total microbial load for second volunteer was found to be 119 x 10^3 for morning session palm washes and 85 x 10^3 for evening palm washes (Table 6, Fig. 2). Reduction was observed in total microbial load on palms from first to third palm wash in case of morning palm washes while no reduction was observed for evening session. It was 140 x 10^3, 128x103 and 76x10^3 cfu/ml for first, second and third palm washes respectively in case of morning session palm washes. And 83 x 10^3, 188 x 10^3 and 315 x 10^3 for evening session palm washes (Table 6). For the third volunteer, reduction was observed in total microbial load from morning to evening session. Total average microbial load counted during morning session was 116 x 10^3 and during evening session it was found to be 103 x 10^3 (Table 6,fig. 3) for all the three volunteers. For the entire three volunteer it was observed that at the morning time microbial flora was more than evening. Table 6 and fig. 5

represents the total average microbial flora on palms of all the three volunteers. Average bacterial flora was found to be 108 $x10^3$ cfu/ml. Previous studies also showed that the transient flora varies considerably from person to person (Ayliffe et. al., 1988). The subungal spaces have an average log 10 cfu of 5.39 (McGinley et. al., 1988).

Table 6: Viable bacterial count in cfu/ml

Volunteer	Morning				Evening			
	Hand wash no.				Hand wash no			
	1	2	3	Mean	1	2	3	Mean
1	$132x10^3$	$136x10^3$	$95x10^3$	$129x10^3$	$118x10^3$	$108x10^3$	$66x10^3$	$95x10^3$
2	$140x10^3$	$128x10^3$	$76x10^3$	$119x10^3$	$83x10^3$	$188x10^3$	$315x10^3$	$85x10^3$
3	$154x10^3$	$89x10^3$	$132x10^3$	$116x10^3$	$133x10^3$	$109x10^3$	$80x10^3$	$103x10^3$
	$121.3x10^3$				$94.3x10^3$			
Mean	$108x10^3$							

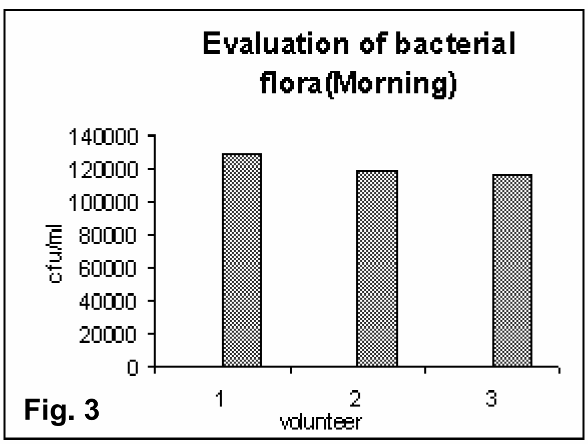


Fig. 3

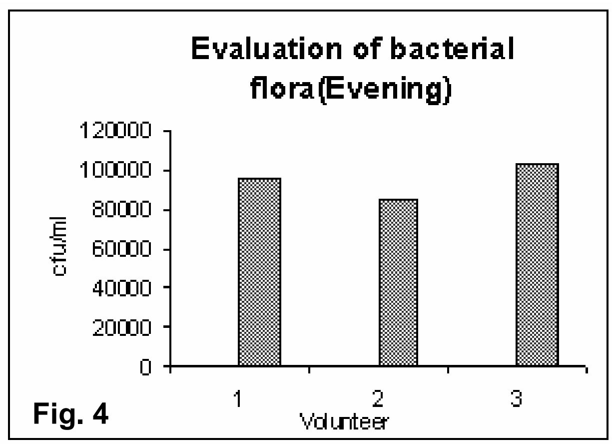


Fig. 4

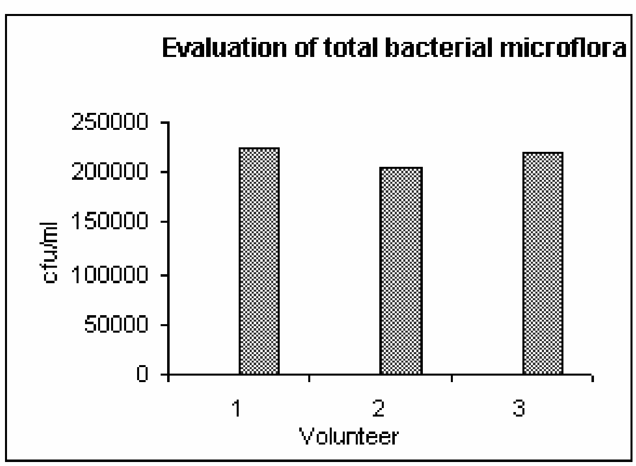


Fig.5

Table 7: *S. aureus* microflora in cfu/ml			
Volunteer	Morning	Evening	Mean
1	67×10^3	57×10^3	61.66×10^3
2	48×10^3	66×10^3	56.7×10^3
3	39×10^3	59×10^3	48.8×10^3
Mean	51.3×10^3	61×10^3	56×10^3

Table 8 : *E. coli* microflora in cfu/ml			
Volunteer	Morning	Evening	Mean
1	15.33×10^3	21.33×10^3	18.33×10^3
2	2.66×10^3	2.73×10^3	2.69×10^3
3	7.4×10^3	12.5×10^3	9.95×10^3
Mean	8.46×10^3	12.18×10^3	10.32×10^3

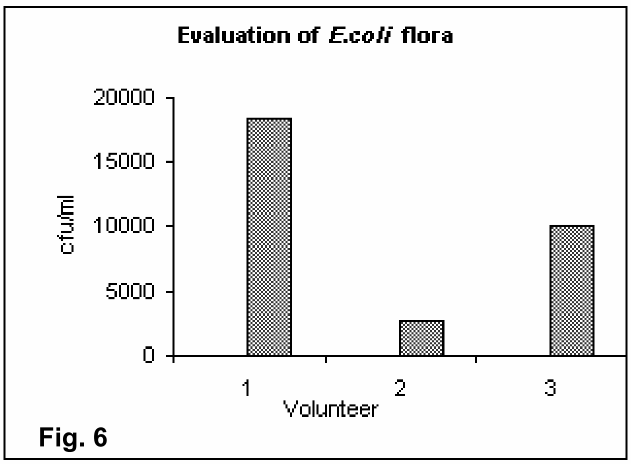


Fig. 6

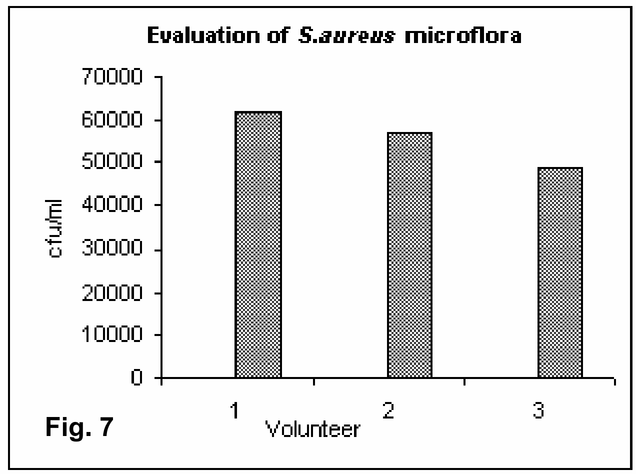


Fig. 7

Table 7 and fig. 7 represents the average *S.aureus* (56 x 10^3cfu/ml) for all the three volunteers. It was found that *S.aureus* cfu/ml were higher during evening as compared to morning. Total average *S.aureus* flora was 51 x 10^3 cfu/ml (Table 2) and 61 x 10^3 cfu/ml (Table2) respectively for morning and evening session. *S. aureus* flora was observed to be highest in case of first volunteer followed by second and third. *S.aureus* cfu/ml counts for 50% of the total microflora. 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 (Larson *et. al*., 1988). Colonization of health care worker hands with *S.aureus* has been described to range between 10 to 76.3% (Ayliffe *et. al*., 1988). Table 8 and fig. 6 shows the average *E.coli* microflora of all the three volunteers. It was observed that total average *E.coli* cfu/ml were $10x10^3$ (Table 8, fig. 6). *E.coli* flora was found to be very less as compared to *S.aureus* flora. *E.coli* counts 10% of the total microbial load. The results show that the average cfu/ml in the morning was less then that of evening for all the three volunteers. The average cfu/ml for morning and evening were 9×10^3 and 12×10^3 cfu/ml. It was concluded that *S.aureus* is the major part of the total general palm bacterial flora. It accounts for more then 50% of the total bacterial flora on palms. *E.coli* was observed to be only 10% of the total bacterial flora on palms.

5.2 *In vivo* efficacy of soaps

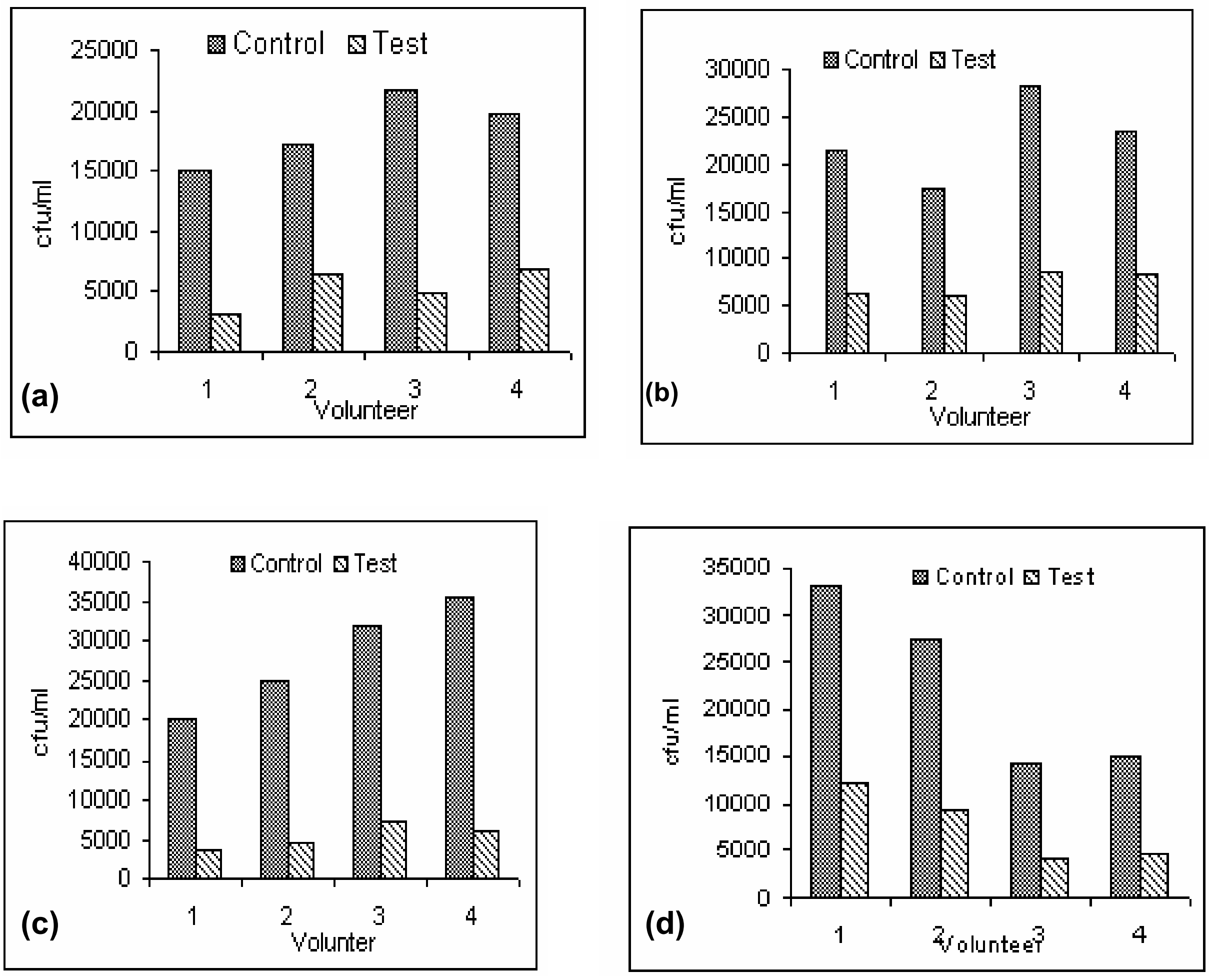
In vivo efficacy for total microbial load was estimated by using different commercial antiseptic and herbal soaps.

Table 9 and fig.10 represents the % reduction in bacterial flora after use of medimix, savlon, indigenous herbal soap (Fig.8) and control soap (Fig.9). Highest reduction was observed by indigenous herbal soap followed by medimix, savlon and control soap. % Reduction shown by indigenous herbal soap was 80.57% (Table 9 and fig.8) while it was found to be to be least in case of control soap (67.74%) (Table 9 & 10). In case of commercial antiseptic soaps medimix showed more reduction then savlon (68.29%) (Table 9, fig. 10).

Table 9. % Reduction in bacterial flora

Soap	Average Cfu/ml	Volunteer				
		1	2	3	4	Mean
Medimix	Control	$15.1x10^3$	$17.1x10^3$	$21.6x10^3$	$19.8x10^3$	$18.4x10^3$
	Test	$3.1x10^3$	$6.4x10^3$	$4.9x10^3$	$6.9x10^3$	$5.32x10^3$
	% Reduction	77	67	77	68	72
Savlon	Control	$21.4x10^3$	$17.4x10^3$	$28.8x10^3$	$23.4x10^3$	$22.75x10^3$
	Test	$6.2x10^3$	$6.1x10^3$	$8.5x10^3$	$8.4x10^3$	$7.3x10^3$
	% Reduction	74	64	72	63	68
Indigenous herbal soap	Control	$20.1x10^3$	$24.9x10^3$	$31.8x10^3$	$35.4x10^3$	$28.05x10^3$
	Test	$3.6x10^3$	$4.6x10^3$	$7.2x10^3$	$6x10^3$	$5.35x10^3$
	% Reduction	81	81	79	82	81
Control soap	Control	$33x10^3$	$27.4x10^3$	$14.4x10^3$	$15x10^3$	$22.45x10^3$
	Test	$12.14x10^3$	$9.14x10^3$	$4.14x10^3$	$4.57x10^3$	$7.5x10^3$
	% Reduction	64	68	70	69	68

Fig.10: Effect on general transient bacterial microflora after use of (a) Medimix (b) Savlon (c) Indigenous herbal soap (d) Control soap



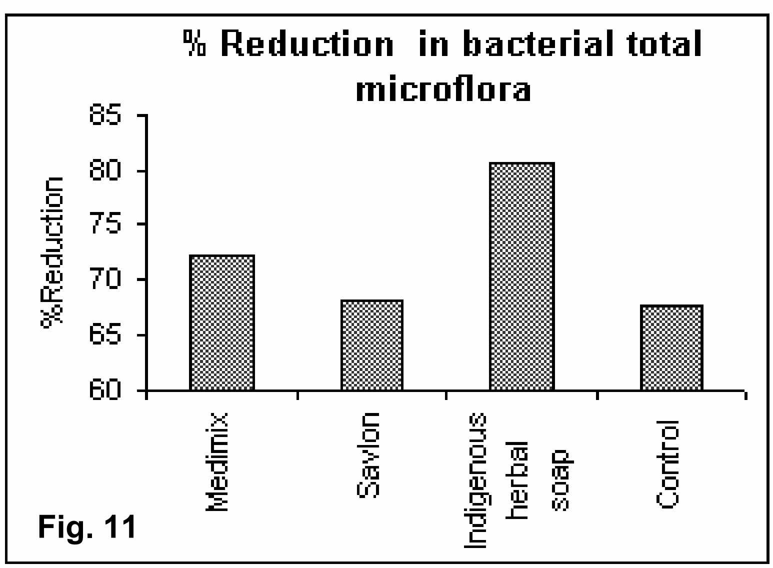
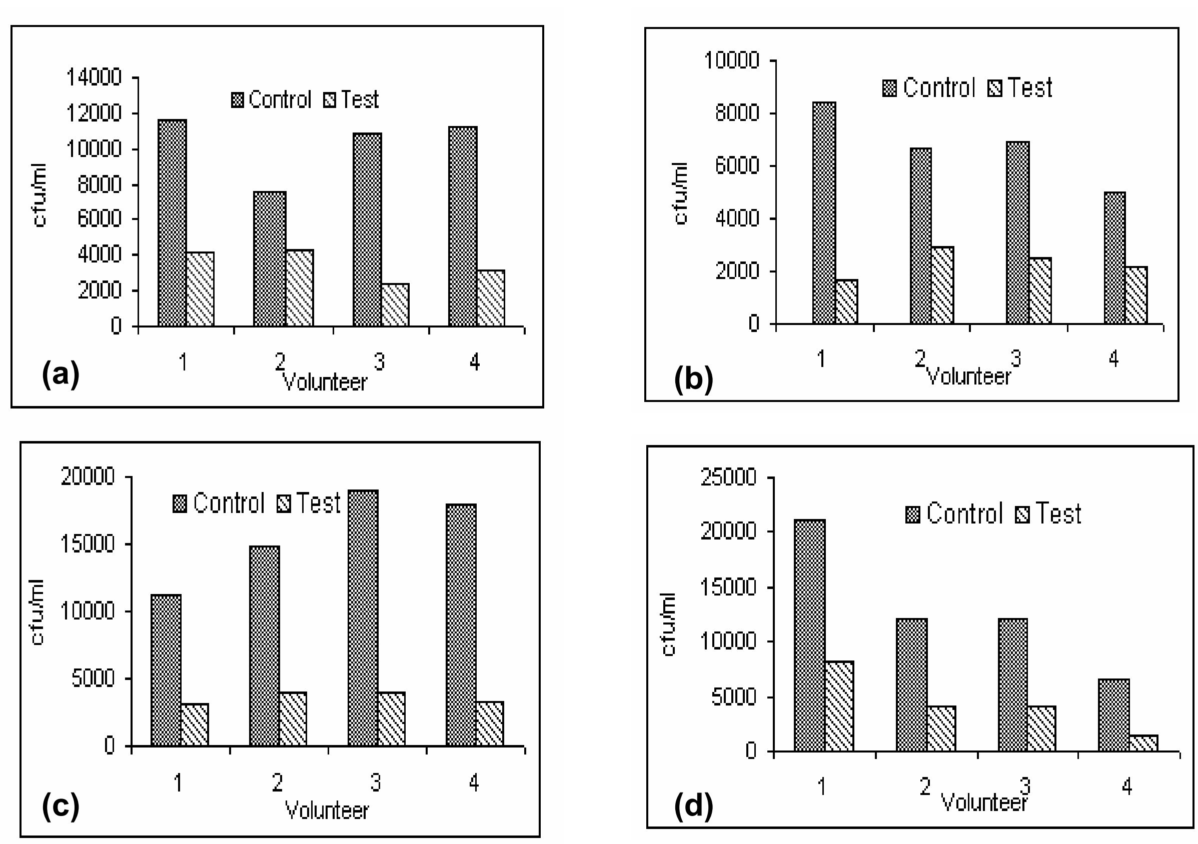


Fig. 11

Table 10. % Reduction in *S.aureus* flora						
		Volunteer				
Soap	**Average cfu/ml**	**1**	**2**	**3**	**4**	**Mean**
Medimix	Control	$11.57x10^3$	$7.6x10^3$	$10.80x10^3$	$11.20x10^3$	$10.29x10^3$
	Test	$4.14x10^3$	$4.25x10^3$	$2.40x10^3$	$3.10x10^3$	$3.47x10^3$
	% Reduction	67.19	79.42	74.306	71.872	73.197
Savlon	Control	$8.4x10^3$	$6.7x10^3$	$6.9x10^3$	$5x10^3$	$6.75x10^3$
	Test	$1.7x10^3$	$2.9x10^3$	$2.5x10^3$	$2.2x10^3$	$2.325x10^3$
	% Reduction	76.42	65.76	47.68	51.68	60.28
Indigenous herbal soap	Control	$11.2x10^3$	$14.9x10^3$	$18.9x10^3$	$17.9x10^3$	$15.7x10^3$
	Test	$2.2x10^3$	$3.1x10^3$	$3.9x10^3$	$3.9x10^3$	$3.2x10^3$
	% Reduction	81.55	77.96	87.83	82.81	82.53
Control soap	Control	$21.14x10^3$	$12.0x10^3$	$12.1x10^3$	$6.58x10^3$	$12.9x10^3$
	Test	$8.14x10^3$	$4.17x10^3$	$4.14x10^3$	$1.4x10^3$	$4.46x10^3$
	% Reduction	59.8	64.47	65.78	68.1	64.53

Fig. 12: Effect on *S.aureus* microflora after use of – (a) Medimix (b) Savlon (c) Indigenous herbal soap (d) Control Soap



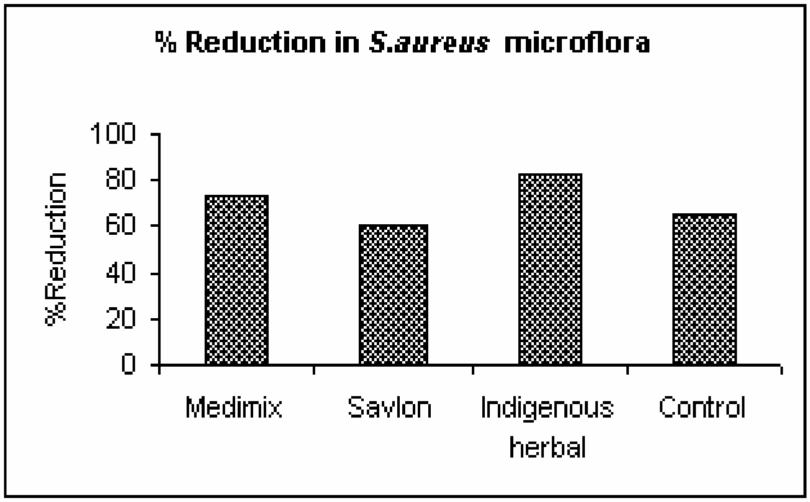


Fig.13

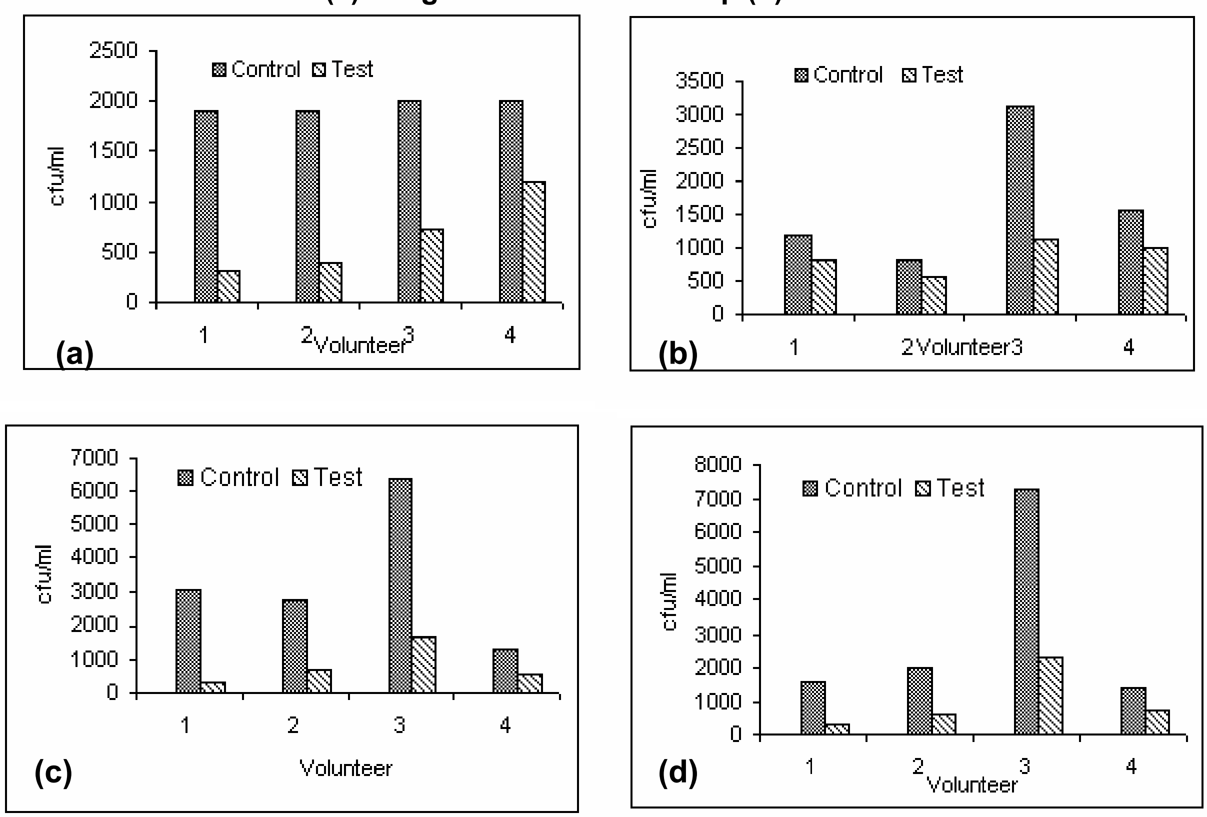
Table 10, fig. 12 and 13 represents the reduction in *S.aureus* cfu/ml after use of all the four soaps. It was observed that *S.aureus* reduction was highest in case of indigenous herbal soap followed by medimix, control and savlon. Indigenous herbal soap showed highest %reduction of 83 %(Fig. 13). While lest reduction was shown by savlon (60 %).

In case of *E.coli,* highest reduction was shown by control soap (Table 12, fig.24) followed by indigenous soap. Least reduction was shown by savlon. But all the four soaps were found to be less effective against *E.coli* reduction. Reduction was observed to be less then 50% in all the four cases. Among commercial antiseptic soaps, medimix (46%) showed more reduction then savlon (34%).

The goal of hand hygiene is sufficient reduction of microbial counts on the skin to prevent cross- transmission of pathogens among people. According to new CDC hand hygiene guidelines, the use of an alcohol based hand rub is preferred over non alcoholic hand rub (CDC, 2002). Earlier studies also showed the higher efficacy of herbal soaps over commercial antiseptic soaps. In a study made by Kavatheker *et. al*. (2004), pure herbal hand sanitizers were found to be more effective in reducing the total microbial load as compared to placebo. Tea tree essential oil is found to be effective in removing transient skin flora (Carson *et. al.,* 1996).

Table 11. % Reduction in *E.coli* flora							
Soap	**Average Cfu/ml**	**Volunteer**					
		1	**2**	**3**	**4**	**Mean**
Medimix	Control	$1.9x10^{3}$	$1.9x10^{3}$	$2x10^{3}$	$2x10^{3}$	$1.95x10^{3}$
	Test	$0.3x10^{3}$	$0.4x10^{3}$	$.714x10^{3}$	$1.2x10^{3}$	$0.65x10^{3}$
	% Reduction	63	28	71	13	44
Savlon	Control	$1.2x10^{3}$	$0.8x10^{3}$	$3.14x10^{3}$	$1.57x10^{3}$	$1.67x10^{3}$
	Test	$0.8x10^{3}$	$0.55x10^{3}$	$1.14x10^{3}$	$1x10^{3}$	$0.87x10^{3}$
	% Reduction	35	26	58	18	34
Indigenous herbal soap	Control	$3.01x10^{3}$	$2.7x10^{3}$	$6.4x10^{3}$	$1.287x10^{3}$	$3.34x10^{3}$
	Test	$0.3x10^{3}$	$0.7x10^{3}$	$1.7x10^{3}$	$0.57x10^{3}$	$0.81x10^{3}$
	% Reduction	40	44	65	29	44
Control soap	Control	$1.6x10^{3}$	$2x10^{3}$	$7.28x10^{3}$	$1.4x10^{3}$	$3.07x10^{3}$
	Test	$0.3x10^{3}$	$0.6x10^{3}$	$2.28x10^{3}$	$0.71x10^{3}$	$0.97x10^{3}$
	% Reduction	35	51	77	24	47

Fig. 14: Effect on *E.coli* microflora after use of – (a) Medimix (b) Savlon (c) Indigenous herbal soap (d) Control



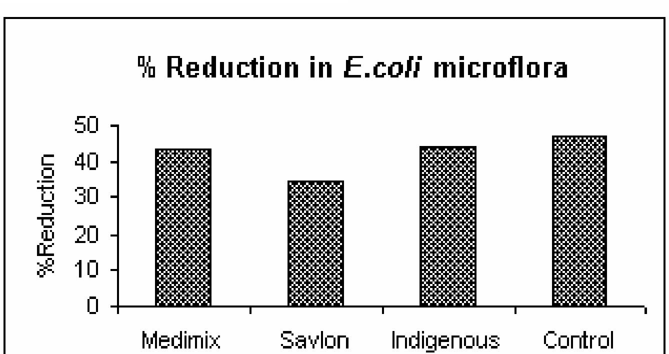


Fig. 15

5.3 Antimicrobial susceptibility testing

Pure isolated *S.aureus* (21*) and E.coli* (5) strains were further investigated for their antimicrobial susceptibility against 33 antibiotics. Table 12 represents the results of antimicrobial susceptibility testing of isolated pure *S.aureus* and *E.coli* strains. Among *S.aureus* strains, most of the strains were found to be resistant towards all the antibiotics except few. Highest resistance was observed against penicillin (81%) followed by cefixime (53%), nalidixic acid (48%), cefpodoxime and tetracycline (43%). All 21 strains were found to be non resistant (susceptible or intermediate) against chloremphenicol, neomycin, ceftrioxone and ofloxacin. Out of total twenty one *S. aureus* strains; seven strains (33.3% of total) namely SauS3, SauM3 (Fig. 21), SauS1, SauH1, SauH2, SauH3 and SauH4 were found to be resistant against methicillin. Their MIZ (Zone of inhibition) were found to be less then 14 mm. SauS1 (Fig. 18), SauS3 (Fig.19), SauM3 (Fig. 17) and SauH2 were found to be resistant against vancomycin**.** SauS1, SauM3, SauH2 and SauS3 were the strains that were found to be resistant against both vancomycin and methicillin. Some methicillin and vancomycin resistant strains were also found to be resistant to other antimicrobial agents like cefdinir, carbenecillin, cefixime, cefpodoxime, erythromycin, fosfomycin, linzolid acid, penicillin, tetracycline and trimethoprim.

Table 12: Culture classification based on susceptibility testing (NCCLS,2002)

Culture	No.	Culture ID
MRSA	7	SauS3, SauM3, SauS1, SauH1, SauH2, SauH3, SauH4
VRSA	4	SauS1, SauS3, SauM3, SauH2
VISA	2	SauH9, SauH6
Multi drug resistant *S.aureus*	21	SauS3, SauM3, SauS1, SauH1, SauH2, SauH3, SauH4, SauH9, SauH6, SauH4, SauS7, SauH8, SauS5,SauM5, SauSH3, SauH11, SauH6, Sau10, SauM1, SauM2, SauM6
Vancomycin & Methicillin resistant *S. aureus*	4	SauS1, SauM3, SauH2, SauS3

In case of *E.coli*, few strains were found to be resistant against used antibiotics.100% reduction was observed against nalidixic acid. Four out of five *E.coli* strains were found to be resistant against tetracycline and cefpodoxime. None of the *E.coli* strain was observed resistant against vancomycin and methicillin.

Earlier studies also showed the presence of resistance in *S.aureus* against common antimicrobial agents.38% of total *S.aureus*, isolated after hand wash were found to be resistant to penicillin, ampicillin and erythromycin (Mc Ginley *et. al*.,1988)

Control Soap

Indigenous Herbal Soap

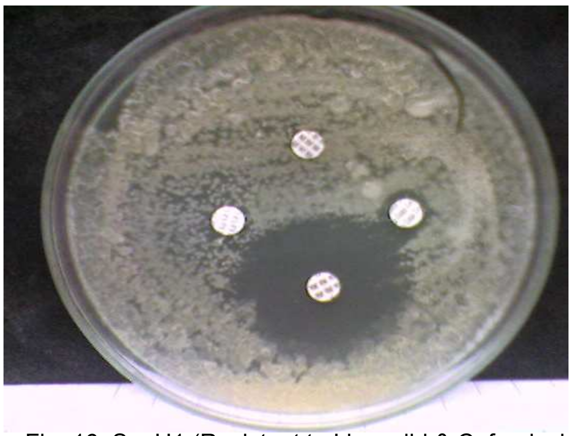


Fig. 16: SauH1 (Resistant to Linezolid & Cefpodoxime)

Fig.17: SauM3 (Resistant to Vancomycin & Fosfomycin

Fig. 18: SauS1 (Resistant to vancomycin)

Fig .19: SauS3 (Resistant to Vancomycin

Fig. 20: SauH1 (Resistant to Linezolid & Cefpodoxime)

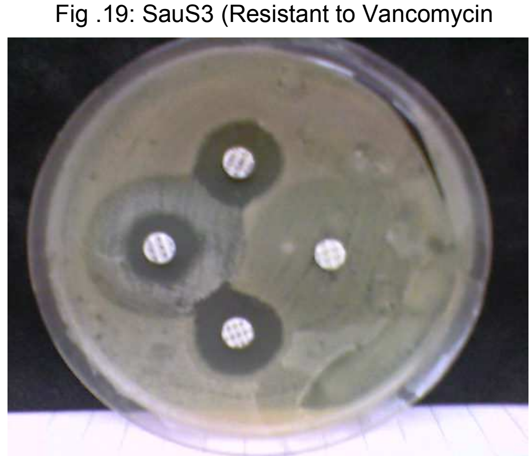


Fig 21: SauM3 (MRSA)

CONCLUSION

The present study reveals potential efficacy of the indigenous herbal soap in maintaining hand hygiene as evaluated by *in vivo* rinse wash method. This is evident from the % reduction of the transient bacterial flora of palm, which was maximum (80%) for the indigenous herbal soap as compared to savlon (68%) and medimix (72%). All soaps were more effective in reducing *S. aureus* (83% reduction) as compared to *E. coli* (44% reduction).

Susceptibility testing of the pure cultures (*S. aureus* and *E. coli*) isolated from the palms of different individuals revealed high resistance among the bacterial isolates. This is evident from the fact that of the twenty- one isolates of *Staphylococcus aureus*, 4 strains were resistant to vancomycin i.e. VRSA and 7 strains were resistant to methicillin i.e. MRSA. Apart from this all the cultures were multidrug resistant. The emergence and spread of these strains in the community is a major threat for community as there may be chances of hand-to-hand transmission of MRSA and VRSA.

The skin on hands is the first defense against infection from pathogenic organisms. Any cuts or lesions are possible sources of entry for bacteria and viruses so its care and hygiene are crucial for reducing the risk of acquiring an infection. Hands are the most likely way in which infections or microorganisms might be spread between patients. The study signifies the importance of hand washing as a method to restrain the spread of multidrug resistant infectious microbes.

Today microbes are getting refractory to antimicrobial drugs and these drug resistant microbes are responsible for occurrence of chronic diseases. The development of newer and effective anti- infective agents is thus the need of the hour. The study provides evidence that plant derived antimicrobial agents have a vast potential to be developed into commercial hand hygiene agents viz. herbal soap and herbal hand rub.

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