

Development and In-Vitro SPF Evaluation of Marine-Derived Lip Balm Formulation: An Approach Using Red Algae Extract for Enhanced UV Protection

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ABSTRACT

Lips are highly susceptible to environmental stress and ultraviolet radiation, which can lead to dryness, pigmentation, and long-term health complications. Conventional lip care products commonly contain synthetic sunscreens and colorants; however, these ingredients may cause irritation, accelerate skin aging, trigger allergic reactions, and some have raised environmental concerns. The growing demand for safe, natural, and eco-friendly alternatives has encouraged the development of natural formulations that combine nourishment, protection, and cosmetic appeal. This study focuses on the formulation and evaluation of natural SPF lip balm designed to provide multifunctional benefits. Cocoa butter was selected as the primary emollient base due to its soothing and moisturizing properties, while beeswax was incorporated to provide stiffness and structural stability. Coconut oil contributed hydration, and almond oil enhanced nourishment and softness. Zinc oxide, carrot seed oil and red algae extract (a source of marine-derived mycosporine-like amino acids) were included for UV protection and as antioxidants to minimize free radical formation. Beetroot extract imparted a natural rosy tint, vitamin E functioned as an antioxidant and formulation stabilizer, and vanilla flavour was added to improve flavour and fragrance. The prepared natural SPF lip balm was evaluated for physicochemical and organoleptic parameters including (texture, pH, melting point, colour, odour), thin-layer chromatography, and in-vitro SPF by UV spectrophotometry. The in-vitro SPF of the final formulation was 7. The formulation aims to provide hydration, nourishment, and natural coloration along with UV protection, evaluated in vitro in the UVB range (290–320 nm). By integrating marine-derived UV filters with antioxidant-rich oils and natural colorants, this formulation suggests the potential of natural-ingredient-based lip care as an alternative to synthetic formulations; further safety, stability and sustainability studies are required. This research highlights the importance of combining cosmetic attractiveness with functional protection.

Keywords: Lip balm, Ultraviolet radiation (UVA/UVB), Sun induced lip damage, Marine derived sun protectant, Mycosporine-like amino acid, Natural SPF balm

INTRODUCTION

Lips are the most sensitive areas of the face. The upper and lower lips are referred to as the labium superius oris and labium inferius oris, respectively [1,2]. The juncture where the lips meet the surrounding skin of the mouth area is the vermilion border [3], and the typically reddish area within the borders is called the vermilion zone [4]. The vermilion border of the upper lip is known as the Cupid's bow [5]. The fleshy protuberance located in the centre of the upper lip is a tubercle known by various terms including the procheilon (also spelled prochilon), the "tuberculum labii superioris", and the "labial tubercle" [6]. The vertical groove extending from the procheilon to the nasal septum is called the philtrum [7].

Compared with other body sites, lips have limited natural protection against ultraviolet radiation. This is because the skin of the lip is highly fragile, delicate and thin. It is made up of only 3 to 5 cellular layers, whereas the rest of the part of the face consist of 15 to 16 protective layers. Lips also contain little melanin (which is a natural pigment, crucial for absorbing harmful UV radiation) and have few or no sebaceous glands (oil producing gland which secrete sebum, which protects the body from environmental pathogens).

ANATOMY OF THE LIPS

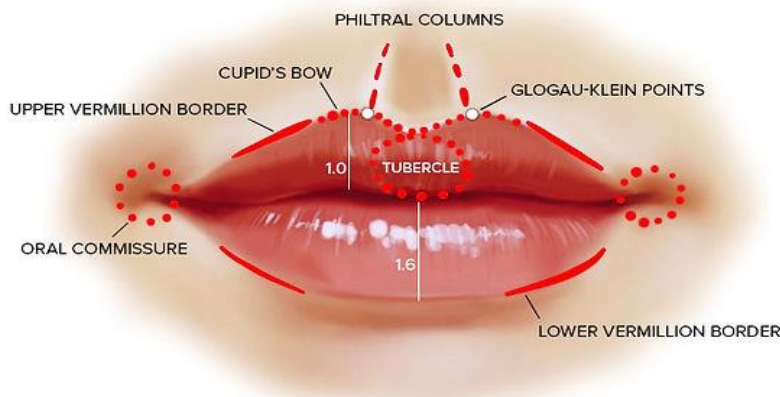


Figure 1: Anatomy of Lips (Source: Abboud^[8])

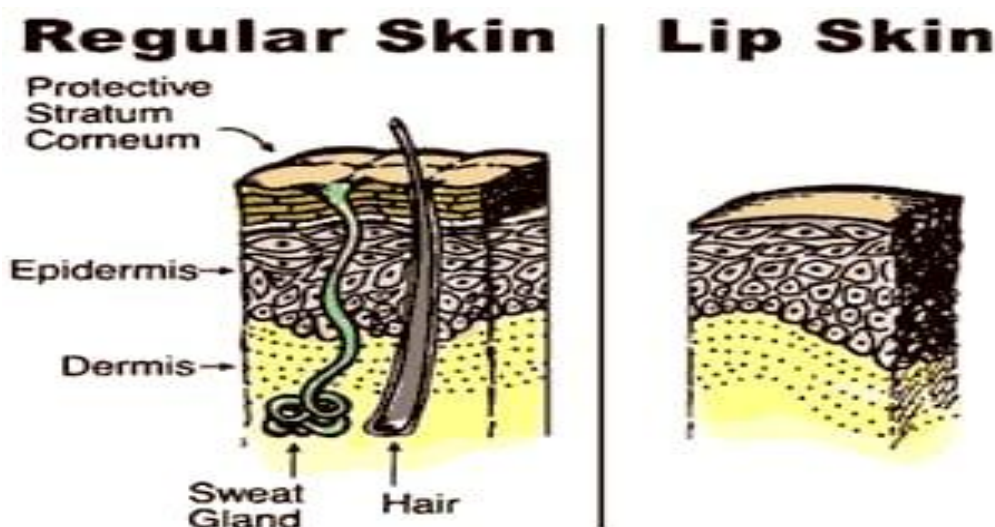


Figure 2: Comparison of regular skin and lip skin (Source: Krishnapriya et. al. ^[9])

Health Concerns Due To Sun Exposure On Lips:

Exposure to ultraviolet radiation can cause both acute and chronic effects on the lips.

1. Immediate effects include dryness, blistering, peeling, swelling and redness on the lips.
2. Repeated and long-term exposure can lead to permanent and harmful effects like pale discoloration, Actinic Cheilitis (Solar Cheilitis) and also lip cancer that is squamous cell carcinoma.

Some mineral lip balms use zinc oxide (ZnO) as the primary filter, which can leave a white cast. To reduce this, ZnO may be lowered and combined with organic filters such as oxybenzone and avobenzone. Some studies suggest these filters can be absorbed systemically and may have endocrine-disrupting effects. There

have been increasing concerns about the use of sunscreens and their safety for both humans and the environment. Published studies have shed light on this topic by investigating the harmful effects of UV filters such as oxybenzone on the hormone system of aquatic animals and humans. Currently, in vitro and in vivo animal models are being used to determine the mechanistic and cellular effects these products produce. With growing awareness of adverse effects posed by UV filters on the environment and exposed organisms, several jurisdictions are prohibiting their use in sunscreens. ^[10]

Natural Alternatives to Synthetic Uv Filters

Natural photoprotective agents are gaining prominence in cosmetic science due to their potential as natural, marine derived alternative. Mycosporine-like amino acids (MAAs), derived from marine sources, act as natural UV-absorber, while carrot seed oil contributes to antioxidant activity and mild sun protection. In combination with ZnO, a well-established sunscreen agent, these agents may offer complementary mechanisms and photoprotection. Enriched with vitamin E, this tinted formulation was designed to combine photoprotection, antioxidant support, and consumer appeal in a multifunctional lip care product.

Mycosporine-Like Amino Acid, A Marine Derived Approach Against Uva & Uvb Radiation

Mycosporine and mycosporine-like amino acids (MAAs) are derived from many marine living sources like algae, fungi, lichens, seaweeds, corals and cyanobacteria. These compounds absorb ultra-violet rays and also have photoprotective and antioxidant functions. MAAs are explored as natural UV filter and antioxidant ingredients in cosmetic products.

MAAs are low-molecular-weight (generally < 400 Da), colourless, water-soluble compounds and highly stable molecules under environmental conditions. ^[11] They were first isolated by Wittenberg et al. (1960) from a siphonophore, *Physalia physalis*. ^[11] MAAs are not found in higher plants or animals since they have a natural UV protectant like flavonoid in the former, and melanin in the latter. MAAs are present especially in organisms that live in environments with high levels of UVR. Their composition varies according to the taxonomic group with the frequent coexistence of several MAAs with different absorption maxima allowing a more effective protective filter. ^[11] There are majorly 2 types of ultraviolet rays that reach the surface of the earth, namely, UVA and UVB rays. UVA rays penetrate deep into the skin and are responsible for premature aging (photoaging), ^[12] wrinkle formation and also in the formation of skin cancer. ^[12] UVB rays produce sunburn and they have a greater role in forming skin cancer like malignant melanoma (i.e. deadly black mole of skin cancer).

MAAs have high molecular absorptivity ($\epsilon = 12.400\text{--}58.800 \text{ M}^{-1}\cdot\text{cm}^{-1}$), ^[11] making them very potent UVA-absorbing substances found naturally in marine ecosystem. They also provide protection against UVB radiation, which highlights their significant role in photoprotection. Since MAAs are highly water soluble, they are primarily located in the cytoplasm, so that they can be distributed evenly.

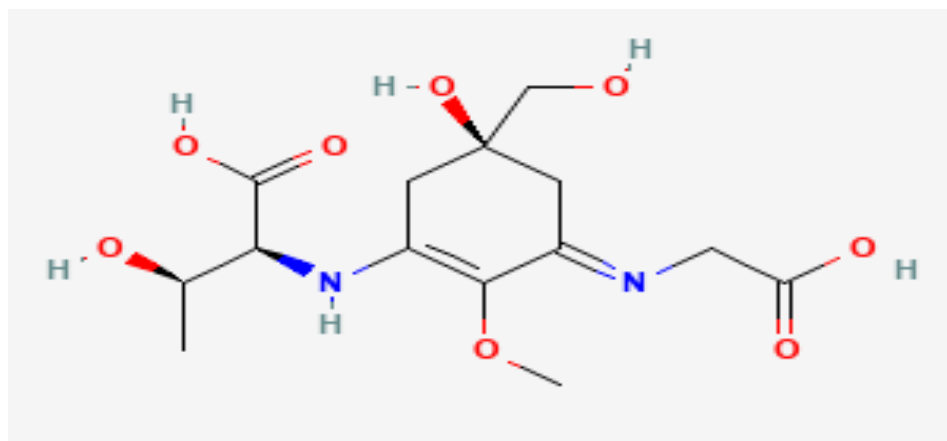


Figure 3: Chemical Structure of Mycosporine-like amino acids (MAAs) - Example of MAA: Porphyrin 334

(2S,3R)-2-[[[(5R)-3-(carboxymethylimino)-5-hydroxy-5-(hydroxymethyl)-2-methoxycyclohexen-1-yl]amino]-3-hydroxybutanoic acid (Source: PubChem [\[13\]](#))

MAAs are derived from various marine sources viz., [\[11\]](#)

1. Dinoflagellates
2. Cyanobacteria
3. Microalgae
4. Seaweeds
5. Sea stars
6. Corals
7. Fungi
8. Lichen

They have many biological functions like: [\[11\]](#)

1. Photoprotection: The photoprotective function is the most important role that MAAs play in nature. This is verified by the abundance of MAAs in organisms exposed to high UVR intensities.
2. Antioxidant property: UVR exposure generates oxidative stress and can produce reactive oxygen species (ROS) and DNA damage. This oxidative DNA damage can lead to mutations and inhibit DNA repair. Antioxidants can reduce the harmful effects of ROS and can, thus, help to prevent oxidative stress.
3. Osmotic balance: In hypersaline environments, cyanobacteria usually contain high concentrations of MAAs suggesting that these compounds may have an osmotic function helping the cells to cope with the high salinity.
4. Thermal stress: There are a few reports of thermal stress protection by an increase in MAA induction in a range of organisms. Michalek-Wagner (2001) stated that MAA content was upregulated under heat stress, and their concentrations were further enhanced during simultaneous exposure to UV. [\[14\]](#)
5. Desiccation stress: Desiccation is responsible for cell damage by affecting cytoplasmic components (such as DNA and proteins) and cell membrane fluidity. The production of polysaccharides and antioxidant compounds are some of the techniques to overcome the consequences of drying.

Their cosmetic applications include: [\[11\]](#)

1. Natural Sunscreens: MAAs absorb UV radiation efficiently and hence act as photo protective agents.
2. Antioxidant properties: They scavenge reactive oxygen species generated by UV exposure and reduce oxidative stress in cells by maintaining cellular integrity and prevent lipid peroxidation.
3. Anti-inflammatory properties: MAAs have been reported in in-vitro studies to inhibit pro-inflammatory cytokines like Tumour Necrosis Factor- α and Interleukin-6.
4. Anti-aging function: MAAs slow down collagen degradation and cellular senescence which preserve skin elasticity and firmness.
5. Inhibition of Protein Glycation: Protein Glycation can cause formation of advanced glycation end products which can cause wrinkles and also stiffen the collagen. MAAs may inhibit this protein glycation.
6. Inhibition of Collagenase Activity: Collagenase enzymes break down collagen fibres which can lead to ageing. MAAs may suppress this activity of collagenase.

These activities were not evaluated in this study.

Mechanism Of Action Of Maas As Uv Protectants:

1. MAAs absorb UVA/UVB rays mainly between about 310 and 362nm. They share a common cyclohexanone (AcyO) or cyclohexanimine (NN) nucleus.

2. MAAs gets excited from ground state (S₀) to first singlet excited state (S₁) due to UV photon absorption via π - π transition. Then it converts back to relaxation state (S₀) by internal conversion. This absorbed energy is released as heat into the surrounding environment that is the 90% of photon energy, which is confirmed by photoacoustic calorimetry.
3. This process is rapid and the non-destructive return to ground state (S₀) helps in minimizing the chances of damaging photoreactions (like reactive oxygen species and photodegradation).
4. In this formulation, MAAs are combined with zinc oxide and carrot seed oil with the aim of complementary photoprotection; synergy was not evaluated in this study.
5. Zinc oxide is a mineral UV filter and acts as a physical barrier that sits on skin's surface rather than getting absorbed into the body. Zinc oxide is an inorganic filter that mainly absorbs UVA and UVB radiation, with a smaller scattering contribution, dissipating the energy as heat; it remains largely on the skin surface.
6. Carrot seed oil absorbs UV rays with the help of its active compounds like umbelliferone that absorb UVB rays to block them from penetrating the skin. It also contains carotenoids and other constituents with antioxidant activity; in this study its in-vitro SPF was negligible (0.26), so it was included mainly for antioxidant and emollient effects.
7. The combination is intended to provide photoprotection while limiting white cast; safety, irritation potential and white cast were not assessed in this study.

MATERIALS AND METHODS

Materials: Zinc Oxide, Red Algae Extract (Source: Seaweed, Part used: Thallus, Supplier: MYOC, Grade: Cosmetic), Carrot Seed Oil, Lecithin, Beeswax, Cocoa Butter, Coconut Oil, Almond Oil, Vitamin E, Vanilla Flavour, Beetroot Extract, and all other reagents used were of pharmaceutical grade.

Preformulation studies

1. Selection of base materials: Beeswax is used to provide structure and hardness and Cocoa Butter provides moisturizing and smooth application of the lip balm.
2. Selection of UV Filters: Red algae extract and carrot seed oil were included as natural photoprotective/antioxidant ingredients; coconut oil was used as an emollient.
3. Selection of emulsifier: Since waxes and oils were oil phase and red algae extract was aqueous phase, phase separation could occur. Hence, to avoid that Lecithin was used as an emulsifying agent to prepare an emulsion of both phases.
4. SPF determination: SPF of each active ingredient was determined individually using UV-Visible spectrometry.
- 5.

TABLE 1: Composition of SPF Lip Balm

SR. NO.	INGREDIENTS	PHASE	QUANTITY FOR 100 g	ROLE
1.	Red Algae Extract	Aqueous Phase	32g	Natural UV Protectant
2.	Zinc Oxide	Oil Phase	15g	Mineral UV Filter
3.	Carrot Seed Oil	Oil Phase	2.5g	Mild UV Protectant, Antioxidant
4.	Beeswax	Oil Phase	20g	Structuring Agent

5.	Cocoa Butter	Oil Phase	15g	Emollient
6.	Coconut Oil	Oil Phase	6g	Emollient
7.	Almond Oil	Oil Phase	6g	Emollient
8.	Lecithin	Oil Phase	2g	Emulsifier
9.	Vitamin E	Oil Phase	0.5g	Antioxidant
10.	Vanilla flavour (artificial)	Aqueous Phase	0.5g	Flavour & Fragrance
11.	Beetroot Extract	Aqueous Phase	0.5g	Natural Pigment
	TOTAL		100g	

METHODOLOGY

The lip balm was formulated using Emulsion-based incorporation technique.

1. The ingredients which are in the aqueous phase that is Red Algae and Beetroot extract along with Artificial Vanilla were weighed and mixed in a beaker. The temperature of this mixture is then maintained at 60°C.
2. The oil phase ingredients that are the Beeswax, Cocoa Butter, Coconut Oil, Almond Oil along with the emulsifying agent Lecithin were melted and stirred to make a uniform mixture.
3. Now, small amount of oil phase mixture was taken and Zinc Oxide was dispersed slowly into it. A smooth paste was made and this paste was then added in the remaining oil phase mixture.
4. The aqueous phase mixture at 60°C was slowly added in the oil phase mixture. Phase separation will start to occur; hence it was continuously mixed using overhead stirrer until a smooth paste is formed. Carrot seed oil and Vitamin E are also added while stirring.
5. Final mixture was added in the mould and it was allowed to set at room temperature and then it was refrigerated to gain proper shape. (Figure 4)



Figure 4: Final Product image

Evaluation Parameters

1. pH of the formulation [\[15\]](#): pH meter was used to carry out this evaluation. Determining the pH of the lip balm is essential to check its compatibility with the human lips. Small amount of lip balm was mixed with water. The pH meter probe was cleaned with distilled water. The probe was immersed fully in the test sample without letting it touch the bottom or sides of the container. The pH value was recorded from the screen.
2. Melting Point [\[15\]](#): The melting point of the formulation was determined to assess thermal stability during storage and use of the lip balm. To check the melting point, the assembly was formed where the lip balm was first filled into a capillary and then attached to a thermometer which is further dipped in liquid paraffin filled in a thiele tube. The point at which the sample melts are noted.
3. Homogeneity [\[16\]](#): Homogeneity is carried to check whether the formulation has uniform distribution of API and bases. It is carried out by smearing the sample on a glass slide and placing another slide on top of that and checking whether it shows any coarse grains.
4. Physical evaluation:
 - Colour [\[15\]](#)- The colour of formulated lip balm was evaluated visually.
 - Odour [\[15\]](#)- The Odour was evaluated by smelling the lip balm.
 - Consistency- To assess the texture and stability, consistency was evaluated.
5. Thin-layer chromatography (TLC): TLC was performed for red algae (RA) extract and carrot seed oil. Silica plate was used as stationary phase and Chloroform: Acetic acid: Methanol: Water = 15:8:3:2 (v/v) as mobile phase. Spotting was done and it was detected in UV at 254 nm. Retention factor (Rf) was calculated by the following formula-

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by the solvent}}$$
 This confirms the presence of RA and carrot seed oil in the given sample.
6. In vitro SPF determination using UV-Visible spectrometry [\[16\]](#): All samples (1g) were weighed, transferred to a 100 mL volumetric flask, diluted to volume with ethanol, mixed for 15 min and then filtered through Whatman filters. A 5.0 mL sample was transferred to a 25mL volumetric flask and diluted to volume with ethanol. The absorption values were obtained in the range of 290 to 320 nm (every 5 nm) in 1cm quartz cuvettes using UV-Vis Spectrometer (Make: Shimadzu, Model: UV 1900). Each measurement was conducted three times and the value given is the mean value at each point. Then, Mansur equation was used to determine the SPF values of the formulations. Mansur et al. (1986) developed a very simple mathematical equation, which in conjunction with substitutes the in vitro method proposed by Sayre et al. (1979), UV spectrophotometry. The introduced equation is as follows:

$$\text{SPF (in vitro)} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times \text{abs}(\lambda)$$

Figure 5: Mansur equation (Source: Asrori MR)[\[17\]](#)

Here,

CF: Correction factor (set to 10).

EE(λ): Erythral effect spectrum at wavelength λ .

I(λ): Solar intensity spectrum at wavelength λ .

Abs(λ): Spectrophotometric absorbance of the sunscreen solution at wavelength λ .

λ : Wavelength ranging from 290 nm to 320 nm in 5 nm increments.

A marketed lip balm (Deconstructing Brightening Lip Balm, labelled SPF 30) as reference sunscreen, and the base with only red algae, zinc oxide and carrot seed oil were analysed individually under identical conditions. This is a screening method, and not a substitute for ISO 24444 (in vivo).

RESULT AND CONCLUSION

1. pH of the formulation: The pH of the lip balm formulation was determined using a calibrated digital pH meter. 1 gm of the sample was dispersed in 10 mL distilled water. The measured value was $\text{pH } 6.81 \pm 0.02$ at room temperature (25°C).
2. Yosipovitch et al. reported a mean surface pH value of 6.61 for the lips of healthy controls; however, these measurements were made on the lower lip mucosa, not on the vermilion. [\[18\]](#)
3. The pH is close to the reported lip mucosal surface pH (6.61); irritation testing was not performed.
4. Melting point: The melting point of the lip balm formulation was determined using the capillary method and thiele tube. The temperature at which the balm transitioned from solid to semi-liquid was recorded. The measured melting point was $62.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ suggesting the balm will remain solid at typical ambient temperatures.
5. Homogeneity: The formulation was homogenised properly due to continuous stirring using the over-head stirrer. No coarse particles were suspended in the final formulation.
6. Physical evaluation: The organoleptic property of the lip balm was evaluated visually and sensorially. The formulation had a brownish-pink colour due to the presence of beetroot extract. The odour was mild, pleasant and slightly sweet due to incorporation of vanilla essence. The consistency was smooth and uniform no phase separation or grittiness immediately after preparation.



Figure 6: Physical evaluation of lip balm

TLC

The R_f value of red algae extracts and carrot seed oil was found to be 0.370 and 0.118 respectively which provided preliminary evidence for the presence of corresponding constituents in the tested sample.

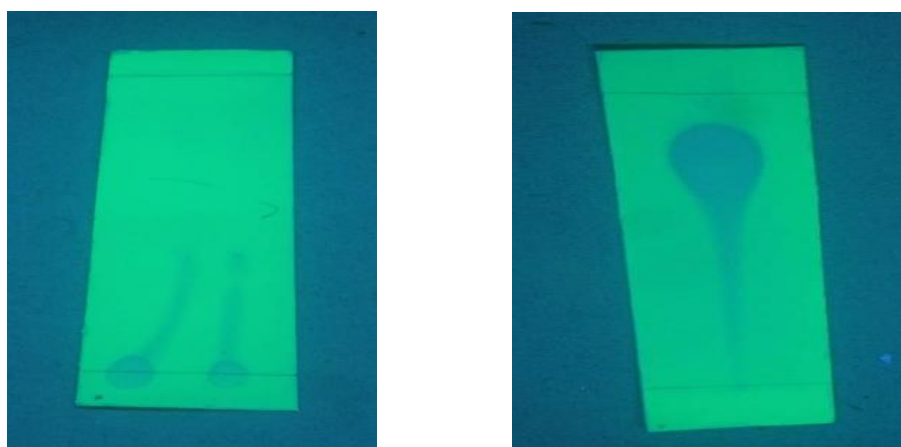


Figure 7: a. TLC of Red Algae,

b: TLC of Carrot seed oil

1. In vitro SPF determination using UV-Visible spectrometry:

TABLE 2: SPF Determination of Red Algae Extract

WAVELENGTH (nm)	EE (λ)*I(λ) EMPLOYED	ABSORBANCE (A)	EE (λ)* I(λ)*ABSORBANCE(A)
290	0.0150	0.629	0.009435
295	0.0817	0.503	0.0410951
300	0.2874	0.479	0.1376646
305	0.3278	0.473	0.1550494
310	0.1864	0.468	0.0872352
315	0.0837	0.467	0.0390879
320	0.0180	0.466	0.008388
TOTAL			0.478072
SPF (TOTAL*10)			4.78

TABLE 3: SPF Determination of Zinc Oxide

WAVELENGTH (nm)	EE (λ)*I(λ) EMPLOYED	ABSORBANCE (A)	EE (λ)* I(λ)*ABSORBANCE(A)
290	0.0150	0.7844	0.011766
295	0.0817	0.7813	0.063832
300	0.2874	0.7817	0.22466
305	0.3278	0.7776	0.25489
310	0.1864	0.7644	0.1424841
315	0.0837	0.7594	0.063713
320	0.0180	0.7409	0.013336
TOTAL			0.7746811
SPF (TOTAL*10)			7.77

TABLE 4: SPF Determination of Carrot Seed Oil

WAVELENGTH (nm)	EE (λ)*I(λ) EMPLOYED	ABSORBANCE (A)	EE (λ)* I(λ)*ABSORBANCE(A)
290	0.0150	0.06	0.0009
295	0.0817	0.04	0.003268
300	0.2874	0.03	0.008622
305	0.3278	0.025	0.008195
310	0.1864	0.02	0.003728
315	0.0837	0.015	0.0012555

320	0.0180	0.01	0.00018
TOTAL			0.026148
SPF (TOTAL*10)			0.26148

TABLE 5: SPF Determination of Final Formulation

WAVELENGTH (nm)	EE (λ)*I(λ) EMPLOYED	PREDICTED ABSORBANCE (A)	EE I(λ)*ABSORBANCE(A)
290	0.0150	0.7433	0.01
295	0.0817	0.7349	0.06
300	0.2874	0.7327	0.21
305	0.3278	0.7260	0.24
310	0.1864	0.7232	0.13
315	0.0837	0.7163	0.06
320	0.0180	0.7091	0.01
TOTAL			0.73
SPF (TOTAL*10)			7.3

TABLE 6: SPF Determination of Marketed Formulation (Deconstructing Brightening Lip Balm, labelled SPF 30)

WAVELENGTH (nm)	EE (λ)*I(λ) EMPLOYED	ABSORBANCE (A)	EE I(λ)*ABSORBANCE(A)
290	0.0150	2.4281	0.0364215
295	0.0817	2.5734	0.21024678
300	0.2874	2.7004	0.77609496
305	0.3278	2.9650	0.971927
310	0.1864	2.8575	0.532638
315	0.0837	2.8949	0.2518563
320	0.0180	2.9115	0.052407
TOTAL			2.8315
SPF (TOTAL*10)			28.315

The in-vitro SPF values of red algae extract, zinc oxide and carrot seed oil (0.1 mg/mL) were 4.78, 7.77 and 0.26 respectively. The final formulation showed an SPF of 7.3, and for the marketed product SPF was found to be 28.315. Within the limits of the Mansur method (UVB range, screening only), these results indicate that the ingredients used suggest a potential contribution as photoprotective agents. Stability, safety/irritation, microbial quality and MAA content were not evaluated and need further study.

Conflict of Interest

The authors declare no conflicts of interest.

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