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Antimicrobial Activities of Some Commercial Cosmetics on Selected Cutaneous Microflora

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Authors' contributions

This work was carried out in collaboration between all authors. Authors TVA and DJA designed the study. Author DJA supervised the research. Author TVA performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors TVA and TCE managed the analyses of the study. Author TVA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The antimicrobial activities of twenty-two cosmetics on selected cutaneous microflora were investigated. The microorganisms isolated from the human skin were *Staphylococcus epidermidis, Staph. aureus, Micrococcus luteus, Bacillus subtilis, Proteus mirabilis, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger* and *A. fumigatus.* It was observed that those cosmetics that did not inhibit some specific microorganisms at 100 mg/ml did not also inhibit the microorganisms at 400 mg/ml. Ten (45.45%) of the cosmetics had antimicrobial effect on *Staph. epidermidis,* nine (40.91%) of the cosmetics had antimicrobial effect on *Staph. aureus,* six (27.27%) of the cosmetics had antimicrobial effect on *Micrococcus luteus,* four (18.18%) of the cosmetics had antimicrobial effect on *Bacillus subtilis,* only one (4.55%) of the cosmetics had antimicrobial effect on *Proteus mirabilis.* Also five (22.73%) each of the cosmetics had antimicrobial effect on *Pseudomonas aeruginosa* and *Candida albicans,* none of the cosmetics was able to inhibit *A. niger* and *A. funmigatus.* Most of the cosmetics employed in the course of the research could cause diseases in immune competent patient.

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1. INTRODUCTION

A cosmetic product is defined in European Union (EU) law as any substance or preparation intended to be placed in contact with the various external parts of the human body or with the teeth and the mucous membranes of the oral cavity, with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, and/or correcting body odours, and/or protecting them or keeping them in good condition [1].

Humans have a basic need to change their appearance. The vastness of today's cosmetics and toiletries industry clearly indicated this widespread and basic need for cosmetics [2]. Perhaps this need arises because cosmetics allow us to make ourselves unique for rituals or societal reasons [2]. Human beings apparently have a primal need for cosmetics to provide for our well-being and serve as cures for the disease of being someone we prefer not to be [2]. It is most important that majority of the present day topical products pose serious threat to the normal flora of skin and ultimately to human health. In spite of this, the demand for cosmetics is increasing worldwide, particularly among the youth [3].

Fransway [4] defined the perfect preservative as a colourless, odourless, water soluble, nontoxic, non allergenic, non irritating chemical capable of inhibiting the growth of a broad spectrum of bacteria and fungi. So far no preservative fulfils all these demands [5]. Cosmetic preservatives are molecules that are toxic for the consumer as well as potential sources of allergies and skin disorders [6]. Virtually all cosmetic preservatives, including disinfectants, are effective against both prokaryotic and eukaryotic cells, as, unlike antibiotics, they do not act against a defined target cell [6]. When the skin conditions are changed by the use of topical applications such as creams, powders, lotions, sprays and other cosmetics, the chemicals that are present in the preservatives may alter the population of skin biota [7]. However, the function of preservative is to maintain the shelf life of a product, they may be absorbed into the inner parts of skin microbiota or sometimes even into the blood without undergoing contamination [7]. This may induce variations on the superficial part of the skin resulting in decrease in the number of microbes [7]. When the effect of a chemical is neutralized or weakened, then again there will be an increase in the microbial number. The regeneration of colonies may be either from native microbes or from foreign source. This change in colonization with different microbes will also change the composition of normal microbiota, thus leading to the diseases in immune competent cases [7].

In cosmetics microbiology, the use of preservatives could reproduce the experience of clinical microbiology after the emergence of antibiotics [6]. The addition of higher concentrations of preservatives to products (always according to regulations) in order to avoid this kind of contamination could solve the problem in some instances, but this approach is not practical since it could generate toxicity for the consumer. Preservatives should never be used to mask poor manufacturing practices [6]. Preservatives must be used at the lowest concentration that ensures their efficacy and this must be determined during the product development process [8].

The objective of this study was to examine the antimicrobial activities of some commercial cosmetics on selected cutaneous microfora.

2. MATERIALS AND METHODS

2.1 Samples Collection and Storage

Twenty two different brands of cosmetics were randomly purchased from shops and drug stores at Oja oba within Akure metropolis. The cosmetics include five lotions, two Vaseline, eight creams, five powder and two natural cosmetics. All the samples collected were stored in the refrigerator at 4℃ in the microbiology laboratory of The Federal University of Technology, Akure, Nigeria. Prior to storage the samples were inspected for any physical defects and organoleptic characteristics. The container label information such as batch number, expiry date, manufacturing date, directions for use and composition, which should be disclosed as per the Good Manufacturing Practice Certification (GMPC), were recorded [9].

2.2 Collection of Swabs and Isolation of Normal of the Skin

Isolation of normal flora of the human skin was done as described by Ikpoh et al. [10] with a

slight modification. Sterile swab sticks were purchased from Pharmaceutical Stores in Akure. Swab samples were collected from 100 Microbiology students, at FUTA age range from 18 to 35 years. One swab stick was used for each student to collect samples. The sterile swabbed sticks were damped with sterile peptone water before the samples were collected from the hand, armpit, face and legs. The area of skin to be swab was first swabbed with methylated spirit so as to remove some of the transcient microflora. The samples collected with swab sticks were then used to inoculate already poured Nutrient agar, potato dextrose agar, Mannitol salt agar, 5% sheep blood agar and MacConkey agar (Oxoid, UK). The culture plates were then incubated in an inverted position to prevent condensed moisture from dripping into the media or bacteria colony and fungal colony. The plates were incubated at 37℃ for 24 hours and 27℃ for 48 hours for bacteria and fungi respectively. After 48 hrs, plates with no growth were observed for another 72 hrs to observe if there will be growth. Subculturing of the isolates was done on nutrient agar, blood agar and Sabouraud dextrose agar media. Bacterial isolates were maintained on agar slant at 37℃ for 48 hours while the fungal isolated were maintained and stored on Sabouraud dextrose agar slants in the refrigerator.

2.3 Identification of Microorganisms Isolated from Human Skin

2.3.1 Identification of bacteria isolates

Parameters used in differentiating each isolate include colonial characteristics (edges, texture, elevation, colour, pigmentation, and size etc, cell morphology (Shape, arrangement and Gram reaction). The bacteria were further analyzed by conventional biochemical test.

2.3.2 Identification of fungi isolates

Fungal isolates were characterized and identified based on macroscopic and microscopic details with reference to Barnett and Hunter [11].

2.3.3 Standardization of test microorganisms

2.3.3.1Standardization of test bacteria

A loopful of the bacterial culture was aseptically inoculated onto freshly prepared sterile nutrient broth and incubated for 24 hours. Zero-point-two millimetre was pipetted from the 24 hours broth culture of the test organism, dispensed into 20 ml sterile nutrient broth and incubated for another 4 hours. This was to standardise the culture to 0.5 McFarland's standard (10^6 cfu/ml) before use as described by Oyeleke et al. [12].

2.3.4 Standardization of test fungi

A loopful of the fungal culture was aseptically inoculated into freshly prepared sterile sabouraud dextrose agar plate and incubated for 48 hours at 28±2℃. A loopful of the fungal culture was suspended in saline solution (0.85% NaCl) and adjusted to match a turbidity of 10^6 Cfu/ml.

2.3.5 Antimicrobial activities of cosmetics

2.3.5.1 Reconstitution of cosmetic

One gram and four gram of each of the cosmetic were dissolved in 10 ml of tween 20 respectively and each of these concentrations was subjected to antimicrobial activity test. Tween 20 was used to enhance the solubility of the tested cosmetics.

2.3.6 Determination of preservative capacity by cup plate technique

Antimicrobial susceptibility test was carried out according to the method of Mwambete and Simon [13]. Two concentrations (100 mg/ml and 400 mg/ml) of each sample were subjected to antimicrobial efficacy testing against some selected normal flora of the skin using Mueller-Hinton and Sabouraud's dextrose agar-plates for bacterial and fungal isolates respectively. Each of these microorganisms was separately inoculated onto the agar plates and left for 15 minutes before being cup-plated with each of the cosmetic concentrations. Observation and determination of zones of inhibition (ZI) were preceded with an aerobic overnight incubation at 37℃ for 24 hrs and at 28±2℃ for 48 hrs for bacteria and fungi respectively.

2.3.7 Minimum inhibitory concentration (MIC)

The Minimum inhibitory concentration test was carried out using tube dilution method using Mueller Hinton broth. The tube dilution susceptibility test was used to determine the MIC values for the cosmetics samples. A series of Mueller-Hinton broth tubes containing varying two-fold concentrations of the various cosmetics samples in the range of 100 mg/ml to 6.25 mg/ml was prepared and incubated with a previously

standardized density of the test organisms (0.5 ml). The lowest concentration of the cosmetics sample resulting in no growth after 18-24 hrs of incubation for bacteria and 24-72 hrs for yeasts and moulds using spectrophotometer was recorded as the MIC.

2.3.8 Statistical analysis of data obtained

Data obtained were subjected to one way analysis of variance, while the means were compared by Duncan's New Multiple Range Test at 95% confidence interval using Statistical Package for Social Sciences version 16.0. Differences were considered significant at $p \leq 0.05$.

3. RESULTS

Table 1: Seven parameters of cosmetic samples were considered which include, manufacturing date, expiry date, NAFDAC number, batch number, seal lining, and type of closure or container of each of the cosmetics. Fifteen (75%) of the specific synthetic cosmetic products disclosed the date of manufacture and also Fifteen (75%) indicated the expiry dates of their products out of twenty synthetic cosmetics used in the course of the study. Seventeen (85%) out of the twenty manufacturers gave indications of inclusion of preservative(s) but not the type of preservative used and none of the manufacturers disclosed the type of preservative(s) used. Three manufacturers (15%) did not even state whether a preservative was included at all. Eight manufacturers (40%) gave the batch numbers of the products, with regard to seal lining only four (20%) of the cosmetics had seal lining and those that contain seal linings were creams. All the synthetic cosmetics used in this study showed the composition of the product on the container label.

Table 2: The antibacterial activities of some cosmetics at 100 mg/ml against normal bacteria flora of the skin are shown in Table 2. The antibacterial activities of some cosmetics at 100 mg/ml against *Staphylococcus epidermidis, Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis, Proteus mirabilis* and *Pseudomonas aeruginosa* are reported in the Table 2. Some of the specific cosmetics had antimicrobial activities on some specific normal flora of the skin.

Table 1. Container label disclosures on the cosmetics employed in course of the study

Sample	Manufacturing date	Expiry date	NAFDAC no	Preservative		Batch no	Seal lining	Type of closure/ container
				Any	Type			
S1	+	+	+	+	-	+	-	Flip cap
S2	+	+	+	+	-	+	-	Pump top
S3	+	+	+	+	-	-	-	Flip cap
S4	+	+	+	+	-	+	-	Open Screw cap
S5	-	-	-	+	-	-	-	Flip cap
S6	-	-	+	-	-	-	-	Cup
S7	+	+	+	-	-	-	-	Cup
S8	+	+	+	+	-	+	-	Cup
S9	+	+	+	+	-	+	+	Cup
S10	+	+	+	+	-	+	+	Cup
S11	+	+	-	+	-	-	+	Cup
S12	+	+	+	+	-	-	-	Cup
S13	+	+	+	+	-	+	+	Cup
S14	+	+	-	+	-	-	-	Cup
S15	+	+	+	+	-	-	-	Cup
S16	+	+	+	+	-	-	-	Flip cap
S17	+	+	+	+	-	+	-	Dispenser
S18	-	-	-	-	-	-	-	Dispenser
S19	-	-	-	+	-	-	-	Cup
S20	-	-	-	+	-	-	-	Flat

Key: + Implies label disclosure provided, - Implies label disclosure not provided, S1-S20= Cosmetics sample 1 to 20

Table 2. Antibacterial activities (mm) of some cosmetics at 100 mg/ml against bacteria normal flora of the skin

Samples	*S. epidermidis*	*S. aureus*	*M. luteus*	*B. subtilis*	*P. mirabilis*	*P. aeruginosa*
S1	0.00±0.00^{a}	9.33±0.58^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S2	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S3	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	7.67±1.15^{b}	0.00±0.00^{a}	3.67±0.58^{b}
S4	0.00±0.00^{a}	12.67±1.15^{e}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S5	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S6	0.00±0.00^{a}	11.67±0.58^{c}	0.00±0.00^{a}	0.00±0.00^{a}	10.00±0.00^{b}	0.00±0.00^{a}
S7	13.00±1.00^{e}	0.00±0.00^{a}	8.67±0.58^{b}	8.67±0.58^{c}	0.00±0.00^{a}	13.33±0.58^{d}
S8	17.00±0.00^{h}	13.67±0.58^{f}	14.33±1.52^{c}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S9	12.00±0.00^{d}	12.33±0.58^{de}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S10	15.67±0.58^{g}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S11	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S12	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	7.33±0.58^{b}	0.00±0.00^{a}	3.67±0.58^{b}
S13	16.00±0.00^{g}	9.00±0.00^{b}	13.67±0.58^{c}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S14	15.67±0.58^{g}	0.00±0.00^{a}	15.67±0.58^{d}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S15	13.67±0.58^{f}	16.67±0.58^{g}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	8.00±1.00^{c}
S16	0.00±0.00^{a}	12.00±0.00^{cd}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S17	0.00±0.00^{a}	0.00±0.00^{a}	15.33±0.58^{d}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
518	0.00±0.00^{a}	13.67±0.58^{f}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	17.67±0.58^{f}
S19	8.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S20	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S21	7.67±0.58^{b}	0.00±0.00^{a}	0.00±0.00^{a}	11.67±0.00^{d}	0.00±0.00^{a}	0.00±0.00^{a}
S22	8.67±0.58^{c}	0.00±0.00^{a}	9.00±0.58^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
C	24.33±0.58^{i}	14.00±0.00^{f}	23.67±0.58^{e}	14.00±0.00^{e}	19.67±1.15^{c}	17.00±0.00^{e}

Data are presented as Mean±S.D (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05). Key: S1-S22= Cosmetics sample 1to 22, C= Cloramphenicol

Table 3. Antibacterial activities (mm) of some cosmetics at 400 mg/ml against bacteria normal flora of the skin

Samples	*S. epidermidis*	*S. aureus*	*M. luteus*	*B. subtilis*	*P. mirabilis*	*P. aeruginosa*
S1	0.00±0.00^{a}	10.33±0.58^{c}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S2	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S3	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	8.33±0.58^{b}	0.00±0.00^{a}	4.67±0.58^{b}
S4	0.00±0.00^{a}	13.33±0.58^{d}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S5	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S6	0.00±0.00^{a}	13.00±1.00^{d}	0.00±0.00^{a}	0.00±0.00^{a}	11.00±1.00^{b}	0.00±0.00^{a}
S7	13.67±0.58^{d}	0.00±0.00^{a}	9.57±0.58^{b}	9.67±0.58^{c}	0.00±0.00^{a}	14.33±0.58^{d}
S8	18.67±1.53^{h}	14.67±0.58^{f}	15.33±0.58^{e}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S9	13.67±0.58^{d}	13.67±0.58^{de}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S10	15.67±0.58^{ef}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S11	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S12	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	8.67±0.58^{b}	0.00±0.00^{a}	4.33±0.58^{b}
S13	17.00±1.00^{g}	9.67±0.58^{b}	14.33±0.58^{d}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S14	16.33±0.58^{fg}	0.00±0.00^{a}	16.33±0.58^{f}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S15	15.00±1.00^{e}	17.67±0.58^{h}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	8.67±0.58^{c}
S16	0.00±0.00^{a}	13.00±0.00^{d}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S17	0.00±0.00^{a}	0.00±0.00^{a}	16.33±0.58^{f}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
518	0.00±0.00^{a}	15.33±0.58^{g}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	18.33±1.15^{f}
S19	8.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S20	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S21	7.67±0.58^{b}	0.00±0.00^{a}	0.00±0.00^{a}	12.67±0.00^{d}	0.00±0.00^{a}	0.00±0.00^{a}
S22	9.00±1.00^{c}	0.00±0.00^{a}	10.33±0.58^{c}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
C	24.33±0.58^{i}	14.00±0.00^{e}	23.67±0.58^{g}	14.00±0.00^{e}	19.67±1.15^{c}	17.00±0.00^{e}

Data are presented as Mean±S.D (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05). Key: S1-S22= Cosmetics sample 1 to 22, C= Cloramphenicol

Table 3: The antibacterial activities of some cosmetics at 400 mg/ml against normal bacteria flora of the skin are shown in Table 3. The antibacterial activities of some cosmetics at 400

mg/ml against *Staphylococcus epidermidis, Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis, Proteus mirabilis* and *Pseudomonas aeruginosa* are reported in the Table 3. Those cosmetics that did not inhibit some specific bacteria at 100 mg/ml does not also inhibit those bacteria at 400 mg/ml.

Table 4: The antifungal activities of some cosmetics at 100 mg/ml on fungal normal flora of the skin are shown in Table 4. The antifungal activities of some cosmetics at 100 mg/ml against *Candida albicans, Aspergillus niger* and *Aspergillus fumigatus* are reported in the Table 4. Some of the specific cosmetics were able to inhibit *Candida albicans*, while none of the specific cosmetics were able to inhibit *Aspergillus niger* and *Aspergillus fumigatus.*

Table 5: The antifungal activities of some cosmetics at 400 mg/ml on fungal normal flora of the skin are shown in Table 5. The antifungal activities of some cosmetics at 400 mg/ml against *Candida albicans, Aspergillus niger* and *Aspergillus fumigatus* are reported in the Table 5. Some of the specific cosmetics had antimicrobial activities on some specific normal flora of the skin. Some of the specific cosmetics were able to inhibit *Candida albicans*, while none of the specific cosmetics were able to inhibit *Aspergillus niger* and *Aspergillus fumigates.*

Table 6 is showing the Minimum inhibitory concentration (mg/ml) of the cosmetics that inhibit some specific normal flora of the skin. The Minimum inhibitory concentration (mg/ml) of the cosmetics range from 12.5 mg/ml to 100 mg/ml.

4. DISCUSSION

Antimicrobial activities of the cosmetics at 100 mg/ml and 400 mg/ml against cutaneous microflora were carried out. It was observed that those cosmetics that did not inhibit some specific microorganisms at 100 mg/ml did not also inhibit the microorganisms at 400 mg/ml. Eighty one-point-eight-one percent of the cosmetic use in the course of the research were able to inhibit one or more of the skin microflora, this could probably due to the fact that many cosmetics are formulated to contain antimicrobial agents or antibiotics which can effectively change the microbial ecology on application to the skin surface as reported by Varghese et al. [3], this correlate with the report of Orus and Leranoz [6] who reported that virtually all cosmetic preservatives, including disinfectants,

Table 4. Antifungal activities (mm) of some cosmetics at 100 mg/ml on fungi normal flora of the skin

Sample	*C. albicans*	*A. niger*	*A. funmigatus*
S1	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S2	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S3	6.67±1.15^{c}	0.00±0.00^{a}	0.00±0.00^{a}
S4	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S5	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S6	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S7	5.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}
S8	7.67±0.58^{d}	0.00±0.00^{a}	0.00±0.00^{a}
S9	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S10	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S11	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S12	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S13	9.67±1.15^{e}	0.00±0.00^{a}	0.00±0.00^{a}
S14	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S15	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S16	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S17	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S18	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S19	6.00±1.00^{c}	0.00±0.00^{a}	0.00±0.00^{a}
S20	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S21	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S22	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
C	15.33±0.58f	19.67±0.58^{b}	17.00±0.00^{b}

Data are presented as Mean±S.D (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05). Key: S1-S22= Cosmetics sample 1to 22, C= Fluconazole

Table 5. Antifungal activities (mm) of some cosmetics at 400 mg/ml on fungi normal flora of the skin

Samples	*C. albicans*	*A. niger*	*A. funmigatus*
S1	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
S2	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
S3	7.33±0.58[b]	0.00±0.00[a]	0.00±0.00[a]
S4	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
S5	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
S6	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
S7	7.33±0.58[b]	0.00±0.00[a]	0.00±0.00[a]
S8	8.67±0.58[c]	0.00±0.00[a]	0.00±0.00[a]
S9	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
S10	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
S11	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
S12	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
S13	10.33±0.58[d]	0.00±0.00[a]	0.00±0.00[a]
S14	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
S15	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
S16	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
S17	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
S18	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
S19	7.67±1.15[b]	0.00±0.00[a]	0.00±0.00[a]
S20	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
S21	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
S22	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
C	15.33±0.58[e]	19.67±0.58[b]	17.00±0.00[b]

Data are presented as Mean±S.D (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05). Key: S1-S22= Cosmetics sample 1to 22, C= Fluconazole

are effective against both prokaryotic and eukaryotic cells, as, unlike antibiotics, they do not act against a defined target cell. When the skin conditions are changed by the use of topical applications such as creams, powders, lotions, sprays and other cosmetics, the chemicals that are present in the preservatives may alter the population of skin biota [7]. Eighty one-point-eight-one percent of the cosmetic use in the course of the research could alter skin microbiota i.e four out of twenty two products (Tables 2 to 5). Stecher and Hardt [14] reported that altered skin microbiota diversity may result in disease, from 'species diversity/ microbial community structure' to 'health outcomes', include inflammation, absence of necessary members of the microbial community, and a decrease in microbial antagonistic interactions.

The use of cosmetic preservative in the preservation of cosmetics to prevent the growth of microorganism in cosmetics might make the cosmetic to have antimicrobial activities on the normal flora of the skin. Some ingredients in cosmetics, such as certain detergents, alcohols, and plant oils can irritate the skin if there is enough of the ingredient in the product. If these ingredients are used in small amounts, they may have no health effects at all and are of little concern. Ingredients with very serious health effects or those that can build up in our bodies or the environment can be a problem even in small amounts. Preservatives must be used at the lowest concentration that ensures their efficacy and this must be determined during the product development process as reported by Detmer et al. [8]. Though the function of preservative is to maintain the shelf life of a product, they may be absorbed into the inner parts of skin microbiota or sometimes even into the blood stream. The regeneration of colonies may be either from native microbes or from foreign source. This change in colonization with different microbes will also change the composition of normal microbiota, thus leading to the diseases in immunocompetent patients [7]. Several types of diseases including scabies, acne, eczema, dyschromia and other skin diseases have been reports upon usage of cosmetic [15]. Cosmetics can pose various short-term hazards, such as flammability (hairspray, deodorant, nail polish remover) or skin irritation (e.g. hair colors). Products contain a wide variety of ingredients, including many different dyes and fragrances. Some ingredients can cause allergic reactions or sensitivity in certain individuals. Others may cause cancer or other serious illness. National Agency for Food and Drug Administration and Control (NAFDAC) reported that mercury and its compounds and corticosteroids are not permitted

Table 6. Minimum inhibitory concentration (mg/ml) of cosmetics against normal flora of the skin

Microorganisms	S1	S3	S4	S6	S7	S8	S9	S10	S12	S13	S14	S15	S16	S17	S18	S19	S21	S22
Staphylococcus epidermidis	NI	NI	NI	NI	25	12.5	25	12.5	NI	25	25	25	NI	NI	NI	50	50	50
Staphylococcus aureus	50	NI	25	25	NI	25	25	NI	NI	50	NI	12.5	25	NI	25	NI	NI	NI
Micrococcus luteus	NI	NI	NI	NI	50	25	NI	NI	NI	25	12.5	NI	NI	25	NI	NI	NI	50
Bacillus subtilis	NI	50	NI	NI	50	NI	NI	NI	50	NI	NI	NI	NI	NI	NI	NI	25	NI
Proteus mirabilis	NI	NI	NI	25	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Pseudomonas aeruginosa	NI	100	NI	NI	25	NI	NI	NI	100	NI	NI	50	NI	NI	25	NI	NI	NI
Candida albican	NI	100	NI	NI	100	50	NI	NI	NI	50	NI	NI	NI	NI	NI	50	NI	NI

Key: NI=No inhibition, S1-S22= Cosmetics sample 1 to 22

in cosmetic products and also that lanolin and boric acid are not permitted in baby products. None of the cosmetics used in the course of this study contain any of the prohibited compounds as stated by NAFDAC. The baby lotion used during the course of this study did not contain lanolin except three of adult synthetic cosmetics that contain lanolin as listed on the container of the cosmetics.

5. CONCLUSION

The use of preservatives to preserved cosmetics could have antimicrobial effect on the normal flora of the skin. Most of the cosmetics used in the course of this research had antimicrobial effects on some selected cutaneous microflora of the skin which could indirectly lead to diseases in immunocompromised and immunocompetent patients.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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