JOURNAL OF APPLIED BIOANALYSIS, November. 2024, p. 60-68
http://doi.org/10.53555/jab.v10i3.045 (ISSN 2405-710X)
Vol. 10, No. 3

OPEN ACCESS

RESEARCH ARTICLE

Formulation Development and Evaluation of Self Preserving Oral Care Cosmeceuticals

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Oral care cosmetic products are used to cleanse the oral cavity, freshen the breath, and maintain good oral hygiene. As the dental industry expands day by day, numerous types of oral care products are available on the market, including toothbrushes, toothpaste, mouthwash, floss, and whitening agents. Among these, toothbrushes and toothpaste are the most widely used oral care products. The microbiological protection of oral care cosmetics is of extreme importance in the industry because microbial contamination can harm the product, damage the skin, or introduce pathogens to injured skin, endangering the consumer's health and spreading infection. Preservatives are antimicrobial compounds included in cosmetics to shield them from microbial infections brought on by ingredients, manufacturing processes, and user interaction. Despite the effectiveness of chemical preservatives in preventing microbial growth and extending shelf life, an increasing number of consumers are beginning to doubt their safety. As a result, there is growing interest in cosmetics that are self-preserving or preservative-free. The use of multifunctional ingredients with antimicrobial capabilities as alternatives to conventional preservatives has been studied. This article reports on the formulation of self-preserving oral care cosmeceutical products using multifunctional ingredients and other cosmetic ingredients. We identified ternary mixtures of multifunctional actives that act synergistically and validated the potential of these formulations to deliver microbiologically safe, self-preserving products equivalent to those preserved with approved preservatives. Glyceryl caprylate, inulin, and zinc lactate in the ratios of 1:6.3:1.3, 0.5:12.5:35 and 1:12.5:35 showed a synergistic interaction. Anti-decay toothpaste, anti-decay tooth gel, and anti-decay mouth rinse dosed with these formulations at 0.5% and 0.75% were prepared. The treated cosmeceutical personal care formulations were compared against approved conventional preservative and non-preserved formulations. All three antimicrobial compositions were effective in preserving the cosmetic formulations for up to 28 days (PCT study).

Keywords: antimicrobial, self-preservation, oral care formulations, conventional preservatives, multifunctional ingredients.

INTRODUCTION

Oral care cosmeceutical products are used to clean the mouth, freshen the breath, and maintain good oral hygiene. As the dental industry grows, various types of oral care products are available on the market, including toothbrushes, toothpaste, mouthwash, floss, and whitening agents. However, toothbrushes and toothpaste remain the most commonly used oral care items [1]. Oral hygiene is regarded as the most important aspect in the prevention of oral diseases and the maintenance of oral health [2].

Product deterioration due to microbial contamination is a serious concern in oral care cosmeceutical products. Although these products are not required to be sterile, they are expected to be safe for human use. Preservatives are antimicrobials used in cosmeceutical products to prevent microbial decomposition, extend product shelf life, and protect consumers from harmful

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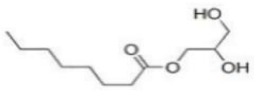
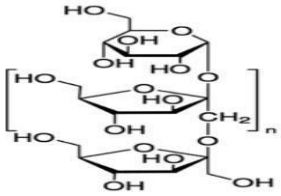
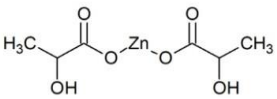
microbial infections [3, 4]. Common preservatives used in cosmeceutical products include parabens, formaldehyde releasers, isothiazolinones, and others. However, in recent years, concerns about the safety of such widely used preservatives have grown. As a result, consumer preference for "preservative-free" products is increasing. The term 'preservative-free' indicates that the product does not include any preservatives as defined by cosmetic legislation. A more accurate term is 'self-preservative.' 'Multi-functional ingredients' (MFI) can be used to create self-preserving formulations that not only provide the primary cosmetic benefit but may also have antimicrobial properties [5, 6, 7, 8].

We explored the synergistic interactions of MFIs to prepare specific combinations to be used as self-preserving ingredients. The present study examined the use of these identified synergistic combinations of multifunctional ingredients to develop self-preserving cosmeceutical oral care products and assessed the formulations' microbial safety. The study reports a comparative analysis of the self-preserved formulations against control formulations preserved using approved preservatives.

MATERIALS AND METHODS

Materials: The multifunctional cosmetic ingredients listed in Table 1, along with other cosmetic ingredients, including preservatives used in this study, were obtained from a variety of reputable dealers and suppliers, including Simson Pharma Ltd., India; Brenntag Ingredients Pvt. Ltd., India; Gangwal Chemicals Pvt. Ltd., India; Ashland Pvt. Ltd., India; Merck Specialities Pvt. Ltd., India; Clariant Ltd., India; Confiance Life Sciences Pvt. Ltd., India; Schulke & Mayr GmbH, Germany; Sigma Aldrich, USA; Inolex CC, USA; Symrise Pvt Ltd, India; Dow Chemicals, India; Maya Chemtech Pvt. Ltd., India; Lonza India; Galaxy Surfactants Ltd, India; Wacker Chemie India Pvt. Ltd., India; Vivimed Labs Ltd., India; Hayashibara Co. Ltd., Japan; Kumar Organic Products Ltd., India; Croda Chemicals Ltd., India; NK Industries Ltd., India; Simson Pharma Ltd., India and BASF India.

Table 1: Ingredients with INCI name, structure, form, benefits, and vendor/supplier

S.No.	Multifunctional Ingredients INCI Name	Structure	Form	Benefits	Vendor/Supplier
1	Glyceryl Caprylate		Powder	Wetting agent, Moisturiser, Emulsifer, Anti-microbial	Evonik India Pvt. Ltd., Mumbai
2	Inulin		Powder	Moisturizing agent, prebiotic Preservative booster	DKSH India, Mumbai
3	Zinc Lactate		Powder	Dietary Nutrient, Anti-microbial	Gangwal Chemicals Pvt.Ltd., Mumbai

Microbial Strains: The standard microbial culture strains recommended for the screening studies were obtained from official cell culture collections, such as the American Type Culture Collection (ATCC), as suggested by the Personal Care Products Council (PCPC) of the United States, and were supplied by Microbiologics Inc., USA. Gram-negative bacteria like *Escherichia coli* ATCC 8379 and *Pseudomonas aeruginosa* ATCC 9027 were the most commonly used test strains in this study, followed by potentially pathogenic Gram-positive bacteria like *Staphylococcus aureus* ATCC 6538, mold like *Aspergillus brasiliensis* ATCC 16404, and yeast like *Candida albicans* ATCC 10231.

Inoculation of Samples: After adjusting the number of starting cells, the inoculum was used to inoculate the test samples. Bacterial cell cultures were grown in Tryptone Soy Agar slants for 18–24 hours at 36°C ± 1°C. The fungal strains were inoculated onto Sabouraud Dextrose Agar/Potato Dextrose Agar and cultured for five to seven days at 23°C ± 1°C. All cultures were harvested after incubation and diluted to 1 x 10^8 CFU/ml in sterile saline.

Screening of Multifunctional Ingredients with Antimicrobial Efficacy: Different cosmetically approved ingredients, including antioxidants, microbial preservative boosters, glycols, biomimetic

phospholipids, esters, emollients, sugars, polysaccharides, fatty acids, surfactants, chelating agents, moisturizers, and multifunctional actives, were assessed for their MIC (Minimal Inhibitory Concentration) against the microbial strains mentioned. In total, about three ingredients and seventy-five ternary combinations were studied. Approved conventional preservatives for use in cosmeceuticals as controls were also examined. The tests were performed in quadruplicate, and the average scores were determined.

Minimal Inhibitory Concentration and FIC Index Determination: The Minimal Inhibitory Concentration (MIC) of an antimicrobial agent is the lowest concentration that prevents visible growth of the microorganism in an agar or broth dilution test. The antimicrobial characteristics were tested using the MIC macro-dilution method for both antibacterial and antifungal activity in accordance with CLSI recommendations [9]. The inhibitory concentration of the test materials was determined by incubating them along with the specific microorganisms at varying concentrations, both individually and in combinations. The tests were repeated four times, and the average values were calculated. To determine synergy/additive/antagonism activity, the FIC index endpoints of antimicrobial agents were calculated alone and in combinations.

24-hour fresh bacterial cultures and 120-hour fungal cultures were used as inoculums. The turbidity of the inoculum was adjusted to a 0.5 McFarland standard with sterile saline or Soybean Casein Digest Medium for bacterial cultures and Sabouraud Dextrose Agar for fungal cultures, to obtain an inoculum size of 1-2 x 10^8 CFU/ml for bacterial cultures and 1-2 x 10^6 CFU/ml for fungal cultures. Stock solutions of the antimicrobial agents were then prepared at concentrations of at least 1,000 mg/ml or ten times the highest concentration to be tested, whichever was greater.

Suitable antimicrobial concentrations were diluted twofold (1000 mg, 500 mg, 250 mg, 125 mg, 62.5 mg, etc.) using the macro dilution method, and inoculums were introduced to separate tubes for each bacterial and fungal culture. For each organism to be investigated, a control tube containing broth devoid of antimicrobial concentrations was used. All inoculation tubes were incubated for 24 hours at 35 ± 2°C, and the experiments were carried out in triplicates/quadruplicates. The culture strains were tabulated in Figs. 1–5.

Fig-1: Gram-positive bacteria *Staphylococcus aureus* ATCC 6538	**Fig-2: Gram-negative bacteria *Escherichia coli* ATCC 8379**	**Fig-3: Gram-negative bacteria *Pseudomonas aeruginosa* ATCC 9027**	**Fig-4 Mold *Aspergillus brasiliensis* ATCC 16404**	**Fig-5 Yeast *Candida albicans* ATCC 10231**
				

The FIC index is calculated by multiplying the synergy index ratio by the number of available techniques [10].

Qa/QA + Qb/QB = Synergy Index

QA represents the concentration of chemical A in PPM that caused an endpoint when acting alone, whereas Qa represents the concentration of compound A in PPM that produced an endpoint in the mixture. QB is the concentration of component B in PPM that produces an endpoint when operating alone, while Qb is the concentration of chemical B in PPM in the final combination.

The results were interpreted using the following criteria:Less than 1.0: Synergy; Equal to 1.0: Additive impact; Greater than 1.0: Antagonism

Cosmeceutical Oral Care Formulations and Processes [13, 14, 15, 16, 17]: Twelve personal care cosmeceutical formulations were prepared, including:

I. Anti-decay toothpaste (ATP 1, 2, 3, and 4) with four different preservation strategies. II. Anti-decay toothgel (ATG 1, 2, 3, 4) with four different preservation strategies and III. Anti-decay mouthrinse (AMR 1, 2, 3, and 4) with four different preservation strategies were prepared as listed in Table 2 with conventional preservatives. (positive control) code: ATP1, ATG1, and AMR1, placebo base without preservative (negative control) code: ATP2, ATG2, and AMR2, synergistic combination of multifunctional ingredients Glyceryl caprylate, inulin, and zinc lactate (synergistic antimicrobial composition 1: 6.3: 1.3) at 0.5% and 0.75% in anti-decay toothpaste ATP3 and ATP4, Glyceryl

caprylate, inulin, and zinc lactate (synergistic antimicrobial composition 0.5: 12.5: 35) at 0.5% and 0.75% in anti-decay toothgel ATG3 and ATG4, and Glyceryl caprylate, inulin, and zinc lactate (synergistic antimicrobial composition 1: 12.5: 35) at 0.5% and 0.75% in anti-decay mouthrinse AMR3 and AMR4 along with Stannous Fluoride cosmeceutical oral care active

Table 2: Cosmeceutical oral care products: anti-decay toothpaste (ATP), anti-decay toothgel (ATG), and anti-decay mouthrinse (AMR) formulations and processes

Cosmeceutical anti-decay toothpaste composition (ATP 1,2,3,4)			Cosmeceutical anti-decay toothgel composition (ATG 1,2,3,4)			Cosmeceutical anti-decay mouthrinse composition (AMR 1,2,3,4)		
Phase	**INCI Name**	**Dosage (%)**	**Phase**	**INCI Name**	**Dosage (%)**	**Phase**	**INCI Name**	**Dosage (%)**
A	Water	QS to 100	A	Water	QS to 100	A	Water	QS to 100
	Sorbitol	25.00		Sorbitol	50.00		Sorbitol	10.00
	Magnesium Aluminum Silicate	1.75		Betaine	1.75		Betaine	1.00
B	Glycerin	10.00		Propylene Glycol	2.00		Propylene Glycol	6.00
	Sodium Carboxymethylcellulose	1.00	B	Glycerin	10.00	B	Glycerin	10.00
C	Calcium Carbonate	40.00		Cellulose Gum	1.00	C	Panthenol	0.25
D	Sodium Saccharin	0.20	C	Hydrated Silica	20.00	D	Sodium Saccharin	0.20
E	Benzyl alcohol, water,sodium benzoate, potassium sorbate* (positive control with conventional preservative) ATP1	0.50	D	Sodium Saccharin	0.20	E	Benzyl alcohol, water,sodium benzoate, potassium sorbate * (positive control with conventional preservative) AMR1	0.50
	Placebo base without preservative (negative control without preservative) ATP2	0	E	Benzyl alcohol, water,sodium benzoate, potassium sorbate* (positive control with conventional preservative) ATG1	0.50		Placebo base without preservative (negative control without preservative) AMR2	0
	Glyceryl caprylate, inulin and zinc Lactate (syngeristic antimicrobial composition 1 : 6.3 : 1.3) ATP3	0.50		Placebo base without preservative (negative control without preservative) ATG2	0		Glyceryl caprylate, inulin and zinc Lactate (syngeristic antimicrobial composition 1: 12.5: 35) AMR3	0.50
	Glyceryl caprylate, inulin and zinc Lactate (syngeristic antimicrobial composition 1 : 6.3 : 1.3) ATP4	0.75		Glyceryl caprylate, inulin and zinc Lactate (syngeristic antimicrobial composition 0.5: 12.5: 35) ATG3	0.50		Glyceryl caprylate, inulin and zinc Lactate (syngeristic antimicrobial composition 1: 12.5: 35) AMR4	0.75
F	Stannous Fluoride**	0.34		Glyceryl caprylate, inulin and zinc Lactate (syngeristic antimicrobial composition 0.5: 12.5: 35) ATG4	0.75	F	Stannous Fluoride**	0.34
G	SodiumLaurylglucosides Hydroxypropylsulfonate	4.00	F	Stannous Fluoride**	0.34	G	SodiumLaurylglucosides Hydroxypropylsulfonate	1.00

H	Flavour	0.25	G	SodiumLaurylglucosides Hydroxypropylsulfonate	5.00	H	Flavour	0.25
I	Citric acid/Sodium Hydroxide	Q.S	H	Flavour	0.25	I	Citric acid/Sodium Hydroxide	Q.S
Manufacturing Procedure: Combine Phase A ingredients and mix until uniform. In a separate vessel, combine Phase B ingredients as a premix. Add the premix to Phase A and mix well for 30 minutes to ensure complete hydration. Add Phase C ingredients and continue mixing until well combined. Add Phases D, E, F,G, and H one by one, mixing well after each addition to ensure uniformity. Finally, add Phase I, adjusting the pH as required. * conventional preservative ** cosmeceutical active			I	Citric acid/Sodium Hydroxide	Q.S	Manufacturing Procedure: Combine Phase A ingredients and mix until uniform. Add Phases B, C, D, E, F, G, and H one by one, mixing well after each addition to ensure uniformity. Finally, add Phase I, adjusting the pH as required. * conventional preservative ** cosmeceutical active		
			Manufacturing Procedure: Combine Phase A ingredients and mix until uniform. In a separate vessel, combine Phase B ingredients as a premix. Add the premix to Phase A and mix well for 30 minutes to ensure complete hydration. Add Phase C ingredients and continue mixing until well combined. Add Phases D, E, F, G, and H one by one, mixing well after each addition to ensure uniformity. Finally, add Phase I, adjusting the pH as required. * conventional preservative ** cosmeceutical active					

Preservative Challenge Test:

The PCT (Preservative Challenge Test) helps assess the formulation's ability to preserve the product. Base formulations including preservatives were used as controls. Unfortunately, there is no universally accepted technique for challenge testing and interpreting results. Various pharmacopoeias prescribe different procedures; however, CTFA (Cosmetic, Toiletries, and Fragrance Association) — now PCPC (Personal Care Products Council) — ISO 11930 requirements are commonly used for cosmetic items. According to CTFA recommendations, the PCT consists of a challenge study with pathogenic bacterial, yeast, and mold cultures were used. The plate count method determines the initial concentration of bacterial or fungal load (CFU/ml) in the test product by counting the number of viable microorganisms in the inoculum suspension and analyzing the microbial level. The inoculated samples are checked at intervals of one, two, seven, fourteen, twenty-one, and twenty-eight days after inoculation. The growth in the number of microorganisms (CFU/ml) is calculated at each time interval, with the percentage of microorganisms assessed relative to the initial concentration.

A preservative challenge test is performed with additional essential details, in which 10 g of sample material is weighed into different sterile containers and spiked with a known amount of microorganisms included in the study. An initial mixed culture of all three bacterial strains—*S. aureus, E. coli, P. aeruginosa*—and fungal strains—*C. albicans and A. brasiliensis*—was prepared. An inoculum size of 11 x 10^6 CFU/ml was created for bacterial cultures, and 15 x 10^5 CFU/ml for fungal cultures. 10 µl of each bacterial culture was added to the container with the sample marked for bacteria, and 100 µl of the fungal inoculum was inoculated into the container marked for fungi. The samples were left at room temperature under sterile environmental conditions. At each predefined time interval (1st, 2nd, 7th, 14th, and 28th day), 1 g of sample from the inoculated containers was weighed and mixed with 9 ml of a sterile neutralizer like Modified Letheen Broth for bacterial sampling and Sabouraud Dextrose Broth for fungal sampling. Further dilutions were made and plated out separately.

3. RESULTS & DISCUSSION

The MIC of the selected three multifunctional ingredients—glyceryl caprylate, inulin, and zinc lactate—along with conventional preservatives (benzyl alcohol, water, sodium benzoate, and potassium sorbate) against five organisms *Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Staphylococcus aureus (S. aureus), Candida albicans (C. albicans),* and *Aspergillus brasiliensis (A. brasiliensis*) were tested based on the macro broth double dilution method and are tabulated in Table 1. The selected multifunctional compounds demonstrated good antimicrobial activity when compared to traditional preservatives commonly used in cosmetic oral care products.

The first constituent of the compositions was glyceryl caprylate. Two substances, inulin and zinc lactate, were chosen as the second and third

components of each composition to determine their ability to aid in synergistic interaction. As a result, three compositions were created based on their MIC data. Composition-1 (three ratio combinations) consisted of glyceryl caprylate, inulin, and zinc lactate. These compositions' components were created in a range of ratios. The ratio concentrations of two of the elements were doubled, while the ratio concentration of the third component was increased to at least 35 times the starting concentration. The concentration range was chosen to achieve a cost-effective composition of the selected elements.

As a result, the concentration ratio of the first specified element in the composition was increased from 0.5 to 1. The concentration ratio of the second ingredient was increased from 6.3 to 12.5, and the concentration ratio of the third ingredient was increased from 1.3 to 35.

Seventy-five composition combinations were produced and tested for MIC. These combinations were evaluated for their MIC value against the five organisms listed above. Table 3 displays the MIC values of a synergistic mixture of multifunctional ingredients with antimicrobial effectiveness. In comparison to the individual MIC values of the multifunctional compounds, synergistic ternary combinations demonstrated superior antimicrobial effectiveness.

The FIC index of the combinations was calculated, and based on the FIC index data, three combinations were identified as synergistic, as shown in Table 3.

Table 3: MIC data of multifunctional ingredients, synergistic composition of multifunctional ingredients, and FIC index of synergistic composition of multifunctional ingredients with antimicrobial efficacy

MIC data of multifunctional ingredients with antimicrobial efficacy						
S. No.	Ingredients	Challenged Organisms				
		Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Candida albicans*	*Aspergillus brasiliensis*
		MIC µg/ml	**MIC µg/ml**	**MIC µg/ml**	**MIC µg/ml**	**MIC µg/ml**
1	Glyceryl caprate	2500	500	250	1000	500
2	Inulin	1250	2500	2500	1250	1250
3	Zinc acetate	1250	2500	625	250	500
4	**Benzyl alcohol,water, sodium benzoate, potassium sorbate** * * Conventional preservative	1250	1250	1000	2000	1000
MIC and FIC data of synergistic composition of multifunctional ingredients with antimicrobial efficacy						
S. No	Composition , ratio, MIC µg/ml & FIC index	Challenged organisms				
		Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Candida albicans*	*Aspergillus brasiliensis*
1	Glyceryl caprylate: inulin: zinc lactate (1 : 6.3 : 1.3)					
	MIC µg/ml	1250	1250	625	625	500
	FIC index	0.94	0.73	0.63	0.82	0.56
	Glyceryl Caprylate: Inulin: Zinc Lactate (0.5 : 12.5 : 35)					
	MIC µg/ml	1250	500	625	250	500
	FIC index	0.99	0.21	0.82	0.78	0.84
2	Glyceryl Caprylate: Inulin: Zinc Lactate (1 : 12.5 : 35)					
	MIC µg/ml	1250	500	625	250	250
	FIC index	0.98	0.21	0.81	0.78	0.42

* conventional preservative

PRESERVATIVE CHALLENGE TEST- Evaluation of preservative efficacy of the cosmeceutical formulations as per PCPC/ISO 11930 Guidelines [11,12] Twelve oral care

cosmeceutical formulations anti-decay toothpate (ATP 1,2,3,4) , anti-decay toothgel (ATG 1,2,3,4), and anti-decay mouthrinse (AMR 1,2,3,4) were prepared as listed in the Table 2 with conventional preservative (positive control) code: ATP1,ATG1 & AMR1, placebo base without preservative (negative control) code: ATP2,ATG2 & AMR2 synergistic combination of multifunctional ingredients at different dosages along with cosmeceutical actives (ATP3,ATG3,AMR3, ATP4,ATG4 & AMR4). All these twelve formulations were evaluated for the preservative challenge test as per PCPC/ ISO 11930 guidelines for 28 days. The results of the preservative challenge test are given below in table 4.

Table 4: Preservative efficacy testing of selected antimicrobial of the developed cosmeceutical oral care products

Methodology : mixed culture challenge																	
Organisms challenged: Bacteria- *S.aureus* + *E.coli* + *P.aeruginosa* Fungal- *C.albicans* + *A.brasiliensis*																	
Challenge dose: bacterial load = $11x10^6$CFU/ml; fungal load = $15x10^5$ CFU/ml																	
Ex. No	Anti-decay toothpaste (ATP3&ATP4)			Usage of % in formulation	Bacterial Count (CFU/ml)							Fungal Count (CFU/ml)						
					D1	**D2**	**D3**	**D7**	**D14**	**D21**	**D28**	**D1**	**D2**	**D3**	**D7**	**D14**	**D21**	**D28**
1	1	6.3	1.3	0.5	$2x10^2$	40	< 10	< 10	< 10	< 10	< 10	340	20	< 10	< 10	< 10	< 10	< 10
2	1	6.3	1.3	0.75	110	< 10	< 10	< 10	< 10	< 10	< 10	30	< 10	< 10	< 10	< 10	< 10	< 10
3	ATP1 Positive Control (with preservative)			0.5	90	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
4	ATP2 Negative Control (without preservative)			0	$18x10^4$	$5x10^3$	$2x10^3$	$1x10^3$	$8x10^2$	50	< 10	$15x10^3$	$6x10^3$	$4x10^2$	910	70	< 10	< 10
Ex. No	Anti-decay toothgel (ATG3&ATG4)			Usage of % in formulation	Bacterial Count (CFU/ml)							Fungal Count (CFU/ml)						
					D1	**D2**	**D3**	**D7**	**D14**	**D21**	**D28**	**D1**	**D2**	**D3**	**D7**	**D14**	**D21**	**D28**
1	0.5	12.5	35	0.5	$3x10^2$	40	< 10	< 10	< 10	< 10	< 10	630	< 10	< 10	< 10	< 10	< 10	< 10
2	0.5	12.5	35	0.75	$1x10^2$	< 10	< 10	< 10	< 10	< 10	< 10	40	< 10	< 10	< 10	< 10	< 10	< 10
3	ATG1 Positive Control (with preservative)			0.5	100	< 10	< 10	< 10	< 10	< 10	< 10	60	< 10	< 10	< 10	< 10	< 10	< 10
4	ATG2 Negative Control (without preservative)			0	$15x10^5$	$1x10^5$	$2x10^4$	$1x10^3$	90	< 10	< 10	$5x10^3$	$3x10^3$	$4x10^2$	990	200	< 10	< 10
Ex. No	Anti-decay Mouthrinse (AMR3&AMR4)			Usage of % in formulation	Bacterial Count (CFU/ml)							Fungal Count (CFU/ml)						
					D1	**D2**	**D3**	**D7**	**D14**	**D21**	**D28**	**D1**	**D2**	**D3**	**D7**	**D14**	**D21**	**D28**
1	1	12.5	35	0.5	$3x10^2$	90	< 10	< 10	< 10	< 10	< 10	400	20	< 10	< 10	< 10	< 10	< 10
2	1	12.5	35	0.75	<10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
3	AMR1 Positive Control (with preservative)			0.5	560	< 10	< 10	< 10	< 10	< 10	< 10	50	< 10	< 10	< 10	< 10	< 10	< 10
4	AMR2 Negative Control (without preservative)			0	$22x10^4$	$3x10^4$	$2x10^3$	$2x10^2$	40	< 10	< 10	$12x10^4$	$2x10^3$	560	70	20	< 10	< 10

It was observed in our study that when the base formulations of anti-decay toothpaste (ATP3 and ATP4), anti-decay toothgel (ATG3 and ATG4), and anti-decay mouthrinse (AMR3 and AMR4) were incorporated with the synergistic multifunctional ingredients, the preservative efficacy profile was found to be similar to the formulations incorporated with conventional preservatives (control) (ATP1, ATG1, and AMR1) in the preservative challenge test. The results indicate that the synergistically acting composition, when incorporated at 0.5% and 0.75% levels in ATP3,ATP4 ATG3,ATG4,AMR3 and AMR4, delivers (PASS) preservative efficacy as per PCPC/ISO 11930 standards [18,19].

The combination of three antimicrobial multifunctional ingredient mixtures at the ratios given above, when incorporated at 0.5% and 0.75% levels in ATP3,ATP4 ATG3,ATG4,AMR3 and AMR4, imparts preservative efficacy equivalent to

conventional preservatives. Most importantly, all dosage quantities meet regulatory requirements. From Table 4, it is evident that the three synergistic combinations were able to impart antimicrobial preservative potency to the composition of different cosmeceutical oral care products, equivalent to conventional preservatives such as benzyl alcohol, water, sodium benzoate, and potassium sorbate dosed at 0.5% in anti-decay toothpaste (ATP1), anti-decay toothgel (ATG1), and anti-decay mouthrinse (AMR1). Therefore, it can be concluded that formulations incorporating these unique synergistic mixtures were as well-preserved as those with conventional preservatives. The unique synergistic combination of multifunctional ingredients can be an alternative solution to preserve cosmeceutical products from microbial attack. These ingredients are skin-friendly and preferred by consumers. This smart approach to cosmeceutical product preservation helps to avoid the use of conventional preservatives, which might cause skin allergies, irritation, or contact sensitivity.

Many cosmeceutical products are complex compositions comprised of a wide variety of components that provide beneficial characteristics to the substrate while also contributing structural uniqueness to the product. As a result, the formulator's goal is to use the fewest components necessary to achieve the greatest benefit. Managing microbial deterioration is an important consideration for formulators during the development process. Typically, this is addressed by including appropriate preservatives. Legislation governs the selection and dosage of preservatives in cosmeceutical products, which are limited by the number of chemistries available [20].

Formulators are seeking opportunities to employ new preservation principles to create "preservative-free" or "self-preserving" formulations, aiming to go beyond current technology. The use of 'Hurdle Technology' is gaining the most attention in this endeavor. This approach combines various preservation features to prevent the growth of microbes, where the different hurdles may have synergistic rather than merely additive effects [21,22].

To develop self-preserving oral care cosmeceutical formulations, we investigated the use of selected multifunctional ingredients that are approved cosmetic ingredients but are not classified as preservatives according to Annex VI of Commission Directive 76/768/EEC, in combination with surfactant-based biomimetic phospholipids, fatty acids, and esters. Several cosmeceutical substances known for providing distinct functional benefits, such as multifunctional behavior, esters, polysaccharides, and antioxidant agents (glyceryl caprylate, inulin, and zinc lactate), were chosen based on their antimicrobial properties. These multifunctional chemicals, when combined with an ester, a polysaccharide, and an antioxidant, exhibit synergistic antimicrobial properties that help reduce microbiological issues.

The fact that these formulations have successfully passed microbiological challenges due to their preservative efficacy instills great confidence in the products' microbial stability and ensures the stated shelf life for consumers. In this work, we have demonstrated that it is possible to create self-preserving oral cosmeceuticals that are as durable as preservative-containing formulations.

CONCLUSION

Glyceryl caprylate, inulin, and zinc lactate were identified as three distinct multifunctional ingredients based on their minimum inhibitory concentration (MIC) values. To explore their potential synergistic interactions, seventy-five different combinations of these three multifunctional substances were created and tested. Based on the MIC values of the individual multifunctional components and their combinations, as well as the calculated Fractional Inhibitory Concentration (FIC) index, three synergistic antimicrobial compositions were identified. The ratios of glyceryl caprylate, inulin, and zinc lactate at 1:6.3:1.3, 0.5:12.5:35, and 1:12.5:35 demonstrated significant synergistic interactions. All combinations exhibited lower MIC values compared to their individual constituents.

These synergistically active combinations were then incorporated into three different cosmeceutical oral care formulations at various dosages. The treated cosmeceutical oral care formulations were evaluated against formulations containing approved conventional preservatives and non-preserved controls. All three antimicrobial formulations successfully preserved the cosmetic formulations for up to 28 days, as demonstrated in the Preservative Challenge Test (PCT).

This method of product preservation is advantageous as it reduces the reliance on traditional preservatives, which may cause skin irritation or contact sensitivity. As a result, this study demonstrates that the proper application of multifunctional actives can lead to the effective development of self-preserving cosmeceutical formulations. These formulations are capable of protecting themselves from microbial contamination without the need for potentially

harmful preservatives, offering a safer and more skin-friendly alternative for consumers.

ACKNOWLEDGEMENTS

We would like to extend our gratitude to Ms. B. Nithya and Ms. G. Sivaranjani for their contributions to the microbiological work. Additionally, the authors express their sincere thanks to Dr. A.K. Kathireshan, Director of the School of Life Sciences at VISTAS, for his invaluable encouragement and support.

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