STUDENT STUDY PROJECT

ON

"DETERMINATION OF ANTI MICROBIAL ACTIVITY OF DIFFERENT SOAP SAMPLES ON [illegible]RMAL MICROBIAL FLORAOF HUMAN BODY"

Department of Microbiology
Dr. BRR Government Collage, Jadcherla
Mahabubnagar– 509001

Accredited by NAAC with "B^{++}"Grade// An ISO 9001-2015 Institution
Mahabubnagar (Dist.), Telangana State, India – 509301

Affiliated to Palamuru University

SumeraTabassum
Lecturer in Microbiology,
Dr. BRR Government Degree College,
Jadcherla – 509301,
[illegible]bnagar District,
Telangana [illegible] India.

CERTIFICATE

This is to certify that the project work entitled **STUDY OF DETERMINATION OF ANTIMICROBIAL ACTIVITY OF DIFFERENT SOAPS SAMPLES ON NORMAL MICROBIAL FLORA OF HUMAN BODY. Jadcherla, Mahabubnagar District, Telangana State,** is a bonafide work done by the students of III MZC (E/M) Miss K.Jyothi, CH.Aarthi, B.Swetha, K.Saroja, P.Raju under my supervision for the award of project work in Microbiology, Department of Microbiology,Dr.BRR Government college, Jadcherla and the work hasn't been submitted to any other college/ university either in part nor in full, for the award of any degree.

Sumera
Lecturer in Microbiology

Head of the Department
Dept. of Microbiology
Dr. B.R.R. Gov' College,
JADCHERLA 509 301

26/8/23
Signature of external examiner

Sumera
Signature of Internal Examiner

Signature of Principal

PRINCIPAL
Dr. B.R.R. Goverment Degree College
JADCHERLA

DECLARATION

We are hereby declaring that the project work entitled with STUDY OF DETERMINATION OF ANTIMICROBIAL ACTIVITY OF DIFFERENT SOAPS SAMPLES ON NORMAL MICROBIAL FLORA OF HUMAN BODY. Jadcherla, Mahabubnagar District, Telangana State, is Is a genuine work done by us under the supervision of Sumera Tabassum, for the Department of Microbiology, Dr.BRR. Government College and it has not been under the submissionto any other Institute/University either in part or in full, for the award of any degree.

Name of the student	Class	H.T.Number	Signature
K.Jyothi	B.Sc. MZC III	20033006457011	K. Jyothi
CH.Aarthi,	B.Sc. MZC III	20033006457005	Aarthi
B.Swetha,	B.Sc. MZC III	20033006457003	Swetha
K.Saroja	B.Sc. MZC III	20033006457015	K. saroja
P.Raju	B.Sc. MZC III	20033006457021	P. Raju

ACKNOWLEDGMENTS

We would like to convey our heartfelt gratitude to **K. NEERAJA, HOD,** Microbiology, and our mentor **Sumera Tabassum**, for their invaluable advice and assistance in completing our project. They are there to assist us in every step of the way, and their motivation is what enables us to accomplish our task effectively. We would also like to thank all of the other supporting personnel who assisted us by supplying the equipment that was essential and vital, without which we would not have been able to perform efficiently on this project. We would also like to thank the **Dr. CH. APPIYA CHINNAMMA,** Principal Dr. BRR. Government Jadcherla, Mahabubnagar and Palamuru University. We also like to thank my friends and parents for their support and encouragement as we worked on this project work.

INDEX

ABSTRACT

The present study was carried out to isolate the organisms present in the skin surface of students at Dr BRR Govt Degree college, Jadcherla,mahabubnagar dist Telangana. India. The samples were isolated by plating techniques onto different agar medium. The colonies thus formed on the plates were identified using staining and Biochemical tests and confirmed as E.coli, B. subtilis, S. aureus and Pesudomonas .The isolated bacterial samples were maintained as pure culture for further studies. Antiseptic and Herbal soaps such as Dabur soap, Dettol soap, No.1 soap, Medimix, Aloevera,shikakay, Cinthol (antibacterial soaps), lux soaps, lifebuoy soap, and santoor soap (Beauty soaps) available I jadcherla market. Isolated organisms were subjected to sensitivity test using different soaps. The antiseptic soap, Dettol showed better response against the bacterial species when compared to others. Dettol was followed by Medimix and Lifebuoy in controlling all the organisms. Totally 46 isolates of bacteria were obtained from 30 skin swab samples.

Keywords:
skin swab, Dettol, Lifebuoy, Medimix, Lux.

INTRODUCTION:

Soaps and other cleansing agents have been around for quite a long time. For generations, hand washing with soap and water has been considered a measure of personal hygiene. Bacteria are very diverse and present in soil, water, sewage and on human body and are of great importance with reference to health (Johnson et al., 2002). In 1961 the U.S public Health service recommendation 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 (Osborne and Grube, 1982). Although fats and oils are general ingredients of soaps but some detergent additives enhance the antibacterial activities of soaps (Friedman and Wolf, 1996). Transient bacteria are deposited on the skin surface from environmental sources and cause skin infections. Examples of such bacteria are Pseudomonas aeruginosa (Fluit et al., 2001) and Staphylococcus aureus (Higaki et al., 2000). The importance of hand washing is more crucial when it is associated to health care workers because of possible cross contaminating of bacteria that may be pathogenic or opportunistic (Richards et al., 1999). Hand hygiene and prevention of infection through the use of medicated soaps has been well recognized. A large number of chemical compounds have the ability to inhibit the growth and metabolism of microorganisms or kill them. The number of chemicals is enormous, probably at least 10, 000 with 1,000 commonly used in the hospital and homes. Chemicals exist as solids, liquids and gases. Of the many groups of chemicals used to reduce or destroy microbes important groups include hydrogen, phenols, soaps, detergents, ammonia compounds, alcohols, heavy metals, acids and certain special compound. Disinfection, decontamination, antisepsis/sanitization and sterilization, just naming a few are terms that describe the process of cleaning by either soaps/detergents or other cleaning agents. Numerous cleaning agents are available in the market, which are presented in various forms with distinct formulation. Triclosan, trichlorocarbanilide and Pchloro-in-xylenol (PCMX/Chloroxylenol) are the commonly used anti-bacterials in medicated soaps. These are generally only contained at preservation level unless the product is clearly marked as antibacterial, antiseptic, or germicidal (Larson et al., 1989). Scrubbing body or hands, particularly with soaps is the first of defense against bacteria and other pathogens that can cause colds the Flu, skin infection and even deadly communicable diseases (Kimel, 1996). Conceptually, many people consider that an antimicrobial portion of soaps is effective at preventing communicable disease. But now researchers highlight that too much of it can have the opposite effect spreading disease/infection instead of preventing them

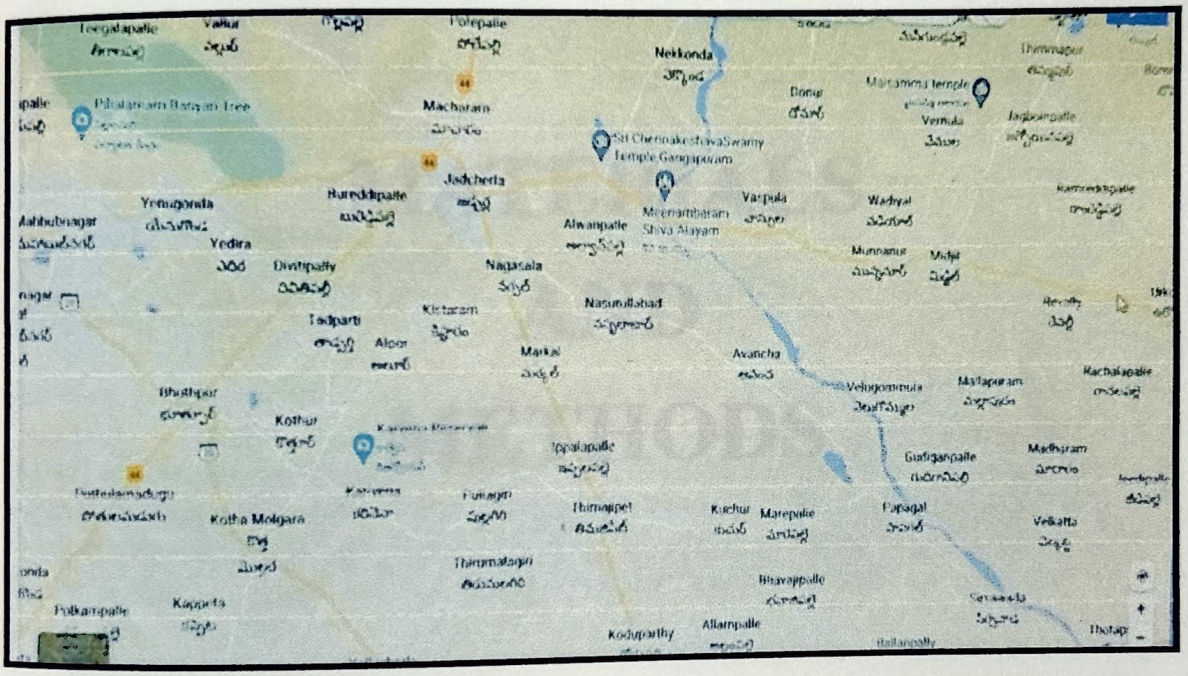
(Poole, 2002). Over-utilization of medicated rendering might result in antimicrobial resistance and even rendering an individual more vulnerable to microbial attacks such as opportunistic skin infections (White and McDermolt, 2001). Unfortunately, in the long run may affect the consumers, because overuse of these agents can ascribe to the emergence of drug, resistant micro organisms. This research work carried out in 2004 was aimed at determining the antibacterial activities of some commonly used medicated soaps in selected human pathogens.

OBJECTIVES

- Isolation and identification of Normal microbial human flora from skin samples.
- Testing of antimicrobial activity of different soap samples on isolated normal microbial flora .

STUDY AREA:

Jadcherla is a town in Mahbubnagar district in the state of Telangana. It is located in Jadcherla mandal in Mahbubnagar revenue division.Jadcherla is located at 16.7738°N 78.1367°E and at an altitude. Jadcherla is located 86 km from Hyderabad 130 km from Kurnool and 21 km from Mahabubnagar.



1. Sample Collection

The medicated and toilet soap samples used for the study were purchased from supermarket from Jadcherla town.

2. Isolation of Microorganism

Sterile swab sticks were moistened with sterile peptone water, and were used to collect skin swabs samples from the neck, armpit, chest, face and hand of students of the department of Microbiology, Dr BRR Govt College, and Jadcherla. The skin areas sampled were first swabbed with methylated spirit to remove the transient micro flora. The samples collected with swab sticks were then used to inoculate already prepared Nutrient and Mcconkey Agar plates. The cultured plates were then incubated at 37°C for 48 hours. Biochemical characterization and identification of the test pathogens were carried out using standard identification methods.

Gram staining

The basic principle of gram staining involves the ability of the bacterial cell wall to retain the crystal violet dye during solvent treatment. Gram-positive microorganisms have higher peptidoglycan content, whereas gram-negative organisms have higher lipid content. A Gram stain helps diagnose harmful bacteria. Under a Gram stain, different kinds of bacteria change one of two sets of colours (pink to red or purple to blue) under a special series of stains and are categorized as "gram-negative" or "gram-positive," accordingly.

- Crystal Violet, the primary stain
- Iodine, the mordant
- A decolorizer made of acetone and alcohol (95%)
- Safranin, the counterstain

Procedure:

- Take a clean, grease free slide.
- Prepare the smear of suspension on the clean slide with a loopful of sample.
- Air dry and heat fix.
- Crystal Violet was poured and kept for about 30 seconds to 1 minutes and rinse with water.
- Flood the gram's iodine for 1 minute and wash with water.
- Then, wash with 95% alcohol or acetone for about 10-20 seconds and rinse with water.
- Add safranin for about 1 minute and wash with water.

- Air dry, blot dry and Observe under Microscope.

Biochemical Tests

Indole Test

Methyl Red Test

Citrate Test

Catalase Test

Urease Test

Indole test

Principle:

Indole tests are a biochemical process, which is used to identify the indole producing organism from trypton. Trypton is an important amino acid which is found in most bacteria cell protein.

Reagents:

- Indole Kovac's reagents
- P-Dimethylaminocinnamaldehyde [DMACA] - 10.0gm
- Hydrochloric Acid,37% -100.0ml
- Amyl alcohol - 750.0ml

Procedure:

Inoculate the tube of tryptone broth with a small amount of a pure culture. Incubate at 37°C for 24 to 48 hours. To test for indole production, add 5 drops of Kovác's reagent directly to the tube. A positive indole test is indicated by the formation of a pink to red colour ("cherry-red ring") in the reagent layer on top of the medium within seconds of adding the reagent. If a culture is indole negative, the reagent layer will remain yellow or be slightly cloudy. Indole positive bacteria: E. coli, Vibrio cholera Indole negative bacteria: Klebsiella, Salmonella, Shigella sp.

Methyl Red Test & Voges Proskauer test

Principle:

Some bacteria have the ability to utilize glucose and convert it to stable acid like lactic acid, acetic acid or formic acid as the end product. These bacteria initially metabolize glucose to pyruvic acid, which is further metabolized the '" mixed acid pathway to produce the stable acid. The type of acid produced differs from species to species depends on the specific enzymatic pathways present in the bacteria. The acid so produced decreases the PH to 4.5 or below, which is indicated by a change in the colour of methyl red from yellow to red.

In the methyl red test, the test bacteria are grown in a broth medium containing glucose. If the bacteria has the ability to utilize glucose with production of stable acid, the colour of methyl red changes from yellow to red, when added in to the broth culture.

The mixed acid pathway gives 4 mol of acidic products (mainly lactic acid and acetic acid), 1 mol of neutral fermentation product, 1 mol of CO2, and 1 mol of H2 per mol of glucose fermented. The large quantity of acids produced causes a significant decrease in the PH of culture medium.

Reagents:

- MRVP broth (pH 6.9)
- Ingredients per litre of deionizer water
- Buffered peptone = 7.0 gm
- Glucose= 5.0 gm
- Dipotassium phosphate = 5.0 gm
- Methyl red solution = 0.02%
- Dissolve 0.1 gm of methyl red in 300 ml of ethyl alcohol, 95%
- Add sufficient distilled after to make 500 ml
- Store at 4 to 8 degree C in a brown bottle. Solution is stable for 1 year.

Procedure:

- Prior to inoculation, allow medium to equilibrium to room temperature.

- Using organisms taken from an 18-24 hours pure culture, lightly inoculate the medium.
- Incubate aerobically at 37 degrees C for 24 hours.
- Following 24 hrs. of incubation, aliquot 1ml of the broth to a clean test tube.
- Re incubate the remaining broth for an additional 24 hrs.
- Add 2 to 3 drops of methyl red indicator to aliquot.
- Observe for red color immediately.

Citrate Utilization Test

Principle:

Sodium citrate is considered as both a carbon source and an energy source. Nitrogen source is NH_4^+. The presence of enzymes such as citrate permease [citrase] facilitates the citrate into bacterium. Bromothymol blue is considered as a PH indicator for the citrate utilization test oxygenises required and the process is done on the slants. The citrate is oxidised from bacteria and is extracted with the release of CO_2 from the medium. Sodium from sodium citrate combines with CO_2 and water, to form an alkaline product [sodium carbonate]. The change in the PH of the solution gives details about the presence and absence of the test. Colour change of the solution to blue indicates the absence of the citrate test. If there is no colour shift, that means there is no growth in the medium which indicates the absence of the citrate test.

Reagents:

- Simmon's citrate agar [PH 6.8]
- Bromothymol blue indicator solution
- Koser'sliquid citrate medium [PH .6.8]

Procedure:

- Prepare Simmon citrate agar in test tubes, taking 5 ml medium by autoclaving for 30 minutes tilt the test tube containing melted citrate medium to prepare distinct slant and butt.
- Inoculate the given sample of organism were on the slant of the media using sterile inoculation loop and label the tubes
- Incubate the tubes at 37°C for 24 -48 hours.
- Observe the color change in the medium

Catalase test

Principle:

The enzyme Catalase mediates the breakdown of hydrogen peroxide into oxygen and water. The presence of the enzyme in a bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide, and the rapid elaboration of oxygen bubbles occurs. The lack of Catalase is evident by a lack of or weak bubble production. The culture should not be more than 24 hours old.

$$2H_2O_2 \rightarrow\rightarrow\rightarrow\rightarrow\rightarrow\rightarrow 2H_2O + O_2 \text{ (gas bubbles)}$$

Bacteria thereby protect themselves from the lethal effect of Hydrogen peroxide which is accumulated as an end product of aerobic carbohydrate metabolism.

Reagents:

3% hydrogen peroxide

Procedure:

Place a microscope slide inside a Petri dish. Keep the Petri dish cover available. Using a sterile inoculating loop or wooden applicator stick, collect a small amount of organism from a well-isolated 18- to 24-hour colony and place it onto the microscope slide. Be careful not to pick up any agar. This is particularly important if the colony isolate was grown on agar containing red blood cells. Carryover of red blood cells into the test may result in a false-positive reaction. Using a dropper or Pasteur pipette, place 1 drop of 3% H2O2 onto the organism on the microscope slide and observe for immediate bubble formation (O2 + water = bubbles). Observing for the formation of bubbles against a dark background enhances readability.

Urease test

Principle:

Urease is the product of decarboxylation of amino acids. Hydrolysis of urea produces ammonia and CO2. The formation of ammonia alkalinizes the medium, and the pH shift is detected by the colour change of phenol red from light orange at pH 6.8 to magenta (pink) at pH 8.1Rapid urease-positive organisms turn the entire medium pink

within 24 hours. Weakly positive organisms may take several days, and negative organisms produce no colour change or yellow as a result of acids production.

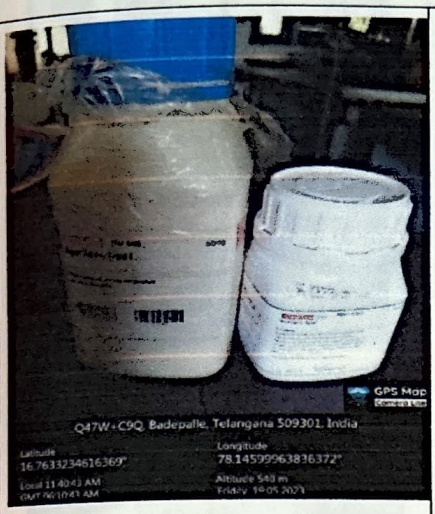
$$(NH_2)_2CO + 2\,H_2O \xrightarrow{\text{Urease}} CO_2 + H_2O + 2\,NH_3$$

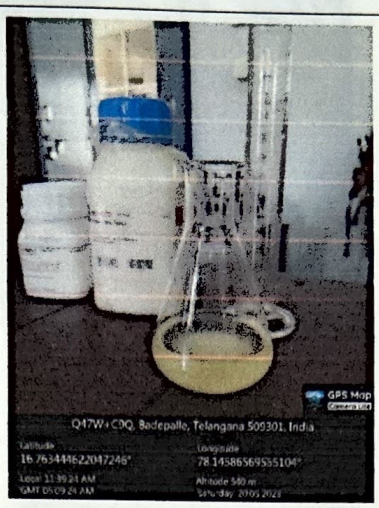
Urea — Carbon dioxide — Water — Ammonia

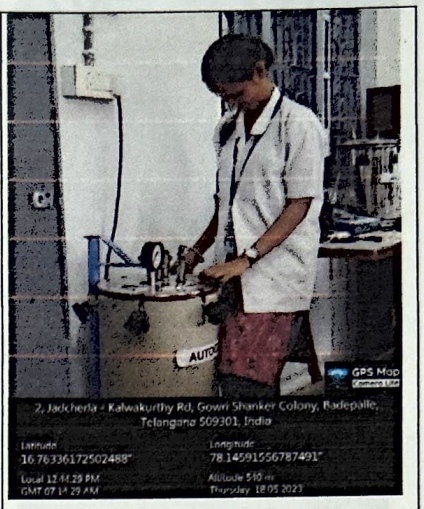
Procedure:

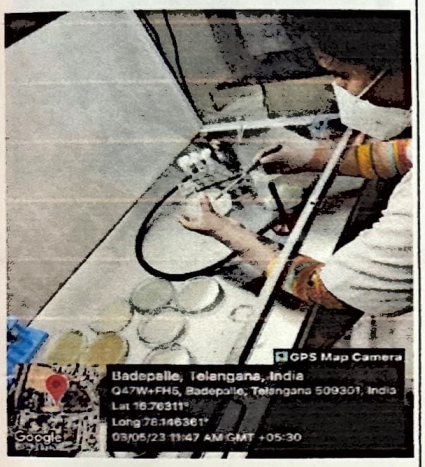
Christensen's Urea Agar (4, 5) Use a heavy inoculum from an 18 to 24 hours pure culture to streak the entire slant surface. Do not stab the butt as it will serve as a colour control. Incubate tubes with loosened caps at 35^0C.Obserbe the slant for a colour change at 6 hours, 24 hours, and every day for up to 6 days. Urease production is indicated by a bright pink (fuchsia) colour on the slant that may extend into the butt. Note that any degree of pink is considered a positive in the medium. To eliminate protein hydrolysis as the cause of a positive test, a control medium lacking urea should be used. Rapidly urease-positive Protease (Proteus spp., Organelle morganii, and some Providencia stuartii strains) will produce a strong positive reaction within 1 to 6 hours of incubation. Delayed-positive organisms (e.g., Klebsiella or Enterobacter) will typically produce a weak positive reacti0on on the slant after 6 hours, but the reaction will intensify and spread to the butt on prolonged incubation (up to 6 days). The culture medium will remain a yellowish colour if the organism is urease negative.

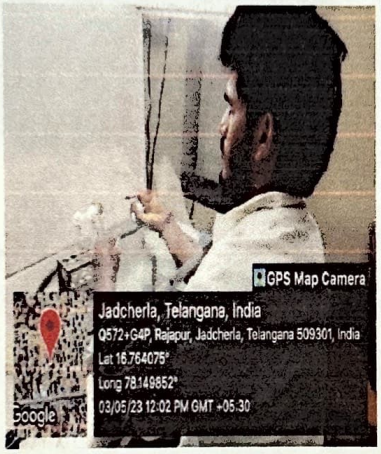
Photographs showing of laboratory experiments

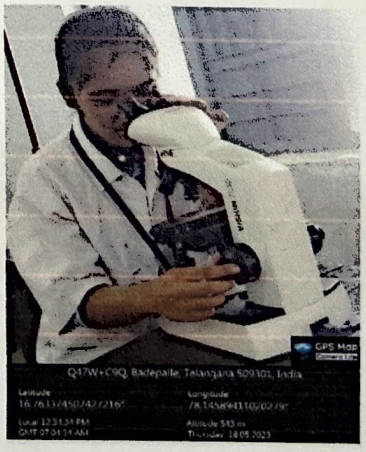


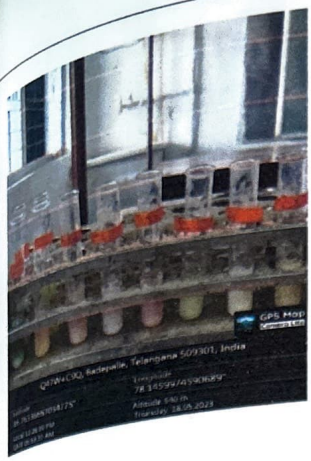


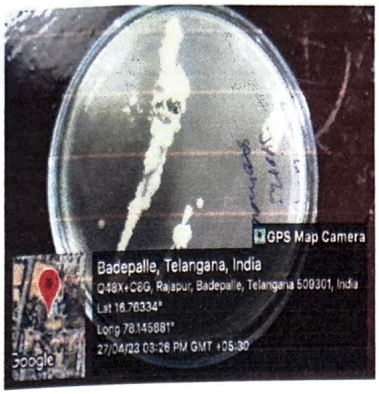


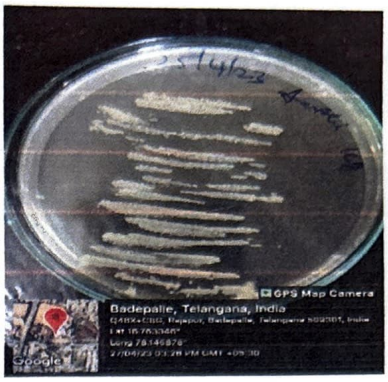


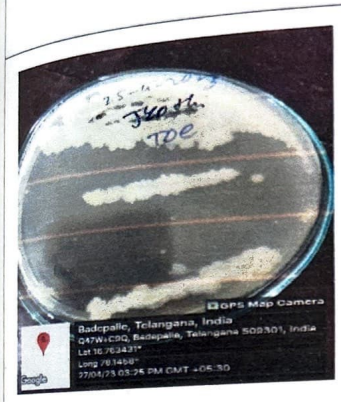


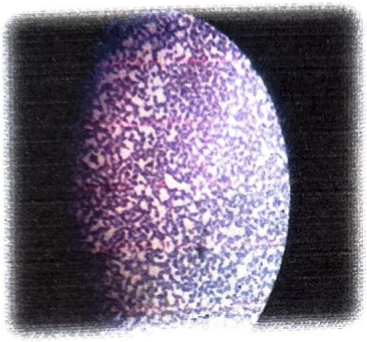


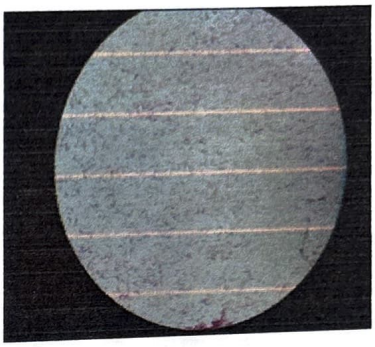


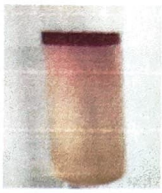
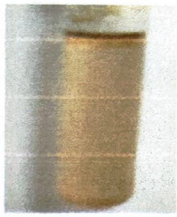
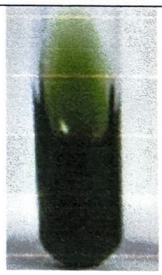
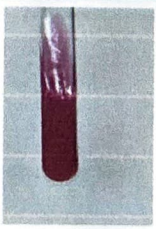
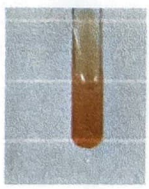

GPS Map Camera
Badepalle, Telangana, India
Q48X+C8G, Rajapur, Badepalle, Telangana 509301, India
Lat 16.76334°
Long 78.145681°
27/04/23 03:26 PM GMT +05:30


GPS Map Camera
Badepalle, Telangana, India


GPS Map Camera
Badepalle, Telangana, India
Q47W+C9Q, Badepalle, Telangana 509301, India
Lat 16.763431°
Long 78.14598°
27/04/23 03:25 PM GMT +05:30





Test	Positive Result	Negative Result
Indole test		
Methyl Red Test		
Voges Proskauer test		
Citrate Utilization Test		
Catalase test		
Urease test		

3. Preparation of Soap Samples:

A sterile blade was used to scrap one gram (1 g) each of the soaps and which quantity was dissolved in 9 mls of sterile distilled water to a give a stock solution of 10-1. These stock solutions were then stored in a refrigerator in well sealed containers for future use.

4. Antimicrobial Susceptibility Testing

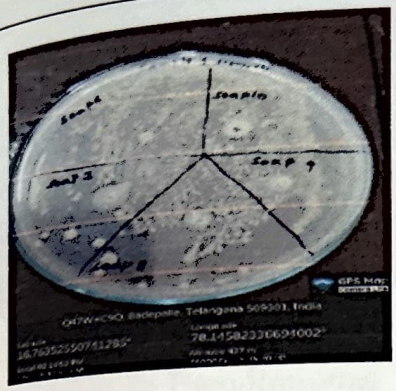
The well diffusion method is basically carried out for antigen and antibody interactions like in precipitation tests; similar way adopted and carried out antimicrobial activity of soap sample on the isolated microorganisms from the human flora. A nutrient agar was prepared and poured on to the Petri plates after solidification the test microorganism is going to inoculate by spread palate method, then by taking a tough sterilized plastic straw have to cut wells in appropriate position on the Petri plate and then place the .2ul of soap sample on to the well by micropipette and allow the soap solution to diffuse and incubated at 35°C for 24 hrs and then were examined for of zone of inhibition around the disk

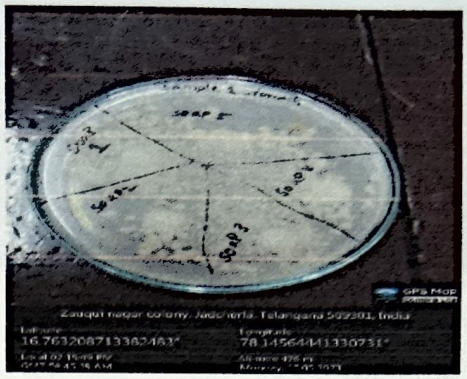
RESULTS

Normal microbial flora isolated and identified from skin surfaces

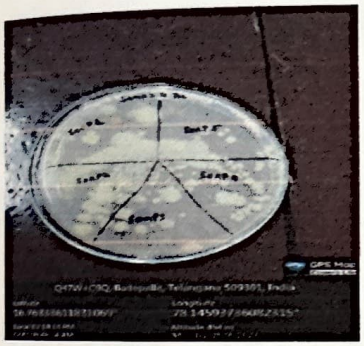
S.No	Characteristics	E.Coli	Bacillus	Staphylococci	Pseudomonas
1	Colony morphology	Smooth, circular, white	Rough, Opaque, white	Large yellow or white	Large, opaque, flat, greenish
2	Gram staining	-	+	+	-
3	Shape	rod	Rod	Round, braches	
4	Indole	+	-	-	-
5	MR	+	+	+	-
6	VP	-	-	-	-
7	Citrate	-	+	-	-
8	Catalase	+	+	+	+
9	Urease	-	-	-	-

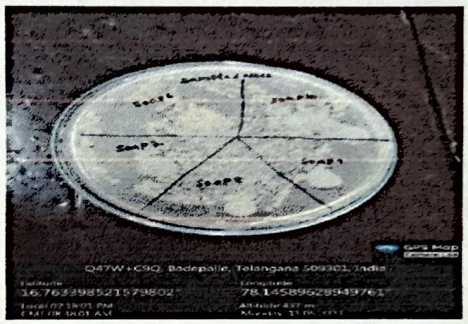
Antimicrobial activity of soap samples on Isolated Microorganisms



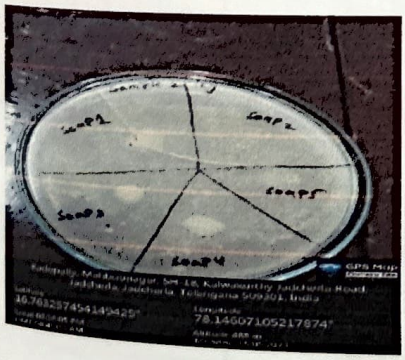


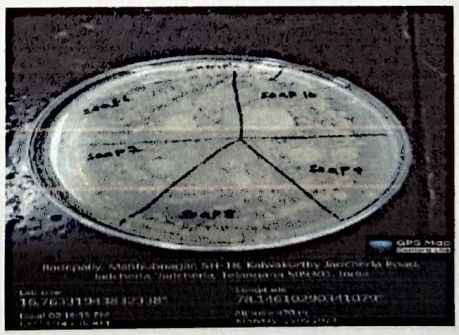
soap samples on E. Coli



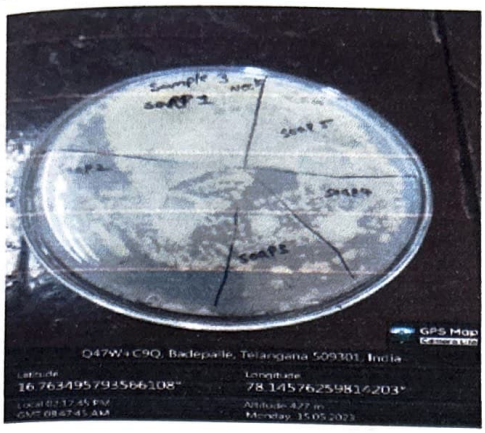


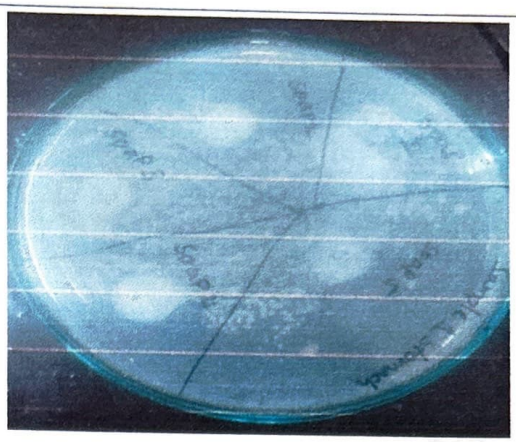
Soap samples on Bacillus





Soap samples on staphylococcus





Soap samples on Pseudomonas

Table showing Zone of inhibition: Expressed in centimeters

Microorganism	Shikakay (S1)	Lifebuoy (S2)	Dabur (S3)	No.1(S4)	Santoor (S5)	Lux(S6)	Cintol (S7)	Aloevera (S8)	Dettol (S9)	Medimix (S10)
E.coli	0.9	1.3	1.2	1.0	1.1	0.4	1.2	0.7	1.7	1.4
Bacillus	0.8	0.9	0.7	0.9	0.8	0.9	0.8	0.5	1.5	1.8
Staphylococci	0.9	1.1	0.5	0.5	0.9	0.6	0.8	0.8	1.8	1.6
Pseudomonas	0.7	0.9	0.7	0.5	0.7	0.4	1.0	0.9	1.3	1.0

Results

Results of this study revealed that most of the assayed medicated soaps have antibacterial activity, though to varying degrees as indicated by the inhibition of the growth pattern of the isolates. When the efficacy of the antibacterial soap were compared using the disc agar diffusion method, Crusader was found to be most effective against all the bacteria strains tested having the highest zone of inhibition (1.8 cm) against Staphylococcus aureus and 1.7cm against Escherichia coli at the highest dilution used.the least antibacterial activity with zone of inhibitions of 0.4 cm, 0.6 cm respectively against pseudomonas and Staphylococcus.Soaps are intended for reduction of the inoculums sizes of pathogenic and non-pathogenic microorganisms, the later include the normal flora. Of these, two types are well known viz resident that are the normal flora of the skin and other human body parts, and transient flora that are usually picked up from objects or other human beings (White house station, 2008). Thus, it is routine practices to wash hands prior to eating, after examining a patient and before surgery, in order to remove some potentially harmful transient flora as well as reduce a number of resident flora, which might cause opportunities infections (Saba Riaz et al., 2009).

Conclusion

The soaps tested in this work showed varied levels of effectiveness against the test isolates. Hence, the antibacterial activity can be used to prevent skin infections and transmission of skin pathogens when used in hand washing. However, prolonged used of these soaps could lead to development of microbial resistance in future.