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RESEARCH ARTICLE

SELF PRESERVING HAIR CARE PRODUCTS WITH HERBAL INGREDIENTS

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ABSTRACT

Herbal based cosmetic products have always been considered a good marketing strategy, in view of their delivery of cosmetic goodness. However, increased use of herbal ingredients in formulations poses a high threat of microbial contamination. Presence of plant material in products which by virtue of their nature have high water content forms an ideal breeding ground for microbes to thrive. Sometimes, even preservatives may not be sufficient to give good shelf life to these products.

Interest in developing 'Preservative – free' or 'Self – Preserving' cosmetic formulations is rising. In self preserving products, the traditional preservatives are replaced by 'Multifunctional' cosmetic ingredients which apart from delivering their main functional property also exhibits antimicrobial property

This paper explores the use of combination of multifunctional and other cosmetic ingredients in herbal based hair care preparations to deliver microbiologically safe product. Products which have been studied are herbal shampoo and henna based hair coloring paste.

INTRODUCTION

Use of herbs for delivering cosmetic benefits on skin and hair has been known since ancient times. In recent times, there is a resurgence in the use of herbal ingredients in personal care formulations, wherein they are combined with regular cosmetic ingredients used for making the product.

Use of herbal preparations are perceived to be safe and without side effects. However, one of the major problems faced by formulators using herbal material in personal care products is the high risk of microbial contamination. Plant materials being organic in nature, provides nutrition to microorganisms and facilitates their multiplication which leads to contamination, deterioration and variation in the composition, thus giving rise to inferior products (Rajapandian *et al*., 2013). By their origin, herbs are subject to contamination by microorganisms from soil, air and water and may be potentially pathogenic to humans. Microbial contamination can be influenced by environmental factors such as temperature, humidity and storage conditions (de Freitas Ariyo *et al*., 2012).

The main microbial contamination of plant materials in general, are attributed to total aerobic mesophilic, enterobacterial, yeast and mold (Kneifel *et al*., 2002). Due to its high propensity for microbial contamination, herbal materials have to be decontaminated before using in cosmetic formulations. Several methods like heat treatment, UV irradiation, Ethylene oxide treatment and Gamma irradiation are being used to decontaminate.

Use of herbal materials is quite prevalent for hair wash, conditioning and for natural hair coloring in India. Several plants have been attributed with such hair beneficial properties. These materials need to be incorporated at sufficiently high doses in the product formulation to exert their beneficial properties. Usually in hair wash products, herbal ingredients of interest are incorporated in a typical surfactant based system with other cosmetic ingredients which form the product base. These base formulas themselves may have limited microbial load. Although preservatives generally control the microbial load in a cosmetic preparation, high herbal load in formulas may lead to overwhelming microbial growth within a short span of time. Herbal material may create a good nutrient source for microbes to grow swiftly, so much so that the preservatives seem ineffective.

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The current paper explores the use of preservative enhancing agents and multifunctional ingredients with antimicrobial property to create microbially safe herbal based hair preparations. Products which have been studied are herbal shampoo and henna based hair coloring paste.

MATERIALS AND METHODS

***Materials*:** The cosmetic ingredients including preservatives used in this study were procured from various leading dealers and suppliers which includes Merck Specialities, India; Schulke & Mayr GmbH Germany; Sigma Aldrich, USA; Inolex CC, USA; Symrise Pvt Ltd, Mumbai; Dow Chemicals, Mumbai; Lonza India; Galaxy Surfactants Ltd, Mumbai; Vivimed Labs Ltd, Hyderabad; Hayashibara Co. Ltd, Japan; Kumar Organic Products Ltd., Bangalore; Croda Chemicals, India; and BASF India.

Microbial Culture: The microbial strains used in this study were *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* MTCC 435, *Escherichia coli* ATCC 8379, *Pseudomonas aeruginosa* ATCC902, *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404.

***Preparation of Inoculum*:** The bacterial cultures were inoculated in slants of Tryptone Soy Agar for 18 – 24 hours at 36°C± 1°C for 48 hours. The fungal strains were inoculated on Sabouraud Dextrose Agar and incubated at 23 ±1°C for seven days respectively. All the cultures were harvested in sterile saline and diluted to obtain counts of $1x10^8$ CFU/ml.

***Selection of Multifunctional actives with anti microbial properties*:** Several cosmetically approved ingredients from among solvents, solubilizers, emollients, penetration enhancers, Functional actives, chelators, Anti oxidants, preservative boosters, etc., were screened for their Minimal Inhibitory Concentrations (MIC) against the micro – organisms listed. In all about 69 ingredients were evaluated. Preservatives approved for use in cosmetics were also evaluated as controls.

***Assessing the Minimal Inhibition Concentration*:** MIC was determined by macro broth double dilution method for both antibacterial and anti fungal activity, as per the NCCLS guidelines (NCCLS, 2000). These assays were done in quadruplicates and inferences were drawn.

***Formulations* :** Herbal based hair wash products like Shampoo and henna based hair coloring paste were developed with different combinations of the above mentioned multifunctional actives as listed in the Table 1.

Preservative Efficacy Test (PET) / Preservative Challenge Test (PCT)

In the present study, the preservative efficacy of the product to microbial challenge is evaluated vs. the control product (base + preservative) by a combination of CTFA and Schulke KoKo method (Narayanan *et al*., 2016; CTFA 1993; Siegert, 2014).

***Statistics*:** The non-parametric Kruskal -Wallis test was conducted to evaluate the differences among the test products (including control) on the median change in the log count of bacterial and fungi in the substrate at each challenge.

Table 1 Formulations of Herbal products studied for PET

		Herbal Shampoo APG base			Herbal shampoo SLES base			Henna Paste		
	Ingredients	CONTROL (Base + Pres.)	TEST (Base + Pres + MF Actives)	TEST (Base + MF Actives)	CONTROL (Base + pres.)	TEST (Base + Pres + MF Actives)	TEST (Base + MF Actives)	CONTROL (Base + pres.)	TEST (Base + Pres + MF Actives)	TEST (Base + MF Actives)
1	APG 2000 - Decyl Glucoside (DGl)	5	5	5	-	-	-	5	5	5
2	APG 818 – Cocoglucoside (CGl)	10	10	10	-	-	-	10	10	10
3	APG 810 – Caprlyl Capryl Glucoside (CCG)	20	20	20	-	-	-	20	20	20
4	APG 1200 – Lauryl Glucoside (LGl)	10	10	10	-	-	-	10	10	10
5	SLES 26%	-	-	-	45	45	45	-	-	-
6	Glycerin	3	3	3	3	3	3	-	-	-
7	Xanthan Gum	0.3	0.3	0.3	0.3	0.3	0.3	-	-	-
8	Sodium Carboxy Methyl Cellulose (SCMC)	0.5	0.5	0.5	0.5	0.5	0.5	-	-	-
9	DM water	QS 100	QS 100	QS 100	QS 100	QS 100	QS 100	QS 100	QS 100	QS 100
10	Kathon CG	0.1	0.1	-	0.1	0.1	-	0.1	0.1	-
11	Fenugreek Powder	15	15	15	15	15	15	-	-	-
12	Henna Powder	-	-	-	-	-	-	25	25	25
13	Tert – Butylhydroquinone (TBHQ)	-	0.01 - 0.1	0.01 - 0.1	-	0.01 - 0.1	0.01 - 0.1	-	0.01 - 0.1	0.01 - 0.1
14	Ethylenediamine tetraacetic acid – TS (EDTA – TS)	-	0.05 - 0.1	0.05 - 0.1	-	0.05 - 0.1	0.05 - 0.1	-	0.05 - 0.1	0.05 - 0.1
15	Ethylenediamine tetraacetic acid (EDTA – DS)	-	0.01 - 0.1	0.01 - 0.1	-	0.01 - 0.1	0.01 - 0.1	-	0.01 - 0.1	0.01 - 0.1
16	Tartaric acid (TA)	-	0.5 - 1.5	0.5 - 1.5	-	0.5 - 1.5	0.5 - 1.5	-	0.5 - 1.5	0.5 - 1.5
17	Citric acid (CA	-	0.5 - 1.5	0.5 - 1.5	-	0.5 - 1.5	0.5 - 1.5	-	0.5 - 1.5	0.5 - 1.5

Due to the sample size, the chi-square statistic for the Kruskal-Wallis test is only approximate. If the Kruskal-wallis test showed statistical significance, it was followed-up with the pair

- wise comparisons and these were conducted using the Mann-Whitney U test. For each pair - wise comparison, controlling for Type I Error was ensured by adjusting the *a priori* alpha level divided by the number of comparisons (**Bonferroni adjustment**). The control (base + preservative) was compared against the different test products in each category. The statistical analysis was done using the statistical package PASW Statistics 18.

RESULTS

Minimal Inhibitory Concentration

9 out of 69 ingredients evaluated, showed MIC values similar to cosmetically approved preservatives which includes emulsifiers (Alkyl Polyglocosides (APG) – Lauroyl glucoside, Cocoglucoside, Decyl Glucoside, Capralyl cayl glucoside); Chelators – EDTA – Di Sodium (EDTA – DS) & EDTA – Tetra Sodium (EDTA – TS); Antioxidants – Citric acid, Tartaric acid & TBHQ (Table 2).

Table 2 MIC of hair care ingredients which showed promising antimicrobial activity

S.No	SAMPLE	MIC (µg / ml)					
		S.aureus	***S.epider***	***E.coli***	***P.aeruginosa***	***C.albicans***	***A.niger***
		PRESERVATIVES					
1	Glydant	390.62	781.25	1562.5	625	1875	625
2	Kathon CG	546.87	703.12	546.87	625	48.82	43.94
		Multifunctional Ingredients / Functional Actives					
3	Lauroyl Glucoside (APG 1200)	97.65	78.12	> 10000	> 10000	8750	87850
4	Cocoglucoside (APG 818)	195.31	156.25	> 10000	> 10000	6562.5	5625
5	Decyl Glucoside (APG 2000)	195.31	136.71	625	390.62	195.31	97.65
6	Capralyl Capryl glucoside (APG 810)	273.43	156.25	2500	937.5	351.56	273.43
		Chelators					
7	EDTA – DS	68.35	136.71	6250	2787.5	24.41	136.71
8	EDTA – TS	156.25	195.31	6250	1328.12	97.65	546.87
		Antioxidants					
9	Citric acid	546.87	1250	3125	3125	8750	> 10000
10	Tartaric acid	2187.5	1093.75	2500	2187.5	> 10000	> 10000
11	TBHQ	24.41	34.17	781.25	390.62	625	781.25

Preservative Efficacy test and multiple challenge Tests

3 types of product categories – herbal shampoo with SLES as surfactant base, Herbal shampoo with APG combination as surfactant base & Henna base hair coloring paste and evaluated for preservative efficacy by multiple challenge studies (Table 1). 8 each out of the 23 SLES & APG based herbal shampoo formulations passed the criteria. 4 options out of the 20 Henna paste formulations showed good preservative efficacy.

The control product (SLES & APG base + Preservative) was not effective even for 1 microbial challenge cycle. Hence the challenge was to make the preservative efficacious in the formula (Table 3).

To achieve this, various combinations of antioxidants and chelators were studied in presence of preservative. A synergistic combination of preservative with 2 antioxidants (one of which has to be TBHQ) and a chelator were found to effectively preserve the SLES & APG based formula for 6 multiple microbial challenges and also controlled further growth for the remaining 28 days of study, thus meeting our pass criteria. (Table3).

Surprisingly, in both SLES base & APG base formulas of herbal shampoos, even in the absence of preservative, the synergistic combination of 2 Antioxidants (one of which has to be TBHQ) with a chelator was effectively protecting the formulation against multiple microbial challenges (Figs. 1 – 4).

The SLES and APG base formulas were individually tested with antioxidants (single or combination), or chelators alone and were found to be inefficacious in protecting the formula against multiple challenges. In Henna paste, the neat control (Henna powder + water) and APG base formula without preservative was not effective in controlling microbial challenge for even 1 cycle.

Table 3 Effective synergistic combinations in different Herbal Hair wash / color products

S.No	Combinations	Effective combinations in products			# of PCT cycles passed	Final outcome Pass / Fail
		Herbal Shampoo APG base	Herbal shampoo SLES base	Henna Paste		
1	Base – Preservative	Base	Base	Base	0	Fail
2	Base + Preservative	Base + Kathon CG	Base + Kathon CG	Base + Kathon CG	0/0/6	Fail / fail / Pass
3	Base + Antioxidant + Antioxidant + Chelator	Base + TBHQ + CA / TA + EDTA – DS / TS	Base + TBHQ + CA / TA + EDTA – DS / TS	Base + TBHQ + CA / TA + EDTA – DS / TS	6	Pass
4	Base + Antioxidant + Antioxidant + Chelator + Preservative	Base + TBHQ + CA / TA + EDTA – DS / TS + Kathon CG	Base + TBHQ + CA / TA + EDTA – DS / TS + Kathon CG	-	6	Pass

between APG base and Kathon CG. Among the various formulation combinations studied without preservatives, the APG base with 2 antioxidants (one of which is TBHQ) and a chelator was found to be efficacious in preserving the formulas against 6 multiple microbial challenges. These combinations without preservatives were as efficacious as the formula with preservative. (Fig 5 – 6)

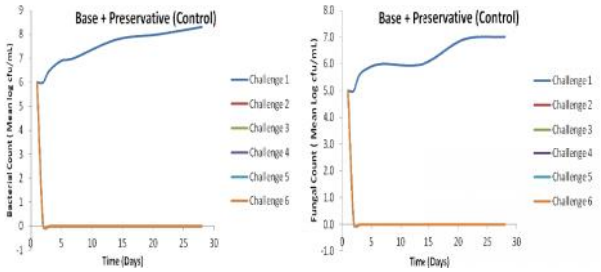

Fig 1 Herbal Shampoo SLES base : Base + Preservative

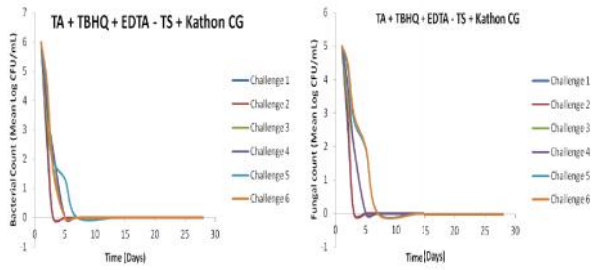

Fig 2 Herbal Shampoo SLES base : Base + TA + TBHQ + EDTA – TS + Kathon CG

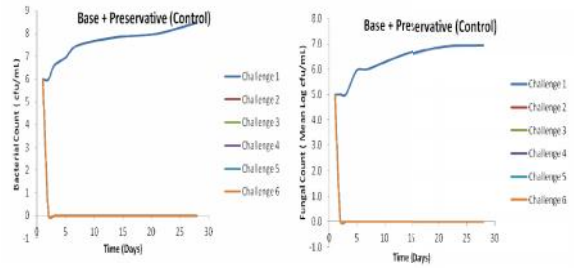

Fig 3 Herbal Shampoo APG base : Base + Preservative

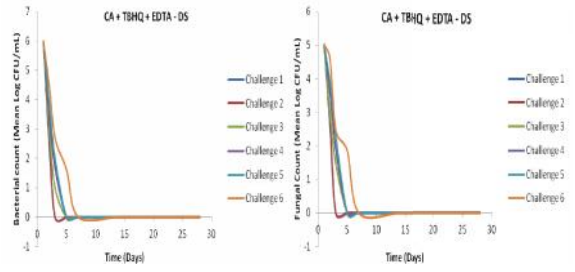

Fig 4 Herbal Shampoo APG base : Base + CA + TBHQ + EDTA – DS

Statistical Evaluations

Statistical evaluation among Herbal shampoo (SLES & APG base) samples (Figs. 7 – 8) for bacterial & fungal counts indicates that there is a significant difference in the median log counts at challenge 1, Day 2. However, pair-wise comparison of control against other test products, after Bonferroni adjustment showed that there is no statistical difference between the median values of each pair. Hence it can be stated that test products are on par with the control.

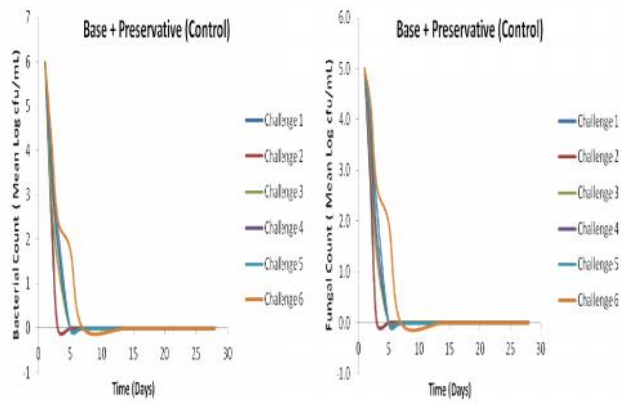

Fig 5 Henna paste: Base + Preservative

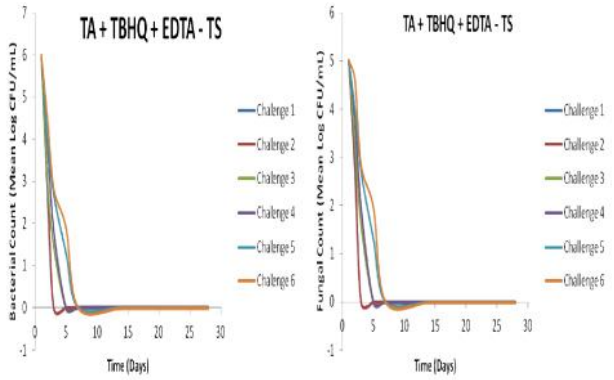

Fig 6 Henna Paste: Base + TA + TBHQ + EDTA – TS

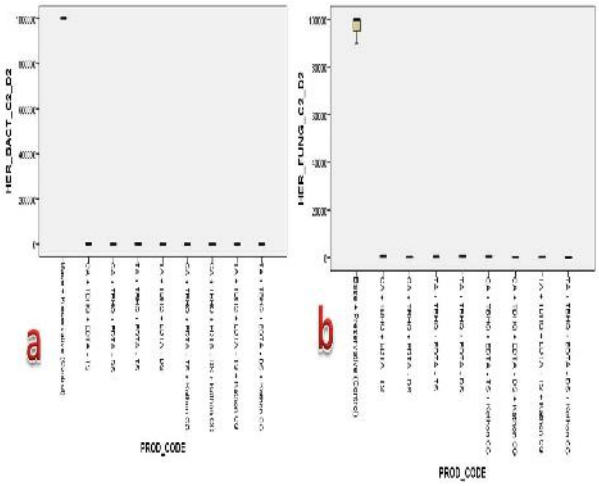

Fig 7 Herbal Shampoo APG Base: Microbial log counts in various combinations in specific challenge numbers & day after challenge a) Bacterial b) Fungal

For Henna paste samples, (Fig. 9) the results of the analysis indicates that there is a significant difference in the median log counts at all challenges excluding Challenge 3 & 5 (for bacterial & fungal counts respectively). However, pair-wise comparison of control against other test products, after Bonferroni adjustment, showed that there is no statistical difference between the median values of each pair. Hence it can be stated that test products are on par with the control.

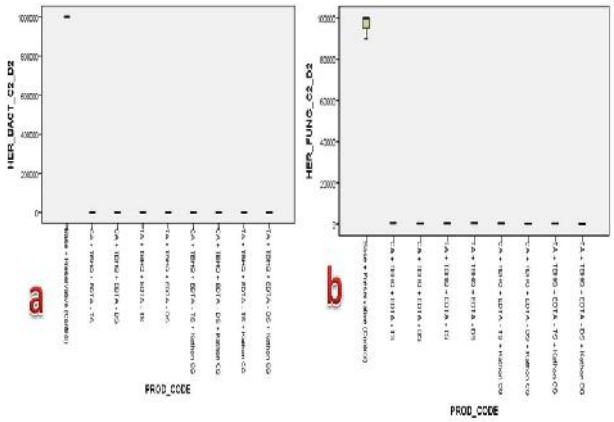

Fig 8 Herbal Shampoo SLES Base : Microbial log counts in various combinations in specific challenge numbers & day after challenge a) Bacterial b) Fungal

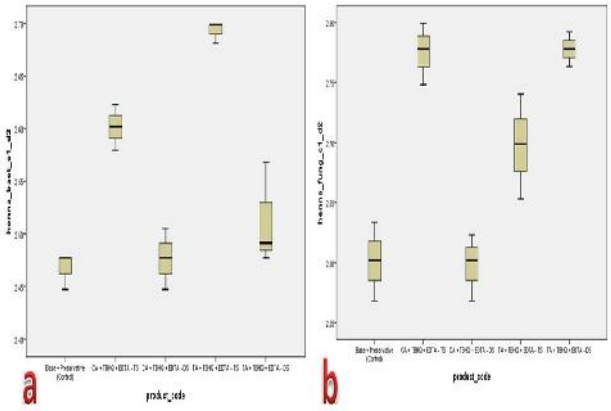

Fig 9 Henna Paste: Microbial log counts in various combinations in specific challenge numbers & day after challenge a) Bacterial b) Fungal

DISCUSSION

Hair care products (like shampoos, coloring products) are complex formulations containing variety of ingredients for giving benefit to hair as well as to give structural identity to the product. Use of herbal / plant materials for hair benefit has been an age old practice in India. Traditionally, fresh herbs / plant materials were used for hair care which needs to be prepared by the user them. But now, in modern times consumers demand readymade products, easily available off the shelf, which are aesthetically pleasing, functionally performing and also stable during its shelf life.

Use of herbal ingredients in personal care products is on the rise, due to consumer preference to such products. However, it is always a challenge to the industry, to develop microbiologically safe products with high herbal load in the product. In spite of using decontaminated herbal raw materials in personal care products, the presence of minimal microbial load in other ingredients makes the formulation a fertile ground for the organisms to proliferate, thereby contaminating the product.

A scan through literature provides ample evidence of the challenge faced not only by the personal care industry, but also by herbal drug business (Rajapandian *et al*., 2013; Justin – Tern *et al*, 2009; Noor *et al*., 2013). Various studies have shown that the microbial contamination most commonly encountered with herbal ingredients involve bacteria and their spores, yeasts and molds. This apart, products of microbial metabolism such as toxic, low molecular weight metabolites from molds are also important chemical contaminants (Kosalec *et al*., 2009). The main microbial contamination of plant materials in general, are attributed to total aerobic mesophilic, enterobacterial, yeast and mold (Kneifel *et al*., 2002). The common microbial species encountered with herbal ingredients include *S.aureus, E.coli, B.cereus*, *Salmonella* spp, *Shigella* spp, *Pseudomonas* spp. *Klebsiella* spp., *B.subtilis* and fungi *Rhizopus stolonifer* (Rajapandian *et al*., 2013; Noor *et al*., 2013; Abba *et al*., 2009; Alwakeel., 2008; Idu *et al*., 2011.)

All across, be it in herbal medicinal preparations or cosmetics or cosmeceuticals, the biggest challenge faced by formulators is to keep the product free from microbial contamination during its shelf life. Presence of microbes can lead to inferior product quality, unstable composition, compromised aesthetics, leading to product spoilage as well as damage to the reputation of the manufacturer.

While it is common to use preservatives to control microbial load in cosmetic formulations; in presence of high herbal load, preservatives may not be adequate. Although, uses of preservatives are regulated, there is an interest among consumers to use preservative free products, as several concerns on safety of preservatives are arising.

By a careful selection of cosmetic ingredients with anti-microbial properties, it is possible to reduce or eliminate the use of regular preservatives and develop self preserving formulas (Varvaresou *et al*., 2009). Termed as 'Multi-functional actives', they are molecules with more than one beneficial effect on the skin or hair. These include glycols, fatty acids and their monoesters; phenethyl alcohol; Ethyl Hexyl Glycerine; phospholipids, etc. (Kabara, 1999; Stoffels 2012; Varvaresou *et al*., 2009).

Chelators like EDTA are known to have potentiating effects on biocides by synergistic action. This potentiating effect is exhibited across a wide range of antimicrobials (including antibiotics, antifungals and antimalarial drugs) (Pradines B *et al*., 2002; Belzalwar P.M. *et al*., 2014 ; Hinton *et al*., 2010; Ayres H.M. *et al*.,1999). Chelators exert a lytic action on the outer lipopolysaccahride layer of the bacterial cell wall and also remove multivalent cations from the cell membrane by forming complexes, weakening the outer surface by reducing the membrane stability; thereby making them more vulnerable to biocides (Kabara, 1999; Siegert, 2014; Boziaris and Adams, 1999). Furthermore, strong chelators may inhibit microbial metabolism by binding trace minerals required for cellular reproduction, growth and survival (Shokri, 2011).

Antioxidants primarily function to delay autoxidation of unsaturated lipids in products. Primary antioxidants are phenolic compounds. Butylated Hydroxyanisole (BHA), Butylated Hydroxytoluene (BHT), and Tert-Butyl Hydroquinone (TBHQ) are commonly used antioxidants in cosmetic products. BHA and TBHQ are effective antimicrobials. Organic acids like citric acid, tartaric acid are

also effective antioxidants. Antioxidants have also been reported to enhance the potency of antimicrobials (Thool *et al*., 2014; Dharmik *et al*., 2012; Ooi *et al*., 2013; Sebastain *et al*., 2009).

Antimicrobial activity of antioxidants like citric acid and tartaric acid may be attributed to inhibitory mechanism like depression of internal pH of microbial cell by ionization of undissociated acid molecules and disruption of substrate transport by altering cell membrane permeability or reduction of proton motive force (Jay, 2005; Davidson *et al.*, 2005; Thool *et al*., 2014). Tartaric acid also prevents the production of malic acid a key intermediate in the process of gluconeogenesis, a primary fuel for the cell (Beggs W.H *et al*., 1978).

Antioxidants are presumed to potentiate efficacy of antimicrobials by protecting them from autoxidation, thereby reducing the rate of spontaneous inactivation and prolonging their biological activity (Hurdle *et al.*, 2011). On the other hand, antioxidant like TBHQ is known for its radical scavenging activity as well as antimicrobial property. Ooi *et al* (2009 & 2013) have demonstrated that TBHQ exhibits antibacterial activity upon degradation and formation of Tert Butylbenzoquinone (TBBQ) which compromises the integrity of bacterial cell membrane without causing cell lysis.

In this study we have explored the use of selected multifunctional ingredients which are approved cosmetic ingredients, but not classified as preservatives according to Annex VI of Commission Directive 76/768/EEC, in combination with chelators and antioxidants to develop self-preserving hair care cosmetic formulations. The study concludes two important results: (1) Multifunctional ingredients along with Chelators and antioxidants in the formulations, synergizes with preservative, enhancing its antimicrobial efficacy in controlling multiple microbial challenges. (2) Even in the absence of preservatives, the synergistic combination of multifunctional ingredient with chelators & antioxidant or combination of 2 antioxidants (one of which has to be TBHQ) also showed good preservative efficacy against multiple challenges.

This study shows that by judicious use of multifunctional actives, chelators and antioxidants, it is possible to develop self preserving cosmetic products with high herbal load, which is microbially stable during its shelf life.

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