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RESEARCH ARTICLE

SURVEY OF MICROBIAL QUALITY AND PRODUCT INTEGRITY OF COSMETICS SOLD IN PORT HARCOURT, NIGERIA

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ABSTRACT

Microbial quality of cosmetic products affects their shelf-life and effectiveness. A total of 30 (thirty) unopened cosmetic samples were studied. The methods employed in the analysis included physicochemical characteristics, microbial challenge test while preservative concentrations was determined by high performance liquid chromatography (HPLC). Results showed that less than 10% of the samples contained microorganisms. *Pseudomonas aeruginosa* was the only isolate recovered from the samples. The preservative capacity of 63.3% of cosmetic samples was ineffective, while 36.7% passed the test. The result of HPLC analysis revealed that values for methyl paraben and propyl paraben ranged from 0.00030 % to 0.134 % and 0.0001% to 0.153% respectively. The values for physiochemical parameters analysed varied between 4727 mPa.S and 19,820 mPa.S for viscosity; and from 2.4 to 5.85 for pH. Moisture contents were between 14.59 % and 22.6 %. Our findings indicate that majority of the cosmetic samples do not contain microorganisms while a few samples were adequately preserved. The challenge test showed that preservatives were active more on the inoculated *Candida albicans* than on bacterial species (*Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus*). This will help in monitoring and reducing deterioration of cosmetic products, while maintaining quality.

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INTRODUCTION

Cosmetics are therefore defined as materials and preparations that are intended for external and oral use in cleansing, care or influencing appearance or smell or in imparting olfactory impressions (Igile, 2009). Microbial contaminations of cosmetic brands has been examined since the year 1960's. Contaminated cosmetic formulations are relatively infrequent but certain of the products cause a health risk because of their incapability to prevent the growth of microbes of possible faecal origin during in-use (Okeke and Lamikanra, 2001). Microbial contaminations of cosmetic brands represent a possible risk to health for two reasons. First, it may produce product spoilage; the metabolic versatility of microorganisms is such that any formulation ingredient ranging from simple sugars to complex aromatic molecules which may undergo chemical transformation in the presence of an appropriate microorganism. Spoilage will not only affect therapeutic quality but may also decrease consumer compliance. Second, product contamination represents a danger to consumer's health, although the degree of the danger to health will vary from product to product and from consumer to consumer, also be dependent on the type and numbers of microorganisms present and the immunity of the buyer to infection. According to Sharif-Abad *et al*. (2015), high degree of microbial contamination in sunscreen creams, can affect consumers; health. It seems that low grade raw materials, and insufficient manufacturing surveillance in production process are the main determinant factors in the contamination. Cosmetic formulations are not required to be sterile but they must of necessity be free of pathogenic microorganisms (such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*) and the total aerobic microbial counts must certainly be low (USP, 2003; Steinberg, 2006). By limiting microbial contamination, the dangers of infection and spoilage to a level is greatly reduced. Preservatives are added to

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products for two reasons: first, to inhibit microbial spoilage and therefore prolong the shelf-life of the cosmetic brand; second, to save the consumer from a potential infection. Cosmetic products provide favourable environment for microbial (bacteria and fungi) growths, unless they contain adequate amounts of suitable antimicrobial agents as preservatives. The contaminating microorganisms if not adequately controlled will constitute a threat to health of consumers of such products. It is indeed important that manufactured cosmetics be appropriately preserved to avoid unpleasant changes in the product. This present study is aimed at evaluating the microbial quality and preservative capacity of cosmetic products available in the region.

MATERIALS AND METHODS

Sample Collection

Thirty (30) commercially available product types of cosmetic creams and lotions were purchased randomly and their bacterial and fungal counts as well as types of microbes evaluated employing standard procedures. Method for isolation from cosmetic products was direct colony counts (Total viable counts) and enrichment culturing (Hitchins *et al*., 1998). Cosmetic products which are insoluble in water were treated initially to make them miscible before isolation procedures were carried out.

Sample Preparation

The cosmetic samples were prepared using the methods described by Hitchins *et al.* (1998). One gram (1g) sample from each cosmetic product were aseptically weighed and placed into screw-capped test tube containing 9ml sterile Locke's Ringers solution (0.25% Tween-80 in salt solution) as diluents. The total content was mixed properly for 5minutes.

Label disclosed information

All sample containers were carefully examined for integrity, content discoloration, label-disclosed information and odour. Label disclosed information was performed by physical examination of label on each cosmetic product to disclose the manufacturing and expiry date, presence and type of preservative agent and also registration with National Agency for Food and Drug Administration and Control (NAFDAC).

Total Viable Aerobic Bacterial and Fungal Counts

Total viable bacterial and fungal counts were determined for each sample formulation purchased using the following procedure: One gram of the cosmetic preparation was dispersed in an autoclaved sterilized Ringer's solution containing Tween 80 (preservative neutralizer) and then 10-fold serial dilutions of the prepared cosmetic sample were performed under aseptic conditions according to the method of Collins and Lyne (1976). Aliquots (0.1ml) of different serial dilutions (10-10^6) were spread-plated on nutrient agar plates and Sabouraud Dextrose Agar (SDA) plates in duplicates. The inoculated plates were incubated at 37^0C for 18 – 24 hours and at an ambient temperature for 5 – 7 days for the bacterial and fungal counts respectively. Visible and discrete colonies in incubated plates were estimated and expressed as Colony Forming Units per gram (cfu/g) of cosmetic samples.

Determination of Microbial Quality of the Samples

Aliquots (0.1ml) of prepared cosmetic samples were inoculated onto MacConkey agar, cetrimide agar, mannitol salt agar and Sabouraud Dextrose Agar plates in duplicates using the spread-plate method of Demain and Davies (1999). The Sabouraud Dextrose Agar plates were incubated at ambient temperature for 5 – 7 days while the MacConkey agar plates, cetrimide agar plates and Mannitol Salt Agar (MSA) plates were incubated at 37^oC for 18 – 24 hours. Developed colonies were obtained in pure culture and identified by routine microbiological methods. Identification tests included: Oxidase, catalase, Gram reaction, shape, sugar fermentation, and other conventional tests.

Physicochemical Analysis

The physicochemical properties of the cosmetic samples were analyzed. The parameters include temperature, pH, colour, odour, moisture content and viscosity. These characteristics were evaluated in compliance with standard methods (ASTM, 2003). The viscosity of cosmetic samples was ascertained with Brookfield's viscometer (Model NDJ-5S Digital, China). This viscometer consists of a T-bar spindle which penetrates the cream on a helicoidally path. Viscosity was shown on a circular dial into 100 units, knowing the rotation speed and the kind of spindle, viscosity in centipoises (cps) was found out with the help of corresponding conversion factor. Spindle C was used at 4rpm. pH measurement was carried out by accurately weighing 5g of the cosmetic samples and dispersed in 45 ml of water to determine the pH of the suspension at 27 ^{0}C using the digital pH meter (Jenway model, 3305). Moisture content of the cosmetic samples was evaluated using a moisture analyzer (MB35 Ohaus model) at the temperature of 60^0C±2 for 10 minutes.

Evaluation of Preservative Constituents

The presence and concentration of the preservatives such as methyl paraben and propyl paraben in the products were evaluated by High Performance Liquid Chromatography (HPLC) method using a standard.

Microbial Challenge Test

The preservative capacity of the cosmetic samples was assessed by challenging the cosmetic samples with microorganism. The following microorganism/ isolates were incorporated in the challenge test viz *Candida albicans, E.coli* (ATCC 25922) *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus*. Suspension of the cultures of microorganisms was made (conc. about $1x10^8$) and a proportion of 0.1ml was mixed with 20ml of each cosmetic product. The cosmetic product and inoculums in bacteriological tubes were stored at ambient temperature for 28 days. Viable count for enumeration of bacterial survivors was examined at 0, 7^{th}, 14^{th}, 21^{st} and 28^{th} days. Un-inoculated samples served as baseline controls.

RESULTS

Microbial quality of sample

Microbiological analysis of 30 unopened cosmetic samples was carried out. This study indicated that less than 10% of samples were contaminated by *Pseudomonas aeruginosa.*

Contamination rate for manufactured cosmetic samples

The percentage contaminant rate for manufactured cosmetic samples is presented in Table I. From the result, creams and lotions had equal percentage contamination. Also, the percentage contaminant rate between foreign and locally made manufactured cosmetic samples is presented in Table I. From the results, 3.3% of the locally made product was contaminated as with 3.3% of the foreign product.

Container label disclosures on some cosmetic creams and lotions

The result of physical examination of product label on cosmetic samples is illustrated in Table II. The label disclosure showed that 86% of cosmetic samples were preserved with methyl-propyl paraben combinations, 53% indicated date of production, 83% showed the expiry date while 40% were registered with National Agency for Food, Drug Administration and Control (NAFDAC), an indigenous agency that monitors fake product.

Physiochemical Characteristics

The physiochemical parameters for the cosmetic samples were analysed and results are presented in Table III. The characteristics include viscosity, pH, moisture content and colour.

Table I: Contamination rates for sampled manufactured cosmetic samples

Product	Percentage contaminated		Total Percentage contaminated
All products	Foreign	Local	
Cream	3.3%	-	3.3 %
Lotion	-	3.3%	3.3 %

Table II: Container label disclosures on some cosmetic creams and lotions

	Product label disclosure				
Sample code	**Date of Production**	**Expiry Date**	**Preservative Any**	**Type**	**NAFDAC Reg. No.**
CO1	+	+	+	+	-
CO2	+	+	+	+	+
CO3	+	+	+	+	+
CO4	+	+	+	+	+
CO5	+	+	+	+	+
CO6	_	+	+	+	+
CO7	_	+	+	+	+
CO8	_	_	_	_	_
CO9	_	_	+	+	_
CO10	+	+	+	+	_
CO11	_	+	+	+	_
CO12	_	_	+	+	_
CO13	+	+	+	+	+
CO14	_	_	+	+	_
CO15	+	+	+	+	_
CO16	_	+	+	+	_
CO17	_	+	+	+	_
CO18	+	+	+	+	+
CO19	+	+	+	+	+
CO20	+	+	+	+	+
CO21	+	+	+	+	+
CO22	+	+	+	+	+
CO23	+	+	_	_	_
CO24	+	+	_	_	_
CO25	+	+	_	_	+
CO26	-	-	+	+	+
CO27	-	-	-	-	-
CO28	+	+	+	+	+
CO29	_	_	+	+	+
CO30	+	+	+	+	+

Table III: Physiochemical characteristics

S/No.	Sample code	Moisture content (%)	pH	Viscosity (mPa.s)	Colour
1	CO1	14.525	2.92	4788	Cream
2	CO2	14.59	5.79	4727	Pink
3	CO3	16.59	5.85	4789	Green
4	CO4	15.35	5.74	4781	Cream
5	CO5	16.99	3.09	4764	Cream
6	CO6	18.65	4.76	4770	Pink
7	CO7	17.99	3.00	4736	White
8	CO8	18.89	4.88	4735	Pink
9	CO9	18.89	4.39	4853	Pink
10	CO10	19.83	4.52	4769	White
11	CO11	21.97	3.38	4771	White
12	CO12	19.40	4.92	4810	Lemon
13	C013	18.12	2.45	4748	White
14	CO14	14.68	5.05	4747	White
15	CO15	18.05	2.4	858.3	White
16	CO16	18.08	5.02	4845	White
17	CO17	17.8	2.3	4801	Yellow
18	C018	22.6	2.37	4815	White
19	CO19	20.37	2.7	4873	White
20	CO20	22.28	2.73	4890	White
21	CO21	20.05	5.12	4930	Lemon
22	CO22	20.37	5.35	4922	White
23	CO23	20.26	4.63	4913	White
24	CO24	21.04	4.74	4931	White
25	CO25	20.9	4.57	4915	Chocolate
26	CO26	19.97	5.21	5014	Pink
27	CO27	20.69	5.12	5007	Pink
28	CO28	20.02	4.98	5022	Pink
29	CO29	20.54	5.01	4907	White
30	CO30	21.71	5.01	19,820	White

Table IV: Bacterial challenge characteristics (*Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus*)

S/N	Sample code	Day 0	Day 7	Day 14	Day 21	Day 28
1	CO1	30 000	2000	1	0	0
2	CO2	1 360 000	TNTC	TNTC	TNTC	TNTC
3	CO3	90 000 000	3 600 000	TNTC	TNTC	TNTC
4	CO4	42 000 000	0	0	0	0
5	CO5	500 000	15	0	0	0
6	CO6	6 400 000	TNTC	TNTC	TNTC	TNTC
7	CO7	11 300 000	100	0	0	0
8	CO8	109 000 000	74 000	18 000	5000	7400
9	CO9	198 000 000	TNTC	TNTC	TNTC	TNTC
10	CO10	16 000 000	7500	2	0	0
11	CO11	3 100 000	30 000	0	0	0
12	CO12	82 000	25	2	0	0
13	CO13	6 000	0	0	0	0
14	CO14	11 000 000	TNTC	TNTC	TNTC	TNTC
15	CO15	200	0	0	0	0
16	CO16	124 000	5600	0	0	0
17	CO17	34 000	0	0	0	0
18	CO18	6 000 000	0	0	0	0
19	CO19	100	0	0	0	0
20	CO20	300	0	0	0	0
21	CO21	17 000 000	TNTC	TNTC	TNTC	TNTC
22	CO22	59 000 000	TNTC	TNTC	TNTC	TNTC
23	CO23	14 200	TNTC	TNTC	TNTC	TNTC
24	CO24	800 000 000	580 000	77	16	0
25	CO25	64 000 000	35 000	14	0	0
26	CO26	230 000 000	20 000	5 000	3	0
27	CO27	310 000 000	0	0	0	0
28	CO28	20 000 000	120 000	TNTC	TNTC	TNTC
29	CO29	9 000 000	100 000	TNTC	TNTC	TNTC
30	CO30	290 000 000	4 600 000	TNTC	TNTC	TNTC

TNTC – Too numerous to count

Table V: Fungal challenge characteristics (*Candida albicans*)

S/N	Sample code	Day 0	Day 7	Day 14	Day 21	Day 28
1	CO1	4 000 000	0	0	0	0
2	CO2	600 000	TNTC	TNTC	TNTC	TNTC
3	CO3	25 000 000	TNTC	TNTC	TNTC	TNTC
4	CO4	73 000 000	0	0	0	0
5	CO5	200	0	0	0	0
6	CO6	23 000 000	TNTC	TNTC	TNTC	TNTC
7	CO7	36 000 000	0	0	0	0
8	CO8	11 000 000	3000	500	11	0
9	CO9	45 000 000	TNTC	TNTC	TNTC	TNTC
10	CO10	4 000 000	3600	0	0	0
11	CO11	600 000	42000	0	0	0
12	CO12	4000	0	0	0	0
13	CO13	500	1	0	0	0
14	CO14	15 000 000	TNTC	TNTC	TNTC	TNTC
15	CO15	100	0	0	0	0
16	CO16	9100	0	0	0	0
17	CO17	42 000	0	0	0	0
18	CO18	11 000 000	0	0	0	0
19	CO19	100	0	0	0	0
20	CO20	200	0	0	0	0
21	CO21	570 000 000	TNTC	TNTC	TNTC	TNTC
22	CO22	370 000 000	TNTC	TNTC	TNTC	TNTC
23	CO23	1 020 000 000	TNTC	TNTC	TNTC	TNTC
24	CO24	280 000 000	20 000	39	0	0
25	CO25	46 000 000	1000	7	0	0
26	CO26	90 000 000	70 000	2000	1	0
27	CO27	62 000 000	0	0	0	0
28	CO28	25 000 000	0	0	0	0
29	CO29	21 000 000	70 000	10 000	1500	200
30	CO30	330 000 000	570 000	1200	197	TNTC

TNTC – Too numerous to count

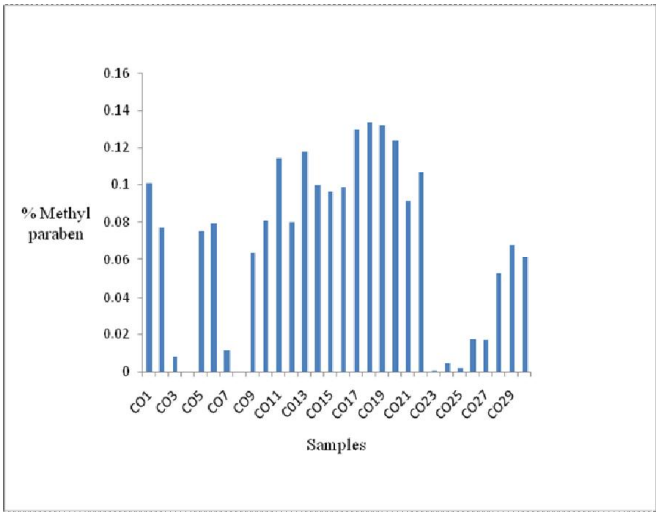

Fig. I: Percent Methyl paraben in cosmetic samples

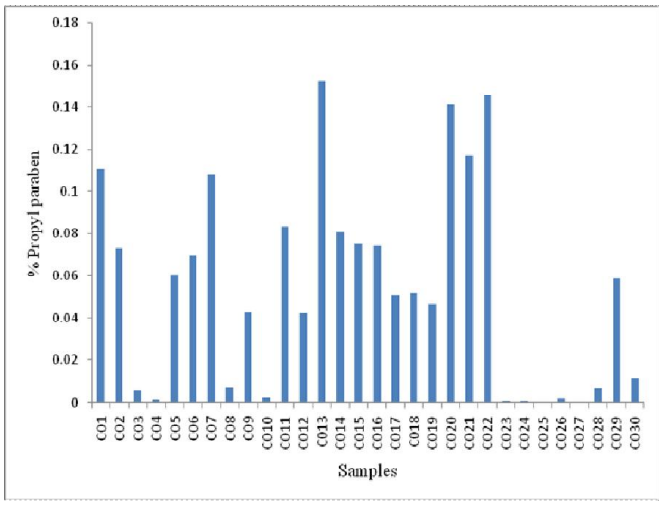

Fig. II: Percent Propyl paraben of samples

Values for viscosity varied between 4727 mPa.S and 19,820 mPa.S; while pH values varied from 2.4 to 5.85. Moisture content values were between 14.59 % and 22.6 %. The colours include cream, white, pink, lemon, yellow, green and chocolate.

Challenge Characteristics

Challenge test results obtained from this study are presented in Tables IV and V. From the results obtained, 33% of the sampled cosmetic products were adequately preserved while 67% were poorly preserved. At the end of the 28-day sampling period, 12 (40%) of samples were seen with microbial growth

Preservative contents of the cosmetic products

The results obtained from the HPLC analysis for the preservative contents of the cosmetic samples for methyl paraben and propyl paraben ranged from 0.00030 % to 0.134 % and 0.0001 % to 0.153 % respectively. The results of Preservative constituents of the cosmetic samples are shown in Figs I and II.

DISCUSSION

Microbial quality

Cosmetics are used every day to cleanse, decorate, perfume or beautify the human body. The complexity of cosmetics

microbiology is because of the broad spectrum of product formulations, manufacturing process and conditions of consumer use. The capability of microorganisms to grow and multiply in cosmetic products has been established. A total of 30 (thirty) unopened samples comprising fifteen body creams and fifteen body lotions were studied. Of the 30 samples, fifteen were locally manufactured while the rest of the fifteen were foreign products. Viable counts were performed to ascertain the microbial quality of the samples. Results obtained indicated that less than 10 % of the cosmetic samples were contaminated with microorganisms. *Pseudomonas aeruginosa* was recovered from the samples and this result is in agreement with other research reports (Becks and Lorenzoni, 1995; Perry, 2001; Okeke and Lamikanra, 2001; Haft-baradarane *et al*., 2014; Keshtvarz *et al.*, 2014).

Product label disclosures (Table II) indicated that all the creams and lotions were preserved with methyl-paraben combinations except five (5) products – CO8, CO23, CO24, CO25 and CO27, where the specific agent was not stated. Of these five products, four (4) of them are locally-made products. Parabens are known good cosmetic preservatives because they possess majority of the desired attributes of a standard preservative system. The label disclosure revealed that 86% of cosmetic samples were preserved with methyl-propyl paraben combinations, 53% indicated dates of production, and 83% reported the expiry dates. Label disclosure also revealed that some cosmetic samples were not registered with the National Agency for Food and Drug Administration and Control (NAFDAC), the regulatory body responsible for drug examination and monitoring of fake products in Nigeria. Result showed that 40% indicated registration number. The physiochemical characteristics of the cosmetic samples (Table III) were also evaluated by the specific analysis. The physiochemical parameters included viscosity, pH, moisture content and colour. Values for viscosity varied between 4727 mPa.S and 19,820 mPa.S; viscosity varies with time, shear and temperature. The values for pH ranged from 2.4 to 5.85. The results demonstrated that nearly all cosmetics were observed to be acidic and hence can pose a potential danger to the health of consumer. Moisture content values were between 14.59 % and 22.6 %. The colours include cream, white, pink, lemon, yellow, green and chocolate. A cosmetic cream with good preservative capacity is one that has the capability of suppressing immediate post-production microbiological contaminants and also to prevent subsequent low inocula of microbiological contaminants during use, and thereby maintains an acceptable low number of microbes in the cosmetic samples (Hugbo *et al*., 2003). The preservative capacity assayed by the challenge tests is reported in Tables 4 and 5. According to Ramp and Witkowski (1975) a well preserved cosmetic can be described as one that would decrease a microbial challenge by at least 99.99 per cent in a comparably short range of time. Challenge test products are adjudged adequately preserved when bacteria decrease by more than 99% (2 log) after 2 days and more than 99.9% (3 log) after 7 days. Yeasts and molds should decrease by more than 99% (2log) for criterion A and (1 log) for criterion B after 14 days. Preservative capacity tests were conducted for the brands that were free of contaminants using Pseudomonas aeruginosa ATCC 9027 (Abu Shaqra and Al-Groom, 2012). This test demonstrated that 28.1% of the sampled cosmetic products tested were inadequately preserved. This result agrees with the findings from this study. Challenge test result recorded in this present study demonstrated that the preservatives were active more on the inoculated *Candida* organisms than bacterial organisms. Parabens do not possess broad limit of activity since they exert inhibitory effect more against fungi and less against bacteria. The efficiency of the preservative methods of two cosmetic samples satisfied the criteria of Official Italian Pharmacopoeia and Cosmetics, Toiletries, and Fragrance Association, while only one tested cosmetic sample respected the Rapid Challenge test criteria (Lynn and Hugo, 1981). Challenge test results according to report by Hugbo *et al*. (2003) revealed that commercial products do possess low potential for inhibiting excessive bacterial growth as may happen during in-use contamination. Contamination of a brand of cosmetic by microorganisms may probably be encountered through in-use and during production. Preservative capacity was analysed by the challenge test while their concentration level in each cosmetic product was examined by High Performance Liquid Chromatography (HPLC) method.

The result of the HPLC analysis (Table Figs I and II) revealed that values for methyl paraben and propyl paraben ranged from 0.00030 % to 0.134 % and 0.0001 % to 0.153 % respectively. The European Economic Community (EEC) Directives stipulate that parabens are permitted in a concentration of up to 0.8% in cosmetics; with a maximum concentration for each individual one of 0.4% (w/w), expressed as p-hydroxybenzoic acid. The results observed indicated that all the cosmetic products investigated contained 0.001% - 0.15% parabens. Nearly all cosmetics were observed to contain parabens. A study similar to this was performed by Rastogi *et al*. (1995). Their results demonstrated that 77% of the sampled products investigated contained 0.01% - 0.87% parabens. Nearly all (99%) of the leave-on cosmetics and 77% of rinse-off cosmetics were seen to contain parabens. A maximum of 0.32% methyl- and propylparabens, 0.19% ethylparaben, and 0.07% butyl- and benzylparabens were observed in paraben-positive cosmetics. A preferred use of combinations of methyl-/ethyl-/propyl-/butyl-/benzyl paraben in several categories of cosmetic care products has been reported. The super scale inclusion of preservatives in cosmetic preparations can cause potential health hazards. Majority of the preservatives may be harmful. The preservative capacity of a cosmetic preparation cannot be foretold in every detail and must be ascertained by microbial challenge testing since the performance of preservative is based on the effect of various individual ingredients and the packaging material in which it is stored.

Conclusion

Cosmetics and topical products need not be sterile but may comprise of limit levels of contaminating microorganisms during in-use by consumers (Hugbo *et al*., 2003). The results obtainable from this research study has demonstrated that the preservatives incorporated in many of these sampled products did not possibly possess adequate preservative activity in so much as to hinder microbial contaminants resulting to an suitable low levels of microbial contamination as required by

regulatory bodies (British Pharmacopoeia, 1993). Preservative capacity was investigated with the challenge test. After an exposure time of 0, 7, 14, 21 and 28 days, viable cell was examined by the plate count method and a decline in the number of each microorganism of 99.9% by 7 days was needed for the formulation to pass the test. After the challenge test analysis, the preservative activity of 63.3% of the sampled cosmetic products was observed to be ineffective because the microbial growth was not limited with a reduction of 99.9%; while 36.7 % of the cosmetic brands passed the test. However, there are few research works in published literature regarding the performance of preservative systems included in cosmetics to control microbial contamination during in- use by buyers (Farrington *et al.*, 1994; Okeke and Lamikanra, 2011).

In order to control growth of contaminating microorganisms and to stabilize any cosmetic product, there is need to add some form of preservative. However, in many of cosmetic samples no expiry date has been indicated and may loose the preservative capacity and therefore become a potential threat to health for microbial contamination. According to data illustrated by FDA, several instances of contamination by microorganisms are due to cosmetic manufacturers using ineffective, poorly designed preservative systems and also not carrying out stability testing on such preservatives during the product's usual shelf life and under normal use conditions (US FDA, 1995). It is essential therefore to enhance the preservative capacity in so much so as to suppress growth of contaminating microorganisms during manufacturing, in-use by consumers and storage (Campana *et al*., 2006).

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