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Preparation of self-preserving personal care cosmetic products using multifunctional ingredients and other cosmetic ingredients

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The development of self-preserving personal care cosmetics represents a significant advancement in the cosmetics industry, offering safer and more natural alternatives to consumers. This study focused on the preparation of such formulations using multifunctional ingredients along with other cosmetic components. Five unique multifunctional ingredients (MFIs) were identified based on their antimicrobial properties: sodium coco PG-dimonium chloride phosphate, ricinoleic acid, palmitoleic acid, raspberry ketone, and sorbitan caprylate. Through meticulous experimentation, 150 combinations of MFIs were prepared and tested to understand their synergistic actions. From these trials, three synergistic antimicrobial compositions were determined: sodium coco PG-dimonium chloride phosphate: ricinoleic acid: raspberry ketone in the ratios 1:6.3:6.3 and 1:6.3:15.7. Sodium coco PG-dimonium chloride phosphate: palmitoleic acid: sorbitan caprylate at a ratio of 1:12.5:37.5. These synergistic compositions exhibited enhanced antimicrobial efficacy compared with their individual components, as evidenced by their lower Minimum Inhibitory Concentration values. Incorporating these formulations into three distinct personal care cosmetic products, including a color protection shampoo, body wash shower gel, and skin-lightening cream, the study further validated their effectiveness. A Preservation Challenge Test study revealed that all three antimicrobial compositions successfully preserved the cosmetic formulations for up to 28 days. This method of product preservation not only ensures consumer safety and stability but also reduces the need for potentially conventional preservatives. In conclusion, the appropriate use of multifunctional ingredients in combination with meticulous formulation techniques has led to the successful development of self-preserving personal care cosmetics. These formulations offer a promising avenue for the cosmetic industry, catering to the rising demand for natural, effective, and consumer-friendly cosmetic products.

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Preservatives are used in cosmetic formulations to prevent microbiological contamination during many stages of production, processing, packaging, and storage. Their primary purpose is to prolong the shelf life of the product and safeguard consumers from potential health risks[1,2]. Preservatives are a prevalent trigger for human allergies, despite evidence that they are frequently employed at low levels[3]. In recent times, cosmetic companies have shifted their attention towards producing preservative-free products, which involve the creation of cosmetic items without the use of any substances that prevent the growth of microorganisms. Preservative-free products available in the market often include antimicrobial compounds that have not been recognized as preservatives by the European Scientific Committee. These compounds are not listed in Annex VI of the Commission Directive 76/768/EEC or in the improving directives codes 2003/15/EC, 2007/17 EC, and 2007/22/EC. These directives allow the use of authorized and traditional preservatives in cosmetic preparations[4–6]. Hence, the phrase 'self-preserving cosmetics' is more suitable for these types of products. Conventional preservatives are substituted by various cosmetic chemicals in self-preserving formulations. These alternative substances not only serve their main purpose, but also possess antibacterial properties. These chemicals are sometimes referred to as 'Multi-functional' ingredients (MFI)[7–10].

We explored the synergistic actions of MFI in the preparation of combinations for use as self-preserving ingredients. The present study explored the use of identified synergistic combinations of MFI to develop self-preserving cosmetic personal care products and to assess the microbial safety of the formulation. Furthermore, this study presents a comparative analysis, pitting self-preserved formulations against control formulations preserved using conventional preservatives. The efficacy and stability of the self-preserving formulations were evaluated through rigorous testing, including Preservation Challenge Tests (PCT). This comparison aimed to provide a comprehensive understanding of the suitability of self-preserving formulations for commercial applications, highlighting their potential advantages over traditional preservatives in the cosmetics industry.

Materials and methods

The multifunctional cosmetic ingredients listed in Table 1 and other cosmetic ingredients, including preservatives used in this study were obtained from various reputed dealers and suppliers which includes Brenntag Ingredients Pvt. Ltd., India; Ashland Pvt. Ltd., India; Merck Specialities Pvt. Ltd., India; Gangwal Chemicals 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, India; Simson Pharma, India; NK Industries Ltd., India and BASF India.

Microbial strains

The specific microbial culture strains recommended for the screening studies were the same as the standard microbial culture strains to be studied for preservative challenge testing, which can be obtained from official cell culture collections, such as the American Type Culture Collection (ATCC), as recommended by the Personal

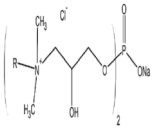
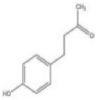
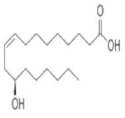
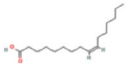
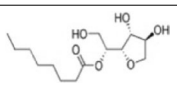
S. No.	Multifunctional ingredients INCI name	Structure	Form	Benefits	Vendor/supplier
1	Sodium coco PG-dimonium chloride phosphate		Liquid	Surfactant multifunctionality, broad antimicrobial enhancement	Brenntag ingredients India Pvt. Ltd., Mumbai
2	Raspberry ketone		Powder	Broad antimicrobial boosting effect, strong antioxidant and free radical scavenger	Ashland India Pvt. Ltd., Mumbai
3	Ricinoleic acid		Liquid	Moisturizer, anti-inflammatory, antimicrobial	NK Industries Ltd., Gujarat
4	Palmitoleic acid		Liquid	Emollient, moisturizer, antimicrobial	Simson pharma, Mumbai
5	Sorbitan caprylate		Liquid	Rheology modifier, emulsifier and preservative booster	Clariant IndiaLtd., Mumbai

Table 1. Multifunctional ingredients, structure, form, benefits and vendor.

Care Products Council (PCPC) of the United States, and were obtained from Microbiologics Inc., USA. The most common test strains used in this study were potentially pathogenic Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538), Gram-negative bacteria (*Escherichia coli* ATCC 8379 and *Pseudomonas aeruginosa* ATCC 9027), mold (*Aspergillus brasiliensis* ATCC 16404), and yeast (*Candida albicans* ATCC 10231).

Inoculation of samples

The inoculum was used to introduce microorganisms into the test samples following adjustment of the initial cell count. The Bacterial cultures were introduced into tryptone soy agar slants and incubated for 18–24 h at a temperature of 36 ± 1 °C. The fungal strains were introduced onto Sabouraud dextrose agar and kept in a controlled environment at a temperature of 23 ± 1 °C for a period of five to seven days. The cultures were collected in sterile saline and diluted to a concentration of 1×10^8 CFU/ml.

Screening of multifunctional ingredients with anti-microbial efficacy

Different cosmetically approved ingredients from biomimetic phospholipids, esters, emollients, sugars, polysaccharides, fatty acids, surfactants, chelating agents, anti-oxidants, microbial preservative boosters, glycols, moisturizers, and multifunctional actives were assessed for their MIC—minimal inhibitory concentrations (MICs) against the microbial strains. In total, approximately five ingredients and 150 ternary combinations were studied. Conventional preservatives approved for use in cosmetics were also studied as controls. The tests were performed in quadruplicate and the average scores were determined.

Determination of minimal inhibitory concentration and FIC index

The MIC refers to the lowest concentration of an antimicrobial agent that completely inhibits growth in a specified tube. Antimicrobial properties were assessed using the MIC macrodilution method for both antibacterial and antifungal properties, according to CLSI guidelines[11]. The test materials were dissolved in the appropriate broth at varying concentrations, both individually and in combination, and the concentrations of antimicrobial agents providing significant endpoints were recorded. The tests were performed in quadruplicate and the average values were determined.

To establish synergy/additive/antagonistic activity of anti-microbial agents, the FIC index endpoints of the anti-microbial agents were calculated both individually and in mixtures. Freshly grown 24 h bacterial cultures and 120 h fungal cultures were used as inoculums. The turbidity of the inoculum was adjusted to 0.5 McFarland standard with sterile saline or Soybean Casein Digest Medium for bacteria, and Sabouraud dextrose agar for fungal cultures was used to obtain an inoculum size of $1–2 \times 10^8$ CFU/ml for bacterial cultures and $1–2 \times 10^6$ CFU/ml. Stock solutions of antimicrobial agents must be prepared at concentrations of at least 1000 mg/ml or ten times the highest concentration to be tested, whichever is greater.

Suitable anti-microbial concentrations were diluted twofold (1000, 500, 250, 125, and 62.5 mg) using the macro dilution method, and inoculums were added to separate tubes for each bacterial culture and fungal culture. For each tested organism, a control tube containing a broth without antimicrobial agents was used. All the inoculated tubes were incubated for 24 h at 35 ± 2 °C. All experiments were performed in triplicate or quadruplicate. The culture strains are shown in Fig. 1.

FIC index

This is calculated by multiplying the synergy index ratio by the number of reported methods[12].

$$\text{FIC index} = Qa/QA + Qb/QB$$

where QA is the concentration of compound A in the PPM that produces an endpoint when acting alone, and Qa denotes the concentration of compound A in the PPM in the mixture, resulting in an endpoint. QB is the

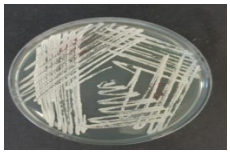
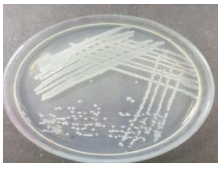
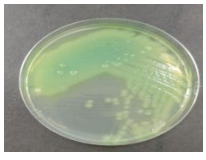
a. Gram-positive bacteria *Staphylococcus aureus* ATCC 6538	b. Gram-negative bacteria *Escherichia coli* ATCC 8379	c. Gram-negative bacteria *Pseudomonas aeruginosa* ATCC 9027	d. Mold *Aspergillus brasiliensis* ATCC 16404	e. Yeast *Candida albicans* ATCC 10231
				

Figure 1. Different culture strains.

concentration of compound B in PPM that produces an endpoint when acting alone, and Qb is the concentration of compound B in PPM in the mixture, which results in an endpoint result. Results were interpreted using the following criteria: (1) synergy < 1.0, (2) additive effect = 1.0, and (3) antagonism > 1.0.

Cosmetic personal care formulations and Process[8,13–16]: Twelve personal care cosmetic formulations were prepared.

I. Color-protection shampoo (CPS 1, 2, 3, 4) using four different preservation strategies.
II. Body wash shower gels (BWSG 1, 2, 3, and 4) with four different preservation strategies.
III. Skin lightening cream (SLL 1, 2, 3, 4) with four different preservation strategies, were prepared as listed in the Table 2 with conventional preservative* (positive control) code: CPS1, BWSG1 & SLL1, placebo base without preservative (negative control) code:CPS2, BWSG2 & SLL2, synergistic combination of multifunctional ingredients Sodium coco PG-dimonium chloride phosphate, ricinoleic acid and raspberry ketone, synergistic antimicrobial composition 1: 6.3: 6.3 at 0.5% and 1% in colour protection shampoo CPS3 & CPS4, Sodium coco PG-dimonium chloride phosphate, ricinoleic acid and raspberry ketone, synergistic antimicrobial composition 1: 6.3: 15.7 at 0.5% and 2% in body wash shower gel BWSG3 & BWSG4 and Sodium coco PG-dimonium chloride phosphate, palmitoleic acid and Sorbitan caprylate synergistic antimicrobial composition 1: 12.5: 37.5 at 1% and 2% in skin lightening lotion SLL3 &SLL4 along with special cosmetic actives **

Preservative challenge test

The preservative challenge test (PCT) has been used to evaluate the capacity of a product to maintain its preservation. For comparison, base formulations containing preservatives were used as the controls. Regrettably, there is currently no universally recognized method for conducting challenge testing and interpreting outcomes. Various pharmacopoeias recommend different methods; however, for cosmetic products, the CTFA (cosmetic, toiletries, and fragrance association), now the Personal Care Products Council (PCPC)/ISO 11930 criteria, are used. According to CTFA guidelines, PCT involves solitary exposure to pathogenic bacteria, yeast, and mold cultures. The plate count method was employed to assess microbial counts by quantifying the initial concentration of colony-forming units per milliliter (CFU/mL) in the test product by enumerating viable bacteria in the inoculum suspension. The inoculated vessels were analyzed at specific time intervals (one, two, seven, fourteen, twenty-one, and twenty-eight-days after inoculation). The number of microorganisms present in each vessel (measured in colony-forming units per milliliter, CFU/mL) was determined at each time point. The percentage of bacteria was calculated relative to the initial concentration[12].

The preservative challenge test was performed with additional details, in which 10 g of the sample was weighed in 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, A. brasiliensis* were prepared. An inoculum size of 11×10^6 CFU/ml was created for bacterial and 15×10^5 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 fungal-marked container separately and left at room temperature under sterile environmental conditions. At each predefined time interval (1st, 2nd, 3rd, 7th, 14th, and 28th days), 1 g of sample was weighed and mixed with 9 ml of neutralizer (modified letheen broth), and further dilutions were made for analysis. One milliliter of each dilution was plated on 15–20 ml of molten agar using the pour plate technique and appropriate growth medium for bacteria and fungi.

Results and discussion

The MIC of the selected five multifunctional ingredients, sodium coco PG-dimonium chloride phosphate, raspberry ketone, ricinoleic acid, palmitoleic acid, and sorbitan caprylate, and two conventional preservatives, methylchloroisothiazolinone and methylisothiazolinone and phenoxyethanol and methyl paraben and ethyl paraben and butyl paraben and propyl paraben and isobutyl paraben, against five organisms *Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa)*, *Staphylococcus aureus (S. aureus),Candida albicans (C. albicans), Aspergillus brasiliensis (A. brasiliensis)* were tested based on the macro bath double dilution method and are tabulated in Table 1. The five selected multifunctional ingredients showed good anti-microbial activity compared to the conventional preservatives normally used in cosmetic personal care products. Sodium coco PG-dimonium chloride phosphate, which has a cationic charge, exhibited a low MIC value. However, this ingredient is costly compared with other ingredients; hence, we decided to maintain its concentration at a minimum level and use it as the first ingredient of the three component compositions prepared. Two ingredients, ricinoleic acid and palmitoleic acid, were selected as the second components, and two other ingredients, raspberry ketone and sorbitan caprylate, were selected as the third components of each composition. Thus, compositions were prepared and tested to determine their ability to aid synergistic interactions. Two of the three components were prepared based on MIC data.

Composition-1 (prepared in two different ratios) was composed of sodium coco PG-dimonium chloride phosphate, ricinoleic acid, and raspberry ketone. Composition-2 was made up of sodium coco PG-dimonium chloride phosphate, palmitoleic acid, and sorbitan caprylate. The two components of these compositions were prepared in various ratios. The ratio of the concentration of the second ingredient was doubled, whereas the concentration of the third component was at least twenty-eight times the initial concentration. This concentration range was chosen to achieve an economically viable composition for the selected ingredients. The concentration

Colour protection shampoo composition (CPS 1,2,3,4)			Body wash shower gel (BWSG1,2,3,4)			Skin lightening lotion (SLL 1,2,3,4)		
Phase	**INCI Name**	**Dosage (%)**	**Phase**	**INCI Name**	**Dosage (%)**	**Phase**	**INCI Name**	**Dosage (%)**
A	Water	Q.S 100	A	Water	Q.S 100	A	Steareth-2	2.00
	Acrylates/c10-30 alkyl acrylate crosspolymer	0.30		Xanthan gum	1.50		Steareth-21	2.00
B	Sodium laureth sulfate	45.00		Propylene gycol	2.00		Cetearyl alcohol	2.00
C	Lauroyl/myristoyl methyl glucamide	5.00		PEG-7 glyceryl cocoate	1.00		Dimethicone	0.50
	Cocamidopropyl betaine	3.00	B	Cocamidopropyl betaine	7.50		Tocopheryl acetate	1.00
D	Water	15.00		Sodium laureth sulfate	25.00		C12-15 alkyl benzoate	4.00
	Hydroxypropyl Guar hydroxypropyltrimonium chloride	0.20		Jojoba esters **	0.25		Butyrospermum parkii (shea) butter	2.00
E	Trideceth-9 PG-amodimethicone and trideceth-12	1.00		Phenoxyethanol &methyl paraben &ethyl paraben &butyl paraben&propyl paraben &isobutyl paraben*(positive control with conventional Preservative) BWSG 1	0.80		77	0.50
				Placebo base without preservative (negative control without preservative) BWSG 2	0			
				Sodium coco PG-dimonium chloride phosphate, ricinoleic acid and raspberry ketone (synergisticantimicrobial composition 1: 6.3: 15.7) BWSG 3	0.50			
				Sodium coco PG-dimonium chloride phosphate, ricinoleic acid and raspberry ketone (synergisticantimicrobial composition 1: 6.3: 15.7) BWSG 4	2.00			
	Dimethiconol & TEA-dodecylbenzenesulfonate	2.00	C	Polquaternium-7	1.00		BHT	0.05

Continued

Colour protection shampoo composition (CPS 1,2,3,4)			Body wash shower gel (BWSG1,2,3,4)			Skin lightening lotion (SLL 1,2,3,4)		
Phase	INCI Name	Dosage (%)	Phase	INCI Name	Dosage (%)	Phase	INCI Name	Dosage (%)
F	Water	2.00	D	Fragrance	QS		Phenoxyethanol & methyl paraben & ethyl paraben & butyl paraben & propyl paraben & isobutyl paraben*(Positive control with Conventional Preservative) SLL 1	0.80
	Benzophenone-4 **	0.20	E	Citric Acid Solution	QS		Placebo base without preservative (negative control without preservative) SLL 2	0
							Sodium coco PG-dimonium chloride phosphate, palmitoleic acid and Sorbitan caprylate (syngeristic antimicrobial composition 1: 12.5: 37.5) SLL 3	1.00
							Sodium coco PG-dimonium chloride phosphate, palmitoleic acid and sorbitan caprylate (synergistic antimicrobial composition 1: 12.5: 37.5) SLL 4	2.00
						B	Water	Q.s to 100
G	Aqua (and) glycol Distearate (and) laureth-4	3.00	Manufacturing Procedure: Phase A, disperse Xanthan gum into propylene glycol and PEG-7 Glyceryl cocoate to form a slurry, and then pour slowly into the vortex of water created by high shear rapid stirring. Continue mixing for 5 min at ambient temperature. Add Phase B ingredients in the order listed to Phase A and Mix. Add Phase C, D and mix Adjust pH with Phase E as required				Xanthan gum	0.20
H	Methylchloroisothiazolinone (and) methylisothiazolinone * (positive control with conventional preservative) CPS 1	0.10						
	Placebo base without preservative (negative control without preservative) CPS 2	0						
	Sodium coco PG-dimonium chloride phosphate, ricinoleic acid and raspberry ketone (synergistic antimicrobial composition 1: 6.3: 6.3) CPS 3	0.50						
	Sodium coco PG-dimonium chloride phosphate, ricinoleic acid and raspberry ketone (synergistic antimicrobial composition 1: 6.3: 6.3) CPS 4	1.00					Glycerin	5.00
I	Fragrance	Q.S					Disodium EDTA	0.10
J	Citric acid/sodium hydroxide	Q.S				C	Hyaluronic Acid **	2.50

Continued

Colour protection shampoo composition (CPS 1,2,3,4)			Body wash shower gel (BWSG1,2,3,4)			Skin lightening lotion (SLL 1,2,3,4)		
Phase	INCI Name	Dosage (%)	Phase	INCI Name	Dosage (%)	Phase	INCI Name	Dosage (%)
K	Sodium Chloride	Q.S				D	Fragrance	Qs
Manufacturing Procedure: Phase A Sprinkle polymer on the surface of the water. When fully hydrated, stir for 30 min. Add phase B to A and stir until solubilized. Add Phase C and stir until solubilized. Add phase D pre dispersed cationic polymer in water and stir well. Add remaining pre-mixed phases E & F in the above order while stirring. Add Phase H, I and mix. Adjust pH with Phase J as required. Adjust viscosity with phase K while stirring slowly						Manufacturing Procedure: Heat part A (oil phase) and part B aqueous phase (disperse Xanthan gum into glycerin to form a slurry, and then pour slowly into the vortex of water created by high shear rapid stirring) to 80℃. When both phases reach same temperature 80℃ add part B to part A under stirring. Down to 50℃, Add the part C under Stirring and homogenization. Add the part D under Stirring at 40℃.Cool down the emulsion to room temperature while stirring		

Table 2. Personal care products colour protect shampoo (CPS), body wash shower gel (BWSG) and skin lightening Lotion (SLL) cosmetic formulations and Process.

ratio of the composition of the first mentioned ingredient increased from 0.5 to 1. The concentration ratio of the secondary ingredients increased from 6.3 to 12.5. The concentration ratio of the third component increased from 1.3 to 37.5.

Seventy-five combinations of each composition were prepared and tested for MIC. The 150 combinations were screened for MIC values against five organisms, as described above. Table 3 shows the MIC data for the two synergistic compositions of multifunctional ingredients with antimicrobial efficacy. The synergistic combination of ternary combinations showed better antimicrobial efficacy than the individual MICs of the multifunctional ingredients. The FIC indices of the combinations were calculated based on the FIC index data, and three combinations were identified as synergistic (Table 3).

Preservative challenge test

Evaluation of the preservative efficacy of cosmetic formulations per CPC/ISO 11930 Guidelines[17]. Twelve personal care cosmetic formulation color-protection shampoos (CPS 1, 2, 3, 4), body wash shower gel (BWSG 1, 2, 3, 4), and skin lightening cream (SLL 1, 2, 3, 4) were prepared as listed in Table 2 with conventional preservative (positive control) codes CPS1, BWSG1, and SLL1, placebo base without preservative (negative control) codes

MIC data of multifunctional ingredients with antimicrobial efficacy						
		Challenged Organisms				
		Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Candida albicans*	*Aspergillus brasiliensis*
S. No	Ingredients	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	Raspberry ketone	2000	2000	2000	2000	4000
4	Palmitoleic acid	1250	1250	2500	2500	2500
5	Sorbitan caprylate	2500	2500	2500	2500	2500
6	Methylchloroisothiazolinone & methylisothiazolinone *	500	250	125	1000	250
7	Phenoxyethanol &methyl paraben & ethyl paraben &butyl paraben & propyl paraben &isobutyl paraben *	500	500	250	500	1000
MIC and FIC data of two synergistic composition of multifunctional ingredients with antimicrobial efficacy						
		Challenged organisms				
S. No.	Composition, ratio, MIC µg/ml & FIC index	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Candida albicans*	*Aspergillus brasiliensis*
1	Sodium coco PG-dimonium chloride: Ricinoleic acid: Raspberry ketone (1: 6.3: 6.3)					
	MIC µg/ml	500	187.5	125	187.5	250
	FIC index	0.53	0.2	0.13	0.31	0.13
	Sodium coco PG-dimonium chloride: Ricinoleic acid: Raspberry ketone (1: 6.3: 15.7					
	MIC µg/ml	125	125	187.5	250	125
	FIC index	0.1	0.1	0.15	0.29	0.05
2	Sodium coco PG-dimonium chloride phosphate: palmitoleic acid:sorbitan caprylate(1: 12.5: 37.5)					
	MIC µg/ml	125	125	187.5	250	125
	FIC index	0.56	0.22	0.17	0.22	0.2

Table 3. MIC data of multifunctional ingredients & two synergistic compositions of multifunctional ingredients & FIC index of two synergistic composition of multifunctional ingredients with antimicrobial efficacy.

CPS2, BWSG2, and SLL2 synergistic combination of multifunctional ingredients at different dosages along with special cosmetic activities (CPS3, BWSG3, SLL3, CPS4, BWSG4, and SLL4). All 12 formulations were evaluated for the preservative challenge test according to the CPC/ISO 11930 guidelines for 28 d. The results of the preservative challenge tests are presented below.

In our study, when the base formulation of shampoo CPS3 & 4, shower gel BWSG 3 & 4, and lotion SLL3 & 4 were incorporated with the synergistic multifunctional ingredients, the preservative efficacy profile was found to be similar to that of formulations incorporated with conventional preservatives (control) CPS1, BWSG1, and SLL1 formulations in the preservative challenge test. The results indicate that the synergistically acting composition when incorporated at 0.5% and 1% levels for CPS1, 0.5% and 2% levels for BWSG1, and 1% and 2% for SLL1 levels delivers (PASS) preservative efficacy as per PCPC/ISO 11930 standards[18,19].

The combination of three antimicrobial multifunctional ingredient mixtures at the above given ratios when incorporated at 0.5% and 1% levels for CPS1, 0.5% and 2% levels for BWSG1, and 1% and 2% for SLL1 levels imparts preservative efficacy equivalent to conventional preservatives, most importantly, at all dosage quantities, meets the regulatory requirements. From Table 4, it is evident that the three synergistic combinations were able to impart antimicrobial preservative potency to different cosmetic personal care product compositions equivalent to conventional preservatives (methylchloroisothiazolinone and methylisothiazolinone dosed at 0.1% in color protection shampoo (CPS1), phenoxyethanol, methyl paraben, ethyl paraben, butyl paraben, propyl paraben, and isobutyl paraben dosed 0.8% in body wash shower gel (BWSG1) and lightening lotion (SLL1). Therefore, it can be concluded that the formulators incorporating the unique synergistic mixtures were well preserved and equivalent to conventional preservatives. The unique synergistic combination of multifunctional ingredients can be an alternative solution to protect cosmetic products from microbial attack, which are skin-friendly and preferred by consumers. This smart approach to cosmetic product preservation helps avoid the use of conventional preservatives, which may cause skin allergies, irritation, or contact sensitivity.

Many cosmetic products have complicated compositions that comprise a diverse range of materials that provide beneficial properties to the substrate while also providing structural identity to the product. Consequently, the formulator's ingredient selection would be to use the minimum amount of materials necessary to provide the most beneficial effect. An essential criterion that formulators should consider during formulation development is the control of microbial deterioration. This is usually accomplished through the addition of appropriate preservatives. Preservative selection and dosing in cosmetic products are mandated by legislation and are limited by the number of chemistries available[20].

In our study, when the base formulation of shampoo CPS 3 & 4, shower gel BWSG 3 & 4, and lotion SLL 3 and 4 were incorporated with the synergistic multifunctional ingredients, the preservative efficacy profile was found to be similar to that of formulations incorporated with conventional preservatives(control) CPS1, BWSG1, and SLL1 formulations in the preservative challenge test. The results indicate that the synergistically acting composition when incorporated at 0.5% and 1% levels for CPS1, 0.5% and 2% levels for BWSG1, and 1% and 2% for SLL1 levels delivers (PASS) preservative efficacy as per PCPC/ISO 11930 standards[18,19].

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Methodology: mixed culture challenge																		
Organisms challenged: Bacterial—*S. aureus* + *E. coli* + *P. aeruginosa* Fungal—*C. albicans* + *A. brasiliensis*																		
Challenge dose: bacterial load = 11×10^6 CFU/ml; fungal load = 15×10^5 CFU/ml																		
Ex. No.	**Colour Protection Shampoo (CPS 3 & 4)**			**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	6.3	0.5	1×10^2	60	< 10	< 10	< 10	< 10	< 10	340	60	< 10	< 10	< 10	< 10	< 10
2	1	6.3	6.3	1	30	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
3	CPS1			0.1	240	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
4	CPS2			0	2×10^5	5×10^4	3×10^4	2×10^4	1×10^3	70	< 10	15×10^3	8×10^3	7×10^2	890	180	< 10	< 10
1	1	6.3	15.7	0.5	2×10^2	80	< 10	< 10	< 10	< 10	< 10	340	60	< 10	< 10	< 10	< 10	< 10
2	1	6.3	15.7	2	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
3	BWSG1			0.8	2×10^3	< 10	< 10	< 10	< 10	< 10	< 10	30	< 10	< 10	< 10	< 10	< 10	< 10
4	BWSG1			0	26×10^4	5×10^4	3×10^4	3×10^3	760	< 10	< 10	7×10^3	4×10^3	580	990	200	< 10	< 10
Ex. No.	**Skin lightening lotion (SLL3&4)**			**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	37.5	1	1×10^2	80	< 10	< 10	< 10	< 10	< 10	40	80	< 10	< 10	< 10	< 10	< 10
2	1	12.5	37.5	2	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
3	SLL1			0.8	90	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
4	SLL2			0	2410^4	5×10^4	5×10^3	3×10^3	600	< 10	< 10	12×10^3	5×10^4	580	60	20	< 10	< 10

Table 4. Preservative efficacy testing of selected antimicrobial of the developed cosmetic personal care products.

impart antimicrobial preservative potency to different cosmetic personal care product compositions equivalent to conventional preservatives (methylchloroisothiazolinone and methylisothiazolinone dosed at 0.1% in color protection shampoo (CPS1), phenoxyethanol, methyl paraben, ethyl paraben, butyl paraben, propyl paraben, and isobutyl paraben dosed 0.8% in body wash shower gel (BWSG1) and lightening lotion (SLL1).Therefore, it can be concluded that the formulators incorporating the unique synergistic mixtures were well preserved and equivalent to conventional preservatives.

The unique synergistic combination of multifunctional ingredients can be an alternative solution to protect cosmetic products from microbial attack; these ingredients are skin-friendly and are preferred by consumers. This smart approach to cosmetic product preservation helps avoid the use of conventional preservatives, which may cause skin allergies, irritation, or contact sensitivity. Many cosmetic products have complicated compositions that comprise a diverse range of materials that provide beneficial properties to the substrate while also providing structural identity to the product. Consequently, the formulator's ingredient selection would be to use the minimum amount of materials necessary to provide the most beneficial effect. An essential criterion that formulators should consider during formulation development is the control of microbial deterioration. This is usually accomplished through the addition of appropriate preservatives. Preservative selection and dosing in cosmetic products are mandated by legislation and are limited by the number of chemistries available[20].

To explore beyond present technologies, formulators are looking for chances to use new preservation principles to generate 'Preservative—free' or 'Self—preserving' formulas. The application of 'Hurdle Technology' is gaining the majority of attention in this effort. This method combines several preservation properties to limit the growth of microorganisms. These hurdles may have synergies rather than additive consequences[21,22]. We investigated the utilization of specific multifunctional ingredients, which are authorized cosmetic components but are not categorized as preservatives according to Annex VI of Commission Directive 76/768/EEC. These ingredients were combined with surfactant-based biomimetic phospholipids, fatty acids, and esters to create self-preserving personal-care cosmetic formulations. The selection of several cosmetic ingredients (sodium coco PG-dimonium chloride phosphate, ricinoleic acid, palmitoleic acid, raspberry ketone, and sorbitan caprylate) was based on their antimicrobial efficacy and ability to provide various functional benefits, such as multifunctional surfactant behavior, emollient properties, antioxidant effects, moisturizing effects, and anti-inflammatory properties. When combined with fatty acids, esters, and antioxidants, these versatile chemicals exhibit synergistic antibacterial activity that effectively prevents microbial threats.

The successful resistance of these formulations to microbiological challenges, as demonstrated by their effective preservation, instills strong confidence in the products' microbial stability and ensures that the stated shelf life for consumers is guaranteed. In this study, we successfully demonstrated the feasibility of developing personal care cosmetics with self-preserving properties comparable to those of preservative-containing formulations.

Conclusion

Based on MIC values, five unique multifunctional ingredients (MFIs) were identified: sodium coco PG-dimonium chloride phosphate, ricinoleic acid, palmitoleic acid, raspberry ketone, and sorbitan caprylate. A total of 150 combinations of MFIs were meticulously prepared and tested to explore their synergistic actions. Through extensive experimentation and calculation of the Fractional Inhibitory Concentration (FIC) index, three synergistic antimicrobial compositions were determined. The following synergistic combinations were found to exhibit enhanced antimicrobial efficacy compared to their individual constituents: sodium coco PG-dimonium chloride phosphate: Ricinoleic acid: Raspberry ketone in the ratios 1:6.3:6.3 and 1:6.3:15.7. Sodium coco PG-dimonium chloride phosphate: palmitoleic acid: sorbitan caprylate at a ratio of 1:12.5:37.5. All these combinations demonstrated lower MIC values than their individual MFIs, indicating their potent antimicrobial effects when working synergistically. Encouraged by these findings, these synergistically active components were incorporated into three distinct personal care formulations. In a rigorous Preservation Challenge Test (PCT), the effectiveness of these antimicrobial compositions in preserving cosmetic formulations was evaluated. These results were promising, as all three formulations successfully maintained product stability and prevented microbial contamination for up to 28 days. This approach to product preservation not only ensures the safety and longevity of cosmetic formulations but also presents a significant step towards reducing the reliance on potentially harmful preservatives. By demonstrating the efficacy of these multifunctional activities, we have shown the potential for developing self-preserving personal care formulations that can protect themselves from microbial contamination without compromising consumer safety or skin health. In summary, this study highlights the promising potential of synergistic combinations of multifunctional activities in the development of self-preserving personal care products. This study provides valuable insights into creating safer, more effective, and consumer-friendly cosmetic formulations, aligned with the growing demand for natural and preservative-free options in the cosmetics industry.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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References

1. Epstein, H. Cosmetics preservation: Sense and nonsense. *Clin. Dermatol.* **24**, 551–552. https://doi.org/10.1016/j.clindermatol.2006.07.017 (2006).
2. Papageorgiou, S., Varvaresou, A., Tsirivas, E. & Demetzos, C. A. New alternatives to cosmetics preservation. *J. Cosmet. Sci.* **61**, 107–123 (2010).

3. Andersen, K. E. Contact allergy and irritation from preservatives. *J. Appl. Cosmetol.* **11**, 65–68 (1996).
4. Legendre, J. Y. *et al.* Formulation, characterization, and efficacy of an adenosine-containing dissolvable film for a localized anti-wrinkle effect. *J. Cosmet. Sci.* **58**, 147–155. https://doi.org/10.1111/j.1468-2494.2007.00401_3.x (2007).
5. Varvaresou, A. *et al.* Self-preserving cosmetics. *Int. J. Cosmet. Sci.* **31**, 163–175. https://doi.org/10.1111/j.1468-2494.2009.00492.x (2009).
6. Leistner, L. Basic aspects of food preservation by hurdle technology. *Int. J. Food Microbiol.* **55**, 181–186. https://doi.org/10.1016/s0168-1605(00)00161-6 (2000).
7. Petersen, W. Antimicrobial ingredients for self-preserving cosmetics. *Euro Cosmet.* **2**, 28–36 (2002).
8. Kabara, J. J., Swieczkowski, D. M., Conley, A. J. & Truant, J. P. Fatty acids and derivatives as antimicrobial agents. *Antimicrob. Agents Chemother.* **2**, 23–28. https://doi.org/10.1128/AAC.2.1.23 (1972).
9. Kabara, J. J. & Orth, D. S. *Preservative-Free and Self-Preserving Cosmetics and Drugs: Principles and Practice* (Marcel Dekker, 1997).
10. Meenakshi, N., Sekar, P., Pasupathi, M. & Mukhopadhyay, T. Self-preserving skin care cosmetic products. *Int. J. Adv. Biotechnol. Res.* **7**, 22–37 (2016).
11. Wayne, P. A. Clinical and laboratory standards institute & methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. In *CLSI Reference Method for Susceptibility Testing of Yeasts; Approved Standard. 3rd ed. CLSI Document m27-A3. 10th ed. CLSI Document m07-A10*, Vol. 35, No. 2. 28 (Approved Standard, 2008 & 2015) (Clinical and Laboratory Standards Institute, Wayne, PA, 2008 & 2015)
12. Graf, A. S., Joyce, G. G. & McEwen, G. N. CTFA Microbiology Guidelines (ed. Cosmetic, Toiletry, and Fragrance Association) (Washington, DC, 1993)
13. Bergsson, G. *et al.* In vitro killing of *Candida albicans* by fatty acids and monoglycerides. *Antimicrob. Agents Chemother.* **45**, 3209–3212. https://doi.org/10.1128/AAC.45.11.3209-3212.2001 (2001).
14. Rigano, L. & Leporatti, R. Systemic constellations: With or without preservatives?. *SÖWF J.* **129**, 1–9 (2003).
15. Jänichen, J. The quest for the ideal preserving system-reducing traditional preservatives in combination with Dermosoft Octiol. *Euro Cosmet.* **7**(8), 10–16 (2004).
16. Halla, N. *et al.* Cosmetics preservation: A review on present strategies. *Molecules* **23**, 1571–1612. https://doi.org/10.3390/molecules23071571 (2018).
17. ISO 11930:2019. Cosmetics-Microbiology Evaluation of the Antimicrobial Protection of a Cosmetic Product 2, 22 (2019)
18. Anelich, L. E. & Korsten, L. Survey of microorganisms associated with spoilage of cosmetic creams manufactured in South Africa. *Int. J. Cosmet. Sci.* **18**, 25–40. https://doi.org/10.1111/j.1467-2494.1996.tb00133.x (1996).
19. Campana, R., Scesa, C., Patrone, V., Vittoria, E. & Baffone, W. Microbiological study of cosmetic products during their use by consumers: Health risk and efficacy of preservative systems. *Lett. Appl. Microbiol.* **43**, 301–306. https://doi.org/10.1111/j.1472-765X.2006.01952.x (2006).
20. Whitby, D. & Bachmann Roth, C. Challenging times for preservation. *Pers. Care* 28–29 (2010).
21. Kabara, J. J. Hurdle technology: Are biocides always necessary for product protection?. *J. Appl. Cosmetol.* **17**, 102–108 (1999).
22. Stoffels, K. M. Modern and safe antimicrobial stabilization of cosmetic products. *Househ. Pers. Care Today* **7**, 18–21 (2012).

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Author contributions

K.S. and A.V. created the idea, collected the literature and wrote the half of the manuscript. M.J., A.S., P.R. revised the manuscript several times. S.P., S.G. formatted the manuscript according to the journal guidelines. S.A.A., M.J.A. responsible for the funding support. P.R. and A.V. are responsible for the correspondence. The authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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