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Antimicrobial Activities of Soap and Detergents

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ABSTRACT
In the present study five bacterial strains were used to study the antibacterial activity of soap and detergent by disc method and turbidity method. It was found that Staphylococcus aureus was good bactericidal as it unable to grow in any of the detergent concentration and other species showed varied level of minimum inhibition concentration. It is possible that antibacterial soaps and detergents have the antibacterial agents that can either kill or inhibit the bacterial cells. It might be possible that some bacterial strains become resistant which leads to their survival even at high concentrations of soaps. The resistant bacterium against soap in the present study was Pseudomonas aeroginosa.
Key Words: *Soaps, detergents, antibacterial activity, bacterial strains*

INTRODUCTION

For generations, hand washing with soap and water been considered a measure of personal hygiene. Bacteria are very diverse and present every where such as in soil, water, sewage, standing water and even in human body. Bacteria's that attacks on human body is of great importance with reference to health [1]. In 1961 the U.S. public Health Service recommendations directed that personnel wash their hands with soap and water for 1 to 2 minutes before and after client contact. The antibacterial soaps can remove 65 to 85% bacteria from human skin [2]. Although fats and oils are general ingredient of soaps but some detergents are added to enhance the antibacterial activities of soaps [3]. In 1975 and 1985 guidelines on hand washing practices in Hospitals were published by the Centers for Disease Control (CDC), which recommended hand washing with non-anti microbial soap between client contacts and washing with anti microbial soap before and after performing invasive procedures or caring for clients at high risk. Use of waterless antiseptics agents was recommended only in the situations where sinks were not available. Transient bacteria are deposited on the skin surface from environmental sources and causes skin infections. Examples of such bacteria are Pseudomonas aeruginosa [4] and Staphylococcus aureus [5]. The importance of hand washing is more crucial when it is associated to health care workers because of possible cross contamination of bacteria that may be pathogenic or opportunistic [6]. Hand hygiene and prevention of infection has been well recognized [7]. The importance of hand hygiene is also there for food handlers. Food handler includes those who deals with delivers and serve food [8].

Yet a large number of chemical compounds have the ability to inhibit the growth and metabolism of microorganisms or kill them. The number of chemicals in enormous, probably at least 10,000 with 1,000 commonly used in the hospitals and homes. Chemicals exist as solids, liquids and gases. Of the many groups of chemicals use to reduce or destroy microbes important groups include halogens, phenols, soaps, detergents, ammonia compounds, alcohols, heavy metals, acids and certain special compounds.

The environmental movement led to the promotion of "green" products, products said to be "earth friendly." In contrast to general trends toward value pricing, U.S. consumers demonstrated a willingness to pay slightly higher prices for environmentally friendly products. U.S. consumers, however, were not willing to accept "green" products that were inconvenient to use or those with diminished performance capabilities [9].A detergent is a chemical compound that cleans, they are synthetic surfactants. A detergent is an effective cleaning product, because it contains one or more surfactants. Because of their chemical makeup, the surfactants used in detergents can be engineered to perform well under a variety of conditions. Detergent surfactant was developed in response to a shortage of animal and vegetable fats and oils, during World War I and World War II. In addition a substance that was resistant to hard water was

needed to make cleaning more effective. At that time petroleum was found to be plentiful source for the manufacture of these surfactants. Today detergents are made from a variety of petrochemical chemicals (derived from fats and oils) other chemicals (such as sulphur trioxide, sulphuric acid and ethylene oxide) and alkalis. In 1988 and 1995 guidelines for hand washing and antisepsis were published by the Association for Professionals in Infection Control (APIC) that were similar to those listed in the CDC guidelines. The 1995 APIC guidelines included discussions of alcohol-based hand rubs and supported their use in more clinical settings than had been recommended earlier. In 1995 and 1996 the Healthcare Infection Control Practices Advisory Committee (HICPAC) recommended that either anti-microbial soap or water less antiseptic agent be used for cleansing hands upon leaving the rooms of clients with multi-drug-resistant pathogen s such as *Staphylococcus aureus* [10]. Studies have shown that soaps containing antimicrobial active ingredients remove more bacteria as compared to plain soap [11]. To investigate the antibacterial activity of soaps and detergent, the bacterial cultures were brought from Dept. of Microbiology, Kasturba Medical College, Mangalore and necessary biochemical tests were carried out [12]. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against these bacteria were determined. The present studies were aimed to determine the bactericidal activity/efficacy of both the soap and the detergent and to determine, whether the soaps only removes the bacteria from skin or it also kills the bacteria.In the present study antimicrobial activity of commercial detergents and soaps against Bacillus subtilis, Escherchia coli, Micrococcus sp. and Pseudomonas aeruginosa, Staphylococcus aureus were investigated.

MATERIALS AND METHODS

Bacterial cultures

Different bacterial strains were procured from Microbiology Dept. of Kasturba Medical College, Mangalore. All samples were properly diluted and spread on the nutrient agar. The pH was adjusted to 7.0, incubated for 24 hours at 37°C. Then inoculation was made on nutrient agar plates and incubated at 37°C for 24 hours. The most abundant strain of the samples was selected, Gram stained, and then inoculated onto slants containing nutrient agar.

Different biochemical tests such as oxidase test, catalase test, urease test, motility test, acid production from glucose, mannitol, sucrose, lactose, maltose, coagulase test, Dnase test, indole test, eosine methylene blue test, triple sugar iron reactions, methyl red test, voges proskauer test, and nitrate reduction test following Chesseborugh [12] were carried out. For gram negative bacteria, analytical profile index (biomereux) was performed according to manual instructions (Table 1).

Table 1: Characteristics of the bacterial strains

TEST	Bacterial strains				
	S. aureus	***P. aeroginosa***	***E. coli***	***Micrococcus sp.***	***B. subtilis***
Oxidase	NA	+ve	-ve	+ve	+ve
Catalase	+ve	+ve	+ve	+ve	+ve
Motility	NA	+ve	+ve	-ve	+ve
Lactose	NA	+ve	+ve	NA	+ve
EMB	NA	NA	+ve	NA	NA
Indole	NA	-ve	+ve	NA	NA
Citrate	NA	+ve	-ve	NA	+ve
V P	NA	-ve	-ve	NA	NA
M R	NA	+ve	+ve	NA	NA
TSI	NA	R/R/-/-	Y/Y/+/-	NA	NA
Urease	NA	-ve	-ve	NA	NA
Mannitol	+ve	+ve	NA	+ve	NA
Maltose	NA	-ve	-ve	NA	-ve
Pigment	Golden	Green	-ve	Yellow	-ve
Coagulase	+ve	NA	NA	+ve	NA
DNase	+ve	NA	NA	+ve	NA
Sucrose	+ve	-ve	-ve	+ve	+ve

Strain maintenance

All strains were grown on nutrient agar plates at 37°C for 48 hours. Strains were stored at -70°C in 50% sterile glycerol and TSB [13]. Minimum inhibitory and bactericidal concentrations of different soaps to determine minimum inhibitory concentrations of soap, the two different methods used were tube method [14] and microtitration plate method [1]. The bactericidal concentrations of soaps were determined following Cappuccino and Sherman method [14].

Preparation of detergent and soap extract

Detergent powder (Surf excel) is taken. Four different concentrations (w/v) such as 100 mg/ml, 200 mg/ml, 300 mg/ml and 400 mg/ml are prepared in distilled water. Similarly for soap (Lifebuoy Green soap) four different concentrations are prepared in water.

Plating: This method is routinely employed for the isolation of bacteria in pure culture from clinical specimens. A platinum loop is charged with the bacteria to be cultured. One loop full of the bacteria is transferred into the surface of a well-dried agar plate on which it is spread over a small area at a periphery. The inoculum is then distributed thinly over the plate by streaking it with the loop in a series of parallel lines, in different segments of the plate. The loop should be flamed and cooled between the different sets of streaks. After incubation colonies will be seen well distributed throughout the depth of the medium. Thus pure culture of different bacteria was obtained and these were being sub cultured in order to obtain sources of cultures [15].

Testing the antimicrobial activity of extract of detergent and soap against bacteria:

About 30 ml of Nutrient agar media is poured into five cleaned and autoclaved Petri plates, pure strain of E. coli is inoculated on the solidified media and this is the control. To the other four Petri plates 1ml, 2ml, 3ml and 4 ml of the corresponding crude detergents extract are added. Similarly, four other Petri plates are prepared to which 1ml, 2ml, 3ml, and 4 ml of the corresponding crude soap extract are added, the media is allowed to solidify. E. coli is then inoculated into the Petri plates. The Petri plates are flamed to prevent contamination and then incubated over night at 37oC and the results are observed after 24 hours. The observation is continued for a few days and the growth of the organism is recorded.

Turbidity Analysis: This method is based upon comparison of intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a solution. The higher is the intensity of scattered light, higher is the turbidity. Spectrophotometer or colorimeter is used to find the optical density [16].

Testing the antimicrobial activity of extract of detergents and soaps against bacterial culture:

LB broth was prepared and poured into 9 autoclaved conical flasks. Into one of the flasks pure strain of E. coli was inoculated and this is the control. To the remaining conical flasks1ml of corresponding crude detergents extract (100 mg, 200 mg, 300 mg and 400 mg/ml) and soap extract (100 mg, 200 mg, 300 mg and 400 mg/ml) were added. E. coli was then inoculated into the flasks. The flasks were incubated at 37oC for 24-48 hours.

Day 2: At every hour, the optical density (OD) at 590 nm is noted down. 4-5 readings are taken and the results are tabulated.

Disc method

This method is employed to see whether the bacteria are inhibited by the particular concentration of detergent or soap, or not. By using this method we can find out the least inhibitory concentration of detergent or soap for particular bacteria [17].

Testing the anti-microbial activity of crude extract of detergent or soap against bacterial culture

About 30 ml of Nutrient Agar is taken, to which 5 ml of the E. coli suspension is added. It is poured into two Petri plates. The agar is allowed to solidify. Now the autoclaved disc is dipped in 100 mg/ml detergent solution and is placed on the plate. Likewise, all the discs of particular concentration (200 mg, 300 mg and 400 mg/ml) are placed on the agar plate. These plates are kept in refrigerator for 20 min. and are incubated at 37oC for 24-48 hours and the results are observed.

RESULTS

The effect of different concentrations of surf excels on growth of different species of bacteria showed remarkable variations (Table 2). Among five strains of bacteria, Staphylococcus aureus unable to grow in any of the detergent concentrations indicates it is good bactericidal. For E. coli and Micrococcus sp. the MIC was 400mg/ml while at other concentrations (lower levels) luxurious colonies were observed. In case of Bacillus subtilis the MIC was 100mg/ml and for Pseudomonas aeroginosa it was 200mg/ml. Among bacterial strains the growth of Staphylococcus aureus was completely inhibited even at 100 mg/ml of Lifebuoy Green. Among other species Micrococcus sp., E. coil and Pseudomonas aeroginasa the MIC was 100mg/ml, 300mg/ml 200mg/ml respectively.

Comparing to control, the higher growth was observed in the plates inoculated with E. coli, Pseudomonas and Micrococcus at 100mg/ml concentration, while other species showed either moderate, little or no growth. On other hand 100mg/ml soap showed higher colony growth of E. coli, Pseudomonas and Staphylococcus, whereas 300mg/ml soap inhibited the growth of all bacteria except E. coil and Micrococcus sp. (Table 3).

Table 2: Variation in growth of different species of bacteria under different concentration of detergent

Microorganism\Media	Control	100mg/ml	200mg/ml	300mg/ml	400mg/ml
Escherchia coli	Maximum	High	Moderate	Low	Very little
Pseudomonas aeroginosa	Maximum	High	Moderate	No growth	No growth
Bacillus subtilis	Maximum	Little	No growth	No growth	No growth
Staphylococcus aureus	Maximum	No growth	No growth	No growth	No growth
Micrococcus sp.	Maximum	High	Moderate	Low	Very little

Table 3: Variation in growth of different species of bacteria under different concentration of soap

Microorganism\Media	Control	100mg/ml	200mg/ml	300mg/ml	400mg/ml
Escherchia coli	Maximum	High	Moderate	Low	Very little
Pseudomonas aeroginosa	Maximum	High	Moderate	Low	Very little
Bacillus subtilis	Maximum	No growth	No growth	No growth	No growth
Staphylococcus aureus	Maximum	High	Moderate	Low	Very little
Micrococcus sp.	Maximum	Low	No growth	No growth	No growth

Turbidity analysis method

The growth of the microorganisms in the broth medium with varied concentrations of soaps and detergents were determined by turbidity analysis and the optical density method was showed variation (Table 4 -13). Minimum turbidity was recorded in the culture of E. coli in which the turbidity was maximum as the time interval of incubation increased from 0900 am to 1200 noon, while in other bacterial cultures used, there was a great difference to this organisms as compared, the optical density also showed greater value (Fig. 1-10). In all the bacteria maximum optical density was recorded at lower concentration of soaps and detergents except a few whereas the minimum value was recorded at higher concentration of detergents and soaps.

Disc method

The effect of detergent on growth of different species of bacteria was measured by the presence/absence of clear inhibition zones by using disc method. For each bacterium it was varied and the zone of inhibition was greater at maximum concentration while it was minimum at lower concentration. As a whole E. coli showed less inhibition as compared to other species with both soap and detergent (Table 14 and Table 15). The minimum inhibitory concentration of detergents and soaps for E. coli is 3% and 2% respectively. For other bacteria minimum inhibition was observed at 2% soap and 1% detergent.

DISCUSSION

In the present study the bacteria which were used to study their role against soaps and detergents showed wide variation. Such variations indicate their survival in the particular constituents and their antimicrobial property. Of these five bacteria, E. coli showed lesser antimicrobial property against both soap and detergent. This may explain its wide distribution as well as number of strains.

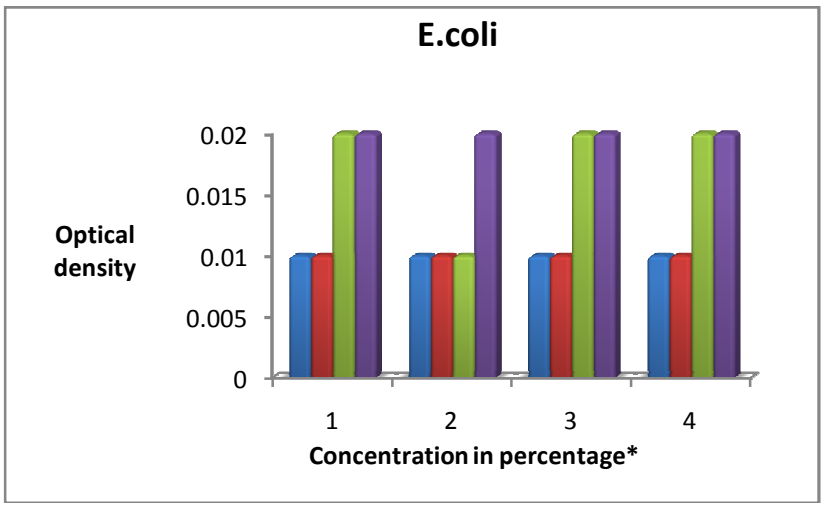

Fig.1: Turbidity analysis of *E. coli* by different concentration of detergent
*1- 100mg/l, 2- 200mg/l, 3- 300 mg/l, 4- 400 mg/l

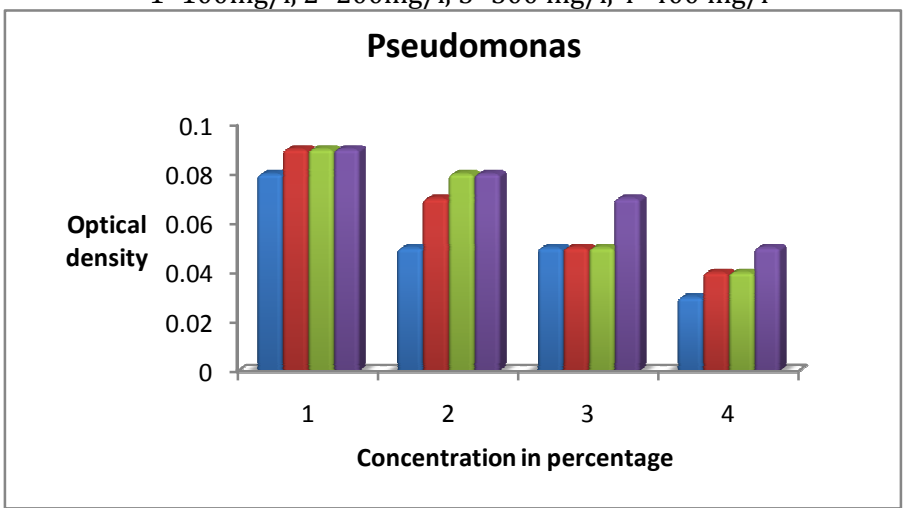

Fig. 2: Turbidity analysis of Pseudomonas by different concentration of detergent

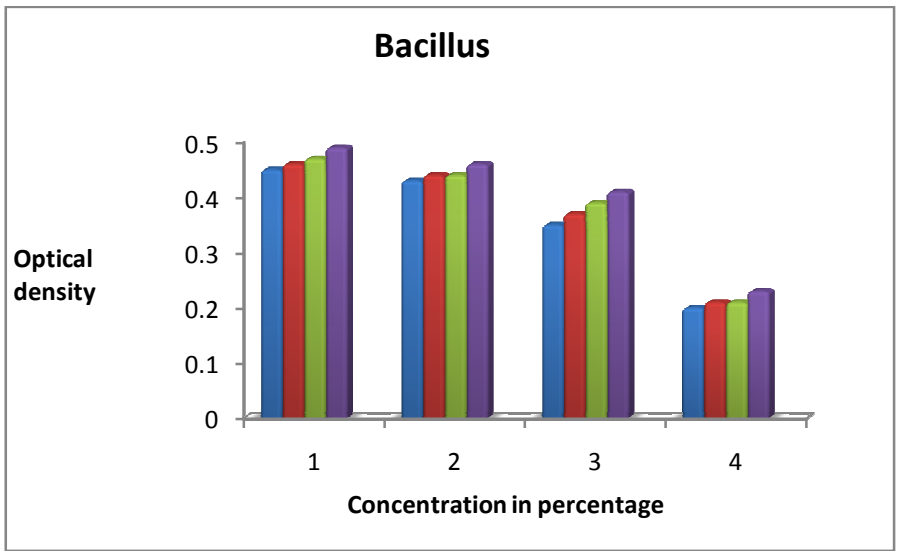

Fig. 3: Turbidity analysis of Bacillus by different concentration of detergent

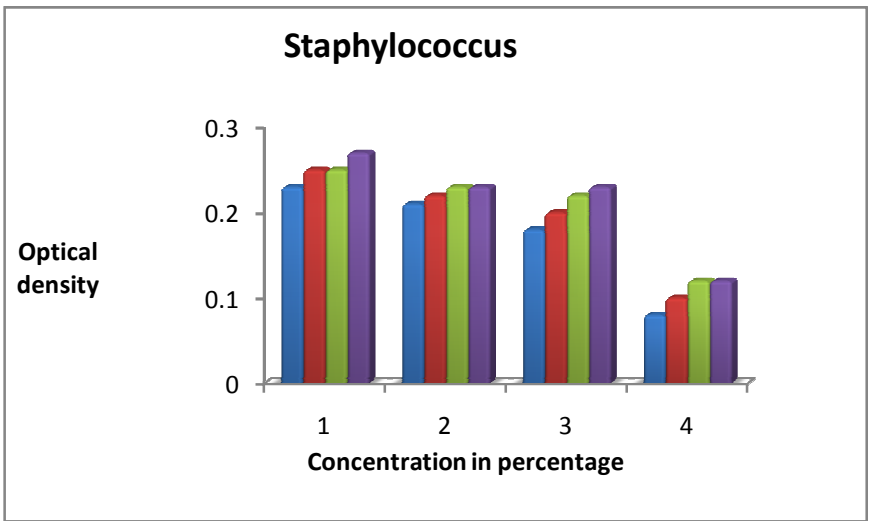

Fig. 4: Turbidity analysis of Staphylococcus by different concentration of detergent

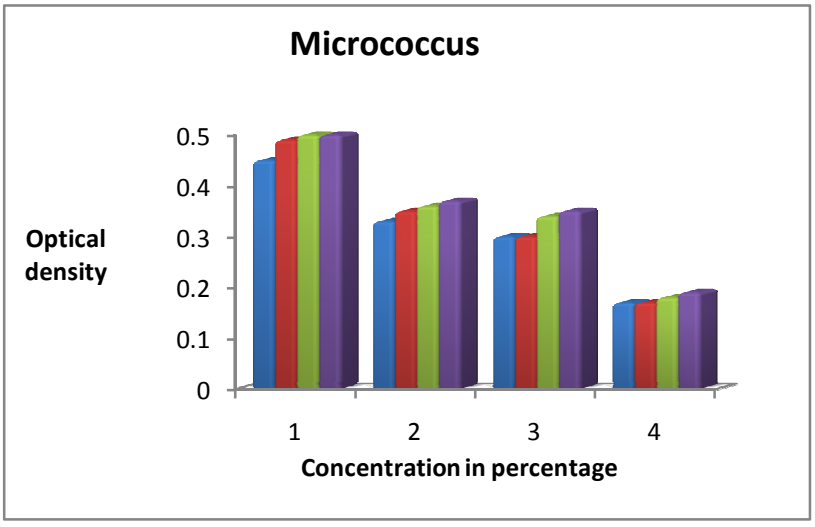

Fig. 5: Turbidity analysis of Micrococcus by different concentration of detergent

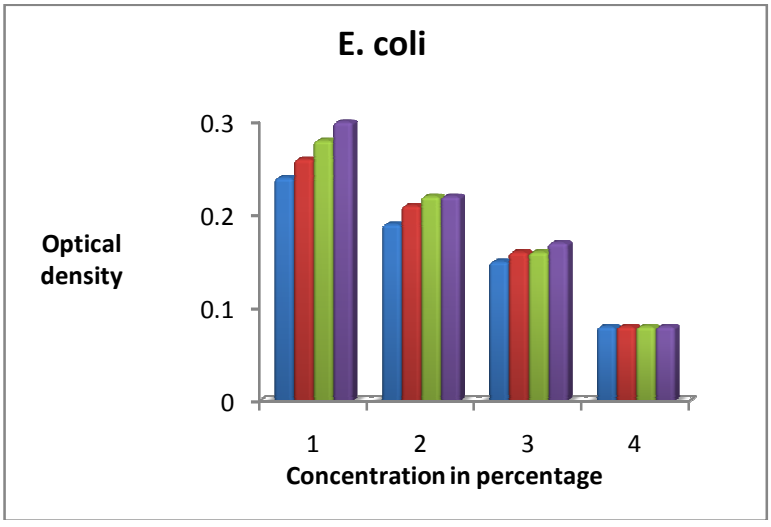

Fig. 6: Turbidity analysis of E. coli by different concentration of soap

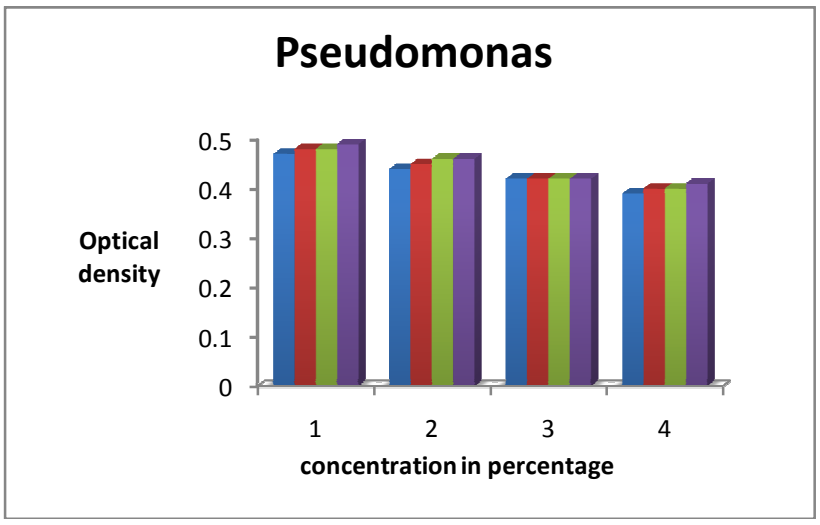

Fig. 7: Turbidity analysis of Pseudomonas by different concentration of soap

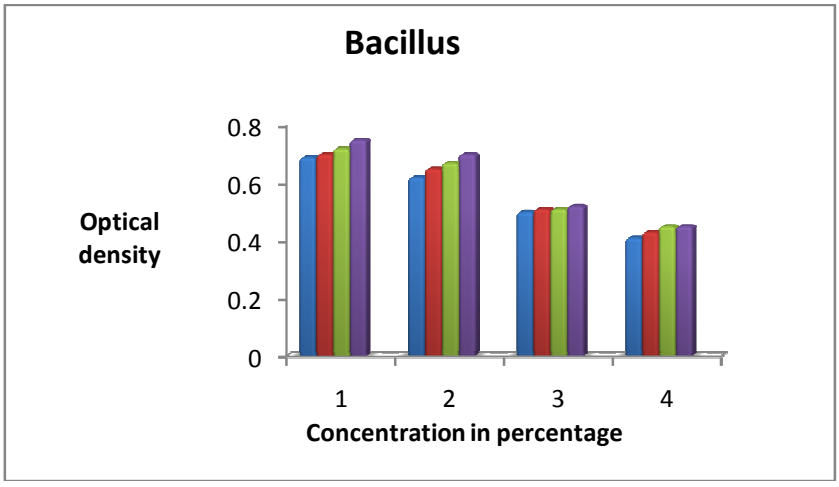

Fig. 8: Turbidity analysis of Bacillus by different concentration of soap

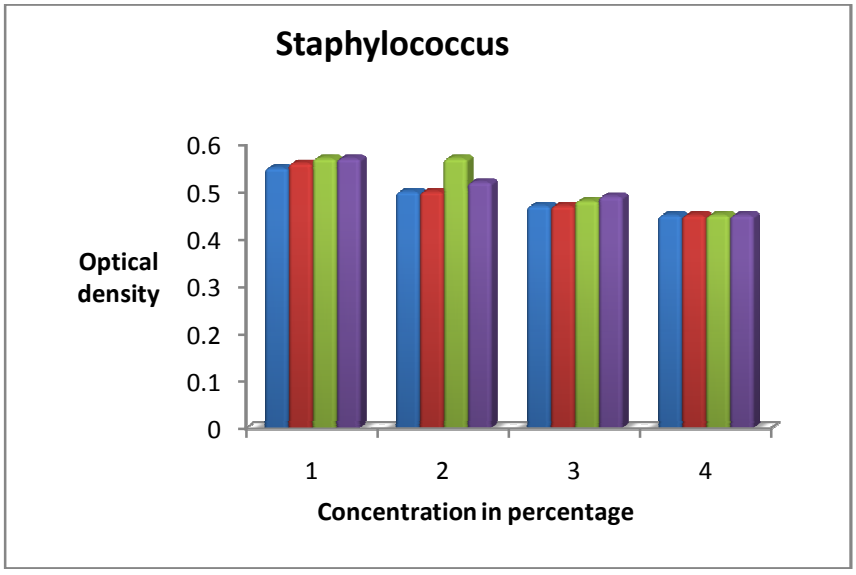

Fig. 9: Turbidity analysis of Staphylococcus by different concentration of soap

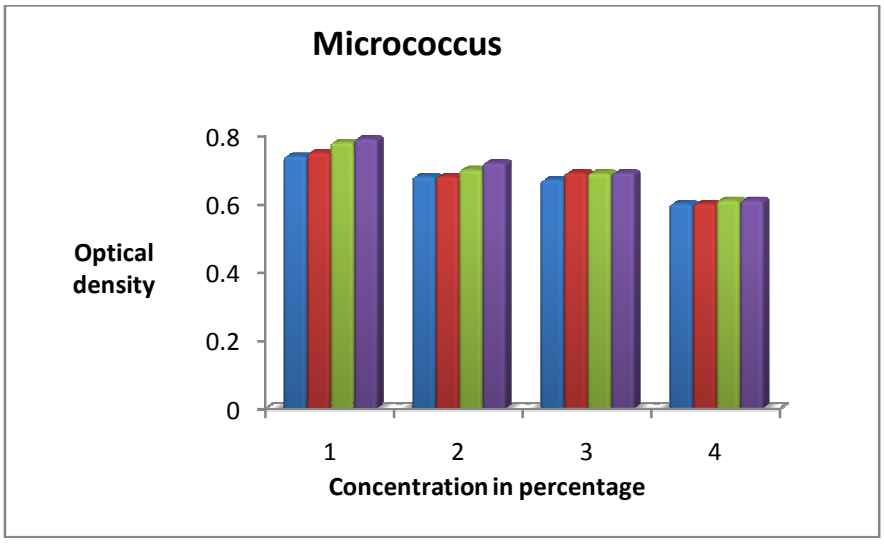

Fig.10: Turbidity analysis of Micrococcus by different concentration of soap

Table 4: The effect of detergent on growth the of E. coli by turbidity analysis Method

Time	Conc. (%)	1	2	3	4
09.00 am		0.01	0.01	0.01	0.01
10.00 am		0.01	0.01	0.01	0.01
11.00 am		0.02	0.01	0.02	0.01
12.00 am		0.02	0.02	0.02	0.02

Table 5: The effect of detergent on growth the of Pseudomonas by turbidity analysis method

Time	Conc. (%)	1	2	3	4
09.00 am		0.08	0.05	0.03	0.03
10.00 am		0.09	0.07	0.05	0.03
11.00 am		0.09	0.08	0.05	0.04
12.00 am		0.09	0.08	0.07	0.05

Table 6: The effect of detergent on growth the of Bacillus by turbidity analysis method

Time	Conc. (%)	1	2	3	4
09.00 am		0.45	0.43	0.35	0.20
10.00 am		0.46	0.44	0.37	0.21
11.00 am		0.47	0.44	0.39	0.21
12.00 am		0.49	0.46	0.41	0.23

Table 7: The effect of detergent on growth the of Staphylococcus by turbidity analysis method

Time	Conc. (%)	1	2	3	4
09.00 am		0.23	0.21	0.18	0.08
10.00 am		0.25	0.22	0.20	0.10
11.00 am		0.25	0.23	0.22	0.12
12.00 am		0.27	0.23	0.23	0.12

Table 8: The effect of soap on growth the of Micrococcus by turbidity analysis method

Time	Conc. (%)	1	2	3	4
09.00 am		0.45	0.33	0.30	0.17
10.00 am		0.49	0.35	0.33	0.17
11.00 am		0.51	0.36	0.34	0.18
12.00 am		0.53	0.37	0.35	0.19

Table 9: The effect of detergent on growth the of Micrococcus by turbidity analysis method

Time	Conc. (%)	1	2	3	4
09.00 am		0.74	0.68	0.67	0.60
10.00 am		0.75	0.70	0.69	0.60
11.00 am		0.78	0.70	0.69	0.61
12.00 am		0.79	0.72	0.69	0.61

Table 10: The effect of soap on growth the of E. coli by turbidity analysis method

Time	Conc. (%)	1	2	3	4
09.00 am		0.24	0.19	0.15	0.08
10.00 am		0.26	0.21	0.16	0.08
11.00 am		0.28	0.22	0.16	0.08
12.00 am		0.30	0.22	0.17	0.08

Table 11: The effect of soap on growth the of Pseudomonas by turbidity analysis method

Time	Conc. (%)	1	2	3	4
09.00 am		0.47	0.44	0.42	0.39
10.00 am		0.48	0.45	0.42	0.40
11.00 am		0.48	0.46	0.42	0.40
12.00 am		0.49	0.46	0.42	0.41

Table 12: The effect of soap on growth the of Bacillus by turbidity analysis method

Time	Conc. (%)	1	2	3	4
09.00 am		0.69	0.62	0.50	0.41
10.00 am		0.70	0.65	0.51	0.43
11.00 am		0.72	0.67	0.51	0.45
12.00 am		0.75	0.70	0.52	0.45

Table 13: The effect of soap on growth the of Staphylococcus by turbidity analysis method

Time	Conc. (%)	1	2	3	4
9.00 am		0.55	0.50	0.47	0.45
10.00 am		0.56	0.50	0.47	0.45
11.00 am		0.57	0.57	0.48	0.45
12.00 am		0.57	0.52	0.49	0.45

Table 14: Variation in extent of inhibition of different species of bacteria under different concentration of detergents

Conc. of detergent	Microorganism	E. coli	Pseudomonas	Bacillus	Staphylococcus	Micrococcus
1%		-	-	+	+	+
2%		+	+	++	+++	++
3%		+	++	+++	+++	++
4%		++	+++	++++	++++	++

Table15: Variation in extent of inhibition of different species of bacteria under different concentration of soaps

Conc. Of soap	Microorganism	E. coli	Pseudomonas	Bacillus	Staphylococcus	Micrococcus
1%		-	+	-	-	+
2%		-	+	+	+	++
3%		+	++	+	++	++
4%		+	+++	+	++	++

-No inhibition + Little inhibition ++ Moderate inhibition +++ High inhibition ++++ Maximum inhibition

The higher growth of E. coli, Pseudomonas and Micrococcus was observed at 1% soap concentration, while other species showed either moderate, little or no growth. On other hand 1% soap showed higher colony growth of E. coli, Pseudomonas and Staphylococcus, whereas 3% soap inhibited the growth of all bacteria except E. coil and Micrococcus. It was seen clearly that Gram positive bacteria were killed at low concentration of soaps than Gram negative bacteria. The best of all the soaps used is lifebuoy white (antibacterial) because the calculation of the efficiency of all the soaps revealed that this soap is more efficient than Saba Riaz et al. [18] who carried out their experiment on branded soaps against ten bacterial strains and found that the most resistant bacterium of all the soaps is K. pneumoniae following P. aeruginosa. In the present investigation P. aeruginosa observed as most resistant bacteria. They have also concluded that antibacterial soaps showed better MIC in comparison with beauty soaps and the MIC was varied from 62.5mg/ml to 250 mg/ml.

In the present study all the bacteria showed maximum optical density at lower concentration of soaps and detergents except a few whereas the minimum value was recorded at higher concentration of detergents. At lower detergent or soap concentration the optical density values are high because the turbidity is high and hence there is maximum growth. Whereas at higher detergent concentration the optical density decreases, turbidity is less and hence there is very little or no bacterial growth. This supports the earlier work of Isenberg [16]. According to him the concentration of the detergent and soaps increases the turbidity decreases and vice versa. Hence the results showed that the detergent contains a number of chemicals which enhances their anti-microbial property.

Jokik [17] made an experiment on antimicrobial activity of some soaps and detergents and found that as the concentration of detergent or soap increases the intensity of inhibition also increases. Similar results were obtained in the present study also. The minimum inhibitory concentrations of detergents and soaps for E. coli are 3% and 2% respectively and for other bacteria minimum inhibition was observed at 2% soap and 0.1% detergent. The antimicrobial property of soaps and detergents are very helpful against some pathogenic organisms such as multi-drug-resistant pathogens such as Staphylococcus aureus [10]. Antibacterial soaps considered to be more effective than beauty (plain) soaps and deodorant [19]. This study suggests that antiseptic soaps were more effective against Gram-negative and Gram-positive bacteria than were plain soaps. Present work showed that plain soaps also possessed antibacterial activity although lesser than that of antibacterial soaps. Garner and Favero [20] studied the hand washing with plain soaps removes millions of microorganisms. Most of the research has been focused on hand washing and hand disinfectants for personnel in health care settings where patients are immune compromised and are at high risk [21, 22].

It is proved experimentally that antibacterial soaps kill the bacteria at a specific concentration; they also have bacteristatic activity and can inhibit the growth of bacteria. Beauty soaps contain some natural and plant extracted ingredients in their composition which have the ability to inhibit or kill the bacteria so they also gave some bactericidal activity. Micro-titration plate method is efficient than tube method and easier to perform. This study suggests that selection of soaps should depend on to the working environment. The soap should have good ingredients which have the ability to kill bacteria but not to damage body tissues. Health care workers should use soaps according to criteria of Health and Hygiene. In this way many immuno-compromised or low immunity patients can be protected from transfer of pathogenic or opportunistic pathogens. This area of research requires attention of scientists and people from soap industry, because quality of soaps is very important as they are the need of every home.

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