Natural Composition with Antioxidant and Antimicrobial Activities as Cosmetic Preservatives

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Abstract

The present study was designed to devise a preservative system of natural origin for perishable commodities, since some of the currently used synthetic preservatives are known to cause undesirable effects. Using high vacuum distillation, colorless and less aromatic fractions of essential oils were obtained and evaluated through GC-MS for the percentage of actives. Fractions with highest percentages were combined in specific proportions with garcinol, monolaurin and magnolol to obtain four blends. These blends were screened for antimicrobial activities against Staphyloccocus aureus, Escherichia coli and Candida albicans. The respective minimum inhibitory concentrations clearly delineated the synergy when compared with individual components. The blends incorporated at concentrations between 0.5% and 4.0% in aqueous cream formulations showed effective preservation at 2.0% and 4.0% when subjected to challenge testing. However, using a combination of pasteurization and the incorporation of synergistic blends, high microbial load in challenged cream bases were controlled to bare minimal levels even at the concentration as low as 0.5%. The above preservative blends were also evaluated for their antioxidant effects and were found to be effective. All the blends were found to be stable over a period of three months demonstrated by the estimation of peroxide value.

Key Words: Preservatives, essential oils, garcinol, monolaurin, magnolol, antimicrobial, antioxidant, peroxide value.

Introduction

Microorganisms are ubiquitous, occurring in large numbers in most natural environments and are capable of producing desirable and undesirable changes. They play a vital role in the deterioration of quality of a wide range of products resulting in degradative processes like discoloration, putrefaction, fermentation or rancidity [1]. Cosmetics and food commodities are prone to microbial spoilage as they are moisture rich and laden with nutrients such as carbohydrates, peptides, oils which favor growth and proliferation of microorganisms. In the absence of protective measures, microorganisms would multiply rapidly and cause biodegradation of the product raising the risk of infection in consumers. Product contamination may arise from ingredients used in formulation or accidentally during use of the formulation [2].

The Food and Drug Administration (FDA) enjoins cosmetic manufacturers to provide safe products. A product is not to contain unacceptable levels of microorganisms at the time of purchase and in some instances during use [3]. Products formulated *entirely* without preservatives must be manufactured in an aseptic environment and refrigerated once opened. This makes the process tedious and expensive. To prevent these commodities from microbial spoilage, preservation techniques must be adopted which may be physical or chemical in nature or a combination of both.

Chemical preservatives may be synthetic or natural. The extensive use of synthetic preservatives is due to their easy availability and cost effectiveness which makes large scale production and warehousing easier. Commonly used synthetic preservatives are paraben esters, formaldehyde, formaldehyde releasers, methylchloroisothiazolinone, methylisothiazolinone etc of which paraben esters are most widely used. However, concern is continuously raised about the safety of synthetic preservatives. For example: formaldehyde, formaldehyde releasers, methylchloroisothiazolinone and methylisothiazolinone are known to cause cosmetic related contact allergy [4-5]. Parabens such as propyl, butyl and ethyl parabens are found to have oestrogenic potencies [6] and their significant increasing permeation in skin layers provokes the accumulation of these molecules as a cause for skin toxicities and carcinogenicity [7] Though research is ongoing to confirm the role of parabens in cancer, it still calls for alternate preservatives.

Current global trends indicate a shift from synthetic to natural products due to increasing awareness of safety concerns associated with former. The rising demand for plant derived products in health and personal care is owing to their non-toxicity, lower side effects and easy affordability [8].

The popularity of phytochemicals as natural antimicrobial agents, also called biocides, is developing at a fast pace [9]. The antimicrobial properties of essential oils are well established and are found to have applications in the field of pharmacology, pharmaceutical botany, phytopathology, medical and clinical microbiology, food preservation etc [9]. In traditional medicine, many essential oils are claimed to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties [10-12]. Essential oils used in present study i.e. thyme, lemon grass, clove, and cinnamon are reported to have potent antimicrobial activities [13-14]

The present study demonstrates an effective preservative system which is a combination of synergistic compositions of natural origin and physical modes of preservation, in particular pasteurization.

Materials and Methods

Essential oil and compounds

Cinnamon bark, clove leaf, lemon grass and thyme oils were purchased from Falcon-Bangalore, India. Standards of cinnamaldehyde, eugenol, *cis*-citral & *trans*-citral and thymol were purchased from Sigma Aldrich (St. Louis,U.S.A.). Monolaurin (90%), garcinol (97%) and magnolol (99%) were from in-house R & D.

Chemicals involved

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Dimethyl sulphoxide (DMSO) was procured from Thomas Baker (Mumbai, India) and sodium chloride was from E-Merck (Mumbai, India).

Media

Nutrient Agar (M001), Mueller-Hinton Agar (M173), Yeast Malt Agar (M424), Tryptone Soya Agar with Lecithin and Polysorbate (M449), Sabouraud Dextrose Agar (M063) and Dey Engley Broth (M1062) media were procured from Hi Media Laboratories Pvt. Ltd. (Mumbai, India).

Microbial strains and culture conditions

The test microbial cultures *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 8739 were purchased from American Type Culture Collection (ATCC), Rockville, USA. *Pseudomonas aeruginosa* NCIM 2200, *Candida albicans* NCIM 3471, *Aspergillus niger* NCIM 1196 were obtained from National Collection of Industrial Microorganisms (NCIM), (Pune, India).

Prior to use, each microbial culture was verified using appropriate cultural and biochemical tests. The microbial strains were maintained as glycerol stocks at low temperatures in the range of -70°C to -80°C for long term storage.

Standards: Antibiotic Chloramphenicol RM 218 (Chloromycetin) with potency of 900 µg/mg and antifungal agent Nystatin RM 212 (Mycostatin) with potency of 4400-5000 USP units/mg were procured from Hi-Media Laboratories Pvt. Ltd (Mumbai, India).

Synthetic preservative standards as methyl paraben and propyl parabens were procured from Salicylates and Chemicals Pvt. Ltd. (Hyderabad, India).

Instrumentation

Vaccum Pump: Remi High VP 200, (Mumbai, India), GC: Agilent 6890N (Illinois, U.S.A), MS: Agilent 5975-G3171A, (Illinois U.S.A) and Rotatory vacuum evaporator: Heidolph (Laborata 4000) Instruments Gmbh & Co.KG, (Germany).

Methods

GC-MS: Model: Column: HP-1 Methyl siloxane, 29.5m × 250.00 um × 0.25 µm, FID Detector Temp: 250°C, MS Detector Temp: 250°C, Temp Programming: 100-290 @ 10°C, Split mode: 200:1, Inlet Temp: 270°C.

Formulation

Essential oils were subjected to high vacuum distillation to obtain the colourless and less aromatic fractions.500 gm of each of the oils were distilled at high vacuum of 1mm to get various fractions at different temperatures. All the fractions were analyzed by GC-MS for estimating the percentage of active constituents (Fig 1-4). Fractions with highest percentages as mentioned in Table 1 along with monolaurin (90%), garcinol (97%) and magnolol (99%) were used for the formulations shown in Table 2.

Table 1 High vacuum distilled fractions used in the blends.

S.No.	Essential oil	Yield of the fraction (%)	% of active constituent	Vapour temp.°C
1.	Thyme oil	20-25	Thymol -95-98	60-85
2.	Lemmon grass	35-50	Citral-95-99	61-75
3.	Cinnamon	35-50	Cinnamaldehyde-85-90	80-85
4.	Clove leaf	25-35	Eugenol- 90-95	75-85

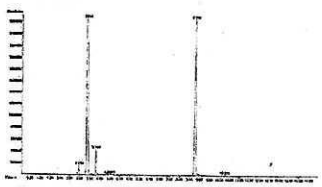

Fig. 1

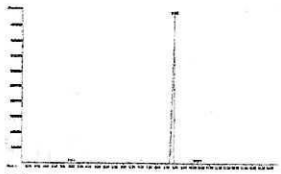

Fig. 2

Antimicrobial activity study and determination of minimum inhibitory concentration

Disc diffusion method was employed to study the antimicrobial activity and subsequently, the minimum inhibitory concentration (MIC) of the natural compounds, essential oils and their blends. The inoculum was prepared from 18-24 hour old bacterial cultures harvested from nutrient agar slants using normal saline. The turbidity of the cell suspensions was adjusted to yield not less than 1×10^6 cfu/ml for use as inoculum whose strength was verified using plate count method. A fresh, sterile cotton swab was dipped into the standardized inoculum suspension and pressed against the side of the test tube to remove excess liquid from it and swabbed on the surface of dry and sterile agar plates of Mueller-Hinton medium (for bacteria) and Yeast Malt medium (for yeasts). The inoculated plates were dried at 35°C for 15-20 minutes. Varying concentrations of the essential oil fractions, herbal extracts and the essential oil- extract blends in the range of 10% w/v to 0.03% w/v were prepared in suitable solvents like water and DMSO. Sterile filter paper discs of 6mm diameter impregnated with these varying dilutions of the oils, actives and blends were placed on the seeded agar surface.

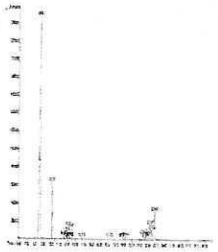

Fig.3

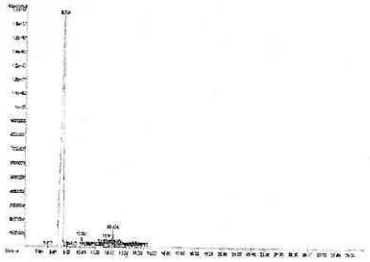

Fig.4

The plates with bacteria were incubated at 35°C± 2°C for 18-24 hours and that of the fungal at 20-25°C for 48 hours followed by measuring the diameter of zones of inhibition around the discs. The lowest concentration showing a zone of inhibition was considered as the minimum inhibitory concentration (MIC). Chloramphenicol and Nystatin were used as positive controls in the range of 0.1% to 0.01%. DMSO, used as diluent was also checked for any antimicrobial activity against the test organisms. Three replicates were studied to ensure reproducibility of the results.

Challenge test/Preservative efficacy test

Among various approaches to study the effect of preservative, microbial challenge test is most commonly used and accepted evaluation criterion. The prolonged four week test cycle was adopted in the present study to evaluate the preservative efficiency of the above mentioned synergistic natural blends. Test microbial cultures of strength 10^5 to 10^6 colony forming units (cfu) or spores in case of molds were introduced into aqueous cream formulation containing blends and assayed for microbial recovery at multiple test points *viz.* 0, 7, 14,21 and 28 days. The inoculated preparations were stored at ambient temperature. Microbial recovery studies were performed using plate count method, particularly pour plate method. 1 gram of the challenged cream was diluted with 9ml of Dey Engley broth medium to neutralize the antimicrobial activity of the preservatives. The 10^{-1} dilution was further serially diluted and those dilutions which yielded countable colonies were plated on Tryptone Soya Agar with Lecithin and Polysorbate for bacteria, Yeast Malt Agar for yeasts and Sabouraud Dextrose Agar for molds. The bacteria and fungi were incubated at 35-37°C for 24-48

hours and 20-25°C for 3-5 days respectively. By extending the test time, slow growing or affected microorganisms would have ample opportunity to recover and grow if they were capable. Methyl and propylparaben combinations were incorporated in cream and challenge tested as positive control within the allowed limits of the study. The ability of the test blends to eradicate or control artificially generated contamination was compared with the preservative action of the positive control. If no colonies are recovered in the initial 1:10 dilution, the microbial count was expressed as <10cfu/g in plate count method. Three replicates were studied to ensure reproducibility of the results.

Pasteurization and incorporation of natural preservative blends

All the four blends were used in combination with pasteurization to enhance the shelf life of the product. Conventional method of pasteurization was followed wherein the product under study was exposed to a temperature of 63°C for 30 minutes, but the subsequent step of quick cooling to 4°C was omitted. A quantity of aqueous cosmetic formulation was inoculated with a prescribed volume of standardized inoculum containing not less than 10^5 cfu/ml or cfu/g in screw capped glass bottle and subjected to a temperature of 63°C for 30 minutes in a water bath. On completion of pasteurization, the formulation was cooled to room temperature and tested for the microbial count by plate count method. Subsequently, the blends were incorporated into the above cosmetic formulation at concentrations varying from 0.5% to 4%, mixed thoroughly and maintained at room temperature. The samples were removed for microbial enumeration after 7, 14, 21, 28, 60 and 90 days. A set of blanks were also maintained without the addition of blends and observed for microbial load similar to that of test.. Three replicates were studied to ensure reproducibility of the results.

Antioxidant activity

The blends were also studied for their antioxidant potential *in vitro*. DPPH radical scavenging activity was employed as used by Hang., 1997 [15]., Oxygen Radical Absorbance Capacity Assay was performed according to Ou *et al.*, 2001 [16]., inhibition of lipid peroxidation was determined according to Duh & Yeh., 1997 [17]. and Paraskevopoulou and Kiosseoglou, 1994 [18]. Three replicates were studied to ensure reproducibility of the results

Table 2 Composition of the blends (% w/w)

S. No.	Plant material	Active Essential Oil fractions/Extracts	% composition (w/w of the blend I)	% composition (w/w of the blend II)	% composition (w/w of the blend III	% composition (w/w of the blend IV
1.	Cinnamon	Cinnamaldehyde 90-95%	21.88	17.47	-	-
2	Thyme	Thymol 95-98%	14.36	11.47	61.04	19
3.	Clove	Eugenol 95-99%	15.26	12.18	-	21
4.	Lemon grass	Citral E & Z 85-90%	17.62	14.067	-	23
5.	Monolaurin	Monolaurin (90%)	28.05	44.80	38.00	37
6.	Garcinol	Garcinol (97%)	2.80	-	-	-
7.	SCFE extract of Magnolia officinalis containing magnolol and honokiol	Magnolol (99%)			0.95	-

Peroxide value

Peroxide values of the blends were determined according to official monograph [19].. Three replicates were studied to ensure reproducibility of the results.

Results and Discussion

Antimicrobial activity and challenge test

The aforementioned preservative blends showed a tenfold increase in antimicrobial activity against the tested microbial cultures in comparison with the individual components, which clearly delineated synergy (Table 3 and 4). Synergism is the expression used when the inhibitory action of the combination is reached at a concentration lower than that of constituent substances separately.

Our findings confirmed that the improved antimicrobial action of the natural preservative combinations over the individual components is determined by more than one active. In such cases, the major component is not only responsible for the activity and the synergistic effect is due to interplay between all the components [20].

Quality deterioration of perishables by microorganisms can result in spoilage of various degrees ranging from reduction in shelf life to presence or growth of infectious or toxinogenic microorganisms [21]. Cosmetics are prone to microbial contamination during manufacture or

Table 3 Minimum inhibitory concentration of the blend constituents (%w/v)

S. No.	Active Essential oil fractions/Extracts	Minimum Inhibitory Concentration		
		S. aureus	*E. coli*	*C. albicans*
1	Cinnamaldehyde 90-95%	0.7	0.3	0.7
2	Thymol 95-98%	0.3	0.3	0.7
3	Eugenol 95-99%	0.3	0.3	0.7
4	Citral E & Z 85-90%	6.25	0.3	0.7
5	Monolaurin 90%	0.12	Not active	0.25
6	Garcinol 97%	0.07	Not active	Not Active
7	Magnolol 99%	0.08	Not active	0.08

at the end user level. This can cause undesirable changes in the composition, odour or colour of products, posing a risk of infection or deterioration of the product.

The assessment of microbial contamination resistance of preserved personal care formulations is a critical element in the development of safe and effective consumer products. To ensure that the product is sufficiently preserved, challenge testing is adopted. In the present study, the preserved test system was evaluated at multiple test points for the survivability of selected microorganisms that were purposely introduced into it. The blends demonstrated the preservative potency effectively at higher concentrations ranging from 2.0% to 4.0% whereas synthetic preservative combination of methyl paraben and propyl paraben were effective at concentration of 0.5%.

Pasteurisation combined with natural preservative for increased shelf life

The use of multiple preservation techniques is a common practice to improve stability of shelf stable foods. It advocates the intelligent use of combinations of different preservation factors or 'hurdles' to achieve multi-target, mild but reliable preservation effects [22].One of the main reasons for toxic effects of preservatives in cosmetics is the exposure dose making it necessary to optimize the preservative system to lessen the said effects [4] As a solution to this, the present study demonstrates that the 'hurdle concept' which is alien to the cosmetic industry, can be used to prolong the shelf life of the product. The present investigation revealed that the addition of the natural preservative blends subsequent to pasteurisation by omitting the conventional deep freezing step controlled the microbial population at bare minimal levels thereby improving the shelf life. Our findings showed that the synergistic blends which were strong preservatives at 2.0% and 4.0% may cause skin irritation in susceptible individuals. However, use

Table 4 Minimum inhibitory concentration of the blends (%w/v)

Target Organisms	MIC concentration (% w/v) of Preservative Blends			
	BLEND I	BLEND II	BLEND III	BLEND IV
S.aureus	0.070	0.070	0.075	0.070
E.coli	0.070	0.070	0.40	0.070
C. albicans	0.070	0.070	0.075	0.070

of combination of physical and chemical means of biocontrol i.e. pasteurization followed closely by incorporation of synergistic preservative blends at much lower concentration ranges (0.5% - 1.5 %) effectively inhibited the growth and multiplication of the existing microbial load in cream formulations (Table 10) thereby bestowing a satisfactory shelf life which is characteristic of a product of high quality and safety.

Table 5 Preservative efficacy of the blends against *S. aureus* ATCC 6538

Plain Base With Preservative Blends	Cell count (cfu/ml) at below test intervals			
	0 days: 10.0×10^5 cfu/ml			
	7 days	14 days	21 days	28 days
Blend I (0.5%)	85	<10	<10	<10
Blend I (1.0%)	75	<10	<10	<10
Blend I (1.5%)	55	<10	<10	<10
Blend I (2.0%)	40	<10	<10	<10
Blend I (4.0%)	15	<10	<10	<10
Blend II (0.5%)	90	<10	<10	<10
Blend II (1.0%)	75	<10	<10	<10
Blend II (1.5%)	65	<10	<10	<10
Blend II (2.0%)	50	<10	<10	<10
Blend II (4.0%)	15	<10	<10	<10
Blend III (1.5%)	850	80	<10	<10
Blend III (2.0%)	90	<10	<10	<10
Blend III (4.0%)	35	<10	<10	<10
Blend IV (0.5%)	95	<10	<10	<10
Blend IV (1.0%)	80	<10	<10	<10
Blend IV (1.5%)	60	<10	<10	<10
Blend IV (2.0%)	45	<10	<10	<10
Blend IV (4.0%)	15	<10	<10	<10
Blank	7.45×10^5	11.7×10^5	12.3×10^5	15.25×10^5
Synthetic preservative blend	15	<10	<10	<10

The blanks which were pasteurized and maintained without incorporation of blends showed increase in the microbial load over a period of three months (Table 9) confirming that the combination gives better results when compared to use of pasteurization alone. This shows that hurdle technology is a better means of preservation. Effect of challenge test and pasteurization combined with synergistic blends on microbial strains

S. aureus is one of the common members of the normal human microflora. But, it can become an opportunistic pathogen and form the commonest causes of localised suppurative lesions in human beings in addition to causing food poisoning, skin infections and even critical conditions like toxic shock syndrome [23]. The tested blends showed strong antimicrobial activity against *S. aureus* at MIC in the range of 0.070%-0.075%. All the blends were efficacious in reducing the cell count by 5.0 log in 4 weeks at concentrations of 1.5% to 4.0% except for the blends I, II and IV which showed strong preservative action even at still lower concentrations of 0.5% and 1.0% (Table 5). However, all the preservative blends incorporated in the pasteurized cream base elicited a potent preservative effect and controlled the microbial load to bare minimal levels for upto three months at concentration ranging from 0.5% to 4.0% (Table 10).

E. coli is a gram negative bacterium belonging to Enterobacteriaceae family and found widely distributed in the intestine of humans and other warm blooded animals. It is regarded as an opportunistic pathogen responsible for infections caused in immunocompromised individuals [24]. Certain pathogenic strains of this organism are responsible for gastrointestinal diseases. The blends I, II and IV showed antimicrobial activity with MIC of 0.070% and blend III with MIC of 0.40% (Table 4). Blends I, II showed 5.0 log reduction in the bacterial count at the end of 28 days in the range of 0.5%-4.0% and hence a more effective preservative compared to blend IV which showed efficacy at higher concentrations of 2.0% - 4.0% (Table 6). Blend III was not effective at any of the tested concentrations. *E.coli* was found to be controlled at minimal levels of less than 10 cfu/g of cosmetic cream base, when subjected to pasteurisation temperatures and prolonged preservation by addition of preservative blends for a period as long as 3 months at room temperature (Table 10).

P. aeruginosa is a common opportunistic pathogen causing both invasive and chronic non-invasive infections in several organs resulting in a diversity of infectious diseases [25].

The preservative blends, when used in combination with the pasteurized moisture rich creams effectively controlled the growth and

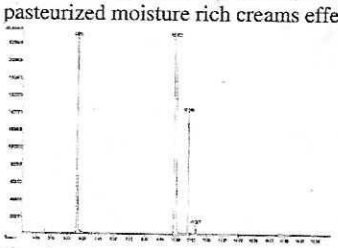

Fig. 5

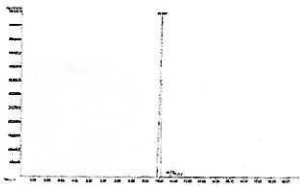

Fig. 6

multiplication of this highly resistant organism for as long as three months at concentrations ranging from 0.5% - 4.0 %(Table 10). But, the blends as such were not potent enough in controlling the proliferation of *P. aeruginosa.*

A. niger, apart from being a common air borne fungi, produces aflatoxins and sterigmatocystin which can have serious implications on human and animal health. It is also responsible for a serious health condition called aspergillosis comprising several categories of infections like invasive aspergillosis, chronic necrotizing aspergillosis, aspergilloma or fungus ball and allergic bronchopulmonary aspergillosis [26].Blends I and II at 0.5% showed a slight increase in the fungal load after 14 days. However, concentrations of 1% to 4% showed potent preservative action against *A. niger* (Table 7). Blends III and IV effectively controlled the fungal growth for two weeks. But, there was an increase in the count for the next two test intervals. However, the objective of long term preservation was accomplished with the preservative blends incorporated in the pasteurized cream base which reduced the microbial numbers to bare minimal levels (<10 cfu/ml) even in case of the spore forming filamentous fungi (molds) like *A. niger* (Table 10).

Table 6 Preservative efficacy of the blends against *E. coli* ATCC 8739

Plain Base With Preservative Blends	Cell count (cfu/ml) at below test intervals			
	0 days: 37.0×10^6 cfu/ml			
	7 days	14 days	21 days	28 days
Blend I (0.5%)	95	<10	<10	<10
Blend I (1.0%)	85	<10	<10	<10
Blend I (1.5%)	60	<10	<10	<10
Blend I (2.0%)	45	<10	<10	<10
Blend I (4.0%)	20	<10	<10	<10
Blend II (0.5%)	90	<10	<10	<10
Blend II (1.0%)	70	<10	<10	<10
Blend II (1.5%)	60	<10	<10	<10
Blend II (2.0%)	50	<10	<10	<10
Blend II (4.0%)	20	<10	<10	<10
Blend IV (2.0%)	85	70	55	50
Blend IV (4.0%)	55	40	<10	<10
Blank	99×10^4	532×10^5	445×10^6	1430×10^6
Synthetic preservative blend	15	<10	<10	<10

C. albicans, the fungal organism belonging to the yeast group is a commonly occurring pathogen responsible for a variety of infections

Table 7 Preservative efficacy of the blends against *A. niger* NCIM 1196

Plain Base With Preservative Blends	Cell count (cfu/ml) at below test intervals			
	0 days : 3.6 x 10^6cfu/ml			
	7 days	14 days	21 days	28 days
Blend I (0.5%)	70	<10	125	150
Blend I (1.0%)	65	<10	<10	<10
Blend I (1.5%)	50	<10	<10	<10
Blend I (2.0%)	35	<10	<10	<10
Blend I (4.0%)	15	<10	<10	<10
Blend II (0.5%)	95	15	90	105
Blend II (1.0%)	80	<10	<10	<10
Blend II (1.5%)	70	<10	<10	<10
Blend II (2.0%)	55	<10	<10	<10
Blend II (4.0%)	25	<10	<10	<10
Blank	2.5×10^6	18×10^6	54×10^6	68×10^6
Synthetic preservative blend	15.5×10^3	34×10^3	650	460

Table 8 Preservative efficacy of the blends against *C. albicans* NCIM 3471

Plain Base With Preservative Blends	Cell count (cfu/ml) at below test intervals			
	0 days count: 7.7 x 10^6cfu/ml			
	7 days	14 days	21 days	28 days
Blend I (0.5%)	85	<10	<10	<10
Blend I (1.0%)	80	<10	<10	<10
Blend I (1.5%)	65	<10	<10	<10
Blend I (2.0%)	30	<10	<10	<10
Blend I (4.0%)	15	<10	<10	<10
Blend II (0.5%)	95	<10	<10	<10
Blend II (1.0%)	80	<10	<10	<10
Blend II (1.5%)	60	<10	<10	<10
Blend II (2.0%)	40	<10	<10	<10
Blend II (4.0%)	20	<10	<10	<10
Blend III (1.0%)	1660	180	624	2830
Blend III (1.5%)	980	65	45	<10
Blend III (2.0%)	525	80	30	<10
Blend III (4.0%)	80	<10	<10	<10
Blend IV (2.0%)	950	75	<10	<10
Blend IV (4.0%)	250	<10	<10	<10
Blank	15.4×10^5	4×10^5	19×10^5	22×10^5
Synthetic preservative blend	800	<10	<10	<10

Table 9 Blank-pasteurized cream without blends
Initial count of test organisms: *S. aureus* ATCC6538: 9 x 10^6cfu/ml, *E.coli* ATCC8739: 38 x10^6 cfu/ml, *P. aeruginosa* NCIM2200: 26 x 10^6cfu/ml, *A. niger* NCIM1196: 7 x 10^5cfu/ml, *C. albicans* NCIM3471: 4.6 x 10^6cfu/ml

Plain base without preservative	Test intervals						
	0days	7days	14 days	21 days	28 days	2months	3 months
S.aureus	<10	145	2.1×10^3	3.9×10^3	7.4×10^3	15×10^3	33×10^3
E.coli	<10	200	3.7×10^3	5.1×10^3	10.6×10^3	19.5×10^3	45×10^3
P.aeruginosa	<10	165	3.5×10^3	6.2×10^3	11.1×10^3	18.6×10^3	51×10^3
C. albicans	<10	95	3.2×10^3	5.6×10^3	10.2×10^3	15.8×10^3	44×10^3
A. niger	<10	110	3.0×10^3	5.1×10^3	9.3×10^3	16.3×10^3	49×10^3

Table 10 Effect of challenge test and pasteurization combined with the blends on microbial strains.
Initial count of test organisms: *S. aureus* ATCC6538: 9 x 10^6cfu/ml, *E.coli* ATCC8739: 38 x10^6 cfu/ml, *P. aeruginosa* NCIM2200: 26 x 10^6cfu/ml, *A. niger* NCIM1196: 7 x 10^5cfu/ml, *C. albicans* NCIM3471: 4.6 x 10^6cfu/ml

Plain base with preservative	PASTEURIZATION	Test intervals						
		0days	7days	14 days	21 days	28 days	2months	3 months
Blend I (0.5%)		<10	10	<10	<10	<10	<10	<10
Blend I (1.0%)		<10	<10	<10	<10	<10	<10	<10
Blend I (1.5%)		<10	<10	<10	<10	<10	<10	<10
Blend II (0.5%)		<10	<10	<10	<10	<10	<10	<10
Blend II (1.0%)		<10	<10	<10	<10	<10	<10	<10
Blend II (1.5%)		<10	<10	<10	<10	<10	<10	<10
Blend III (0.5%)		<10	<10	<10	<10	<10	<10	<10
Blend III (1.0%)		<10	<10	<10	<10	<10	<10	<10
Blend III (1.5%)		<10	<10	<10	<10	<10	<10	<10
Blend IV (0.5%)		<10	<10	<10	<10	<10	<10	<10
Blend IV (1.0%)		<10	<10	<10	<10	<10	<10	<10
Blend IV (1.5%)		<10	<10	<10	<10	<10	<10	<10

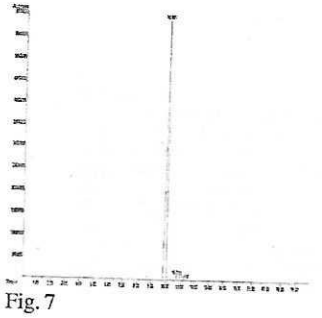
Fig. 7

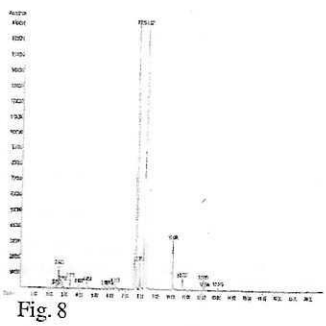
Fig. 8

Table 11 Anti-oxidant effect of the blends.

BLENDS	ORAC VALUES µmol TE/g	DPPH (IC_{50}) µg/ml	Lipid peroxidation Potential(IC_{50}) µg/ml	Peroxide value Initial	Peroxide value at 3rd month
I	4349.75 ± 1202	9.394	5.27	10.58	3.48
II	3729 ± 1076	14.04	5.99	0.57	NIL
III	6964 ± 990	57.31	6.42	10.75	4.24
IV	5006 ± 783	8.149	7.02	4.25	NIL

ranging from localized cutaneous candidiasis in healthy individuals to life threatening systemic candidiasis in immunocompromised hosts [27]. All the blends showed an MIC of 0.070%and were potent preservatives at 1.5% to 4.0%. Blends I and II showed strong inhibitory effects even at concentrations as low as 0.5% (Table 8). The natural preservative blends in pasteurized creams effectively preserved the formulation for as long as 3 months as evident from Table 10.

Anti-oxidant Activity and Peroxide Value

Antioxidants play a vital role in maintaining the quality, integrity and safety of cosmetic products as they retard the oxidation of fats and oils which causes rancidity during storage. Peroxide values of the blends showed that they are stable with the course of time and thus can prevent the products from rancidity. All the synergistic blends showed remarkable antioxidant property as evidenced by experimental data (Table 11). Thus, these blends can prevent oils and fats from oxidative deterioration and rancidity. Blends were also evaluated for their peroxide values initially and then after a period of three months. The peroxide values were found to decrease with the course of time (Table 11). Since microbes are sensitive to oxidative stress, lipid peroxidation at the cellular level enhances their multiplication. Thus, inhibition of lipid peroxidation improves the anti-microbial effect of a compound [28].

Conclusion

Preservatives are an integral part of cosmetic formulations. Traditionally used synthetic preservatives combat microbial contamination and spoilage of complex formulations, but concern is continuously raised about their safety. This has led the cosmetic industry to develop alternate means of preservation such as natural preservatives. A good preservative contains ingredients that have both antimicrobial and antioxidant properties. The preservative blends of essential oils, garcinol, monolaurin and magnolol are efficient synergistic antimicrobial agents and strong antioxidants, thus preventing microbial spoilage and oxidative deterioration. On their own, these may not be as efficacious as synthetic ones at lower concentrations. However, in combination with other inhibiting factors like pasteurization, they provide for effective long term preservation. The present study emphasizes the use of low concentrations of preservatives in combination with pasteurization as multiple hurdles to enhance the microbiological stability. In conclusion, a combination of means of preservation reduces the overall preservative concentration in cream formulations and such combinations can be used

for replacing the classical ones to increase the shelf life of products in a natural way.

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