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ANTISEPTIC SOAPS AND BODY CLEANSING AGENTS AND ITS EFFECTS ON THE NORMAL FLORA OF THE HUMAN SKIN

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ABSTRACT

Studies have shown that plain and antiseptic soaps are effective in keeping the skin from microbial contaminants. However, prolong use of antimicrobial soaps has been implicated with potential health challenges. This present research was carried out to determine the effects of some selected antiseptic soaps and cleansing agents on the bacterial flora of the skin based on its potential influence on the total aerobic bacterial flora on five skin sites. Based on treatment exposure, the result showed that subjects exposed to antiseptic soaps alone had mean aerobic bacterial count ranging from $137\pm40.0Cfu/cm^2$ to $461\pm75.51Cfu/cm^2$. Furthermore, it was observed that individuals who were exposed to antiseptic soaps + cleansing agents had mean aerobic counts ranging from $90\pm50.83Cfu/cm^2$ to $307\pm83.93Cfu/cm^2$, while the mean aerobic bacterial count from the control subjects ranged from $282\pm83.93Cfu/cm^2$ to $834\pm118.82Cfu/cm^2$. ANOVA result revealed that there was significant differences ($p<0.05$) in the mean aerobic bacterial count across the treatment groups. Altogether 120 bacterial isolates were characterized from 180 subjects. Among them, 80 isolates (66.7%) were Gram positive while 40 isolates (33.3%) were Gram negative bacteria. Coagulase negative Staphylococci (35%) was the most dominant skin bacterial flora, followed by Coagulase positive Staphylococci (20%), *Escherichia coli* (16.7%), *Bacillus* spp (11.7%), *Proteus* spp (6.7%), *Klebsiella* (5.8%) and *Pseudomonas* spp (4.2%). This study reveals that antiseptic products should be carefully used because its over- utilization may reduce the resident skin flora, thereby creating a void for opportunistic organisms which could lead to several attendant skin and detrimental health effects.

KEYWORDS: Antiseptic soaps, Cleansing agents, Normal flora, Bacteria, Skin, Triclosan.

INTRODUCTION

Normal flora can be described as microorganisms frequently found in various sites of the body in healthy individuals.[1] The forms, composition and numbers of normal flora vary in various areas of the body and sometimes factors such as physiological states and age affects their distribution. They are composed of microorganisms whose genetic, physiologic, and morphologic properties enable their colonization and multiplication in a particular anatomical site under certain favorable conditions aimed at preventing invading pathogens. It is estimated that the number of microflora exceeds the number of body cells by a factor of about 10.[2]

The human skin which is a vital organ is largely colonized by diverse groups of microbial flora and essentially functions as a physical barrier, priming of the immune system as well as preventing entry of unwanted pathogens or their toxins.[3,4] As an ecosystem, the skin is composed of about 1.8 m^2 of various habitats with large quantity of folds, specialized niches and invaginations that provide support for several groups of microorganisms.[3] Some of the normal microflora of the skin includes *Staphylococcus epidermidis*, *Staphylococcus aureus, Micrococcus luteus*, *Candida albicans*, *Mallasseiza furfur*, *Mycobacterium spp*, *Acinetobacter spp* etc.[5,6] Although, the skin microflora serves as defense, they may become opportunistic pathogens when the skin architecture is breached, allowing their entrance into the bloodstream which may lead to life-threatening diseases.[7]

Chemically, soaps are compounds resulting from the interaction of fatty acids, oils and salt made through saponification process and for centuries, humans have exploited soaps and cleansing agents for various purposes such as hand washing, bathing, washing of utensils, and as facial cleansers etc.[8] In addition, soaps and cleansing agents have been used in healthcare

settings and has shown efficacy in limiting the presence and spread of germs.[9] According to their constituents, soaps are classified either as non-antimicrobial soaps or antimicrobial soaps, otherwise referred to as antiseptic or medicated soaps.[9,10]

According to Ihuma *et al*., (2013) antiseptic soaps and cleansing agents contain chemical components capable of killing (cidal effect) or inhibiting (static effect) the growth of bacteria and other microbes.[4] Furthermore, antiseptic soaps have been reported to be capable of removing about 65% to 85% of microbes from the human skin.[11]

It is well known that soaps and cleansing agents play important roles in promoting skin hygiene. Interestingly, in spite its usefulness, overutilization of antimicrobial soaps and cleansing agents for skin care may not only reduce the microbial community of the skin but could also predispose the skin to colonization by transient pathogenic organisms.[11,12] Therefore, this present research aims at determining the effect of some selected antiseptic soaps and cleansing agents on the normal flora of the human skin.

SAMPLING AREA

MATERIALS AND METHODS

This study was conducted in two Universities campuses, viz, Cross River University of Technology (CRUTECH), and University of Calabar (UNICAL). Both universities were selected for the study because of easy accessibility of volunteers and demographic representation of the study population.

INCLUSION AND EXCLUSION CRITERIA
All participants in the present study were included based on possession of a healthy skin. Those with evidence of skin injury, infections or diseases were excluded from the study. The control group comprised of individuals with no recent history of topical use of antiseptic soaps or cleansing agents. Informed consent was obtained from all eligible individuals.

STUDY SUBJECTS AND EXPERIMENTAL DESIGN
One hundred and eighty (180) human subjects (males and females between the ages of 18 to 59 years) were enrolled in the present study. Each university (CRUTECH and UNICAL) where subjects were enrolled in the study had 90 volunteers. The soaps and cleansing agents used in the present study were purchased from standard cosmetic shops and pharmaceutical outlets in Calabar, with their expiry dates ascertained. The products codes and their active ingredients are presented in (Table 1). Participants from CRUTECH were shared into three categories based on treatment exposure (i.e. experimental or control). Subjects in the experimental groups were divided into two; those exposed to topical use of antiseptic soaps provided (30 participants), and those exposed to topical use of antiseptic soaps in combination with cleansing agents provided (30 participants), while subjects in the control group (30 participants) were exposed to soaps and cleansing agents containing no antimicrobial properties. The experimental set up described above was repeated for participants in UNICAL.

The subjects were instructed to use the provided products twice daily (i.e. morning and evening) for five weeks. In addition, to ensure the validity of results, the subjects were advised to use only the products provided when bathing during the study period, and to avoid applying cosmetic products other than the one provided. Subjects were also requested to disuse their assigned products on the day of sampling, until after samples collection as recommended by a previous study.[13]

Table 1: Cosmetic Products Assayed and Their Active Ingredients As Disclosed By Label.

Products Code	Active Ingredients	Category	Expiring Date	Indication
CP_{Sg}	Triclosan 0.20%, Trichrolocarbanilide 0.5%	Soap	11/2023	Antibacterial
CP_{Pr}	None	Soap	04/2022	None
CP_{Sa}	Tetra sodium EDTA	Soap	03/2022	Medicated
CP_{Me}	Triclosan 0.50%	Soap	07/2022	Antibacterial
CP_{Ev}	None	Soap	02/2023	None
CP_{Jt}	None	Soap	11/2012	None
CP_{Dp}	Triclosan, TCC (0.5%)	Soap	07/2023	Antibacterial
CP_{Dt}	Lemon grass oil 0.05% w/w, Chloroxylenol 0.5%	Soap	08/2022	Antibacterial
CP_{Tu}	0.28% triclosan	Soap	09/2023	Germicidal
CP_{Ni}	None	Facial wash	08/2022	None
CP_{Hh}	Neem	Facial wash	11/2022	Antibacterial
CP_{Av}	None	Facial wash	None	None
CP_{Gm}	Neem	Facial wash	None	Germicidal

SAMPLE COLLECTION AND TRANSPORTATION
Skin swab samples were collected from all study participants from various skin sites (face, neck, armpit, forearm and thigh) as described by Wallen-Russell and Chaudhari.[10,13] The sites were gently scrubbed with

sterile swab sticks moistened in normal saline and labeled properly. Collected samples were carefully placed in sterile bags and transported within 30 minutes of collection to a Microbiology laboratory, for bacteriological evaluation.

SAMPLES PREPARATION AND INOCULATION

Swap samples were inserted into sterile test tubes containing nutrient broth and incubated at 37°C for about 24 hours. After incubation, dilution of 1: 10, 1: 100 and 1: 1000 were made from the broth, and about 0.5 ml aliquot of an appropriate dilution was platted in triplicate on freshly prepared Nutrient agar, MacConkey agar and Mannitol salt agar plates using spread plate technique. Bacterial growth was observed after 18-24 hours of incubation at 37°C and total aerobic counts on nutrient agar plates were expressed as colony forming unit per square centimeter (cfu/cm^2) of skin surface.

ISOLATION AND CHARACTERIZATION OF ISOLATES

The colonial morphology of colonies formed were noted; distinct and identical colonies were purified by sub-culturing in fresh medium using streaking technique, and incubated at 37°C for 24 hours. The pure cultures obtained were examined macroscopically and microscopically and characterized accordingly. The bacterial isolates were further subsequently identified by means of biochemical tests as contained in standard laboratory identification protocols.[14]

Gram Staining

A loopful of distilled water was placed on a grease-free slide, and a smear of the test isolate prepared. It was then allowed to air dry and heat fixed. The smear was flooded with crystal violet and allowed for 60 seconds and washed off with water. Lugol's iodine was applied and allowed for 60 seconds before being rinsed off. The smear was decolourized with few drops of acetone-alcohol for 3 seconds and was immediately rinsed with water. Safranin was applied to the smear as counter stain and allowed for 30 seconds before rinsing off and blot drying the slide. The preparation was then examined under a microscope using x100 oil immersion objective.

Catalase Test

Two drops of 3% freshly prepared hydrogen peroxide solution was placed on a grease-free slide, and a 24 hours test isolate was transferred onto the slide and observed immediately for gas bubbles.

Coagulase Test

A colony of the test bacterial was emulsified in a drop of normal saline on a glass slide. A drop of plasma was placed on the bacterial suspension and rocked to observe for agglutination.

Motility Test

Semi-solid nutrient agar was used. With the aid of a sterile inoculating needle, the medium was inoculated with the test isolate by making a fine stab to a depth of about 1-2 cm short of the bottom of the tube. The tubes were then incubated at 37°C for 24 hours.

Methyl- red Test

Methyl red-Voges proskaure (MR-VP) broth was inoculated with the test isolate and incubated at 37°C for 24 hours. About 5 drops of methyl-red reagent was added to the broth culture and observed for colour change.

Vougues-proskeaur Test

MR-VP broth was inoculated with test isolate and incubated at 37°C for 24 hours. 3 ml of 5% α- naphthol was added, followed by 0.5 ml of KOH. The tube was shaken gently and remained undisturbed for 5 minutes and observed for a red surface layer within 2-5 minutes.

Oxidase Test

2 to 3 drops of oxidase reagent was added to a piece of filter paper in a Petri dish. The filter paper was allowed to absorb the reagent, and a sterile wooden applicator stick was used to pick a test isolate for smearing onto the moistened filter paper. A purple colour formation at the region of bacteria smear within 10 seconds was observed for.

Citrate utilization Test

A loopful of the test organism from nutrient agar was inoculated onto Simmons' citrate agar slant in test tubes, and incubated at 37°C for 24 hours. The development of a deep blue colour was observed for.

Indole Test

The test isolate was grown in 5ml sterile peptone water at 37°C for 48 hours. After incubation, 0.5 ml of Kovac„s reagent was added. The broth was observed for the development red colour layer.

Triple sugar iron (TSI) Test

The surface of TSI agar was streaked with the test isolate and the butt was stabbed before being incubated at 37°C for 24 hours. The TSI tubes were observed for the production of gas, acid and hydrogen sulphide.

Sugar fermentation Test

1ml of 10% sugar (maltose, lactose, mannitol, glucose, fructose, sucrose and sorbitol) solution was added to 10ml of the basal medium containing the indicator phenol red and Durham tube. The media were inoculated with test isolates and incubated at 37°C for 2-5days and observed daily for color change. Acid and gas production were observe for.

STATISTICAL ANALYSIS

The data on geometric aerobic bacterial counts in relation to treatment exposure was analyzed using the Statistical Package for Social Sciences (SPSS), v20. Descriptive statistics were applied to find frequencies, percentages and means. When ANOVA indicated a

significant result (P<0.05), the significantly different means were separated using "Duncan post hoc test". Significant level was set at $\alpha < 0.05$.

RESULTS

The study revealed that the aerobic bacterial count recorded varies with the skin sites. Table 2 represents the mean aerobic bacterial counts obtained from study subjects in CRUTECH. The result based on treatment exposure showed subjects treated with AS had mean aerobic bacterial counts ranging from $137 \pm 40.0 \text{Cfu/cm}^2$ to $311 \pm 110.23 \text{Cfu/cm}^2$. It was observed that individuals who received AS+CA treatment had mean aerobic counts ranging from $90 \pm 50.83 \text{Cfu/cm}^2$ to $207 \pm 79.18 \text{Cfu/cm}^2$, while the mean aerobic bacterial counts from control subjects ranged from $292 \pm 75.51 \text{Cfu/cm}^2$ to $806 \pm 118.82 \text{Cfu/cm}^2$ (Table 2). ANOVA result revealed

that there was significant differences (p<0.05) in the mean aerobic bacterial counts across the treatment group.

Result of the mean aerobic bacterial counts recorded from subjects in UNICAL is illustrated in Table 3. The result obtained showed that subjects treated with AS, had mean aerobic bacterial counts ranging from $141 \pm 26.29 \text{Cfu/cm}^2$ to $461 \pm 75.51 \text{Cfu/cm}^2$. Also, individuals who received AS+CA treatment had mean aerobic counts ranging from $94 \pm 50.83 \text{Cfu/cm}^2$ to $307 \pm 83.93 \text{Cfu/cm}^2$. Furthermore, the mean aerobic bacterial counts from control subjects ranged from $301 \pm 110.23 \text{Cfu/cm}^2$ to $834 \pm 118.82 \text{Cfu/cm}^2$ (Table 3). ANOVA result showed that there was significant differences (p<0.05) in the mean aerobic bacterial counts across the treatment group.

Table 2: Comparative Effects of Antibacterial/Non-antibacterial Products on the Geometric Means of Total Aerobic Bacterial Counts (CFU/cm^2) at 10^3 on Different skin sites from Subjects in CRUTECH.

Skin Site	AS		Treatment Exposure AS+CA		N(AS+CA)	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
Face	259±73.37[b]	231 ± 75.48[b]	169 ± 65.5[bc]	154 ± 26.29[bc]	501 ± 173.77[b]	462 ± 173.77[b]
Neck	278±108.3[b]	253 ± 83.93[bc]	192 ± 59.02[c]	181 ± 78.46[cd]	545 ± 166.53[b]	576 ± 173.77[c]
Armpit	302±119.34[b]	311 ± 110.23[c]	201 ±59.02[c]	207 ± 79.18[d]	806 ± 118.82[c]	789 ± 118.82[d]
Forearm	137±40.0[a]	154 ± 48.39[a]	90 ± 50.83[a]	102 ± 40.27[a]	292 ± 75.51[a]	309 ± 119.34[a]
Thigh	195±62.59[a]	273 ± 84.81[bc]	129 ± 37.8[ab]	115 ± 58.63[ab]	379 ± 75.51[a]	349 ± 75.51[a]

*Data are expressed as mean of three replicate ± SD.
*Different superscript letters in the same column indicate significant difference (p<0.05).
*Values with same superscript letter on the same column are not significantly different at 5% level of significance.

KEY

AS = Antiseptic soap alone; AS+CA = Antiseptic soap + cleansing agent; N(AS+CA) = Non (antiseptic soap + cleansing agent) (Control).

Table 3: Comparative Effects of Antibacterial/Non-antibacterial Products on the Geometric Means of Total Aerobic Bacterial Counts (CFU/cm^2) at 10^3 on Different skin sites from Subjects in UNICAL.

Skin site	AS		Treatment Exposure AS+CA		N(AS+CA)	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
Face	224±72.85[ab]	198±57.51[b]	149±37.81[b]	132±37.80[a]	417±75.51[b]	396±110.23[b]
Neck	299±80.80[b]	318±110.23[c]	197±48.95[c]	212±59.02[b]	455±173.77[b]	436±173.77[bc]
Armpit	446±173.78[c]	461±75.51[d]	291±65.52[d]	307±83.93[c]	834±118.82[c]	821±118.82[d]
Forearm	304±110.23[b]	297±84.81[c]	213±75.48[c]	198±59.02[b]	480±75.51[b]	494±75.51[c]
Thigh	154± 48.95[a]	141±26.29[a]	102±50.83[a]	94±50.83[a]	301±110.23[a]	282±83.93[a]

*Data are expressed as mean of three replicate ± SD.
*Different superscript letters in the same column indicate significant difference (p<0.05).
*Values with same superscript letter on the same column are not significantly different at 5% level of significance.

KEY

AS = Antiseptic soap alone; AS+CA = Antiseptic soap + cleansing agent; N(AS+CA) = Non (antiseptic soap + cleansing agent).

Total Frequencies and Percentage Occurrence of the Isolates per study location

Figure 1 presents the results of frequency and percentage occurrences of bacteria isolates from the study. The result showed that Coagulase negative Staphylococci had the highest percentage of occurrence (35%) compared to

other bacterial isolates while the least isolated species was *Pseudomonas* spp (4.2%).
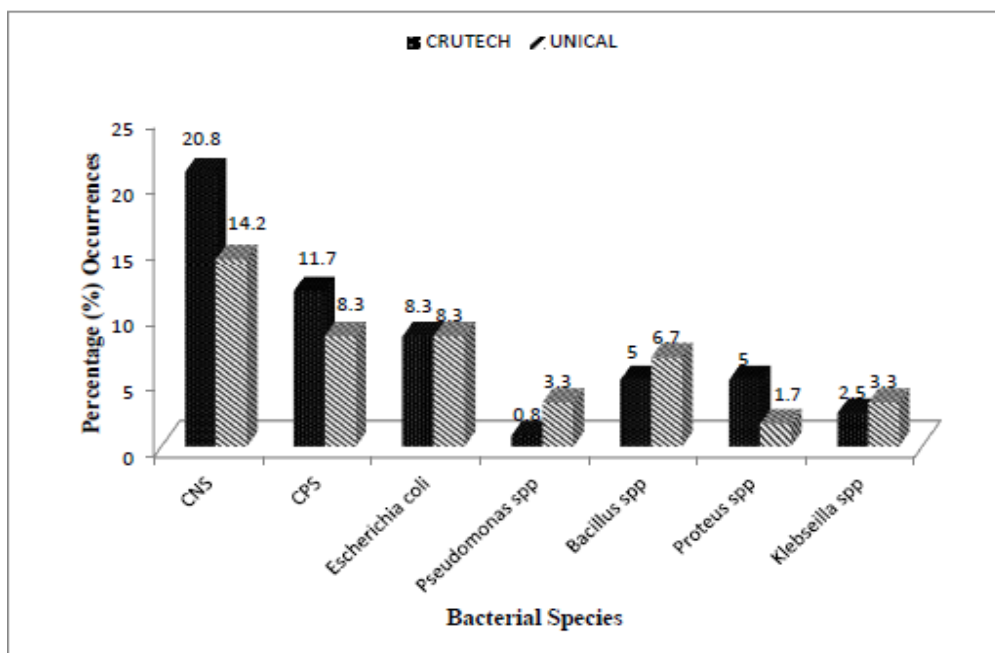
Figure 1: Prevalence of bacterial species isolated from the study in relation to location.

KEY
CNS = Coagulase negative Staphylococci; CPS = Coagulase positive Staphylococci

Effects of Treatment Exposure on Bacterial Diversity of Human skin

Table 4 showed the effects of treatment exposure on bacterial diversity on the skin of volunteer subjects. The result revealed that 26.7%, 23.3% and 50% of the isolates were recovered from subjects in treatment group AS, AS+CA and N(AS+CA) respectively.

Table 4: Effects of Antiseptic Soaps and Cleansing agents on Microbial Flora of Human skin.

	AS		AS+CA		N (AS+CA)		
Bacterial species	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Total Frequency/%**
CNS	7 (5.8)	6 (5.0)	5 (4.2)	6 (5.0)	11 (9.2)	7 (5.8)	42 (35)
CPS	4 (3.3)	4 (3.3)	4 (3.3)	3 (2.5)	4 (3.3)	5 (4.2)	24 (20)
Eschericha coli	3 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)	12 (10.0)	5 (4.2)	20 (16.7)
Pseudomonas spp	2 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)	2 (1.7)	5 (4.2)
Bacillus spp	2 (1.7)	3 (2.5)	3 (2.5)	2 (1.7)	2 (1.7)	2 (1.7)	14 (11.7)
Proteus spp	0 (0.0)	1 (0.8)	0 (0.0)	1 (0.8)	4 (3.3)	2 (1.7)	8 (6.6)
Klebsiella	0 (0.0)	0 (0.0)	2 (1.7)	2 (1.7)	2 (1.7)	1 (0.8)	7 (5.8)

KEY

AS = Antiseptic soap alone; AS+CA = Antiseptic soap + cleansing agent; N(AS+CA) = Non (antiseptic soap + cleansing agent); CNS = Coagulase negative Staphylococci; CPS = Coagulase positive Staphylococci

DISCUSSION

Soaps are classified as non-antibacterial or antibacterial based on its active composition. Notwithstanding its formulation, they are effective in removing microbial contaminants from the human skin.[15,16] Consequently, this present research was carried out to determine the effects of some selected antiseptic soaps and cleansing agents on the bacterial flora of the human skin based on its potential influence on the total aerobic bacterial flora on five skin sites.

Result obtained for total aerobic bacterial count, revealed a significant reduction in the sampled sites of the test groups compared to the control group (Table 2 and 3). This finding correlates with a previous study by Voss.[17]

who reported that antibacterial soaps containing 0.5% trifluoromethyldichlorocarbanilide and 1.0% trichlorocarbanilide produced significant reductions effects in geometric mean counts of the total bacterial aerobic flora on various body sites compared with soaps without antimicrobial formulation. Similarly, Montville and Schaffner.[18] revealed in their study that antiseptic soaps resulted in around 0.5 log reductions in bacterial counts than non- antibacterial soaps. The consequence of this reduction could lead to colonization of human skin by undesirable microbial entities.

One hundred and twenty (120) distinct colonies representing seven genera of bacteria were observed and characterized from the study. The results revealed that

56.7% were isolated from males subjects whereas 43.3% from females subjects. This result revealed higher isolates from male than female subjects. However, the difference was not significant. This finding is in consonance with the report from a similar study by Ying *et al*.[19] that The slightly lower isolates number in female may be as a result of the application of some skin cosmetics which are antibacterial. Other factors such as skin pH, hormone metabolism, and perspiration rate, can also account for gender wise differences of bacterial flora.[20]

The bacterial isolates identified were Coagulase negative Staphylococci 42(35%), Coagulase positive Staphylococci 24(20%), *Escherichia coli* 20(16.7%), *Pseudomonas* spp 5(4.2%), *Bacillus* spp 14(11.7%), *Proteus* spp 8(6.7%) and *Klebsiella* spp 7(5.8%) (Figure 1). Most bacteria isolated in this study have previously been reported by researchers.[12,17,20] Undoubtedly, the normal gram-positive flora of the skin serves a beneficial function in inhibiting the growth of other less desirable organisms.[21] However, there are some indications that the effect of the antibacterial soap on the prevalence of Gram positive organisms may favour the establishment of unwanted Gram negative flora over a period of time. This study indicates that the regular use of antiseptic soaps and cleansing agents reduces gram-positive flora but does not cause overgrowth of gram-negative species. In unusual cases when the normal gram-positive flora has been sharply depressed by intensive use of antibacterial or antibiotics, this is followed by the overgrowth of gram-negative bacteria.[22] Triclosan, an active constituent in antimicrobial products have been reported to have antibacterial activity against different microbial groups. Triclosan use however, remains a matter of controversy due to several reported adverse effects associated with triclosan formulated products. Such effects includes allergies, antibiotic resistance, endocrine disruption, acute/chronic toxicity, bioaccumulation, including being potentially carcinogenic.[16,23,24,25] According to US FDA, the maximum triclosan concentration permissible by law is 0.3%.[16] Also, in Canada, Australia and Europe, 0.3% concentration level is legally permissible. Whereas in Japan, the maximum concentration permitted is 0.1%. Although, the cosmetic products employed in this study pose no health related challenges on the study subjects, some of the antimicrobial products employed in the study had triclosan concentration above the acceptable level by regulatory authorities.[26] This support the view that the use of cosmetics products with triclosan concentration level >0.3% may pose both skin and health related challenges and should therefore be discouraged.[24, 25]

CONCLUSION

The skin is an important organ of the body that serves for protection against infections by germs and shields delicate underlying tissues against injury. The forms, composition and numbers of normal flora vary in various areas of the body and sometimes factors such as physiological states and age affects their distribution. The results obtained from this study suggest that, the use of antiseptic products should be minimized for non-medical reasons because, over-utilization may reduce the resident micro flora thereby giving way to transient micro flora, which may grow opportunistically above the normal threshold level, creating several attendance skin and detrimental health effects.

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