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ANTIMICROBIAL EFFICACY OF LOW LEVEL COSMETIC PRESERVATIVES

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ABSTRACT

Preservatives are used in cosmetics to inhibit the development of microorganisms either previously existing or introduced during the use. An ideal preservative could be one that controls the microbial growth effectively at optimum concentration, while many preservatives cause allergic contact dermatitis and also show other side effects when employed on a sensitive human body. Since no single preservative could control the proliferation of microorganisms at lower concentrations for long period, use of a mixture of preservatives can potentially have synergistic effects against microorganisms. One more advantage of using a combination of preservatives is that, they can address a wide spectrum of microorganisms. In the present study the antimicrobial efficacy of the preservatives Phenoxyethanol (PE), Potassiumsorbate (PS), Sorbicacid (SA), Methylparaben (MP), Propyl paraben (PP) and Sodiumbenzoate (SB) were investigated. The Minimum Inhibitory Concentrations (MIC) of the preservatives singly and in combinations against *Micrococcus luteus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* were studied. A series of challenge tests was used to explore Antimicrobial efficacy of preservatives in certain cosmetic products to verify the effectiveness of different combinations of preservatives.

Key words – preservative, allergic, low level concentrations, wide spectrum, challenge tests.

INTRODUCTION

Cosmetics need preservation to prevent microbial growth and spoilage since water, oils, peptides, and carbohydrates that are present acts as good medium for growth of microbes.The

most frequent problem with cosmetics is repeated challenge while applying with hands and water.[1]The hands may contain normal micro biota or contaminated with external pathogenic or non-pathogenic biota.[2]Water used for cosmetic application should be sterile otherwise it acts as another source of contamination. If a cosmetic becomes contaminated with microorganisms either during manufacture or use, they may directly or indirectly affect the human health. [3] Microbes in cosmetics can cause infection, discoloration, may produce gas and odour. *Enterobacter*spp., *Klebsiella*spp., *Serratia*spp., and *Pseudomonas* spp are some of the classic contaminants of cosmetics.[1,4,5]. Various synthetic preservatives are employed to prevent contamination by pathogens or spoilage microorganisms. In doing so the preservatives extends the shelf life of the products. Frequently used preservatives include benzyl alcohol, boric acid, sorbic acid, chlorhexidine, formaldehyde, parabens, quaternary ammonium compounds, phenol, imidazolidinyl compounds [1,6-8].

A perfect preservative is a "colourless, odourless, water soluble, nontoxic, non-allergenic, non-irritating chemical capable of inhibiting the growth of a broad spectrum bacteria and fungi" [9]; so far no such preservative is in action.A few studies have investigated the use concentration of preservatives in cosmetics[10,11,12].

According to U.S.Food and Drug Administration, Parabens are among the most commonly used preservatives in cosmetic products. Chemically, parabens are esters of p-hydroxybenzoic acid. The U.S. Food and Drug Administration (FDA) and the Cosmetic Toiletries and Fragrance Association (CTFA) in 2004 proclaimed them safe and effective for use in cosmetic formulations[13]. But the use of parabens is becoming increasingly controversial, since they have been found in breast cancer tumors (an average of 20 ng/g of tissue). Parabens have also displayed the ability to slightly mimic estrogen (a hormone known to play a role in the development of breast cancer).[14]Currently the Parabens are the part of over 22000 cosmetics[15].

The most common parabens used in cosmetic products are methylparaben and propylparaben. The methylparaben and propylparaben have been identified as carcinogens causing breast cancer. The chemical components of the body care cosmetics of the body care cosmetics absorps into underlying breast tissues through continuous dermal exposure.[16].The systemic absorption of parabens from environmental exposures is evident through the measurement of intact esters of parabens in human urine. The presence of paraben esters in urine confirms human systemic absorption and detection of intact esters that escaped metabolism. The

parabens detected in human urine at the highest levels were methylparaben and propyl paraben at median concentrations of 43.9 and 9.05 ng mL^{-1}.[17] As reported in US methylparaben is also found up to 1% of the lipsticks.[18]

Methylparaben penetrates the skin to the greatest extent despite having the lowest lipophilicity.[19]. Numerous genotoxicity studies indicate that Methylparaben did increase chromosomal aberrations in Chinese Hamster ovary cell assay. Propylparaben in the diet produced cell proliferaton in the stomach of rats, with the activity directly related to chain activity directly related to chain length of the alkyl chain.[20].Parabens are capable of accumulating in the skin. From an *in-vitro* study it was found that 60% of methyl paraben is remaining on the skin even after eight hours after application and thus leading to an increased risk for paraben sensitization.[21] In some cases paraben sensitization was seen in immune compromised patients. They show different reactions in normal and in sensitized areas of skin. [22]Most often various parabens are merged in products in the form of a mixture. In 2006, the Scientific Committee on Consumer Products(SCCP) concluded that paraben can be safely used in cosmetic products at concentrations of 0.4% for any individual paraben and 0.8% for total paraben concentrations.[23]

Phenoxyethanol has been widely accepted as an ingredient because of its positive reputation as a relatively gentle preservative that is considered non-irritating; it is also one of the few preservatives that does not release formaldehyde. The CIR approves it for use and it is most often used as a preservative in combination with parabens because of its ability to kill bacteria and stabilize formulations, extending their shelf life and making them safe for use even at low levels.[24]A Study in turkey reported the use of 0.9% PE and was found to cause contact dermatitis.[25]

In spite of these protective measures, still ingredients for the preservation of personal care products require high standards of safety and compatibility, hence a few of the well-known and permitted preservatives are used in the majority of products.[26] Organic acids are becoming popular for the preservation of cosmetics.[27]

Sorbic acid is a naturally occurring fatty acid and is non-irritating to the skin. As a result sorbates are often used in baby care products, creams and lotions. Sorbates are non-photosensitizing so they are also appropriate as preservatives for sun care products. Sorbates are environmentally safe. Even though they function as

automatically they are rapidly and completely broken down in biological waste water treatment plants. Sorbicacid and its salt form potassiumsorbate have general acceptance for almost all types of foods and are accepted for use in cosmetics in accordance with the International Cosmetic Ingredient Dictionary and Handbook, CFTA.[28]A survey in 2006 of industry use patterns reported that Sorbicacid and potassiumsorbate were being used in cosmetics at concentrations up to 3% and 0.3% respectively.[29]Sodiumbenzoate is a preservative that is widely used in the food industry. Sodiumbenzoate is a safe, economical preservative in certain toilet preparations such as creams, lotions, gum solutions, and toothpaste. The advantages of sodium benzoate in these applications are that it is colorless, odorless, readily soluble, and generally is compatible other ingredients. Sodium benzoate has been generally reported to be used at concentrations below 3%. The preservative is also most efficient at lower pH values. The inhibitory level of sodiumbenzoate in emulsions increases with oil content. Sodiumbenzoate has found antimicrobial applications in mouthwashes, dermatological creams and ointments, and deodorants.[30]The SCCP is of the opinion that sorbicacid is safe for use as preservative in cosmetic rinse–off products at a maximum concentration of 2% and in cosmetic oral–care products at a maximum concentration of 1.7% and in leave on products up to 0.5%.[31]

The present study focuses on the antimicrobial efficacy of six preservatives; Methylparaben, Propylparaben,Phenoxyethanol, Sorbicacid, Sodiumbenzoate and Potassiumsorbate on selected bacteria.

METHODOLOGY

Antimicrobial efficacy of preservatives

The cosmetic products should be adequately preserved, otherwise microbial contamination may cause alterations in the composition, odour or colour of the product. This can lead to an expensive withdrawal of the products. If the contamination is pathogenic in nature this can have serious consequences on immunosuppressed individuals.FDA recommends for preparation cosmetics with Good Manufacturing Practices (GMPs) rather than addition of preservatives.Since, GMP does not require sterility, addition of preservatives to minimise intrinsic contamination and especially to avoid consumer-based contamination during use is necessary. Improper preservation of cosmetic products or if a microorganism is resistant to the preservative, the products can become contaminated. The efficacy of preservatives and

antimicrobials is measured as the MIC defined as "The lowest concentration of agent that completely inhibits the growth of the test organism".[32]

Minimum Inhibitory Concentration (MIC):- MIC of each preservative was determined for the six preservatives with *Pseudomonasaeruginosa*, *Staphylococcus epidermidis* and *Micrococcus luteus.* The three organisms were considered for testing based on the previous studies that these are included in the frequently present normal flora of human body.[33]The incubation was carried at 25 ± 2^0C for 48hrs with 0.1,0.2,0.3,0.4,0.5,0.6,0.7,0.8,0.9 & 1%concentrations for PE,PS,SB,MP,PP and SA. The Minimum inhibitory concentration of the Preservatives was carried out using challenge tests. [34]

Challenge tests: A series of challenge tests was used to investigate the antimicrobial efficacy of preservatives in a cosmetic cream to compare the effectiveness of combinations and individual preservatives. A facial cream with methyl and propyl parabens as preservatives has been procured from the local market and used in the experiment. Tween 80 was added to the cream to neutralize the effect of preservatives. The cream was inoculated with standard suspension of the microorganism corresponding to 10^5-10^6 CFU. Preservatives were added in different concentrations to the cream containing inoculum and then incubated at 25^0C for 14 days. Samples were drawn on 0, 7 & 14 days and dispersed in NaCl-peptone solution. The samples were inoculated on Nutrient Agar medium, incubated at 35 ± 2^0C for 18-24hrs and observed for the growth of *Pseudomonas aeruginosa, Micrococcus luteus* & *Staphylococcus epidermidis.* A control sample without adding preservatives to the cream was inoculated and included in each challenge test.

RESULTS AND DISCUSSION

In the present study, the minimum inhibitory concentrations (MICs) of PE, PS, SA, SB, MP and PP preservatives both singly and in various combinations was investigated against *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Micrococcus luteus* that are present in a cream. The data presented in Tables 1-3 indicate the growth pattern of the three experimental microorganisms; *Pseudomonasaeruginosa, Micrococcus luteus and Staphylococcus epidermidis* on the individual preservatives; PE, PS, SA, SB, MP and PP. The results presented in Table 4 represent the growth pattern of the three microorganisms on the combinations of 0.1% preservatives

Table 1 representing the response of *Pseudomonas aeruginosa* towards different concentrations of preservatives on day 7 and day 14

MTCC-1688: *Pseudomonas aeruginosa*																				
	DAY-7										DAY-14									
%	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**
PE	+	+	+	+	--	--	--	--	--	--	+	+	+	+	+	+	+	+	--	--
PS	+	+	+	+	--	--	--	--	--	--	+	+	+	+	+	+	+	+	+	+
SA	+	+	+	--	--	--	--	--	--	--	+	+	+	+	+	+	+	+	+	--
SB	+	+	+	--	--	--	--	--	--	--	+	+	+	+	+	+	+	+	+	+
MP	+	+	+	+	+	--	--	--	--	--	+	+	+	+	+	+	+	+	+	+
PP	+	+	+	+	+	+	--	--	--	--	+	+	+	+	+	+	+	+	+	+

%- concentrations of preservatives in 0.1%,0.2%,0.3%,0.4%,0.5%,0.6%,0.7%,0.8%,0.9% and 1%
PE – PHENOXYETHANOL, PS – POTASSIUM SORBATE, SA-SORBIC ACID, SB-SODIUM BENZOATE, MP-METHYL PARABEN, PP-PROPYL PARABEN
+ -growth observed after incubation time i.e, 7days and 14 days

Table 2 representing the response of *Micrococcus luteus* towards different concentrations of preservatives on day 7 and day 14

MTCC-4428: *Micrococcus luteus*																				
	DAY-7										DAY-14									
	0.1	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**
PE	+	+	+	+	--	--	--	--	--	--	+	+	+	+	+	+	+	--	--	--
PS	+	+	+	--	--	--	--	--	--	--	+	+	+	+	+	+	+	+	--	--
SA	+	+	--	--	--	--	--	--	--	--	+	+	+	+	+	+	+	+	--	--
SB	+	+	+	--	--	--	--	--	--	--	+	+	+	+	+	+	--	--	--	--
MP	+	+	+	+	+	+	--	--	--	--	+	+	+	+	+	+	--	--	--	--
PP	+	+	+	+	+	+	--	--	--	--	+	+	+	+	+	+	--	--	--	--

%- concentrations of preservatives in 0.1%,0.2%,0.3%,0.4%,0.5%,0.6%,0.7%,0.8%,0.9% and 1%
PE – PHENOXYETHANOL , PS – POTASSIUM SORBATE, SA-SORBIC ACID, SB-SODIUM BENZOATE, MP-METHYL PARABEN, PP-PROPYL PARABEN
+ -growth observed after incubation time i.e, 7days and 14 days

Table 3 representing the response of *Staphylococcus epidermidis* towards different concentrations of preservatives on day 7 and day 14

MTCC-435: *Staphylococcus epidermidis*																				
	DAY-7										DAY-14									
	0.1	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**
PE	+	+	+	+	--	--	--	--	--	--	+	+	+	+	+	+	+	+	+	--
PS	+	+	+	+	+	--	--	--	--	--	+	+	+	+	+	+	+	+	+	+
SA	+	+	+	--	--	--	--	--	--	--	+	+	+	+	+	+	+	+	--	--
SB	+	+	+	+	--	--	--	--	--	--	+	+	+	+	+	+	+	+	+	+
MP	+	+	+	+	+	+	+	--	--	--	+	+	+	+	+	+	+	+	+	+
PP	+	+	+	+	+	+	+	+	--	--	+	+	+	+	+	+	+	+	+	+

%- concentrations of preservatives in 0.1%,0.2%,0.3%,0.4%,0.5%,0.6%,0.7%,0.8%,0.9% and 1%
PE – PHENOXYETHANOL , PS – POTASSIUM SORBATE, SA-SORBIC ACID, SB-SODIUM BENZOATE, MP-METHYL PARABEN,PP-PROPYL PARABEN

+ -growth observed after incubation time i.e, 7days and 14 days

Table 4 representing the response of bacteria by combinations of 0.1% preservatives

S.NO.	Orgnsm→ Sample↓	*Pseudomonas aeruginosa*			*Micrococcus luteus*			*Staphylococcus epidermidis*		
		D0	**D7**	**D14**	**D0**	**D7**	**D14**	**D0**	**D7**	**D14**
1.	**PE+PS**	+	+	+	+	+	+	+	+	+
2.	**PE+SA**	+	-	-	+	-	-	+	-	-
3.	**PE+SB**	+	+	-	+	+	-	+	+	-
4.	**PE+MP**	+	-	-	+	-	-	+	-	-
5.	**PE+PP**	+	-	-	+	-	-	+	-	-
6.	**PS+SA**	+	-	-	+	-	-	+	-	-
7.	**PS+SB**	+	+	+	+	+	+	+	+	-
8.	**PS+MP**	+	-	-	+	+	+	+	+	-
9.	**PS+PP**	+	-	-	+	-	-	+	-	-
10.	**SA+SB**	+	-	-	+	-	-	+	-	-
11.	**SA+MP**	+	-	-	+	-	-	+	-	-
12.	**SA+PP**	+	-	-	+	-	-	+	-	-
13.	**SB+MP**	+	-	-	+	+	-	+	-	-
14.	**SB+PP**	+	-	-	+	-	-	+	-	-
15.	**MP+PP**	+	-	-	+	-	-	+	-	-
16.	**ALL 6**	+	-	-	+	-	-	+	-	-

PE – PHENOXYETHANOL, PS – POTASSIUM SORBATE, SA-SORBIC ACID, SB-SODIUM BENZOATE,
MP-METHYL PARABEN, PP- PROPYL PARABEN

D0 – Growth of organisms on day 0; D7 – Growth of organisms on day 7; D14 – Growth of organisms on day14

+- growth of bacteria

Preservatives are added to cosmetic products to avoid microbial contamination and spoilage, thereby reducing infections to consumers. Most of the preservatives are involved in allergic contact dermatitis; lowest possible concentrations of preservatives are applied in cosmetic products, without losing the antimicrobial effect. Development of allergic contact dermatitis is dose dependent, hence lowering the preservative concentration may lead to fewer cases of allergic contact dermatitis and safer products. Combinations of preservatives can potentially have synergistic or additive effects against wider spectrum microorganisms, and prevent against allergic contact dermatitis and reduce infections.

The data presented in Tables 1-3 suggest that on day 0, growth was observed in all the samples, on day 7 growth was observed only in samples with low concentration of preservatives while inhibition was noticed at high concentration. On day 14, growth was regenerated, may be suggesting that a single preservative could not be effective against bacteria for long time. Though at higher concentrations i.e., at 1%, the regeneration was not possible but the chances of the preservative absorption by the body may be more, hence possible side effects. The results in Table 4 show that the combination of preservatives even at low concentration i. e, 0.1% was just enough to control the growth since the compounds exhibit synergism, hence low levels are safe for use in any personal care application.

The data of the study suggests that the growth pattern of the experimental microbes with the selected preservatives is more or less the same. Among the six preservatives, propyl paraben, methylparaben and phenoxyethanol are more frequently used preservatives in cosmetics and are familiar for the cause of allergic reactions. The other three preservatives, sorbicacid, potassiumsorbate and sodium benzoate are notified food preservatives. Though all the six are showing same growth pattern, the three preservatives, sorbicacid, potassium sorbate and sodium benzoate are comparatively safe in terms of side effects since they are notified food preservatives.

CONCLUSION

The desire to have germ-free consumer products with long shelf-lives has necessitated the use of preservatives. The optimal preservative for such use should be effective at low concentrations with potency against a wide range of micro-organisms. It should be toxic to neither humans nor the environment, and should not hinder the action of the product being preserved. Parabens initially appeared to fulfil these criteria and were thus incorporated without reservation into a variety of personal care, cosmetic, pharmaceutical, and food products many years ago. But due to the side effects perceived from various studies of safety organisations, their usage is now debatable; hence personal care products are being labelled as “paraben free”. The experimental data suggests that among the six preservatives studied, sorbicacid, potassiumsorbate and sodiumbenzoate are safe cosmetic preservatives compared to propylparaben, methylparaben and phenoxyethanol since the side effects are less in the former compounds.

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The lower level of free C16:1Î”6 seemed to correlate with the higher numbers of Staphylococcus aureus in the skin of AD patients. A pilot clinical test using topically applied C16:1Î”6 yielded reduced S. aureus numbers in 75% of the AD patients tested. Therefore, we hypothesize that free C16:1Î”6 may be involved in the normal human defense against S. aureus. In the present paper, we characterize the antimicrobial properties of C16:1Î”6 against the resident and transient microbial flora of humans.Â To evaluate the antimicrobial efficacy of C16:1Î”6 under these application conditions, model liquid lip glosses with different preservative components were assayed using a preservative efficacy test (a general laboratory testing method [15]) and a home-use test. The antimicrobial efficacy of cosmetic preservatives and known allergens of various potency [diazolidinyl urea, methylchloroisothiazolinone/methylisothiazolinone (MCI/MI), methylisothiazolinone (MI) and phenoxyethanol] was tested alone and in various combinations of two or three preservatives together. The preservatives were tested for minimum inhibitory concentration (MIC) values and possible synergy using fractional inhibitory concentration. MCI/MI was the only preservative showing low-level MIC against all four tested microorganisms: Staphylococcus aureus, Pseudomonas aeruginosa, Candida al