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MICROBIAL CONTAMINATION OF USED HERBAL COSMETIC PRODUCTS

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ABSTRACT

Five types and five brands of used and brand new herbal cosmetic samples (face creams, lipsticks, body lotions, powders and shampoos) were collected from randomly selected 25 beauty parlors and 25 residential areas of different colonies of both economically well-off and economically weaker sections situated at Lashkar, City Center and Thatipur areas of Gwalior (M.P.) from the consumers. All the five types of brand new and used herbal cosmetic samples were examined for their physical appearance and alterations in the physical appearance due to contamination during storage at room temperature. These samples were inoculated on different bacteriological media and a number of biochemical tests were performed for the confirmation of isolates. Total 16 microbial species have been isolated from 25 used cosmetic samples, out of which 10 bacterial and 6 fungal species were documented.

Key Words: *Herbal formulations, Cosmaceuticals, Microbiology*

INTRODUCTION

The word cosmetic originated from the ancient Greek word 'kosmein', which means decoration. Cosmetics are the articles that someone applies to, sprinkles on, or rubs into their body to cleanse, beautify, or promote attractiveness or alter their appearance; the product must not affect normal bodily function or structure (Brannan, 1992). Microbial spoilage of cosmetics is significant from both health and economic viewpoints. The ability of microorganisms to grow and reproduce in cosmetic products has been known for many years. Microorganisms may cause spoilage or chemical changes in cosmetic products and injury to the user (de Navarre, 1941; Baker, 1959; Dunningan, 1968; Iglewski, 1989; Madden, 1984; Morse *et al*. 1967; Smart and Spooner, 1972; Wilson and Ahearn, 1977 and Wilson *et al*. 1975). Microbial spoilage can be caused by bacteria, yeasts or fungi, which are extremely versatile in their metabolic activities. Most of the natural organic and synthetic ingredients are susceptible to degradation due to microbial activity. Cosmetic products are also recognized as substrates for the survival and development of a large variety of microorganisms, since they possess some of the nutrients such as: lipids, polysaccharides, alcohol, proteins, amino acids, glucosides, esteroids, peptides, and vitamins that facilitate the growth of microorganisms. In addition, the conditions of readiness (oxygenation, pH, temperature, osmotic degree, superficial activity, perfume, and essential oils) present in the cosmetic products also favor the microbial multiplication (Herrera**,** 2004). Since, a cosmetic emulsion is usually a complete medium for the growth of microorganisms, the presence of only a new unobserved viable cell in the finished item can cause its complete destruction during storage in a warehouse or on store shelf (Manowitz, 1961).

The increased fascination about the cosmetics for beautification in teenagers, adults and senior citizens in rural as well as city dwellers, the demand of cosmetics have also been increased in India. It is also a bare fact that instead of promoting attractiveness, these cosmetics are also creating severe skin problems of contact dermatitis. Considering the above fact it is necessary to be acquainted with the aspect of microbial contamination of cosmetics during use.

MATERIALS AND METHODS

All the samples were collected separately in sterilized containers and stored at room temperature. The collected samples were properly labeled with the date and place of the collection, type and brand of the sample, ingredients of the samples, manufacturing date and the shelf-life of the samples.

All the used herbal cosmetic samples were examined carefully for their physical appearance, their consistency, discoloration, and any type of smell/ odour, changes in viscosity, formation of gas, changes in texture, any type of growth during shelf life and other changes related to the product. pH values of the sample (used cosmetics) were recorded and compared with brand new cosmetic samples.

Sample preparation for microbiological examination –

Sample preparation from face cream, powder and lipstick - One gram aseptically removed stored sample of face cream was suspended into 20×150mm sterile screw-cap test tube containing 1ml sterile 10% Tween 80. Total contents were mixed by vortexing and total volume was adjusted to 10ml with sterile nutrient broth (8ml) for the 1:10 dilution (stock solution).

Sample preparation from body lotion and shampoo - One ml aseptically removed body lotion sample was suspended into 9ml sterile nutrient broth in 20×150mm sterile screw-cap test tube for 1:10 dilution (stock solution).

The stock solutions of all the brand new samples were prepared and used as control. All the prepared stock solutions of the samples were serially diluted. One ml aliquots of each dilution (10^{-2} to10^{-7}) were immediately inoculated on Letheen agar (modified), Nutrient agar, MacConkey agar and Potato dextrose agar media in triplicate for the isolation of microflora. The culture plates were incubated at 37 ± 2^{0}C. The plates were observed after 24 h and at different intervals upto 7 days of incubation period. The number and cultural characteristics of microbial colonies were recorded at different intervals of incubation period. All the values were analyzed statistically.

RESULTS

All the inoculated samples of used herbal cosmetics were showing the 100% growth of bacteria, whereas, fungal growth was 68%. Total 16 microbial species have been isolated from 25 used cosmetic samples, out of which 10 bacterial and 6 fungal species were documented (Table 1). Out of five types of used herbal cosmetics, body lotions were found highly contaminated, showing maximum percentage of growth of bacteria (13.5% and 23.46% in body lotions after 24 h and 7 days, respectively) and fungi (5.8% after 7 days incubation period) (Fig. 1). The variance of bacterial and fungal colonies among the five types of used cosmetic samples has been statistically analyzed by one-way ANOVA. A significant difference was noted in the bacterial colonies. While in case of fungal colonies the difference was found to be non-significant.

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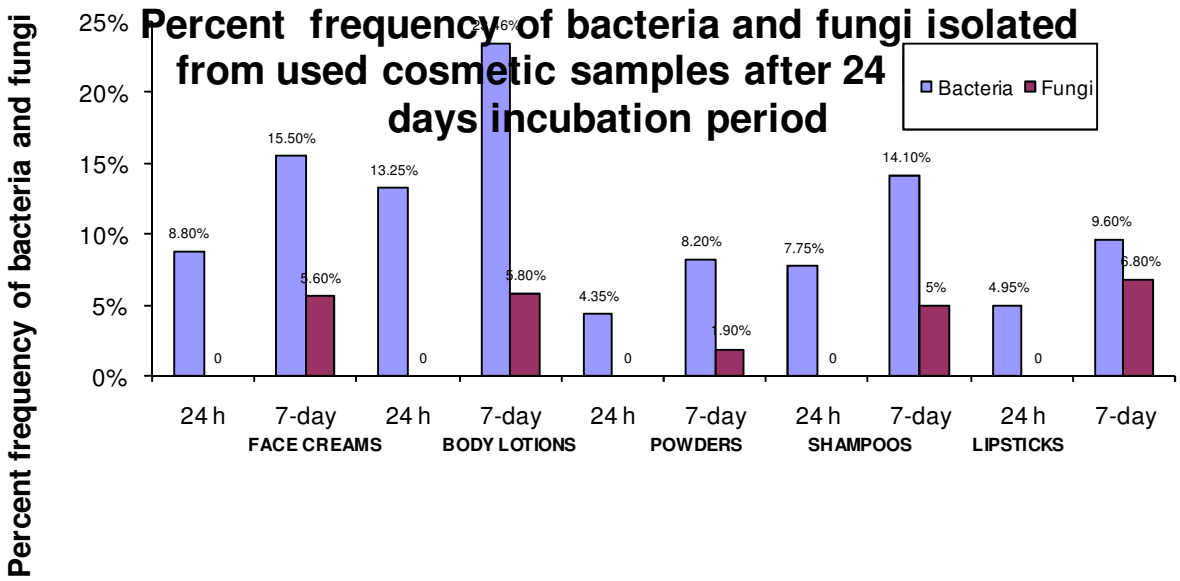
Table-1 Microflora associated with used cosmetic samples

Microorganisms	USED COSMETIC SAMPLES																			
	FACE CREAMS				LIPSTICKS				BODY LOTIONS				POWDERS				SHAMPOOS			
	LA	NA	MA	PDA	LA	NA	MA	PDA	LA	NA	MA	PDA	LA	NA	MA	PDA	LA	NA	MA	PDA
Bacteria																				
1. *Bacillus subtilis*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2. *Corynebacterium xerosis*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
3. *Escherichia coli*	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
4. *Lactobacillus* sp.	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	-
5. *Micrococcus* sp.	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+
6. *Pasteurella* sp.	+	+	+	-	-	-	-	-	+	+	+	-	+	+	+	-	+	+	+	-
7. *Proteus* sp.	+	+	+	-	-	-	-	-	+	+	+	-	+	+	+	-	+	+	+	-
8. *Pseudomonas aeruginosa*	-	-	-	-	+	+	+	-	+	+	+	-	-	-	-	-	+	+	+	-
9. *Staphylococcus aureus*	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+
10. *Streptococcus* sp.	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+
Fungi																				
1. *Alternaria* sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
2. *Aspergillus niger*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3. *Mucor* sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4. *Penicillium* sp.	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+
5. *Rhizopus* sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6. *Trichophyton* sp.	+	-	-	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-	+

LA = Letheen Agar (modified) **NA** = Nutrient Agar **MA** = MacConkey Agar **PDA** = Patato Dextrose Agar
[+] Presence
[-] Absence



Amongst all the 10 bacterial species, *S. aureus* was found to be one of the most dominant and frequently occurring species in all the samples, showing 61% growth followed by *B. subtilis* (52%), while other bacterial species were present in the range of 27-42%. The minimum occurrence (23%) was recorded for the *Proteus* sp. While in the case of fungal species *A. niger* was found to be the most frequently occurring species in 17 samples, showing 48% growth followed by *Alternaria* sp. (39%). Other fungal species were present in the range of 32-36%. The minimum occurrence (22%) was recorded for the *Trichophyton* sp. Enumeration studies in all the used cosmetic samples indicate that maximum growth of contaminants with highest number of bacterial and fungal population was recorded in body lotions on Letheen agar (modified) medium. A fluctuation in the number of total viable microbial cells was observed in body lotions i.e. 1.9×10^6 - 3.6×10^6 after 24 h and 2.5×10^6 - 4.5×10^6 after seven days incubation period on Letheen agar (modified) media. The results clearly indicate that Letheen agar (modified) and PDA medium were found to be the best media for the growth of bacteria and fungi, respectively. The variation in the mean values, Standard Deviation (S.D.) and significant difference in the number of bacterial colonies after 24 h and seven days incubation period on all the culture media were also statistically analyzed by‘t’ test. A significant difference was noted in all the five types of used cosmetic samples.

DISCUSSION

The literature reviewed in the study provides evidences that all the herbal cosmetic products are prone to microbial contamination, mainly due to bacteria and fungi during consumer’s use or in storage. This study also indicates that the retailed brand new herbal cosmetic samples are free of contaminants. Contamination may arise from raw materials or water used in the formulation or accidentally, during use. Similar findings have also been reported by Hugbo *et al*. (2003). The various organic components and the presence of the large quantities of water in these products provide an excellent environment for the growth of microorganisms. According to Wilson *et al*. (1975), during normal use, changes in temperature and humidity provide moisture, which support the microbial growth. The findings of Smart and Spooner (1972) have also revealed that the cosmetic products are also found susceptible to changes in physicochemical conditions due to microbial contamination.

The results of present studies have strongly suggested that most of the herbal cosmetics may be contaminated with microorganisms (bacteria and fungi) during use. *Alternaria* sp., *A. niger*, *B. subtilis*, *C. xerosis*, *E. coli*, *Micrococcus* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp., *S. aureus* and *Streptococcus* sp. were the predominant species found in contaminated used cosmetic samples. Similar findings on contamination of shampoos by gram-negative water borne bacteria (Smart and Spooner, 1972); involvement of gram-negative bacilli as the predominant strains in bacterial contamination of shampoo and rinse fluid (Amemiya and Taguchi, 1994); contamination of shampoos, hand creams, hair cream and hair tonic by *P. aeruginosa*, *E. coli*, *S. aureus*, *B. subtilis* and *Enterobacter* bacteria (Ergun *et al*. 1987); aerobic bacterial, coliforms, fungal counts and potentially hazardous bacteria from body lotions and talcum powders from Egypt (Ashour *et al*. 1989); microbial contamination of eye shadows, mascaras and face creams (Abdelaziz *et al*. 1989); contamination of lipsticks by bacteria, mould and yeast (Akin and Altanlar, 1989) have also been reported. The incidence of contamination by gram-positive *bacilli*, *S. aureus* and *E. coli* gram-negative organisms has been reported from used cosmetic creams (Behravan *et al*. 2000).

CONCLUSION

The findings of the studies indicate that all the cosmetic products used in the investigation are prone to microbial contamination, mainly due to bacteria and fungi during consumer's use or in storage. Herbal and chemical ingredients of the cosmetic products, microbial contaminants, mode of use and the warm and humid environmental conditions of storage are the main factors responsible for the degradation and ultimate spoilage of the cosmetic products.

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