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Assessment of Deodorants And Medical Cosmetics For Antimicrobial Activity on The Normal Flora of Human Skin

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ABSTRACT

The present study is based on Antimicrobial activity of commercially available cosmetics and Deodorants available in the market on selected skin microflora. The bacterial strain used consists of skin microorganisms like *Staphylococcus aureus,Pseudomon*as, Clostridium,*E. coli* and *Bacillus subtilis*. Antibacterial activity of cosmetics and deodorants were done on the selected bacterial strains was done by Disc Diffusion method. The zone of inhibition was found to be maximum for C2 (22mm) and minimum for C3 (6mm) in case of E. coli. Similarly, in case of *Staphylococcus aureus*, the maximum zone of inhibition was found to be maximum for C2 (20 mm), whereas C3 have not shown any antimicrobial activity against *Staphylococcus aureus*. All the Deodorants have shown significant antibacterial activity against the tested strain. The zone of inhibition was found to be maximum for D1 (25mm) in case of *Staphylococcus aureus*, and minimum for D3 (5mm). Similarly, in case of *E. coli* the maximum zone of inhibition was found to be maximum for D3 (20 mm), Similarly for *Pseudomonas sp*. the maximum zone of inhibition was also shown by D1 (12mm) followed by D2 and less by D3

Keywords: Cosmetics, Zone of inhibition, Deodorants, Antimicrobial

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INTRODUCTION

Foul odour caused by bacteria on the body is mainly due to the breakdown of various sweat components under arms and in feet. Deodorants are substances used for the body odour. Deodorants and antiperspirants change odour as well as prevent bacteria and microorganism to grow. Antiperspirants are generally applied in the underarm areas, while deodorants are applied on other areas in the form of under arm spraysand on the feet as well [1]

The terms "antiperspirants" and 'deodorants' are being describe as two different products often used interchangeably. The major component of antiperspirants are found to be Aluminium zirconium tetrachlorohydrate. Triclosan as an antimicrobial agent has been used in addition to Aluminium chloralhydrate in deodorants Ferne sole, chlorophyllin copper complex, Paraben, Zinc ricinolate, trialkyl citrate, chlorophyllin copper and other metal, chelates compounds that retard bacterial growth are also been incorporated in Deodrants. Another difference between antiperspirants and deodorants is that, Antiperspirants have been used to affect the environment of the body because they are drugs, while deodorants are considered to have liquid preparation products because they only control body odour. [2]. Antiperspirants are used for reducing the quantities of sweat. In most cases these are salts of heavy metals based on manganese or aluminium. The most frequently-used salts are of aluminium hydroxy chloride, aluminium sulphate and aluminium bromo hydrate. These products reduce the body sweat by narrowing the ducts which are secreting the sweat. The sweat glands are blocked due to protein mass created with water and small scales of skin by Antiperspirants. Therefore, the large amount of sweat production is reduced. Furthermore, studies have reported that these antiperspirants have some adverse effects on skin,constricting the sweat glands which are further narrowed. [3]. Reports have been suggested that largest organ in the body is skin and the secretions from sebaceous glands and sweat constantly fed skin surface. The long-term use of cosmetics products may alter skin environment or modify it, ultimately effecting the microflora of the skin. Some of the cosmetics formulations contain antibiotics or antimicrobial agents. In the resident microflora, such products may develop resistance due to prolonged use. The use of cosmetics, body sprays and deodorants and other products such as antiseptic, medicated soaps control the microorganism and protect the skin [4]

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MATERIALS AND METHODS

Chemicals and reagents: All the chemicals and reagent used were of AR grade purchased from Hi-media.

Collection of bacterial strains:

Selected bacterial cultures were procured from the IMTECH (Institute of microbial technology). The culture was preserved in a lyophilized form at -80^{0}C.

Collections of Deodrants and Cosmetics

All the samples used in present study were purchased from local market differing in composition.

Deodorants (D)	**Composition**
D1	Propellant , ethly alcohol , fragrance , propylene glycol , diethylphthatate , triclosan, Alcohol (90% v/v) , content 45% w/w , contains 1% w/w ,diethylphthalate
D2	Butane (propellant) , Ethyl alcohol denatured , Fragrance , Aqua , Propylene glycol , diethyl phthalates , tricosan. Alcohol content 36% w/w , ethyl alcohol 95% v/v with 1% diethyl phthalate.
D3	Propellant,Ethyl alcohol , Perfume , Isopropyl Myristate , Cyclomethycaine , Diethyl Phthalate , Triclosan , Alcohol (95% v/v) diethyl phthalate (1% w/v)

Cosmetics (C)	**Composition**
C1	Tankan amla (boric acid)-1% w/w Bhaishajya ratnavali and rastrangni, lanolin
C2	Betamethasone valerate IP, Clioquinol, Chlorocresol
C3	Sandalwood oil, Turmeric extract
C4	Polymyxcin B-Sulphate, Bacitacin Zinc, Neomycin sulphate.

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Table:1: Showing composition of Deodorants and cosmetics used in current study

Anti -bacterial activity of cosmetics on selected bacterial strain

Antibacterial activity of cosmetics on the selected bacterial strains was done by Disc Diffusion methodwith slight modifications [5]. Sterile disc was prepared from Whatman filter paper.1, the diameter of disc was 6 mm. Discs were dipped in cosmetics for 12 minutes and were analysed for antimicrobial activity.

Antibacterial activity was carried out in Muiller Hinton agar medium. 10 ml of MHA medium was added into sterile Petri plates and waskept to solidify. Pure culture of bacterial strains (50µl) was spreaded on the solidified MHA medium using sterile spreader. In order to evaluate antibacterial activity, disc carrying the cosmetics sample were impregnated on the seeded agar plate. The plates were kept for incubation at 37^0C for 24 hrs and were analysed for zone of inhibition of growth of microorganismsin the Petriplates. The diameter of the zone of inhibition was measured in mm

Minimum inhibitory concentration of cosmetics (MIC):

MIC studies of the selected cosmetics were studied by making the dilutions of cosmetics i.e 1:2 1:4, 1:8; 1:16, 1:32. The minimum inhibitory concentration was calculated in microlitres as the lowest concentration that completely inhibits bacterial growth.

Assessment of deodorants for Antimicrobial activity on selected bacterial strains

Three types of deodorants were assayed for antimicrobial activity against the selected bacterial strains. The deodorants were sprayed into a sterilized Eppendorf tubes as deodorants were liquid based. The solidified MHA plates were inoculated with a given culture of bacteria and sterile disc of 6mm dipped in deodorant for about 15 minutes were impregnated on the lawn of bacterial culture in the Petri plate. The plates were than incubated at 37^0C for 24 hrs and diameter of zone of inhibition was measuredin mm

Bacteriostatic effect of Deodorants:

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The bacteriostatic effect of deodorant was also determined [6]. Skin microflora from respondent were cultured and quantified after every two hours. An area of $5cm^2$ on the right hand just below the elbow region of the person was marked. Sterile water was used to wash the marked area. Saline solution having Sterile cotton swab were two to three times on the marked area to isolate microorganisms from the skin outer surface. The swabs containing the microorganisms were suspended in 9 ml of sterile saline solution to give 10^{-1} dilution. 1ml of suspension from this solution were transferred into 9 ml of saline solution to give 10^{-2} dilution and from this dilution again 1 ml of solution was transferred in 9 ml of saline solution to give 10^{-3} dilution. 0.1 ml of last dilution was added into the Nutrient agar plate and spreaded on the entire plate, with the help of spreader. The plated were kept to incubate at 37^0C overnight. The colonies developed on the medium were counted and recorded. Deodorant was then sprayed on the $5cm^2$ are and swabs contained microorganism was collected after 1, 2 and 4 hr and assayed with the same above procedure. The colony was counted and No. of colony forming units per ml was calculated.

RESULTS AND DISCUSSION

Anti -bacterial activity of cosmetics on selected bacterial strain:

In the present study three types of cosmetics were tested for the antibacterial activity. All the cosmetics have shown significant antibacterial activity against the tested strain. The zone of inhibition was found to be maximum for C2 (22mm) and minimum for C3 (6mm) in case of E.coli. (Fig:1-14). Similarly, in case of *Staphylococcus aureus*, the maximum zone of inhibition was found to be maximum for C2 (20 mm), whereas C3 have not shown any antimicrobial activity against *Staphylococcus aureus*. Similarly, for *Psudomonas sp*. the maximum zone of inhibition was also shown by C2 (10mm) followed by C1 and no activity in C3. Therefore, the result clearly shows that C3 is least effective cosmetics and C2 is effective against all range of skin flora, whereas C1 and C4 have moderate activity. (Table-3)

A study was reported in which 212 bacterial and fungal species from neck, armpit and chest of some respondent's isolates were cultured. The majority of bacteria was found to be *Staphylococcus aureus* and some species of *E. coli*. Staphylococcus aureus is found in the follicles of skin and is one of the major resident flora of human skin [7]

Minimum Inhibitory Concentration of cosmetics (MIC)

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Minimum inhibitory concentrations of cosmetics against selected bacterial strain are shown in table-4. From the results it can be observed that C2 has maximum value of MIC against all the bacterial strains with higher against *E.coli and Staphylococcus aureus*. C4 have shown an MIC of 16 and 8 against *Staphylococcus aureus* and *Pseudomonas sp.* The difference in MIC value may be due to the presence of different types of hydroxyacids. The result clearly indicates that even small volumes of C2 cosmetic will be effective against skin flora. Therefore, with the help of MIC values optimum formulation for cosmetics can be done

Assessment of deodorants for Antimicrobial activity on selected bacterial strain

In the present study three types of Deodorants were tested for the antibacterial activity. All the Deodorants have shown significant antibacterial activity against the tested strain. The zone of inhibition was found to be maximum for D1 (25mm) in case of *Staphylococcus aureus,* and minimum for D3 (5mm). Similarly, in case of *E. coli* the maximum zone of inhibition was found to be maximum for D3 (20 mm), Similarly for *Psudomonas sp*. the maximum zone of inhibition was also shown by D1 (12mm) followed by D2 and less by D3 (Fig: 15-17) Therefore, the result clearly shows that D3 is least effective deodorant and D1 is effective against all range of skin flora, whereas D2 have moderate activity. (Table:5).

Egoubi et al. 2013 have reported that commercially available deodorants in Nigeria have antibacterial activity against *Staphylococcus epidermis, Corynebacterium and pseudomonas sp*. Results have also shown that *Pseudomonas sp.* have less susceptibility to all the deodorants tested. This can be due to the efflux of multiple drug that pumps triclosan to expelled out of the cell [8]. Similarly, some studies have also reported that various bacterial species have low level of efficacy triclosan due to some mutations [9]

Bacteriostatic effect of Deodorants:

The bacteriostatic effect of deodorants was studied against all the strains and number of cfu/ml was calculated at a periodic time interval of 2 hrs. (Table:6)The results obtained from bacteriostatic effect reveals that initially the no. of cfu/ml was high but gradually after exposure to deodorant from 2 to 4 hr, the cfu/ ml decreases. This shows the effect of deodorant to prevent the growth of bacteria on the skin and thus generates a long-lasting effect. In the above data, D1 have shown significant activity in reducing cfu/ml from 160 to 10 cfu/ml. Similarly, D2 have also shown a good activity to reduce the number of skin bacteria. By reducing the growth of skin bacteria body odour can also be reduced effectively.

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TABLES AND FIGURES

Cosmetics	***E.Coli***	***Bacillus subtilis***	***Staphylococcus aureus***	***Psudomonas sp.***	***Clostridium acetobutylinium***
Zone of Inhibition (mm)					
C1	13.5	11	10	6	2
C2	22	18	15	10	10
C3	3	8.25	-	-	-
C4	17	23	8	6	12

Table-2: Showing Antibacterial activity of Cosmetics against selected microorganisms

Cosmetics	***E.Coli***	***Bacillus subtilis***	***Staphylococcus aureus***	***Psudomonas sp.***	***Clostridium acetobutylinium***
Minimum inhibitory concentration (µl)					
C1	6	8	8	16	8
C2	32	16	16	8	16
C3	2	4	-	-	-

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C4	8	8	16	8	4

Table-3: Showing minimum inhibitory concentration (MIC)

Deodrant	***E.Coli***	***Bacillus subtilis***	***Staphylococcus aureus***	***Psudomonas sp.***	***Clostridium acetobutylinium***
Zone of Inhibition (mm)					
D1	12	17	25	15	12
D2	12	15	10	15	10
D3	20	10	5	10	8

Table-4: Showing Antibacterial activity of Deodrants against selected microorganisms

Deodorant	**Zero Hour**	**After 2 hours**	**After 4 hrs**
No. of colony forming units per ml (cfu/ml)			
D1	160	84	10
D2	212	80	20
D3	100	40	13

Table-5: Showing Bacteriostatic effect of Deodrants against Skin flora

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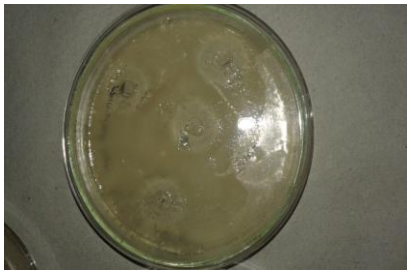

Fig-1: Effect of C1 on *E.coli*Fig-2: Effect of C4 on E.coli

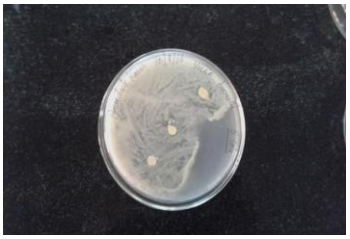

Fig-3: Effect of C3 on E.coli Fig-4:Effect of C2 on E.coli

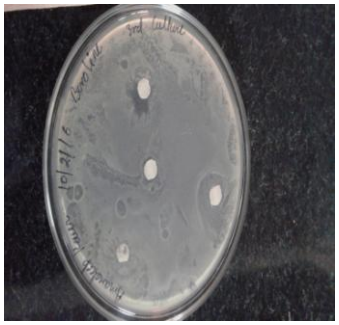

Fig-5: Effect of C1 on *Bacillus subtilis*Fig-6: Effect of C2 on *Bacillus.S*

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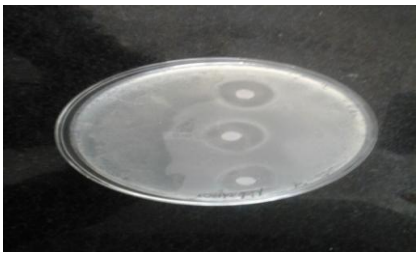
Fig-7: Effect of C3 on *Bacillus subtilis*Fig-8: Effect of C4 on *Bacillus.S*



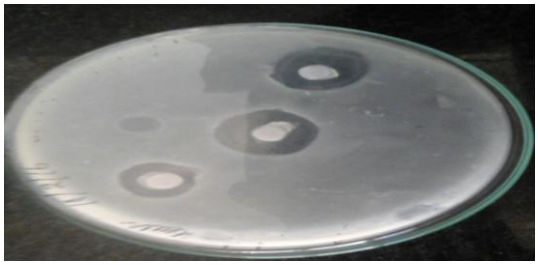


Fig-9 **Fig-10**

Fig-9 Zone of inhibition of C1 on *Staphylococcus aureus*

Fig-10 Zone of inhibition of C2 on *Staphylococcus aureus*

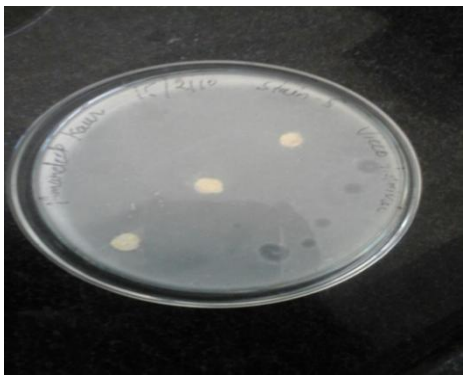


Fig-11 **Fig-12**

Fig-11 Zone of inhibition of C3 on *Staphylococcus aureus*

Fig-12 Zone of inhibition of C4 on *Staphylococcus aureus*

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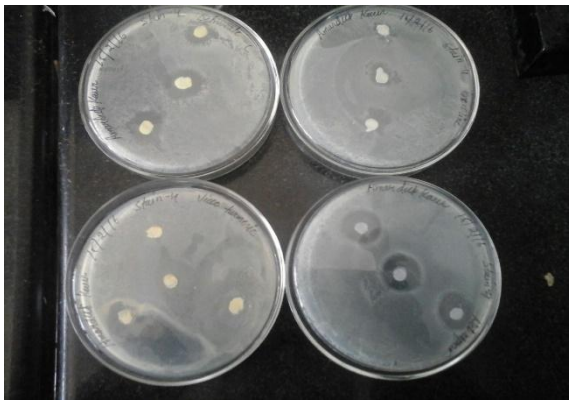

Fig-13 Showing Zone of inhibition of cosmetics against *Pseudomonas Sp*.

Fig-14 Showing Zone of inhibition of Cosmetics against *Clostridium acetobutylium*

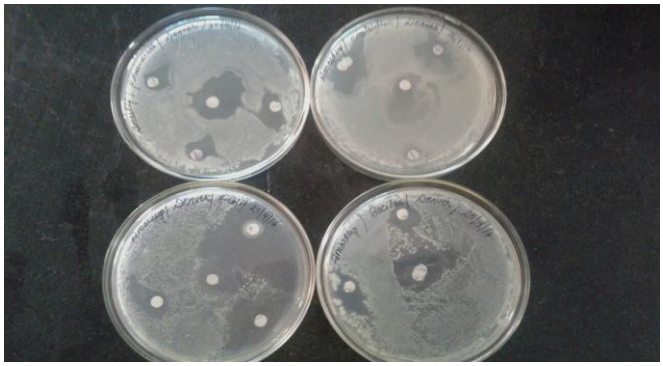

Fig-15: Antimicrobial activity of D1 against selected microorganisms.

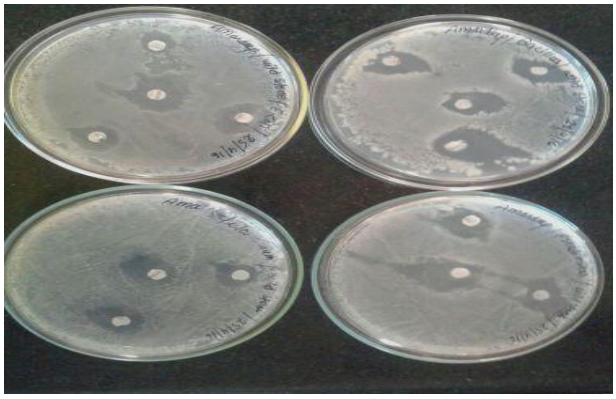

Fig-16: Antimicrobial activity of D2 against selected microorganisms.

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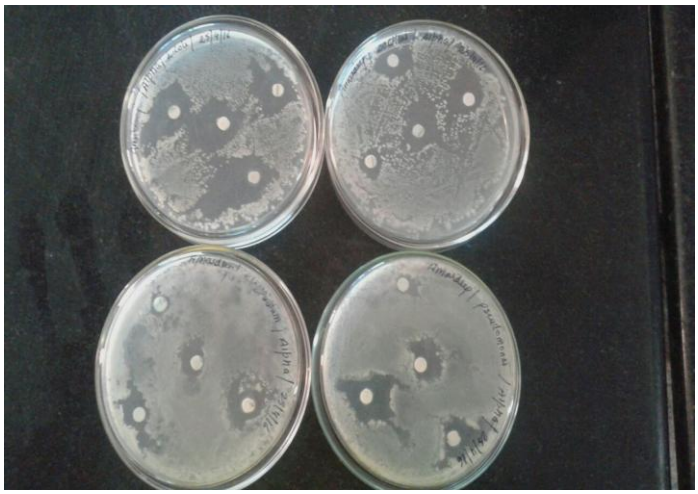

Fig-17: Antimicrobial activity of D3 against selected microorganisms.

Conclusion

In conclusion, it can be stated that Deodorants are effective against bacterial flora which is involving in breakdown of various components of sweat particularly during adult stage of life when various skin glands like apocrine glands bacteriomes active and forms sweat with bad odour. Therefore, it is recommended to use the deodorants. However, a minor drawback can be the inhibition of growth of beneficial bacteria as well. Similarly, various cosmetics are being used by various people as a measure to prevent the growth of microorganism or of having bactericidal activity. Still various medical cosmetics have found to be ineffective against a wide range of bacteria. Therefore, cosmetic to be used should be effective against harmful skin flora.

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