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Microbiological quality and preservative capacity of commonly available cosmetics in Dar es Salaam, Tanzania

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Ten brands of cosmetics were randomly purchased from shops in Dar es Salaam, and were subjected to microbiological assessment for microbial quality, preservative capacity and identification of microbial contaminants. Aliquots of each cosmetic were uniformly spread-plated on agar plates to quantify, isolate and identify microbial contaminants using conventional microbiological methods.
The cup-plate technique complemented by the dilution test was used for evaluation of cosmetic preservative capacity. Microbial contaminants were present in 70% of the cosmetics. The most frequently isolated and identified microbial contaminants were attributable to *Proteus mirabilis, Staphylococcus aureus, Bacillus* and *Trichophyton* species.
The cosmetics displayed inadequate preservative capacity evidenced by inability to lower the inherent bio-burdens to acceptable levels and to inhibit growth of the tested microorganisms. Such products can have detrimental effects on health status of consumers as consequence of their altered stability profiles and secondary microbial infections. Therefore, microbiological quality control of cosmetics available in the Tanzanian market should be re-enforced.

Keywords: Preservative capacity, cosmetics, microbial quality, microorganisms

INTRODUCTION

Quality of cosmetic products largely depends on the quality of starting materials. The guidelines of good manufacturing practice for cosmetic products (GMPC) have clearly depicted the necessity of the starting materials to comply with specifications [1]. This requirement applies equally to both chemical and physical parameters of the products as well as their microbial load. Therefore, starting materials for cosmetics need protection against microbial contamination during their transport, storage and use in production [2, 3]. Contaminated starting materials introduced into production can severely load or overload, a product's preservative capacity, so as to render it ineffective. Consequently, an essential condition for the manufacture of cosmetics is the use of starting materials containing the lowest possible level of microorganisms of less than 10 colony-forming units (cfu) per gram. For most production areas, microbial counts less than 500 cfu/m^3 are recommended [4]

Cosmetics though not required to be sterile, however, require absence of pathogenic microorganisms and low load of non-pathogenic microorganisms [3]. Moreover, they should remain in this condition until use by consumers. Usually, inclusion of preservatives aids in lowering microbial loads within the product to acceptable levels during shelf life. A good preservative is one that is capable of inhibiting immediate postproduction contaminants as well as subsequent low inocula and thereby maintains acceptable low levels of microorganisms in the preparation [5, 6]. Cosmetics that contain more than 10% w/w of ethanol, propylene glycol or glycerol, and those in self-pressurized containers, are usually self-preserved and are unlikely to have microbial contamination [4, 5].

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Women extensively use cosmetics especially skin lighting creams for beauty purposes. However, some of them experience severe life-threatening side effects and/or permanent facial scaring. These side effects have been mainly attributed to toxic ingredients like hydroquinone, hexachlorophene or mercuric compounds. Cosmetologists report that besides the toxicities and other adverse effects associated with usage of cosmetics containing such toxic chemicals, these ingredients also reduce the microbial flora on the skin, particularly the gram-positive bacteria [7, 8], thus increasing vulnerability to pathogenic microorganisms and to secondary microbial infections acquired from the contaminated cosmetics [9, 10]**.** This is the first study to be conducted with regard to microbiological assessment of cosmetics that are available in the Tanzanian market.

MATERIAL AND METHODS

Samples collection and storage

Ten different brands of cosmetics were randomly purchased from shops and drug stores within Dar es Salaam city during a 3-month period (May-July, 2007). Topical pharmaceutical/medicinal cosmetics such as skin and ophthalmological preparations were not included in the study. Due to limited time and resource constraints, only ten most commonly used brands of cosmetics were analyzed. All the samples collected were stored in the microbiology laboratory at the School of Pharmacy until use. 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 GMPC, were recorded[2,6,11].

Microbiological assessment of the samples

The outside surface of each container was swabbed with 70% ethanol before opening. A 100 mg aliquot of each sample was aseptically weighed and using a sterile cotton pad was uniformly spread-plated onto 14 cm diameter-wide agar plates on each of the solid media, Nutrient agar (NA), MacConkey agar (MCA) and Saboraud's dextrose agar (SDA) (Oxoid, UK) for detection of microbial contamination. The inoculated agar plates were aerobically incubated at 37 °C for 24 hours for bacteria and 48 hours for fungi. The resultant colonies were counted and recorded as colony-forming units per milligram of sample (cfu/mg). Each sample was assayed in triplicate and the average value for cfu/mg was calculated. Standard microbial limits were set at 10^3 and 10^2 cfu/mg of sample for bacteria and fungi respectively [2, 5]. Surface plating method with minor modifications was preferred to other methods such as membrane filtration or pour-plating methods because of its cost effectiveness and simplicity in the laboratory settings as well as to avoid the possibility of killing the microorganism with molten agar [12].

Identification of microbial contaminants

Pure cultures of isolated bacteria were preliminarily identified by Gram staining technique and macroscopic observations of growth characteristics on selective, non-selective and differential culture media. The bacteria were further microscopically analyzed and identified by conventional biochemical and physiological characteristics [13, 14]. Malt Extract Agar (Pronadisa) and SDA (Oxoid) media were used to identify fungal species. Light microscope was used for the determination of colony characteristics and morphological structures of fungi.

Determination of preservative capacity by spectrophotometric-dilution method

To investigate the failure of preservative to diffuse through the culture medium matrix, another aliquot of each sample (100 mg and 400 mg) was mixed with a 10 ml broth containing each of the strains of reference microorganisms; *Staphylococcus aureus* (ATCC25923), *Pseudomonas aeruginosa* (ATCC27853), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 90028) and incubated overnight at 37°C. This test was also aimed at evaluating whether some ingredients of the analyzed cosmetics would favor microbial growth, particularly in aqueous

medium (broth). After 24 hours, each of the following volumes: 200, 400, 800 and 1000 µl were drawn and serially diluted with sterile distilled water to 1:19, 1:9, 1:4 and 1:3 respectively. From each of the resultant dilutions, 1 ml of sample was subjected to optical density determination at 280 nm [15, 16] using UV-Vis Spectrophotometer (Janway Co., Dunmow-Essex, UK).

Determination of preservative capacity by cup-plate technique

Two concentrations (100 mg/ml and 400 mg/ml) of each sample were subjected to antimicrobial efficacy testing against the listed four strains of reference microorganisms using Mueller-Hinton and Saboraud'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 °C. The following antibiotic disks were also incorporated as positive controls: tetracycline-(30 µg) (Remedica, Limassol, Cyprus), ampicillin (10 µg), chloramphenicol (30 µg) and Fluconazole (15 µg)-(Pharmathen-SA, Athens, Greece).

Data Analysis

All assays were performed in triplicate for consistency of results and statistical purpose. The data obtained was entered into a database and analyzed using the SPSS Version 15.0 (SPSS, Chicago, USA) computer software. The ZI and optical densities were expressed as mean. The differences in preservative capacity among the analyzed samples were considered significant at $p \leq 0.05$.

RESULTS

Results from the study showed that only 2 out of 10 assayed brands of cosmetics disclosed manufacturing dates, while 6 exhibited expiry dates. Nine of the 10 brands depicted presence of preservatives though only 7 brands specified the preservatives used. Table 1 summarizes label disclosure information that were found or missed on the analyzed samples. All products complied with their original physical characteristics such as color, texture, pH and odor and consistency. cosmetics were observed at varying degrees as shown in Table 2. Seven out of 10 products yielded bacterial contaminants while 4 out of 10 products produced fungal growths within 24 and 48 hours of incubation respectively. The most frequently isolated bacterial contaminants were *Bacillus spp, S. aureus* and *E. coli* while fungal contaminants were found to be *Aspergillus fumigatus* and *Trichophyton spp.*

Table 1: Container label information on collected cosmetics

Product Code	Manufacture Date	Expiry Date	Preservative		Batch Number.
			PI	PS	
S1	-	+	+	+	+
S2	-	-	+	+	-
S3	-	+	+	+	-
S4	+	+	+	+	-
S5	-	-	+	-	-
S6	-	+	-	-	-
S7	-	+	+	+	+
S8	-	-	+	+	+
S9	-	-	+	-	-
S10	+	+	+	+	-

(+) Label disclosure provided, (-) Label disclosure not provided, PI – Presence indicated, PS - Preservative specified

Microbiological contaminations of the cosmetics were observed at varying degrees as shown in Table 2. Seven out of 10 products yielded bacterial contaminants while 4 out of 10 products produced fungal growths within 24 and 48 hours of incubation respectively. The most frequently isolated bacterial contaminants were *Bacillus spp, S. aureus* and *E. coli* while fungal contaminants were found to be *Aspergillus fumigatus* and *Trichophyton spp.*

Table 2: Bacterial and fungal contaminants of the cosmetics

Sample	Bacteria (cfu/mg) x 10^3	Fungi (cfu/mg) x 10^3
S1	None	None
S2	*Staphylococcus spp* (18)	None
S3	*P. aeruginosa* (29)	*Aspergillus fumigatus* (14)
S4	*Proteus mirabilis* (8)	*Trichophyton spp.*(6)
S5	None	None
S6	*Escherichia coli* (11)	*Aspergillus fumigatus* (5)
S7	*Bacillus spp.* (16)	*Trichophyton spp* (3)
S8	None	None
S9	*Bacillus spp.* (26)	None
S10	*Bacillus spp.* (37)	None

The cosmetics' preservative capacity was variable as shown in Table 3. Some cosmetics (S1-S3, S7 and S9) were capable of inhibiting growth of all the tested microorganisms while others (S4-S6, S8 and S10) proved to be inefficacious against *C. albicans*. One cosmetic (S4), exhibited potent antimicrobial activity against *Escherichia coli* (Table 3). The cosmetics were observed to be of equal efficacy ($p<0.05$) with respect to the antibacterial effect (preservative capacity) performed by the dilution test. With exception to samples S1, S6 and S9, all brands manifested a concentration-dependent preservative capacity (Figures 1 and 2).

The most frequently disclosed label information on cosmetic ingredients, showed presence of synthetic preservatives such as methyl-, ethyl-, isopropyl- and butylparaben, organic acids and salts like ethylenediaminetetraacetic acid, triclosan, sodium benzoate and chloracetamide, as well as some halogen-organic derivatives including idopropynyl butylcarbamate and methyldibromo glutaronitrile. Natural preservatives comprised of tocopheryl acetate, ascorbic acid and the essential oils thyme and neem . However, the quantities of the preservatives within those formulations were undeclared. Two cosmetics (S3 and S4) also displayed on the label information that their formulations also contained microbicidal agents like sulphur, zinc oxide and salicylic acid.

DISCUSSION

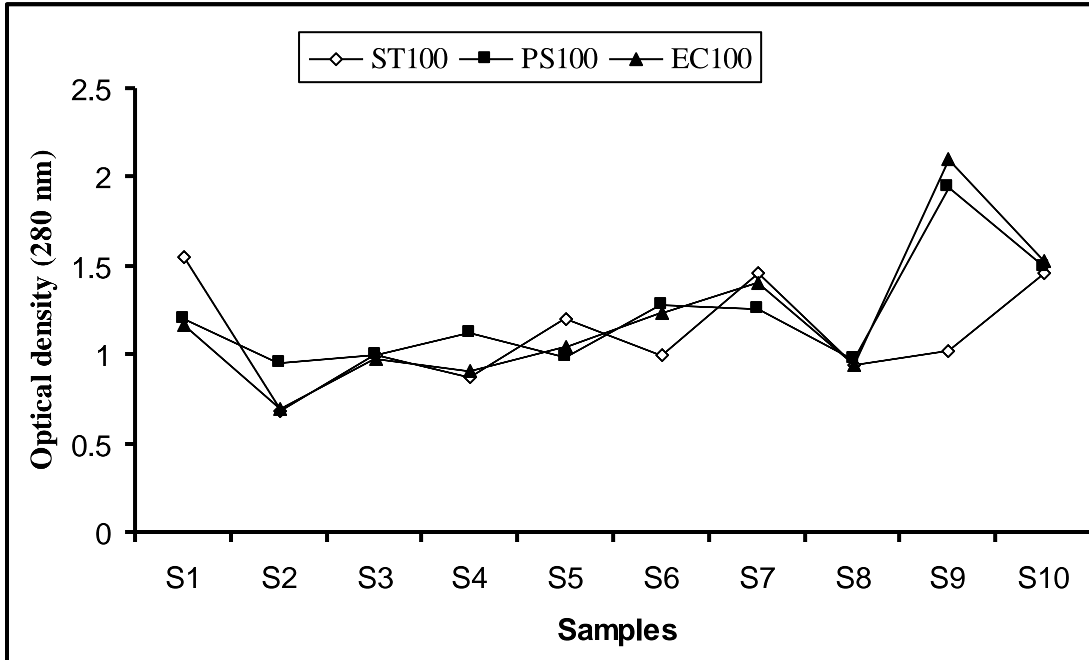
A crucial requirement for the manufacture of cosmetics with low microbial counts is the use of starting materials with low microbial content. The GMPC guidelines also indicate that microbial count of production water, as one of the cosmetic starting materials should be critically analyzed in order to produce products of acceptable microbiological quality [1, 3-5]. This can only be achieved if starting materials have been examined for microbial content as well as conformity with the defined chemical and physical specifications [3-4, 17]. Presently, the cosmetic industry uses numerous ingredients, including preservatives, moisturizers, thickeners, antimicrobials, solvents, emulsifiers and colors. Some of these ingredients support microbial growth. A large microbial load in cosmetics may disturb the ecological balance of the skin normal flora [5, 11].

The study has revealed some inadequacies and inconsistencies in container label information, which are of serious concern, particularly with regard to batch numbers. This means that in the event of defective products, recalls would be extremely difficult to effect [20].

Table 3: Cosmetic preservative capacity on standard microorganisms expressed as zones of inhibition (mm).

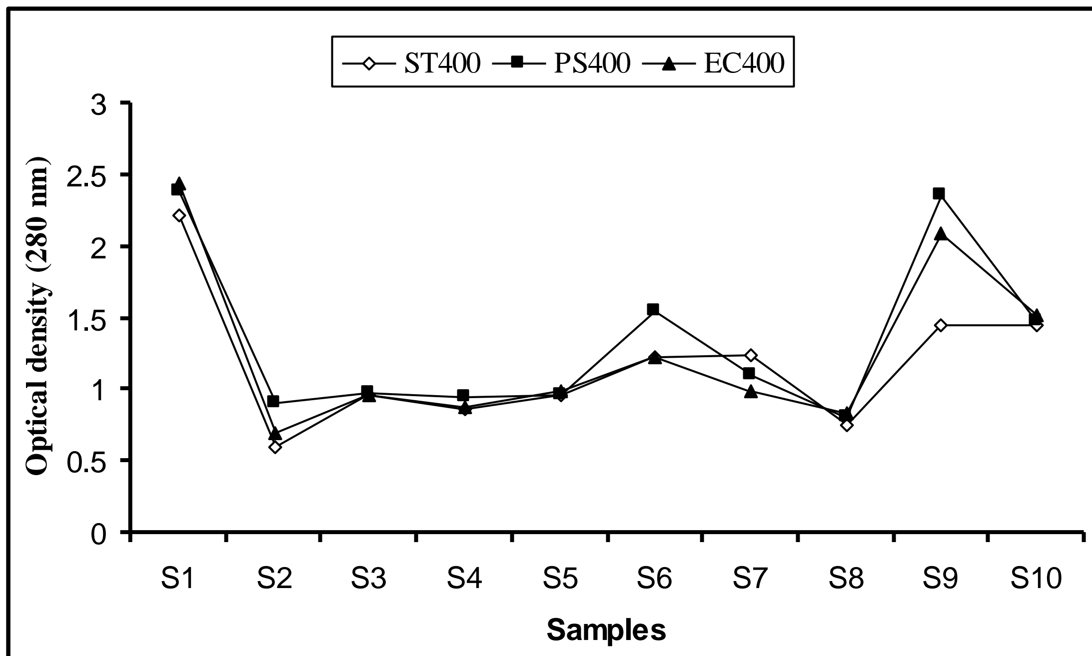
Sample	Concentration (mg/ml)	Inhibition zones (mm)			
		S. aureus	*P. aeruginosa*	*E. coli*	*C. albicans*
S1	400	10.5	12.6	12.5	15.0
	200	10.3	11.2	10.6	9.3
	100	9.3	8.0	8.3	9.3
S2	400	12.0	12.0	12.2	11.0
	200	9.2	11.6	9.55	10.0
	100	8.75	8.8	7.6	8.6
S3	400	13.0	10.8	14.3	10.3
	200	14.5	10.5	12.5	10.0
	100	8.5	10.0	11.6	9.0
S4	400	9.5	12.2	17.6	NZI
	200	9.0	11.0	10.2	NZI
	100	8.0	12.0	12.3	NZI
S5	400	10.9	NZI	14.0	NZI
	200	10.7	NZI	13.3	NZI
	100	10.3	NZI	12.5	NZI
S6	400	11.7	9.7	12.0	NZI
	200	9.65	9.2	9.7	NZI
	100	11.5	8.2	9.0	NZI
S7	400	10.0	9.5	11.0	10.0
	200	9.0	9.2	9.5	9.0
	100	8.5	9.0	9.0	9.0
S8	400	11.0	14.0	13.1	NZI
	200	10.0	13.0	12.2	NZI
	100	10.0	12.5	11.5	NZI
S9	400	10.5	13.5	13.0	12.0
	200	10.0	12.0	12.0	11.8
	100	10.0	9.5	11.5	11.0
S10	400	12.6	12.0	12.5	NZI
	200	12.0	11.5	12.0	NZI
	100	12.0	11.0	10.5	NZI
FLU	15µg/disc	ND	ND	ND	15.0
TCL	30µg/disc	12.7	10.0	10.5	ND
CHL	30µg/disc	11.3	15.6	12.3	ND
AMP	10µg/disc	14.5	ND	11.0	ND

ND - Not done, NZI - No zone of inhibition observed, S1-S10 – Samples 1 to 10,
FLU - Fluconazole, TCL -Tetracycline, AMP - Ampicillin, CHL - Chloramphenicol.



ST = *Staphylococcus*, PS = *Pseudomonas*, EC = *Escherichia coli*

Figure 1: Preservative capacity of cosmetics (100 mg) upon an overnight incubation at 37ºC with broth-containing reference microbial strains.



ST= *Staphylococcus*, PS = *Pseudomonas*, EC= *Escherichia coli*

Figure 2: Preservative capacity of cosmetics (400 mg) upon an overnight incubation at 37ºC with broth-containing reference microbial strains.

Regarding the physical and organoleptic examination of the cosmetics, all products complied with the specifications [6]. There was no apparent deterioration or spoilage of the products though some of them were inadequately preserved.

The hazard of poorly preserved cosmetics to human health has been amply demonstrated by reports of Staphylococcal and fungal infections in hospitals as consequence of using contaminated hand creams and lotions as well as from studies conducted on eye area cosmetics [2, 5].

Regardless of whether a cosmetic becomes contaminated during manufacture or during application, the hazard is mainly attributable to the direct impact of microorganisms on human health and also the indirect effect because of product contamination and spoilage, product separation or formation of harmful microbial metabolites [19].

On the other hand, the environmental conditions that prevail in Tanzania, a tropical country, tend to support the survival and growth of several species of microorganisms. Once a pharmaceutical product is contaminated, rapid microbial growth and multiplication will certainly occur. This might cause biodegradation of the product and hence aggravating the risk of infection to consumers [20].The isolation of enteric bacteria like *Proteus spp* and *E. coli* is a clear evidence of non-adherence to GMPC, because the microbial contaminants presumably have been introduced into the product during manufacturing or packaging process. Previous studies have reported that some ingredients that are usually incorporated into cosmetics tend to reduce the efficiency of preservatives [17-18]. Probably this may explain the observed variability of the cosmetic preservative capacity. The resistance of *P. aeruginosa* to both cosmetics and ampicillin (positive control) is as significant observation. Infections caused by multi-drug resistant *P. aeruginosa* are the most difficult to with conventional antibiotics [21, 22].

The low antimicrobial capacity of the cosmetics can be ascribed to the interaction with the product's ingredients, partition of the active antimicrobial agents into insoluble phases of the cosmetic or presence of agents that create a favorable microenvironment for microbial growth.

Previous research has shown that emulsions (oil/water) which are widely used in cosmetics are occasionally prone to microbial contamination as result of the preservatives partitioning into oily phase of the emulsion while contaminants flourish in the aqueous phase now deprived of preservatives[2,14].

CONCLUSION

In conclusion, the study has revealed inadequacy in cosmetic label disclosure that could make recalls difficult. The results also show that the cosmetics were contaminated and they had inadequate preservative capacity which was evidenced by failure to inhibit or lower the microbial load to the acceptable levels. This calls for incorporation of more efficacious antimicrobial agents in the formulations that will guarantee the microbial quality of cosmetics and adherence to the general guidelines as per GMPC.

The study exposes potential danger and source of microbial infections, which may adversely affect the stability of the products and cause hazards to the health of the consumer.

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