Synergistic Effects of Multifunctional Ingredients in Self-Preserving Body Care Cosmeceuticals

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KEYWORDS

Antimicrobial, self-preservation, body care formulations, conventional preservatives, multifunctional ingredients

ABSTRACT

The evolution of body care cosmetics has been driven by the pursuit of visible benefits that enhance skin health and beauty. Today, body care products include moisturizers, sunscreens, skin brighteners, and chemical peels. Microbiological protection is crucial to preventing contamination that can compromise product quality, harm the skin, and pose health risks by introducing pathogens. To address these concerns, preservatives are commonly added to safeguard cosmetics during production, use, and storage. However, escalating concerns regarding the safety of chemical preservatives in cosmetics have spurred a growing demand for self-preserving or preservative-free alternatives. Consequently, research has shifted toward exploring multifunctional ingredients with inherent antimicrobial properties as viable substitutes for traditional preservatives.

This article presents the development of self-preserving body care cosmeceutical products using multifunctional ingredients alongside other cosmetic components. Through systematic investigation, ternary mixtures of multifunctional actives exhibiting synergistic interactions were identified. The efficacy of these formulations in providing microbiologically safe, self-preserving products equivalent to those preserved with approved preservatives was validated. Specifically, formulations containing sodium coco PG-dimonium chloride, ricinoleic acid, and sorbitan caprylate at specific ratios (1:6.3:10, 1:6.3:12.5, and 1:6.3:37.5) demonstrated notable synergistic effects.

Body wash gel, lotion, and cream formulations incorporating these synergistic compositions were prepared and compared against conventional preservative and non-preserved formulations. Preservation efficacy testing conducted over a 28-day period confirmed the effectiveness of the antimicrobial blends. Overall, this research highlights the feasibility and efficacy of formulating self-preserving body care cosmeceuticals using multifunctional ingredients, addressing the growing consumer demand for safer and more sustainable cosmetic solutions.

Introduction

The evolution of body care cosmetics has increasingly focused on enhancing skin health and beauty through visible benefits. Modern body care products—such as moisturizers, sunscreens, skin brighteners, and chemical peels—are designed to improve both the appearance and health of the skin. This expanding category, known as body care cosmeceuticals, includes products such as body lotions, exfoliants, and anti-aging creams that offer a blend of aesthetic and therapeutic benefits [1, 2].

Ensuring the safety and efficacy of body care cosmeceuticals is crucial, particularly in preventing microbial contamination. Such contamination can compromise product quality, harm the skin, and pose health risks. Traditionally, preservatives have been used to inhibit microbial growth and extend the shelf life of these products. However, growing consumer concerns about the safety of conventional preservatives—such as parabens, formaldehyde releasers, and isothiazolinones—have shifted the focus toward preservative-free alternatives [3, 4, 5, 6].

The term "preservative-free" indicates that a product does not contain these traditional preservatives as defined by cosmetic regulations. A more precise term for these innovative formulations is "self-preserving." These products utilize multifunctional ingredients (MFIs) that not only provide the primary cosmetic benefits but also possess inherent antimicrobial properties [7, 8].

Our study explores how combining multifunctional ingredients can lead to the development of effective self-preserving body care products. We focused on creating these products using specific ingredient combinations that work synergistically. By comparing these self-preserving formulations with traditional products that use standard preservatives, we aimed to evaluate their effectiveness in preventing microbial contamination.

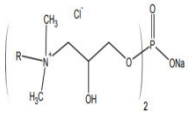
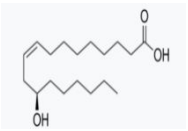
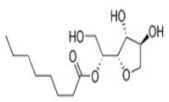
Our findings demonstrate that self-preserving body care products formulated with multifunctional ingredients can effectively address microbial safety concerns while meeting the growing consumer demand for safer and more sustainable cosmetic solutions.

Materials and Methods

Materials:

The materials used in this study included a range of multifunctional cosmetic ingredients and other components, such as preservatives. These ingredients were sourced from several reputable suppliers. Indian suppliers included BASF Ltd., Brenntag Ingredients Pvt. Ltd., Ashland Pvt. Ltd., Merck Specialties Pvt. Ltd., Gangwal Chemicals Pvt. Ltd., Clariant Ltd., Confiance Life Sciences Pvt. Ltd., Symrise Pvt. Ltd., Dow Chemicals, Maya Chemtech Pvt. Ltd., Lonza India, Galaxy Surfactants Ltd., Wacker Chemie India Pvt. Ltd., Vivimed Labs Ltd., Kumar Organic Products Ltd., Croda Chemicals Ltd., Simson Pharma Ltd., and NK Industries Ltd. International suppliers included Schulke & Mayr GmbH (Germany), Sigma Aldrich and Inolex CC (USA), and Hayashibara Co. Ltd. (Japan) [9,10,11,12]

The multifunctional cosmetic ingredients used in this study are detailed in Table 1.

S.No	Multifunctional Ingredients INCI Name	Structure	Form	Benefits	Vendor/ Supplier
1	Sodium Coco PG-Dimonium Chloride Phosphate		Liquid	Surfactant multifunctionality Superior substantivity on hair and skin. Broad antimicrobial enhancement	Brenntag Ingredients India Pvt. Ltd., Mumbai
2	Ricinoleic Acid		Liquid	Moisturizer, Anti-inflammatory, Anti-microbial	NK Industries Ltd., Gujarat
3	Sorbitan Caprylate		Liquid	Rheology modifier, emulsifier and preservative booster	Clariant India Ltd., Mumbai

Microbial Strains:

The microbial strains used for screening were sourced from official cell culture collections, including the American Type Culture Collection (ATCC), as recommended by the Personal Care Products Council (PCPC) of the United States, and were obtained from Microbiologics Inc., USA. The study predominantly utilized Gram-negative bacteria, such as *Escherichia coli* ATCC 8379 and *Pseudomonas aeruginosa* ATCC 9027. Additionally, potentially pathogenic Gram-positive bacteria like *Staphylococcus aureus* ATCC 6538, mold such as *Aspergillus brasiliensis* ATCC 16404, and yeast like *Candida albicans* ATCC 10231 were also included.

Inoculation of Samples:

For inoculating test samples, the initial cell concentration was appropriately adjusted. Bacterial cultures were cultivated on Tryptone Soy Agar slants for 18–24 hours at 36°C ± 1°C. Fungal strains were inoculated onto Sabouraud Dextrose Agar or Potato Dextrose Agar and incubated for five to seven days at 23°C ± 1°C. Following incubation, all cultures were harvested, diluted to a concentration of 1 x 10^8 CFU/ml in sterile saline, and used for testing.

Screening of Multifunctional Ingredients with Antimicrobial Efficacy:

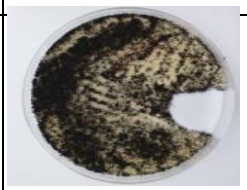
The study evaluated a variety of cosmetically approved ingredients for their antimicrobial efficacy. These included antioxidants, microbial preservative boosters, glycols, biomimetic phospholipids, esters, emollients, sugars, polysaccharides, fatty acids, surfactants, chelating agents, moisturizers, and multifunctional actives. The minimal inhibitory concentration (MIC) of these ingredients against the tested microbial strains was assessed. A total of approximately three individual ingredients and seventy-five ternary combinations were tested. Conventional preservatives approved for cosmetic served as controls. All tests were conducted in quadruplicate, and the average results were calculated.[13]

Minimal Inhibitory Concentration and FIC Index Determination:

The Minimum Inhibitory Concentration (MIC) represents the lowest concentration of an antimicrobial agent required to inhibit visible growth of microorganisms in an agar or broth dilution test. To evaluate the antimicrobial properties, the MIC macro-dilution method was employed for both antibacterial and antifungal activities, following the recommendations of the Clinical and Laboratory Standards Institute (CLSI) [14]. The inhibitory concentrations of the test materials were determined by incubating them with specific microorganisms at various concentrations, both individually and in combinations. Each test was performed in quadruplicate, and the average values were calculated. The Fractional Inhibitory Concentration (FIC) index was calculated to assess the synergistic, additive, or antagonistic effects of the antimicrobial agents, either alone or in combination.[15]

Fresh bacterial cultures (24 hours old) and fungal cultures (120 hours old) were used as inoculums. The turbidity of the inoculum was adjusted to the 0.5 McFarland standard using sterile saline for bacterial cultures, and Sabouraud Dextrose Agar for fungal cultures, to achieve an inoculum size of 1–2 x 10^8 CFU/ml for bacteria and 1–2 x 10^6 CFU/ml for fungi. Stock solutions of the antimicrobial agents were prepared at concentrations of at least 1,000 mg/ml or ten times the highest concentration to be tested, whichever was greater.

The antimicrobial agents were diluted twofold (e.g., 1,000 mg, 500 mg, 250 mg, 125 mg, 62.5 mg, etc.) using the macro-dilution method. The inoculums were then added to separate tubes for each bacterial and fungal culture. A control tube containing broth without any antimicrobial agent was included for each organism. All inoculated tubes were incubated for 24 hours at 35 ± 2 °C. The experiments were conducted in triplicate or quadruplicate. The results for the culture strains were presented 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 eruginosa* ATCC 9027**	**Fig-4 Mold *Aspergillus brasiliensis* ATCC 16404**	**Fig-5 Yeast *Candida albicans* ATCC 10231**
				

The FIC (Fractioal Inhibitory Concentration) Index is a valuable tool for evaluating the interaction between different antimicrobial agents. It is determined by multiplying the synergy index ratio by the number of methods reported [9], as described in the formula

FIC Index= Qa/QA + Qb/QB

In this equation, QA represents the concentration of compound A (in PPM) required to reach the endpoint when used alone, Qa is the concentration of compound A (in PPM) required to acheive

the endpoint when used in combination with another compound. Similarly, QB reprents the concentration of compound B (in PPM) required to reach the endpoint when used alone, and Qb is the concentration of compound B (in PPM) required to achieve the endpoint when combined with compound A. The interpretation of the results is based on the following criteria: an FIC Index lesser than 1.0 indicates synergy, an FIC Index equal to 1.0 signifies an additive effect, and an FIC Index greater than 1.0 suggests antagonism

Cosmeceutical Body care formulations & Process [16-21]. : A total of twelve body care cosmeceutical formulations were prepared and tested, including:

I. Anti-acne body wash (AABW 1,2,3,4) with four different preservation strategies.
II. Anti-aging body lotion (AABL 1,2,3,4) with four different preservation strategies and
III. Anti-dry hydrating body cream (ADBC 1,2,3,4) with four different preservation strategies, were prepared as listed in the Table 2 A,B,C with conventional preservative* (positive control) code: AABW1, AABL1 and ADBC1, placebo base without preservative (negative control) code: AABW2, AABL2 and ADBC2, synergistic combination of multifunctional ingredients Sodium coco PG-dimonium chloride phosphate, ricinoleic acid and sorbitan caprylate (synergistic antimicrobial composition 1 : 6.3 : 10) at 0.4% and 0.6% in body wash AABW3 and AABW4; Sodium coco PG-dimonium chloride phosphate:ricinoleic acid ansd sorbitan caprylate (synergistic antimicrobial composition 1 : 6.3 : 12.5) at 0.4% and 0.6% in body lotion AABL3 and AABL4; sodium coco pg-dimonium chloride phosphate:ricinoleic acid: sorbitan caprylate (synergistic antimicrobial composition 1 : 6.3 : 25) at 0.4% and 0.6% in body cream ADBC3 and ADBC4 along with cosmeceutical actives **

Table 2A: Comeceutical body care product anti-acne body wash (AABW) formulations and Process

Cosmeceutical anti-acne body wash formulation (AABW 1,2,3,4)		
Phase	**Ingredients (INCI)**	**Dosage %**
A	Water	Q.S 100
	Sodium Laureth Sulphate	38.46
	Lauryl Lactyl Lactate	1
	PEG-7 Glyceryl Cocoate	1
B	Propylene Glycol	2
	Salicylic acid **	2
C	PEG-150 Distearate	0.75
D	Phenoxyethanol & Methyl Paraben & Ethyl Paraben & Butyl Paraben & Propyl Paraben & Isobutyl Paraben (positive control with conventional preservative) AABW1	0.8
	Placebo base without preservative (negative control without preservative) AABW2	0
	Sodium coco PG-dimonium chloride phosphate:ricinoleic acid: sorbitan caprylate (synergistic antimicrobial composition 1 : 6.3 : 10) AABW3	0.4
	Sodium coco PG-dimonium chloride phosphate:ricinoleic acid: sorbitan caprylate (synergistic antimicrobial composition 1 : 6.3 : 10) AABW4	0.6
E	Polquaternium-7	1
F	Fragrance	Qs
G	Sodium Hydroxide Solution	QS
H	Sodium Chloride Solution	Qs

Manufacturing Procedure

Phase A: In a suitable vessel, the ingredients of Phase A were charged in the specified order. The mixture was heated to a temperature of 45-50°C while continuously mixing to ensure thorough dispersion. Phase B: A premix was prepared by dissolving salicylic acid in propylene glycol. Once the salicylic acid was fully dissolved, Phase B was added to Phase A while maintaining the temperature at 45-50°C. The mixture was continuously stirred to achieve a uniform blend. Phase C: PEG-150 Distearate (Phase C) was gradually added to the combined Phase AB mixture. The mixture was agitated, maintaining the temperature at 45-50°C, and mixing continued until the solution became completely clear, ensuring the homogeneous incorporation of the components. Cooling:The mixture was cooled to room temperature while mixing continued at a steady pace to prevent any separation or inconsistent texture during the cooling process. Phase D & E: Once the temperature had dropped sufficiently, Phase D and Phase E ingredients were added to the cooled Phase ABC mixture. The components were fully integrated with the rest of the formulation through continued stirring. Phase G & H (pH and Viscosity Adjustment): As needed, pH and viscosity were adjusted using Phase G and Phase H ingredients. The mixture was stirred until the desired pH and viscosity levels were achieved, ensuring the final product consistency met the product specifications.

Table 2B: Comeceutical body care product anti-aging body lotion (AABL) formulations and Process

Cosmeceutical anti-aging body wash formulation (AABL 1,2,3,4)		
Phase	**Ingredients (INCI)**	**Dosage %**
A	Steareth-2	2.25
	Steareth-21	2.25
	Cetearyl Alcohol	2.5
	Dimethicone	1.5
	Tocopheryl Acetate	1
	Caprylic/Capric Triglyceride	6
	Butyrospermum Parkii (Shea) Butter	2.25
B	Water	Q.s to 100
	Xanthan Gum	1
	Glycerin	4
	Acrylates/C10-30 Alkyl Acrylate Crosspolymer	0.2
	Disodium EDTA	0.1
C	Niacinamide **	5
D	Phenoxyethanol & Methyl Paraben & Ethyl Paraben & Butyl Paraben & Propyl Paraben & Isobutyl Paraben (positive control with conventional preservative) AABL1	0.8
	Placebo base without preservative (negative control without preservative) AABL2	0
	Sodium coco PG-dimonium chloride phosphate:ricinoleic acid: sorbitan caprylate (synergistic antimicrobial composition 1 : 6.3 : 12.5) AABL3	0.4
	Sodium coco PG-dimonium chloride phosphate:ricinoleic acid: sorbitan caprylate (synergistic antimicrobial composition 1 : 6.3 : 12.5) AABL4	0.6
E	Fragrance	Qs
F	Potassium Hydroxide Solution	Qs

Manufacturing Procedure

Phase A (Oil Phase) and Phase B (Aqueous Phase) were heated separately to 80°C. Xanthan gum was dispersed into glycerin to form a slurry. This slurry was slowly poured into the vortex of water created by high-shear, rapid stirring in Phase B. Disodium EDTA and Acrylates/C10-30 Alkyl Acrylate Crosspolymer were added to Phase B while stirring continued. Once both Phase A and Phase B reached the same temperature of 80°C, Phase B was added to Phase A under continuous stirring to ensure proper emulsification. The mixture was then cooled to 50°C, at which point Phase C was added under both stirring and homogenization.Phase D and Phase E were added to the mixture at 40°C under stirring.Finally, the pH was adjusted as required using Phase F while maintaining continuous mixing.

Table 2C: Comeceutical body care product anti-dry hydrating body cream (ADBC) formulations and Process

Cosmeceutical anti-dry hydrating body cream formulation (ADBC 1,2,3,4)		
Phase	**Ingredients (INCI)**	**Dosage %**
A	Water	Q.S 100
	Disodium EDTA	0.1
	Glycerin	3
B	Isopropyl Myristate	4
	Caprylic/Capric Triglyceride	4
	Cetearyl Alcohol	5
	Glyceryl Stearate	3
C	Tocopheryl Acetate	0.5
	Propanediol	1
	Panthenol	0.5
D	Sodium Hyaluronate 1% **	0.5
E	Phenoxyethanol & Methyl Paraben & Ethyl Paraben & Butyl Paraben & Propyl Paraben & Isobutyl Paraben (positive control with conventional preservative) ADBC1	0.8
	Placebo base without preservative (negative control without preservative) ADBC2	0
	Sodium coco PG-dimonium chloride phosphate:ricinoleic acid: sorbitan caprylate (synergistic antimicrobial composition 1 : 6.3 : 37.5) ADBC3	0.4
	Sodium coco PG-dimonium chloride phosphate:ricinoleic acid: sorbitan caprylate (synergistic antimicrobial composition 1 : 6.3 : 37.5) ADBC4	0.6
F	Fragrance	0.5
G	Sodium hydroxide Solution	Q.S

Manufacturing Procedure

The ingredients of Phase A and Phase B were weighed separately and heated to 75°C. Phase A was then added to Phase B with continuous stirring, maintaining the temperature at 75°C. The mixture was homogenized for 10 minutes and cooled down to 40°C. The remaining ingredients from Phase C, D, E, and F were added one by one in order, stirring uniformly. Phase G was added while adjusting the pH. The mixture was then cooled down to room temperature while stirring.

Preservative Challenge Test

The Preservative Challenge Test (PCT) is utilized to evaluate a formulation's ability to preserve itself. For control purposes, base formulations containing preservatives are employed. Unfortunately, there

is no universally accepted method for challenge testing and interpreting results. Different pharmacopoeias prescribe various procedures, but for cosmetic products, the guidelines from the Cosmetic, Toiletries, and Fragrance Association (CTFA), now known as the Personal Care Products Council (PCPC) / ISO 11930, are followed. According to CTFA recommendations, the PCT involves a challenge study using pathogenic bacterial, yeast, and mold cultures. The microbial levels are assessed using a plate count method, which measures 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. Inoculated samples are evaluated at intervals of one, two, seven, fourteen, twenty-one, and twenty-eight days after inoculation. The growth of microorganisms (CFU/ml) is determined at each interval, and the percentage of microorganisms is calculated relative to the initial concentration.
In the preservative challenge test, additional relevant details include weighing 10 g of the sample material into separate sterile containers and adding a specified load of microorganisms. A mixed culture of three bacterial strains—*S. aureus, E. coli, P. aeruginosa*—and two fungal strains—*C. albicans and A. brasiliensis*—was prepared. An inoculum size of 15.2 x 10^6 CFU/ml was used for bacterial cultures, and 18 x 10^5 CFU/ml for fungal cultures. Ten microliters of each bacterial culture were added to the containers designated for bacteria, while 100 microliters of the fungal inoculum were added to the containers designated for fungi. The samples were kept at room temperature in sterile conditions. At each planned interval (1st, 2nd, 3rd, 7th, 14th, and 28th day), 1 g of sample was taken from the inoculated containers, mixed with 9 ml of sterile neutralizer—modified Letheen broth for bacterial sampling and Sabouraud dextrose broth for fungal sampling—and further diluted and plated separately.[22]

RESULTS & DISCUSSION

The Minimum Inhibitory Concentrations (MIC) of the three selected multifunctional ingredients—Sodium coco PG-dimonium chloride phosphate, ricinoleic acid, and sorbitan caprylate—along with the conventional preservative blend of Phenoxyethanol & Methyl Paraben & Ethyl Paraben & Butyl Paraben & Propyl Paraben & Isobutyl Paraben, were tested against five organisms: *Escherichia coli* (E. coli), *Pseudomonas aeruginosa* (P. aeruginosa), *Staphylococcus aureus* (S. aureus), *Candida albicans* (C. albicans), and *Aspergillus brasiliensis* (A. brasiliensis). These tests were performed using the macro broth double dilution method, and the results are presented in Table 1. The three multifunctional compounds exhibited strong antimicrobial activity compared to traditional preservatives commonly used in cosmetic body care products. The first component of the compositions was sodium coco PG-dimonium chloride phosphate, while ricinoleic acid and sorbitan caprylate were chosen as the second and third components to evaluate their potential synergistic interaction. Consequently, three compositions were formulated based on their MIC data.

Composition-1 included three ratio combinations of Sodium coco PG-dimonium chloride phosphate, ricinoleic acid, and sorbitan caprylate. These compositions were prepared in a variety of ratios. In two of the combinations, the concentration ratios of the first two components remained the same, while the concentration ratio of the third component was increased to at least 27.5 times the initial concentration. The concentration ranges were selected to create a cost-effective composition of the chosen ingredients.

Thus, the concentration ratio of the first component in the composition was set at 1. The concentration ratio of the second ingredient was 6.3, and the concentration ratio of the third ingredient was increased from 10 to 37.5.

Seventy-five different composition combinations were created and tested for their MIC against the five organisms mentioned earlier. Table 3 presents the MIC values of these combinations, highlighting the antimicrobial effectiveness of the synergistic mixture of multifunctional ingredients. The ternary combinations showed enhanced antimicrobial activity compared to the individual MIC values of the multifunctional compounds.

The Fractional Inhibitory Concentration (FIC) index was calculated for these combinations. Based on the FIC index data, three combinations were identified as synergistic, as indicated 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	Sodium Coco PG-dimonium Chloride Phosphate	125	125	125	62.5	250
2	Ricinoleic Acid	2000	2000	2000	2000	4000
3	Sorbitan Caprylate	2500	2500	2500	2500	2500
4	Phenoxyethanol & Methyl Paraben & Ethyl Paraben & Butyl Paraben & Propyl Paraben & Isobutyl Paraben *	500	500	250	500	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	Sodium Coco PG-dimonium Chloride Phosphate:Ricinoleic Acid: Sorbitan Caprylate (1 : 6.3 : 10)					
	MIC µg/ml	1000	250	500	500	2000
	FIC index	0.93	0.23	0.47	0.7	0.93
2	Sodium Coco PG-dimonium Chloride Phosphate:Ricinoleic Acid: Sorbitan Caprylate (1 : 6.3 : 12.5)					
	MIC µg/ml	1000	1000	500	250	2000
	FIC index	0.88	0.88	0.44	0.32	0.88
3	Sodium Coco PG-dimonium					

	Chloride Phosphate:Ricinoleic Acid: Sorbitan Caprylate (1 : 6.3 : 37.5)					
	MIC µg/ml	500	1000	1000	1000	1000
	FIC index	0.29	0.58	0.58	0.76	0.46

* conventional preservative

PRESERVATIVE CHALLENGE TEST- Evaluation of preservative efficacy of the cosmeceutical formulations as per PCPC/ISO 11930 Guidelines [23]

Twelve body care cosmeceutical formulations- anti-acne body wash (AABW 1,2,3,4) , anti-aging body lotion (AABL 1,2,3,4), and anti-dry hydrating body cream (ADBC 1,2,3,4) were prepared as listed in the Table 2A, B, and C, with the conventional preservative (positive control) coded as: AABW1,AABL1, and ADBC1. The placebo base without preservative (negative control) was coded as: AABW2,AABL2, and ADBC2, while the synergistic combination of multifunctional ingredients at different dosages along with cosmeceutical actives (AABW3,AABL3 & ADBC3, AABW4, AABL4, and ADBC4). All 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 4A-4C.

Table 4A: Preservative efficacy testing of selected antimicrobial of the developed cosmeceutical body care products- Anti-acne body wash (AABW1,2,3 &4)

Methodology : Mixed Culture Challenge											
Organisms Challenged: *S.aureus + E.coli + P.aeruginosa + C.albicans + A.brasiliensis*											
Challenge dose: Bacterial Load = $15.2x10^6$CFU/ml; Fungal Load = $18x10^5$ CFU/ml											
Anti-acne Body wash composition with the most active synergistic mixture of Sodium Coco PG-dimonium Chloride Phosphate:Ricinoleic Acid: Sorbitan Caprylate											
Combination-1 Ratio 1 - 1: 6.3:10											
Ex. No	AABW3 & AABW4			Usage of % in formulation	Bacterial Count (CFU/ml)						
					D1	D2	D3	D7	D14	D21	D28
1	1	6.3	10	0.4	$2x10^2$	50	<10	<10	<10	<10	<10
2	1	6.3	10	0.6	210	<10	<10	<10	<10	<10	<10
3	Positive Control (with preservative) AABW1			0.8	80	<10	<10	<10	<10	<10	<10
4	Negative Control(without preservative) AABW2			0	$21x\ 10^4$	$6x10^3$	$3x10^3$	$2x10^3$	$9x10^2$	90	<10
Ex. No	AABW3 & AABW4			Usage of % in formulation	Fungal Count (CFU/ml)						
					D1	D2	D3	D7	D14	D21	D28
1	1	6.3	10	0.4	340	20	<10	<10	<10	<10	<10
2	1	6.3	10	0.6	30	<10	<10	<10	<10	<10	<10
3	Positive Control (with preservative) AABW1			0.8	<10	<10	<10	<10	<10	<10	<10
4	Negative Control(without preservative) AABW2			0	$15x10^3$	$6x\ 10^3$	$4x10^2$	910	70	<10	<10

Table 4B: Preservative efficacy testing of selected antimicrobial of the developed cosmeceutical body care products- Anti-aging body lotion (AABL1,2,3 &4)

Methodology : Mixed Culture Challenge											
Organisms Challenged: *S.aureus* + *E.coli* + *P.aeruginosa* + *C.albicans* + *A.brasiliensis*											
Challenge dose: Bacterial Load = $15.2x10^6$CFU/ml; Fungal Load = $18x10^5$ CFU/ml											
Anti-aging Body lotion composition with the most active synergistic mixture of Sodium Coco PG-dimonium Chloride Phosphate:Ricinoleic Acid: Sorbitan Caprylate											
Combination-1 Ratio 1 - 1: 6.3:12.5											
Ex. No	AABL3 & AABL4			Usage of % in formulation	Bacterial Count (CFU/ml)						
					D1	D2	D3	D7	D14	D21	D28
1	1	6.3	12.5	0.4	$2x10^2$	40	<10	<10	<10	<10	<10
2	1	6.3	12.5	0.6	200	<10	<10	<10	<10	<10	<10
3	Positive Control (with preservative) AABL1			0.8	80	<10	<10	<10	<10	<10	<10
4	Negative Control(without preservative) AABL2			0	$20x\ 10^4$	$5x10^3$	$3x10^3$	$2x10^3$	$7x10^2$	50	<10
Ex. No	AABL3 & AABL4			Usage of % in formulation	Fungal Count (CFU/ml)						
					D1	D2	D3	D7	D14	D21	D28
1	1	6.3	12.5	0.4	370	10	<10	<10	<10	<10	<10
2	1	6.3	12.5	0.6	60	<10	<10	<10	<10	<10	<10
3	Positive Control (with preservative) AABL1			0.8	<10	<10	<10	<10	<10	<10	<10
4	Negative Control(without preservative) AABL2			0	$17x10^3$	$7x\ 10^3$	$5x10^2$	990	90	<10	<10

Table 4C: Preservative efficacy testing of selected antimicrobial of the developed cosmeceutical body care products- Anti-dry hydrating body creamn (ADBC1,2,3 &4)

Methodology : Mixed Culture Challenge			
Organisms Challenged: *S.aureus* + *E.coli* + *P.aeruginosa* + *C.albicans* + *A.brasiliensis*			
Challenge dose: Bacterial Load = $15.2x10^6$cfu/ml; Fungal Load = $18x10^5$ cfu/ml			
Anti-dry Hydrating Bodycream composition with the most active synergistic mixture of Sodium Coco PG-dimonium Chloride Phosphate:Ricinoleic Acid: Sorbitan Caprylate			
Combination-2 Ratio 2 - 1: 6.3:25			
Ex.	ADBC3& ADBC4	Usage of %	Bacterial Count (CFU/ml)

No				in formulation	D1	D2	D3	D7	D14	D21	D28
1	1	6.3	25	0.4	$2x10^2$	30	<10	<10	<10	<10	<10
2	1	6.3	25	0.6	290	<10	<10	<10	<10	<10	<10
3	Positive Control (with preservative) ADBC1			0.8	50	<10	<10	<10	<10	<10	<10
4	Negative Control(without preservative) ADBC2			0	$22x\ 10^4$	$5x10^3$	$4x10^3$	$3x10^3$	$8x10^2$	70	<10
Ex. No	ADBC3& ADBC4			Usage of % in formulation	Fungal Count (CFU/ml)						
					D1	D2	D3	D7	D14	D21	D28
1	1	6.3	25	0.4	60	<10	<10	<10	<10	<10	<10
2	1	6.3	25	0.6	<10	<10	<10	<10	<10	<10	<10
3	Positive Control (with preservative) ADBC1			0.8	<10	<10	<10	<10	<10	<10	<10
4	Negative Control (without preservative) ADBC1			0	$12x10^3$	$5x\ 10^3$	$3x10^2$	60	20	<10	<10

Our study observed that when base formulations of anti-acne body wash (AABW3 and AABW4), anti-aging body lotion (AABL3 and AABL4), and anti-dry hydrating body cream (ADBC3 and ADBC4) were incorporated with synergistic multifunctional ingredients, the preservative efficacy profile was comparable to formulations using conventional preservatives (control) (AABW1, AABL1, and ADBC1) in the preservative challenge test. The results indicate that the synergistic composition, when incorporated at 0.4% and 0.6% levels for AABW1, AABL1, and ADBC1, delivers preservative efficacy (PASS) as per PCPC/ISO 11930 standards.[24,25]

The combination of three antimicrobial multifunctional ingredient mixtures, when incorporated at 0.4% and 0.6% levels for AABW1, AABL1, and ADBC1, provided preservative efficacy equivalent to conventional preservatives. Importantly, all dosage levels meet regulatory requirements. As shown in Tables 4A, 4B, and 4C, the three synergistic combinations impart antimicrobial preservative potency to the formulations of different cosmeceutical body care products, equivalent to conventional preservatives such as Phenoxyethanol & Methyl Paraben & Ethyl Paraben & Butyl Paraben & Propyl Paraben & Isobutyl Paraben dosed at 0.8% in anti-acne body wash (AABW1), anti-aging body lotion (AABL1), and anti-dry hydrating body cream (ADBC1). Therefore, it can be concluded that the formulations with the unique synergistic mixtures were preserved as effectively as those with conventional preservatives. The unique synergistic combination of multifunctional ingredients can be an alternative solution for preserving cosmeceutical products from microbial attack. These ingredients are skin-friendly and preferred by consumers. This innovative approach to cosmeceutical product preservation helps avoid the use of conventional preservatives, which might cause skin allergies, irritation, or contact sensitivity.[26,27,28,29]

Many cosmeceutical products are complex compositions made up of various components that provide beneficial properties while also giving structural uniqueness to the product. Therefore, formulators aim to use the fewest components necessary to provide the maximum benefit. Managing microbial deterioration is an important requirement 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, limiting the number of available chemistries.

CONCLUSION

Through the analysis of Minimum Inhibitory Concentration (MIC) values, three distinct Multifunctional Ingredients (MFIs)—Sodium Coco PG-dimonium Chloride Phosphate, Ricinoleic Acid, and Sorbitan Caprylate—were identified. Seventy-five different combinations of these MFIs in various ratios were created and tested to explore their synergistic effects. By examining the MIC values and calculating the Fractional Inhibitory Concentration (FIC) index for these MFIs and their combinations, three synergistic antimicrobial formulations were determined. The combinations of Sodium Coco PG-dimonium Chloride Phosphate: Ricinoleic Acid: Sorbitan Caprylate in the ratios of 1:6.3:10, 1:6.3:12.5, and 1:6.3:25 showed significant synergistic interactions, with each combination having a lower MIC than its individual components.

These synergistically active formulations were then integrated into three different cosmeceutical body care products at concentrations of 0.4% and 0.6%. These treated formulations were compared with those containing traditional preservatives and those without any preservatives. All three antimicrobial formulations successfully preserved the cosmetic products for up to 28 days, as shown in the Preservative Challenge Test (PCT). This innovative preservation method minimizes the need for preservatives that could cause skin irritation or contact sensitivity. Thus, this study demonstrates that the strategic use of multifunctional actives can enable the development of self-preserving cosmeceutical formulations, ensuring protection against microbial contamination without relying on harmful preservatives.

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Conflict of Interest

The authors declare no conflicts of interest.

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