JOURNAL OF APPLIED BIOANALYSIS, August 2024, p. 181-191.
http://doi.org/10.53555/jab.v10i2.027 ,(ISSN 2405-710X)
Vol. 10, No. 2

OPEN ACCESS

RESEARCH ARTICLE

Sunshield: Advancing Self-Preserving Sun Care Cosmeceuticals with Multifunctional Ingredients

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Background/Objectives:
UV radiation causes sunburn and prolonged exposure signs. Sunscreens protect by absorbing, reflecting, and scattering UV rays. Advancements include non-traditional UV filters and botanical compounds. Effective sun protection is crucial. Microbiological protection is essential in cosmetics to prevent damage and infections. This study aimed to identify self-preserving preservative complexes for sun care products, comparing their efficacy to conventional preservatives.
Methods:
MICs of cosmetic ingredients were assessed to identify antimicrobial compounds. Synergy indices were determined using combinations of multifunctional components. Formulations for self-preserving sun care products were evaluated using Preservative Challenge Testing.
Results:
Synergistic combinations, e.g., Sodium grapeseedamidopropyl PG-dimonium chloride phosphate: inulin: tetrasodium glutamate diacetate (ratios 0.65:12.5:25, 0.5:12.5:30, and 1:12.5:30), effectively minimized microbiological challenges, comparable to traditional preservatives.
Conclusion:
This study shows self-preserving sun care solutions can be developed by selecting multifunctional components. These products maintain aesthetics, sensory attributes, and resist microbial attacks like traditional preservatives. Exploring multifunctional components offers a potent alternative, promoting cosmetic-friendly preservation while mitigating risks.

Keywords: Antimicrobial, self-preservation, sun care formulations, conventional preservatives, multifunctional ingredients.

INTRODUCTION:

Sunscreens are essential products designed to protect the skin from harmful UV radiation through absorption, blocking, or deflection.[1] UV exposure is a known factor in photoaging, contributing to skin issues such as sunburn, premature aging, and inflammation.[2] With advancements in formulation, sunscreens now incorporate a range of organic, inorganic, hybrid, and botanical UV filters in various product formats like emulsions, gels, aerosols, and sprays, offering consumers diverse options for sun protection.[3-5]

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In the realm of cosmeceuticals, microbial contamination leading to product degradation poses a significant challenge. While these products are not required to be sterile, they must be safe for human use. Cosmetic formulations often contain natural ingredients, increasing susceptibility to microbial growth, including yeasts, molds, and various bacteria.[6]

Preservatives are commonly added to cosmetics to prevent microbial growth, extend shelf life, and safeguard both the product and the consumer from potential infections. However, increasing consumer concerns about the safety of conventional preservatives have led to a demand for preservative-free or self-preserving alternatives.

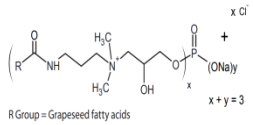
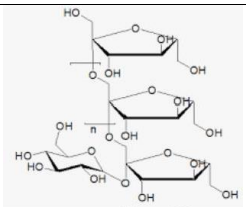
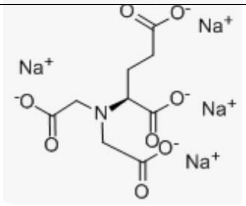
Consumers now seek "Preservative-Free" products, although "Self-Preservative" is a more accurate term. Utilizing multifunctional ingredients (MFI), formulations can be developed to self-preserve while offering additional benefits such as antibacterial properties.[7,8] This study explores synergistic combinations of multifunctional ingredients to develop self-preserving cosmeceutical sun care products and evaluates their microbial safety. Comparative analysis is conducted against control formulations preserved with approved preservatives

Materials and Methods

Materials:

Preservatives, as well as other cosmetic ingredients used in this study, such as multifunctional ingredients listed in **(Table 1),** were purchased from a number of reputable suppliers and dealers, including Brenntag ingredients pvt. ltd., DKSH india, Nouryon chemicals india pvt. ltd., Kumar organic products ltd., Confiance life sciences pvt. ltd., Merck specialities pvt. ltd., Ashland pvt. ltd., Dow chemicals, Evonik pvt. ltd., Lonza, Galaxy surfactants ltd., Wacker chemie india pvt. ltd., Vivimed labs ltd., Hayashibara co. ltd., Gangwal chemicals pvt. ltd., Clariant ltd., Simson pharma, Schulke and mayr GmbH, Sigma aldrich, Croda chemicals, BASF and Inolex CC.[9-11]

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

S.No	Multifunctional Ingredients INCI Name	Structure	Form	Benefits	Vendor/ Supplier
1	Sodium grapeseedamidopropyl PG-dimonium chloride phosphate		Liquid	Anti-oxidant, broad spectrum antimicrobial	Brenntag ingredients india pvt. ltd., mumbai
2	Inulin		Powder	Dietary nutrient, anti-microbial	DKSH india, mumbai
3	Tetrasodium glutamate diacetate		Liquid	Chelating agent, anti-microbial	Nouryon chemicals india pvt. ltd., mumbai

Microbial Strains: According to the Personal Care Products Council (PCPC) of the United States, the official cell culture collections from which the standard microbial culture strains recommended for the screening studies were acquired were the American type culture collection (ATCC) and Microbiologics Inc., USA. The most frequently used test strains in this study were gram-negative bacteria, such as *E. coli* ATCC 8379 and *Pseudomonas aeruginosa* ATCC 9027, followed by potentially pathogenic Gram-positive bacteria, such as *Staphylococcus aureus* ATCC 6538, mold, such as *Aspergillus brasiliensis* ATCC 16404, and yeast, such as *Candida albicans* ATCC 10231.

Inoculation of samples: After adjusting the number of starting cells, the inoculum is used to inoculate test samples. Bacterial cell cultures were grown in Tryptone Soy Agar slants for 18 - 24 hours at 36℃±1℃. The fungal strains were inoculated onto Sabouraud Dextrose Agar/Potato Dextrose Agar and cultured for five to seven days at 23℃±1℃. All cultures were harvested after incubation and were diluted to $1x10^8$ CFU/ml in sterile saline.

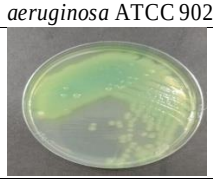
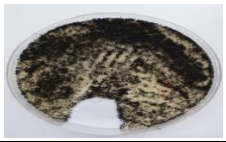
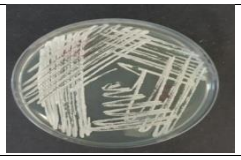
Screening of multifunctional Ingredients with anti-microbial efficacy: The MIC, or minimal inhibitory concentration, of various cosmeceutically authorized substances derived from polysaccharide, chelating agent, and surfactant-based biomimetic phospholipids multifunctional actives was evaluated against the aforementioned microbial strains. In total, about three ingredients and seventy five ternary combinations were studied The use of approved conventional preservatives as controls in cosmeceuticals was also investigated. The tests were repeated four times, and the average scores were calculated.[12]

Determination of Minimal Inhibitory Concentration& FIC Index: The lowest concentration at which an antimicrobial agent totally prevents visible growth of the microorganism in an agar or broth dilution test is known as the minimum inhibitory concentration (MIC). In accordance with the CLSI guidelines,[12] the antimicrobial properties were evaluated using the MIC macro-dilution method for both antibacterial and antifungal activities.[13] The inhibitory concentration of the test materials were conducted by incubating them along with the specific microorganisms at varying concentrations, both individually and in combinations. The tests were conducted four times, and the average results were computed. The FIC index end points of antimicrobial medications were calculated separately and in combinations to identify synergy/additive/antagonism activity.[14]

Inoculums were made using recently generated 24 hour fresh bacterial cultures and 120 hour fungal cultures. The turbidity of the inoculum was adjusted to 0.5 McFarland standard with sterile saline or Soybean Casein Digest Medium for bacteria and Sabourauds Dextrose Agar for fungal cultures were to obtain an inoculum size of 1-2 10^8 CFU/ml for bacterial cultures and 1-2x10^6 CFU/ml. Antimicrobial agents stock solutions were then prepared at concentrations of at least 1,000 mg/ml or ten times the highest concentration to be tested, whichever is greater.

Using the macro dilution method, anti-microbial concentrations were diluted 2 fold (1000 mg, 500 mg, 250 mg, 125 mg, 62.5 mg, etc.), and inoculums were added to distinct tubes for each bacterial culture and fungal culture. A control tube was employed for each organism to be investigated, containing broth devoid of any antimicrobial concentrations. All inoculation tubes underwent a 24-hour period of 35± 2°C incubation and the tests were carried out in quadruplicates.

The culture strains were tabulated in (Figure 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
				

FIC Index: It is calculated by multiplying the synergy index ratio by the number of reported methods.
Qa/QA + Qb/QB = Synergy Index

Where QA is the concentration of compound A in PPM that produces an end point when acting alone, Qa denotes the concentration of compound A in PPM in the mixture that resulted in an end point. QB is the concentration of compound B in PPM that produces an end point when acting alone. & Qb is the concentration of compound B in PPM in the mixture that results in an end result. The results were interpreted using the following criteria: 1) Synergy > 1.0 2) Additive effect = 1.0 3) Antagonism > 1.0

The synergistic combinations evaluated were 0.65:12.5:25, 0.5:12.5:30, 1:12.5:30. These combinations consisted of Sodium grapeseedamidopropyl PG-dimonium chloride phosphate, inulin, and tetrasodium glutamate diacetate.

Cosmeceutical Sun care formulations & Process [15-20]. Twelve sun care cosmeceutical formulations were prepared.

I. Sunscreen cream (SSC 1,2,3,4) with four different preservation strategies.

II. Sunscreen lotion (SSL 1,2,3,4) with four different preservation strategies and

III. Sunscreen spray (SSS 1,2,3,4) with four different preservation strategies, were prepared as listed in the Table 2 with conventional preservative* (positive control) code: SSC1,SSL1 and SSS1, placebo base without preservative (negative control) code: SSC2,SSL2 and SSS2, synergistic combination of multifunctional ingredients Sodium grapeseedamidopropyl PG-dimonium chloride phosphate, inulin and tetrasodium glutamate diacetate (synergistic antimicrobial composition 0.65: 12.5: 25) at 0.5% and 0.75% in sunscreen cream SSC3 and SSC4; Sodium grapeseedamidopropyl PG-dimonium chloride phosphate, inulin and tetrasodium glutamate diacetate (synergistic antimicrobial composition 0.5: 12.5: 30) at 0.5% and 0.75% in sunscreen lotion SSL3 and SSL4; Sodium grapeseedamidopropyl PG-dimonium chloride phosphate, inulin and tetrasodium glutamate diacetate (synergistic antimicrobial composition 1: 12.5: 30) at 0.5% and 0.75% in sunscreen spray SSS3 and SSS4 along with cosmeceutical actives **

Table 2: Comeceutical sun care products sunscreen cream (SSC), sunscreen lotion (SCL) and sunscreen spray (SSS) formulations and Process

Cosmeceutical sunscreen cream composition (SSC 1,2,3,4)			Cosmeceutical sunscreen lotion composition (SSL 1,2,3,4)			Cosmeceutical sunscreen spray composition (SSS1,2,3,4)		
Phase	**Ingredients**	**Dosage (%)**	**Phase**	**INCI Name**	**Dosage (%)**	**Phase**	**INCI Name**	**Dosage (%)**
A	Water	Q.S to 100	A	Water	Q.S to 100	A	Water	Q.S to 100
	Disodium EDTA	0.1		Acrylates/C10-30 Alkyl Acrylate Crosspolymer	0.4		Glycerin	3
	Glycerin	2		Disodium EDTA	0.1		Disodium EDTA	0.1
B	Ethylhexyl Methoxycinnamate and Octocrylene and Ethylhexyl Salicylate and Butyl Methoxytdibenzoylmethane and Benzophenone-3 **	15		Butylene Glycol	3		PEG-7 Glyceryl Cocoate	2
	Isopropyl Myristate	5	B	Ethylhexyl Methoxycinnamate and Octocrylene and Ethylhexyl Salicylate and Butyl Methoxytdibenzoylmethane and Benzophenone-3 **	18		Acrylates/Beheneth-25 Methaacrylate Copolymer	1
	C12-15 Alkyl Benzoate	4		Caprylic/Capric Triglyceride	3.5	B	Ethylhexyl Methoxycinnamate and Octocrylene and Ethylhexyl Salicylate and Butyl Methoxytdibenzoylmethane and Benzophenone-3 **	10
	Cetearyl Alcohol	5		Isopropyl Palmitate	2.5		Isoamyl Laurate	3
	Glyceryl Stearate	3		Cetearyl Alchohol	3		Cetearyl Alcohol	1.5
C	Tocopheryl Acetate	0.5		Glyceryl Stearate	3		Glyceryl Stearate	1.5
D	Panthenol	0.5	C	Tocopheryl Acetate	0.5	C	Tocopheryl Acetate	0.25
	Niacinamide	0.5		Cyclomethicone	4		Cyclomethicone	2.5
E	Sorbitan Caprylate, Phenoxyethanol, Benzyl Alcohol, Benzoic Acid*(positive control with conventional preservative) SSC1	1	D	Panthenol	0.5	D	Panthenol	0.5
	Placebo base without preservative (negative control without preservative) SSC2	0		Sodium Hyaluronate	0.5		Sodium Hyaluronate	0.25
	Sodium grapeseedamidopropyl PG-dimonium chloride phosphate, inulin and tetrasodium glutamate diacetate (synergistic antimicrobial composition 0.65: 12.5: 25) SSC3	0.5	E	Sorbitan Caprylate, Phenoxyethanol, Benzyl Alcohol, Benzoic Acid*(positive control with conventional preservative) SSL1	1	E	Sorbitan Caprylate, Phenoxyethanol, Benzyl Alcohol, Benzoic Acid*(positive control with conventional preservative) SSS1	1

	Sodium grapeseedamidopropyl PG-dimonium chloride phosphate, inulin and tetrasodium glutamate diacetate (synergistic antimicrobial composition 0.65: 12.5: 25) SSC4	0.75		Placebo base without preservative (negative control without preservative) SSL2	0		Placebo base without preservative (negative control without preservative) SSS2	0
F	Fragrance	Q.S		Sodium grapeseedamidopropyl PG-dimonium chloride phosphate, inulin and tetrasodium glutamate diacetate (synergistic antimicrobial composition 0.5: 12.5: 30) SSL3	0.5		Sodium grapeseedamidopropyl PG-dimonium chloride phosphate, inulin and tetrasodium glutamate diacetate (synergistic antimicrobial composition 1: 12.5: 27.5) SSS3	0.5
G	Citric Acid/Sodium Hydroxide	Q.S		Sodium grapeseedamidopropyl PG-dimonium chloride phosphate, inulin and tetrasodium glutamate diacetate (synergistic antimicrobial composition 0.5: 12.5: 30) SSL4	0.75		Sodium grapeseedamidopropyl PG-dimonium chloride phosphate, inulin and tetrasodium glutamate diacetate (synergistic antimicrobial composition 1: 12.5: 27.5) SSS4	0.75
Manufacturing Procedure: Weigh ingredients of phase A & B separately and heat to 75°C. Add phase A to phase B with constant stirring by maintaining the temperature to 75°C. Homegenise for 10 minutes and cool down to 40 °C. Add phase C ingredient and stir it for 5 minutes. Add phase D ingredients and stir it for 5 minutes. Add phase E ingredient and stir it for 5 minutes. Add phase F ingredient and stir it for 5 minutes. Add phase G by adjusting the pH. Cool down to room temperature while stirring. ** Cosmeceutical active			F	Fragrance	Q.S	F	Fragrance	Q.S
			G	Citric Acid/Sodium Hydroxide	Q.S	G	Citric Acid/Sodium Hydroxide	Q.S
			Manufacturing Procedure: Disperse Acrylates/C10-30 Alkyl Acrylate Crosspolyumer and then add other ingredients of Phase A and heat to 80°C. Weigh ingredients of phase B separately and heat to 75°C. Add phase A to phase B with constant stirring by maintaining the temperature to 75°C. Homegenise for 10 minutes and cool down to 40 °C. Add phase C ingredient and stir it for 5 minutes. Add phase D ingredients and stir it for 5 minutes. Add phase E ingredient and stir it for 5 minutes. Add phase F ingredient and stir it for 5 minutes. Add phase G by adjusting the pH. Cool down to room temperature while stirring. ** Cosmeceutical active			**Manufacturing Procedure**: Disperse Acrylates/C10-30 Alkyl Acrylate Crosspolyumer and then add other ingredients of Phase A and heat to 80°C. Weigh ingredients of phase B separately and heat to 75°C. Add phase A to phase B with constant stirring by maintaining the temperature to 75°C. Homegenise for 10 minutes and cool down to 40 °C. Add phase C ingredient and stir it for 5 minutes. Add phase D ingredients and stir it for 5 minutes. Add phase E ingredient and stir it for 5 minutes. Add phase F ingredient and stir it for 5 minutes. Add phase G by adjusting the pH. Cool down to room temperature while stirring. ** Cosmeceutical active		

Preservative Challenge Test

PCT (Preservative Challenge Test) helps to assess the formulations capability to preserve the product. As controls, base formulations with preservatives were utilized. Unfortunately, no globally acknowledged technique of challenge testing and interpretation of results exists. Various pharmacopoeias prescribe different procedures, but for cosmetic products, CTFA (cosmetic, toiletries, and fragrance association) - now PCPC (Personal Care Products Council) /ISO 11930 guidelines are utilized. According to CTFA recommendations, PCT consists of a challenge study with pathogenic bacterial, yeast, and mold cultures. For evaluating microbial levels, a plate count method is used to determine 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. The inoculated samples are examined at an interval of one, two, seven, fourteen, twenty-one, and twenty-eight days after inoculation, and the growth in the number of microorganisms (CFU/ml) is determined at each time interval, with the percentage of microorganisms estimated relative to the initial concentration.[21]

The preservative challenge test is performed with additional relevant details, in which 10 g of sample material is weighed in different sterile containers and spiked with a determined load of microorganisms included in the study. An initial mixed culture of all three bacterial strains - *S.aureus, E.coli, P. aeruginosa* and two fungal strains – *C.albicans and A. brasiliensis* were prepared. An inoculums size of $17x10^6$ CFU/ml was prepared for bacterial cultures and $12x10^5$ CFU/ml for fungal cultures were determined. 10 microlitres of each bacterial culture were added to the container with marked sample for bacteria and 100 microlitres of the fungal inoculums were inoculated to marked sample for fungi container separately and left at room temperature under sterile environmental conditions. At each planned time interval (1st, 2nd, 3rd, 7th, 14th and 28th day), 1 g of sample from the inoculated containers were weighed and mixed with 9 ml of sterile neutralizer like modified letheen broth for bacterial and sabouranuds dextrose broth for fungal sampling were added and further dilutions were made and plated out separately.

Results and Discussion

The MIC of the selected three multifunctional Sodium grapeseedamidopropyl PG-dimonium chloride phosphate, inulin and tetrasodium glutamate diacetate and one conventional preservative Sorbitan Caprylate, Phenoxyethanol, Benzyl Alcohol, Benzoic Acid 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 macrobroth double dilution method were tabulated in table 1. The selected three multifunctional ingredients showed good anti-microbial activity compared to that of conventional preservatives normally used in the cosmeceutical suncare care products. One ingredient with a cationic charge, Sodium grapeseedamidopropyl PG-dimonium chloride phosphate, has shown little MIC value. However, this ingredient is costly compared to other ingredients, hence we decided to keep its concentration at a minimum level and use it as the first ingredient of the three component compositions prepared. Two ingredients, inulin and tetrasodium glutamate diacetate, are selected as the second component and the third component of each composition. Thus, compositions were prepared and tested to know their ability in aiding synergistic interaction among themselves. One of three component compositions were prepared based on their MIC data.

Composition-1 (prepared in three different ratios) is made up of Sodium grapeseedamidopropyl PG-dimonium chloride phosphate, inulin and tetrasodium glutamate diacetate. The one component in these compositions were prepared in a variety of ratios. The ratio concentration of the second ingredient is doubled, while the concentration of the third component was changed to at least thirty times the initial concentration. The concentration range was chosen in order to achieve an economically viable composition of the chosen ingredients.

As a result, the concentration ratio of the composition's first mentioned ingredient was increased from 0.5 to 1.The concentration ratio of the second ingredient 12.5. The concentration ratio of the third ingredient was increased from 25 to 30.

Seventy-five combinations prepared were screened for MIC value against the five organisms as described above. **(Table 3**) showed MIC data of the synergistic composition of multifunctional ingredients with antimicrobial efficacy. Compared to individual MIC value of multifunctional ingredients, synergistic combination of ternary combinations showed better antimicrobial efficacy. FIC index of the combinations were calculated, based on FIC index data three combinations were identified as synergistic showed in **(Table 3)**

Table 3: MIC data of multifunctional ingredients & Synergistic composition of multifunctional ingredients & 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 grapeseedamidopropyl PG-dimonium chloride phosphate	250	250	62.5	62.5	250
2	Inulin	1250	2500	2500	1250	1250
3	Tetrasodium Glutamate Diacetate	3125	2500	62.5	62.5	500
4	Sorbitan Caprylate, Phenoxyethanol, Benzyl Alcohol, Benzoic Acid *	500	500	250	500	1000
MIC and FIC data of one 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 grapeseedamidopropyl PG-dimonium chloride phosphate, inulin and tetrasodium glutamate diacetate(0.65: 12.5: 25)					
	MIC µg/ml	1250	1250	62.5	62.5	500
	FIC index	0.67	0.58	0.68	0.69	0.82
	Sodium grapeseedamidopropyl PG-dimonium chloride phosphate, inulin and tetrasodium glutamate diacetate (0.5: 12.5: 30)					
	MIC µg/ml	1250	1250	62.5	62.5	500
	FIC index	0.63	0.55	0.72	0.72	0.84
	Sodium grapeseedamidopropyl PG-dimonium chloride phosphate, inulin and tetrasodium glutamate diacetate (1: 12.5: 30)					
	MIC µg/ml	1250	1250	62.5	62.5	500
	FIC index	0.68	0.60	0.72	0.73	0.85

Preservative Challenge Test: Evaluation of preservative efficacy of the cosmeceutical formulations as per PCPC/ISO 11930 Guidelines. Twelve personal care cosmeceutical formulations Sunscreen cream (SSC 1,2,3,4), Sunscreen lotion (SSL 1,2,3,4), and Sunscreen spray (SSS 1,2,3,4) were prepared as listed in the Table 2 with conventional preservative (positive control) code: SSC1,SSL1 & SSS1, placebo base without preservative (negative control) code: SSC2,SSL2 & SSS2 synergistic combination of multifunctional ingredients at different dosages along with cosmeceutical actives (SSC3, SSL3 & SSS3, SSC4,SSL4 & SSS4). 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 given below in **(Table 4)**

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

Methodology : Mixed culture challenge																		
Organisms challenged: Bacterial- *S.aureus* + *E.coli* + *P.aeruginosa* Fungal- *C.albicans* + *A.brasiliensis*																		
Challenge dose: bacterial load = $17x10^6$ CFU/ml; fungal load = $12x10^5$ CFU/ml																		
Ex. No	Sunscreen Cream (SSC 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	0.65	12.5	25.0	0.5	$2x10^3$	30	< 10	< 10	< 10	< 10	< 10	780	20	< 10	< 10	< 10	< 10	< 10
2	0.65	12.5	25.0	0.75	$2x10^2$	< 10	< 10	< 10	< 10	< 10	< 10	130	< 10	< 10	< 10	< 10	< 10	< 10
3	SSC1			1	540	< 10	<10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
4	SSC2			0	$2x10^5$	$1x10^5$	$4x10^4$	$1x10^4$	540	70	< 10	$13x10^3$	$4x10^3$	$6x10^3$	560	180	< 10	< 10
Ex. No	Sunscreen lotion (SSL 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	0.5	12.5	30	0.5	$10x10^2$	90	< 10	< 10	< 10	< 10	< 10	380	50	< 10	< 10	< 10	< 10	< 10
2	0.5	12.5	30	0.75	$2x\ 10^2$	< 10	< 10	< 10	< 10	< 10	< 10	120	<10	< 10	< 10	< 10	< 10	< 10
3	SSL1			1	$2x10^1$	< 10	<10	< 10	< 10	< 10	< 10	< 10	< 10	<10	<10	< 10	< 10	< 10
4	SSL1			0	$7x10^4$	$1x10^4$	$3\ x10^4$	$3x10^3$	760	< 10	< 10	$4x10^3$	$2x10^3$	$3x10^2$	900	200	< 10	< 10
Ex. No	Sunscreen spray (SSS3&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	0.5	$1x10^3$	100	<10	< 10	< 10	< 10	< 10	800	70	< 10	< 10	< 10	< 10	< 10
2	1	12.5	37.5	0.75	< 10	< 10	<10	< 10	< 10	< 10	< 10	60	<10	< 10	< 10	< 10	< 10	< 10
3	SSS1			1	90	< 10	<10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	<10	< 10	< 10	< 10
4	SSS2			0	$21x10^4$	$4x10^4$	$4x10^3$	$3x10^3$	400	< 10	< 10	$13x10^3$	$5x10^3$	680	70	30	< 10	< 10

It is observed in our study that, when base formulation of sunscreen cream SSC3 & SSC4, sunscreen lotion SSL3& SSL4 and sunscreen spray SSS3 & SSS4 were incorporated with the synergistic multifunctional ingredients preservative efficacy profile was found to be similar to formulations incorporated with conventional preservatives (control) SSC1, SSL1 & SSS1 formulations in the preservative challenge test. The results indicates that the synergistically acting composition when incorporated at 0.5%, and 0.75 % levels for SSC3 & SSC4, 0.5% and 0.75% levels for SSL3& SSL4 and 0.5% and 0.75% for SSS3 & SSS4 levels delivers (PASS) preservative efficacy as per PCPC/ISO 11930 standards.

The combination of three antimicrobial multifunctional ingredients mixture at the above given ratios when incorporated at 0.5%, and 0.75 % levels for SSC3, SSC4, SSL3, SSL4, SSS3 & SSS 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 synergisitic combinations were able to impart antimicrobial preservative potency to different cosmeceutical personal care products composition equivalent to conventional preservative (Sorbitan Caprylate, Phenoxyethanol, Benzyl Alcohol, Benzoic Acid dosed at 1% in sunscreen cream (SSC1), sunscreen lotion (SSL1) and sunscreen spray (SSS1). Therefore it can be concluded that, the formulators incorporated with the unique synergistic mixtures were well preserved as equivalent to conventional preservatives. The unique synergistic combination of multifunctional ingredients can be an alternate solution to preserve the cosmeceutical products from microbial attack, these ingredients are skin friendly and are preferred by consumers. This smart approach to cosmeceutical product preservation helps to avoid the usage of conventional preservatives which might cause skin allergy, irritation or contact sensitivity.

Many cosmeceutical products are complicated compositions that comprise a diverse range of materials that give beneficial properties to the substrate while also giving the product structural identity. As a result, the formulator's ingredient selection would be to use the minimum materials necessary to provide the most beneficial effect. One essential criterion for formulators to consider during formulation development is microbial deteriorating control. This is usually accomplished by adding appropriate preservatives. Preservative selection and dose in cosmeceutical products are mandated by legislation and limited by the number of chemistries available.[22-25].

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 a number of preservation properties to limit microorganism's growth. The various hurdles may have synergy rather than additive consequences.

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, sugars/polysaccharides and chelating agents to develop self-preserving sun care cosmeceutical formulations. Based on antimicrobial efficacy, several cosmeceutical ingredients known to provide different functional benefits such as multifunctional surfactant behavior, emollient, antioxidant, moisturizer, and anti-inflammatory agent (Sodium grapeseedamidopropyl PG-dimonium chloride phosphate, inulin and tetrasodium glutamate diacetate) were chosen. These multifunctional compounds, in combination with sugars/polysaccharides, chelating agents have synergistic antimicrobial properties in preventing microbial challenges.[26-28].

The fact that these formulations have efficiently survived microbiological challenges by preservative efficacy gives great confidence in the robustness of the products' microbial stability and guarantees the consumer's specified shelf life. We attempted and shown in this work that it is possible to build self-preserving sun care cosmeceuticals that are as durable as formulations with preservatives.

Conclusion

In conclusion, this study identified three unique multifunctional ingredients (MFIs) Sodium grapeseedamidopropyl PG-dimonium chloride phosphate, inulin, and tetrasodium glutamate diacetate based on their Minimum Inhibitory Concentration (MIC) values. Seventy-five combinations of these MFIs were tested to determine synergistic interactions, leading to the discovery of three synergistic antimicrobial compositions. These compositions exhibited significant synergy, with lower MIC values compared to their individual constituents. Incorporating these synergistic compositions into cosmeceutical sun care formulations at varying doses (0.5% and 0.75%) proved effective in preserving the products for up to 28 days, as demonstrated in the Preservative Challenge Test (PCT). This approach offers a promising alternative

to traditional preservatives, reducing the risk of skin irritation or contact sensitivity. By leveraging multifunctional actives, self-preserving cosmeceutical formulations can effectively protect against microbial contamination without the need for harmful preservatives.

ACKNOWLEDGMENTS:
The authors thank Ms. B Nithya, Ms. G. Sivaranjani for microbial work. The authors wish to acknowledge Dr. A.K. Kathireshan, Director, School of Life Sciences, VISTAS for their encouragement and support**.**

Conflict of interest:
The authors report no conflicts of interest

Financial support:
None.

Ethics statement:
None.

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