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Antimicrobial Activity of different Medicinal Soaps and Turmeric Powder against Infectious Microorganisms Isolated from Wound Area of Human

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Abstract: ***The strains of microbes namely Klebsiella pneumoniae,* Pseudomonas aeruginosa*, Escherichia coli,* Staphylococcus aureus *and Bacillus subtilis were isolated from human wound infected swab, collected from SMIMER (Surat Municipal Institute of Medical Education and Research) hospital, Surat. A number of medicated soaps were assayed for antimicrobial efficacy. The medicinal soaps like dettol, ayush, lifebuoy and pantanjali were used and they showed different antimicrobial activity against isolated infectious bacteria. The most common antimicrobial active ingredients were triclosan, trichloroxylenol and tri-chlorocarbanilide. The best antimicrobial activity was observed in ayush soap sample. Microbial resistance to turmeric powder has been reported in minor amount, which make it a very useful in preventing bacterial infection (e.g. Klebsiella pneumoniae, Staphylococcus aureus, Psuedononas aeruginosa, Escherichia coli, Bacillus subtilis (Nakano MM., Zuber P., (1998)) in wounds and in the treatment of infections in chronic wound, it is observed that it may not respond to the antibiotic therapy. Ayush turmeric soap showed higher antimicrobial activity against Klebsiella pneumoniae and other bacteria.***

Keywords: Infectious Microorganisms, Medicated soaps, Resistance, Sensitivity, Turmeric Powder

1. Introduction

Generally the most of dermal wounds are colonized with an aerobic and an anaerobic microorganisms that originate predominantly from the mucosal surfaces same as that seen in the oral cavity and gut. The use of soaps as medicines could be traced as far back as the beginning of human civilization (Josephin Sheeba and Selva Mohab, 2012). From a microbiological perspective, actually the primary function of a normal intact skin is to control microbial populations that basically live on the surface of skin and also to prevent underlying tissue from becoming colonized. Although these microorganisms are also responsible for the wound infections, widely spread controversy still exists regarding the exact mechanisms by which infection is caused and also regarding their unique significance in the nonhealing wounds which may not exhibit clinical signs of infection.

2. Human wound infection

Wound infection can be defined as the accumulation of pus, either within an abscess or exuding from a sinus tract or from a mucocutaneous surface, is one of the most cardinal indicators of local sepsis. Wound healing is one of the most complex multistep physiological process that can involve multitude of events and cells. This interactive process is well dynamic in nature which include soluble mediators, blood cells, extracellular matrix and parenchymal cells.

Pseudomonas aeruginosa is responsible for cause of nosocomial infections as a result of its ubiquitous nature, the ability to survive in the moist environments and also the resistance to many other antibiotics and antiseptics (Nascimento. G, Freitas and Silva. G., 2014). Initially *Escherichia coli* was considered a non-harmful member of the colon flora, but is now responsible for various diseases and infections including meningeal, gastrointestinal, urinary tract, wound and bacteremia infections in all age groups (Desalegn Amenu, 2014).

Staphylococcus aureus is a member of the normal flora of the body, frequently found in the nose, respiratory tract, and on the skin (Masalha M, *et al*., 2001). *B. subtilis* is considered the best studied Gram-positive bacterium and a model organism to study bacterial chromosome replication and cell differentiation (Nakano MM., Zuber P., 1998).

Members of the *Klebsiella* genus typically express two types of antigens on their cell surfaces. The first, O antigen, is a component of the lipopolysaccharide (LPS), of which 9 varieties exist. The second is K antigen, a capsular polysaccharide with more than 80 varieties. Both contribute to pathogenicity and form the basis for serogrouping (Podschun, R; Ullmann. U., October 1998).

3. Medicinal Uses of para-chloro-meta-xylenol (Dettol Soap)

Mostly dettol soap is used in the treatment of fungal infections, bacterial infections, wound-cleansing, Antibacterial and other conditions. These soap contains Chloroxylenol and Triclosan as active ingredients. The Dettol soap works to kill bacteria and also able to reduce inflammation that act against anybacterial infections and fungal infections.

a) Medicinal uses of Pinda Tailam (Ayush Soap)

Ayush purifying turmeric soap is prescribed in ayurveda for purification. As we talk about the turmeric which is known

for its healing and purifying properties and the nalpamaradi tailam is known to cure the skin infections and rashes, which also leave the skin glowing.

b) Medicinal uses of Manglore (Lifebuoy Soap):
As we know that the lifebuoy is one of the world's leading selling antibacterial soap, it is almost sold in 60 countries. Lifebuoy clears the skin's formulation which includes a number of ingredients, one of multani mati that can works well on skin by its natural property such as absorbing excess oil and also been able to eliminating the bacteria.

c) Medicinal uses of Haldi Chandan Kanti (Patanjali Soap):
Patanjali haldi chandan kanti body cleanser soap have all the good properties of turmeric, sandalwood and other herbals. These soap is a medicine that is used for the treatment of skin disorder, inflammation, arthritis, skin problems and other conditions. The most commonly reported use of this medicine is for skin's disorders.

4. Materials and Methods

4.1 Collection of Sample

Human wound infection swab sample was collected from SMIMER (Surat Municipal Institute of Medical Education and Research) hospital, Surat, Gujarat in sterile condition and preserved in refrigerator (20°C) till it was used for the isolation of microorganisms in laboratory.

4.2 Isolation of Microorganisms:

Collected swab sample stick was streaked onto the MacConkey's agar plate (Peptone 20.0 g, Lactose 10.0 g, Nacl 5.0 g, Bile salts 3.0 – 5.0 g, Neutral Red 30.0 mg, Crystal violet 10.0 mg, Distilled water 1000 mL, Agar agar 30.0 g, pH 7.4) and Nutrient agar plate (Peptic digest of animal tissue 5.0 g, Sodium chloride 5.0 g, Beef extract 1.5 g, Yeast extract 1.5 g, Agar agar 30 g, pH (at 25°C) 7.4 ± 0.2, Distilled water 1000.0 mL). After streaking, plate was incubated at 37°C for 48 hrs (Cheesbrough, 2005).

4.3 Identification of microorganisms

Identification of isolated microorganisms were performed according to morphological, cultural and biochemical characteristics. All isolated microorganisms were subjected to Gram's staining, biochemical tests and by analysis VITEK.

Colony morphology:
Bacterial colonies were observed to determine the morphology of selected strains on the basis of size, shape and colour.

Biochemical test:
Isolated microorganisms were subjected to biochemical tests as follow:
Carbohydrate fermentation (Sugar utilization) test, Methyl red (M-R) test, Voges-proskauer (V-P) test, Citrate utilization test, Indole production test, Triple Sugar Iron (TSI) test and Urea hydrolysis test (Patel R., 2009).

4.4 Collection of soaps and turmeric

Different medicinal soaps like, Dettol (para-chloro-meta-xylenol), Ayush (pinda tailam), Lifebuoy (mangalore), Patanjali (haldi chandan kanti) was collected from Super market medical shop, surat, Gujarat. Suspensions of such a medicinal soaps were prepared to check its antimicrobial activity against isolated organisms. Another sample i.e. Turmeric (haldi) was collected from general shop, Surat, Gujarat to check its antimicrobial activity against isolated microorganism.

4.5 Soap sample preparation

Collected soap material (for example, Dettol (para-chloro-meta-xylenol), Ayush (pinda tailam), Lifebuoy (mangalore), Patanjali (haldi chandan kanti) were crushed, using a pestle and mortar, to provide a greater surface area. After crushing, the soap material was dissolved in autoclaved water (121°C, 15 psi).

The universal solvent: Water (50 mL, 20 mL and 10 mL of autoclaved water) was taken into small beaker. A 1 g of crushed soap was mixed with 50 mL, 20 mL and 10 mL of warm autoclaved water separately. The solution was swirled to mix it well (swirling was gentle to avoid foam formation). Solutions were labled accordingly. 20 drops of the liquid soap was mixed with 10 mL of warm autoclaved water. The solution was than swirled to mix it well. For turmeric, 1 g of turmeric was mixed with 50 mL, 20 mL and 10 mL of warm autoclaved water.

pH test: Separate test tubes were labled with accordingly. In the first tube, 10 mL of the soap solution was taken. In the second tube, 10 mL of the commercial soap solution was taken. In the third tube, 10 mL of autoclaved water was taken (as control). One by one, each solution was stirred well for proper mixing and pH was recorded of each solution using pH strips.

Foam Test: The tubes were enclosed properly before shaking each tube continuously for 10 seconds. Observed and recorded the amount of suds or foam produced by each soap solution.

4.6 Antimicrobial activity

The antimicrobial assay was performed by agar well diffusion method and paper disc diffusion method for each soap sample (A. M. Harsulkar, 2009).

Agar well diffusion method:
The media (Mueller Hinton Agar, Peptic digest of animal tissue 5.0 g; Sodium chloride 5.0 g; Beef extract 1.5 g; Yeast extract 1.5 g; Agar agar 15 g; pH (at 25°C) 7.4 ± 0.2; Distilled water 1000.0 mL) was poured into the Petri plate. The isolated microorganisms were cultured in to Mueller Hilton Agar by using spread plate technique (S. D. Jagtap *et al.*, 2009) and a well of 7 mm diameter was made onto the plate for loading the sample. Stock solution of soap sample

in water was used to prepare desired dilutions with different concentration. Each dilutions was poured in to well and the sample were allowed to diffuse properly by keeping the petriplates in refrigerator at 4°C for 30 minutes followed by the incubation at 37°C for 24 hr. Autoclaved water was used as a control in the same manner. The diameter of zone of inhibition was taken as the measure of the antimicrobial activity of a particular soap sample (S. D. Jagtap *et al*., 2009).

Paper disc diffusion method:

Preparation of discs with soap samples:

Discs (Whatman filter paper no.1 is used to prepare discs) of diameter 6 mm were bored using disc borer. The discs were then wrapped in foil paper and sterilized in a hot air oven at 100°C for 1 hr or in a autoclave at 37°C for 15 minutes and were later soaked in the different soap solutions for a period of one hour to ensure full saturation of the soap preparations. The discs were then aseptically removed from soap solution and allowed to dry in an oven at 25°C. They were then packed into sterile bottles and stored in the refrigerator for future use in susceptibility test (Selvamohan and Sandhya, 2012).

Disc diffusion method was suitable for use when there is no requirement to determine the presence of specific concentrations (Bauer *et al*., 1966). The media (Mueller Hinton Agar) was poured into the petri plate. The isolated microorganisms were cultured in to Mueller Hilton Agar by using spread plate technique. The method involves inoculating small filter paper discs dipped in prepared soap samples and placing those discs onto an agar plate that has been inoculated with an indicator organism. The plate was then incubated, typically overnight at 37°C. A sample with sufficient concentration of antimicrobials was responsible to form a zone of bacterial inhibition as it diffuses through the agar. This indicates the presence of antimicrobial activity in the prepared soap sample (Ndukwe, 2005).

5. Results and Discussion

Isolation of unknown collected wound samples was swabbed on to different media (MacConkey's Agar, Nutrient Agar) i.e. shown in Plate 1 and 2.

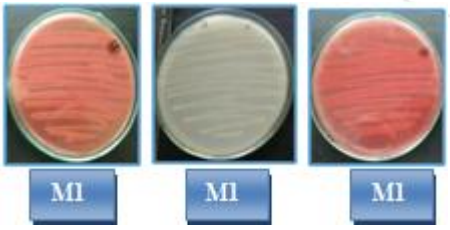

Plate 1: Isolation of microorganisms on MacConkey's Agar Plate and Nutrient Agar Plate.

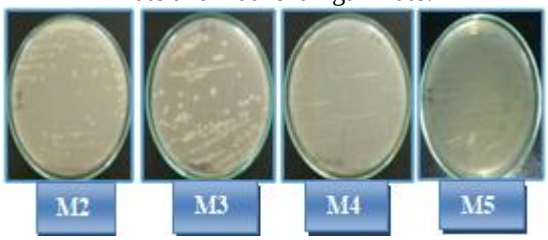

Plate: 2 Isolation of mcroorganisms on Nutrient Agar Plate

Isolation of microorganisms by Gram's staining revealed following result (Table: 1).

Table 1: Result of Gram's staining

Isolate	Characteristics
M1	Gram –ve
M2	Gram +ve
M3	Gram +ve
M4	Gram –ve
M5	Gram –ve

VITEK Analysis:

Unknown bacterial culture submitted to Perfect Diagnostics Laboratory (Bhatar Road, Surat, Gujarat) for identification. According to VITEK result strains were confirmed as follow (Table: 2).

Table 2: Name of identified microorganisms

Isolate	Name of organism
M1	*Klebsiella pneumoniae*
M2	*Staphylococcus aureus*
M3	*Bacillus subtilis*
M4	*Escherichia coli*
M5	*Pseudomonas aeruginosa*

Stock concentration of soap sample and turmeric

Crushed soap in the quantity 1 g was mixed with 50 mL, 20 mL and 10 mL of warm autoclaved water separately. The solution was swirled to mix it well (swirling was gentle to avoid foam formation). Solutions were labeled accordingly. For turmeric, 1 g of turmeric was mixed with 50 mL, 20 mL and 10 mL of warm autoclaved water.

Results of Antimicrobial activities of different Medicinal soaps and turmeric

Table 3: Result of zone of inhibition (For Isolate M1 i.e. *Klebsiella pneumoniae*)

Code	Name of soap sample	Concentration of stock solution	Stock	1	2	3	4
A	Dettol	20/1000 mL autoclaved water	_	2 mm	3 mm	5 mm	4 mm
B	Lifebuoy	20/1000 mL autoclaved water	_	2 mm	2 mm	4 mm	5 mm
C	Patanjali	20/1000 mL autoclaved water	_	2 mm	3 mm	3 mm	2 mm
D	Ayush	20/1000 mL autoclaved water	11 mm	3 mm	4 mm	4 mm	6 mm
E	Turmeric	20/1000 mL autoclaved water	_	1 mm	_	1 mm	_

By performing Agar disc diffusion method of soap sample of Ayush gave 11mm, 6mm, 4mm, 4mm and 3mm diameter zone of inhibition, soap sample of Dettol gave 5mm, 4mm, 3mm and 2mm diameter zone of inhibition, soap sample of Lifebuoy gave 5mm, 4mm, 2mm and 2mm diameter zone of inhibition, Patanjali soap sample gave 3 mm, 3 mm, 2 mm

and 2 mm diameter zone of inhibition and Turmeric sample gave 1 mm diameter zone of inhibition (Table-3). According to this experiment, soap sample of Ayush was showing good zone of inhibition against organism isolate M1 i.e. *Klebsiella pneumoniae* compare to other soap sample (Table-3).

Table 4: Result of zone of inhibition (For Isolate M2 i.e. (*Staphylococcus aureus*)

Code	Name of soap sample	Concentration of stock solution	Stock	1	2	3
A	Dettol	20/1000 mL autoclaved water	_	_	2 mm	4 mm
B	Lifebuoy	20/1000 mL autoclaved water	_	_	2 mm	1 mm
C	Patanjali	20/1000 mL autoclaved water	_	_	2 mm	1 mm
D	Ayush	20/1000 mL autoclaved water	11 mm	8 mm	3 mm	10 mm
E	Turmeric	20/1000 mL autoclaved water	_	_	1 mm	_

By performing Agar disc diffusion and paper disc diffusion method of soap sample of Ayush gave 11mm, 10mm, 8mm and 3mm diameter zone of inhibition, soap sample of Dettol gave 4mm and 2mm diameter zone of inhibition, soap sample of Lifebuoy gave 2 mm and 1 mm diameter zone of inhibition, Patanjali soap sample gave 2 mm and 1 mm diameter zone of inhibition and Turmeric sample gave 1 mm diameter zone of inhibition (Table-4).

According to this experiment, soap sample of Ayush was showing good zone of inhibition against organism isolate M2 i.e. *Staphylococcus aureus* compare to other soap sample (Table-4).

Table 5: Result of zone of inhibition (For Isolate M3 i.e. (*Bacillus subtilis*)

Code	Name of soap sample	Concentration of stock solution	Stock	1	2	3
A	Dettol	20/1000 mL autoclaved water	_	_	_	2 mm
B	Lifebuoy	20/1000 mL autoclaved water	_	2 mm	2 mm	1 mm
C	Patanjali	20/1000 mL autoclaved water	_	_	4 mm	1 mm
D	Ayush	20/1000 mL autoclaved water	13 mm	9 mm	12 mm	6 mm
E	Turmeric	20/1000 mL autoclaved water	_	_	_	1 mm

By performing Agar disc diffusion and paper disc diffusion method of soap sample of Ayush gave 13mm, 12mm, 9mm and 6mm diameter zone of inhibition, soap sample of lifebuoy gave 2mm, 2mm and 1mm diameter zone of inhibition, soap sample of Dettol gave 2 mm diameter zone of inhibition, soap sample of patanjali gave 4 mm and 1 mm diameter zone of inhibition and Turmeric sample gave 1 mm diameter zone of inhibition (Table-5).

According to this experiment, soap sample of Ayush was sawing good zone of inhibition against organism isolate M3 i.e. *Bacillus subtilis* compare to other soap sample (Table-5).

Table 6: Result of zone of inhibition (For Isolate M4 i.e. (*Escherichia coli*).

Code	Name of soap sample	Concentration of stock solution	Stock	1	2	3
A	Dettol	20/1000 mL autoclaved water	_	5 mm	4 mm	1 mm
B	Lifebuoy	20/1000 mL autoclaved water	_	_	1 mm	_
C	Patanjali	20/1000 mL autoclaved water	_	_	4 mm	_
D	Ayush	20/1000 mL autoclaved water	14 mm	13 mm	11 mm	4 mm
E	Turmeric	20/1000 mL autoclaved water	_	_	1 mm	_

By performing Agar disc diffusion and paper disc diffusion method of soap sample of Ayush gave 14mm, 13mm, 11mm and 4mm diameter zone of inhibition, soap sample of Dettol gave 5mm, 4mm and 1mm diameter zone of inhibition, soap sample of Lifebuoy gave 1 mm diameter zone of inhibition, soap sample of patanjali gave 4 mm diameter zone of inhibition and Turmeric sample gave 1 mm diameter zone of inhibition (Table-6).

According to this experiment, soap sample of Ayush was shawing good zone of inhibition against organism isolate M4 i.e. *Escherichia coli* compare to other soap sample (Table-6).

Table 7: Result of zone of inhibition (For Isolate M5 i.e. (*Pseudomonas aeruginosa*).

Code	Name of soap sample	Concentration of stock solution	Stock	1	2	3
A	Dettol	20/1000 mL autoclaved water	_	_	1 mm	_
B	Lifebuoy	20/1000 mL autoclaved water	_	_	_	_
C	Patanjali	20/1000 mL autoclaved water	_	_	_	2 mm
D	Ayush	20/1000 mL autoclaved water	8 mm	6 mm	3 mm	4 mm
E	Turmeric	20/1000 mL autoclaved water	_	_	1 mm	_

By performing Agar disc diffusion and paper disc diffusion method of soap sample of Ayush gave 8mm, 6mm, 4mm and 3mm diameter zone of inhibition, soap sample of Dettol gave 1 mm diameter zone of inhibition, Patanjali soap sample gave 2 mm zone of inhibition, soap sample of Lifebuoy do not shows the zone of inhibition and Turmeric sample gave 1 mm diameter zone of inhibition (Table-7).

According to this experiment, soap sample of Ayush was showing good zone of inhibition against organism isolate

M5 i.e. *Pseudomonas aeruginosa* compare to other soap sample (Table-7).

6. Conclusion

Bacteria are very diverse and present in soil, water, sewage and on human body and are of great importance with reference to health. The medicated soaps tested in this work showed variying levels of effects against the test isolates. The antibacterial soaps can remove 65 to 85% bacteria from human skin. So main purpose of carrying out this experiment was that some medicinal soaps like dettol, ayush, lifebuoy, pantanjali can be used as a medicine. Transient bacteria are deposited on the skin surface from environmental sources and cause skin infections. Examples of such bacteria are *Pseudomonas aeruginosa* and *Staphylococcus aureus*. A large number of chemical compounds have the ability to inhibit the growth and metabolism of microorganisms or kill them. Triclosan, Tri-chlorocarbanilide and P-chloro-in-xylenol (PCMX/Chloroxylenol) are the commonly used anti-bacterials in medicated soaps. According to this experiment, soap sample of Ayush was showing good zone of inhibition against *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Psuedomonas aeruginosa* compare to other soap sample. Ayush turmeric soap showed higher antimicrobial activity against *Klebsiella pneumoniae* and other bacteria. So antibacterial activity can be used to prevent skin infections and transmission of skin pathogens and prolonged used of these soaps could lead to development of microbial resistance in future.

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