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Research Article

Novel Researched Herbal Sunscreen Cream SPF Determination by In-Vitro Model

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A B S T R A C T

INTRODUCTION: Researchers' interest in creating novel cosmetic formulations has increased due to consumer interest in herbal cosmetics and increased patent activity. The rights of indigenous traditional knowledge and benefit sharing are also safeguarded under IPR.

OBJECTIVE: To formulate and evaluate herbal sunscreen with determination of Sun Protection Factor (SPF) and anti-oxidant activity. To compare Sun Protection Factor of developed formulation with marketed formulation.

METHOD: The formulation was developed according to the prepared formula. And multiple tests were done for evaluation i.e., physical observation, spreadability, extrudability, occlusion study, stability study and SPF determination. All the evaluations were found satisfactory. Characterisation of SPF was calculated according to the and UV-Vis Spectrophotometer (LABMAN Scientific instruments Pvt. Ltd.).

RESULTS: The synergistic activity of all herbal compounds utilized in herbal sunscreen formulations, such as *Cucumis sativus*, *Solanum Lycopersicon*, and *Aloe barbadensis* Efficacy of photoprotection found in following order Marketed formulation > F3 > F2 > F1. For prepared formulation F3 provided better results in comparison to Formulation1 and Formulation 2. Formulation 3 was compared with marketed preparation and it showed good SPF value nearer to market preparation. Overall results were satisfactory. These results reveal that the prepared F3 herbal sunscreen have good SPF and good sun protection activity.

CONCLUSION: Formulation 3, which consists of three formulations, has been found to be effective as sunscreen in every way. Since few people use sunscreen, there is a need to raise public knowledge of the risks associated with sun exposure as well as the advantages of using sun protection products on a regular basis to lessen these effects. This kind of research will be useful in offering consumers with an all-inclusive solution or product that will protect them from the damaging effects of sunlight.

Keywords: Herbal, UV rays, sunscreen, skin, SPF, Extraction.

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INTRODUCTION

The term "cosmetic" refers to any item intended to be rubbed, poured, sprinkled, sprayed, introduced into, or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance. This definition also includes any item intended for use as a cosmetic component [1]. There is a need to introduce cosmetics of the highest calibre because the market for herbal cosmetics is expected to develop in the years to come. Along with consumer interest in herbal cosmetics, increased patent activity has increased researchers' interest in developing novel cosmetic formulations. Additionally, access to benefit sharing and the rights of indigenous traditional knowledge are protected under intellectual property rights (IPR) [2].

The following criteria can be used to classify cosmetics [1,3]:

- Accordingto the bodypartwhereitisused : Skin, hair, teeth, eye
- According to the function of cosmetics preparations: Emollients, Cleansing creams, lipsticks.
- According to composition of cosmetics: Powder, lotion, emulsion, gel.

- A variety of medicinal plants have been studied for wound healing activity with the goal of identifying medicines with the lowest toxicity and highest effectiveness [4,5].

Sunlight: Ultraviolet (UV) radiation is described as the range of electromagnetic energy, between 200 and 400 nm, that falls between X-rays and visible light. According to wavelength, there are three groups of ultraviolet light (Figure 1.2): [6,7].

The wavelength of UV-A radiation is between 320 and 400 nm. Due to excessive melanin secretion in the skin, early dermatophilosis, inhibition of immunological activities, mortification of endothelial cells, and even death of dermic blood vessels, UV-A is the radiation that causes skin to instantly darken [8].

UV-B Radiation: The wavelength of this radiation is 280–320 nm. Burning rays, or UV-B radiations, are 1000 times more likely to result in sunburn than UV-A radiation. UV-B radiation is more genotoxic than UV-A radiation because it primarily destroys DNA in the epidermal layer of the skin. Sunburn is mostly caused by ultraviolet B (UVB) rays, which vary with the time of year and season. A major risk factor for both melanoma and non-melanoma skin cancer is sunburned skin [8].

UV-C Radiation: The wavelength of this radiation is 200–280 nm. Due to the steep ozone layers that filter them, UV-C radiation is less harmful and effective [8].

OBJECTIVE

The purpose of this study to prepare an herbal sunscreen cream by using easily available herbal substances like Cucumber (*Cucumis sativus*), Tomato (*Solanum Lycopersicon*), and Aloe-vera (*Aloe barbadensis*). Objective can be stated like;

To formulate and evaluate herbal sunscreen with determination of Sun Protection Factor (SPF) and anti-oxidant activity. To compare Sun Protection Factor of developed formulation with marketed formulation.

So, for this studythree fruits/ parts of different plants were selected those are-

1. Cucumber
2. Tomato
3. Aloe Vera

MATERIALS AND METHODS

All the herbal ingredients such as Cucumber (*Cucumis sativus*), Tomato (*Solanum Lycopersicon*), and Aloe-vera (*Aloe barbadensis*) were collected from the botanical garden of Dr. K. N. Modi University, Newai, Rajasthan. A marketed formulation of 30 SPF was purchased from Krishna Medical Store, Newai, and Rajasthan. Chemicals were purchased from R. S. Enterprises, Jaipur. All solvents were of analytical grade.

Extraction: Here I have selected 3 plants for preparation of this sunscreen cream. Different methods were used for extracting. These are specifically mentioned hereunder:

Extraction from Cucumber (*Cucumis sativus*) :

Cucumber extract can be obtained by macerating the crushed fruit in hydro-alcoholic mixture, then filtering and concentrating it on rotary evaporator.

For preparation of hydroalcoholic solution following reagents and process was used.

Requirement: (This quantity was calculated for 200 ml)

- Alcohol: 833 ml of 96% ethanol or 752 ml of 99.8% isopropanol.
- 42 ml of 3% hydrogen peroxide.
- 15 ml glycerol 98%.
- 110 mL of water. The water should be distilled or boiled and then cooled to minimize contamination.

Process:

- Pour first the alcohol into a container, followed by hydrogen peroxide and then glycerol. When handling glycerol, use a measuring spoon with a large opening due to the high viscosity of glycerol.
- Add distilled water up to 1 L.
- Mix thoroughly by stirring or with a spatula.
- Wait 72 hours before using the hydro-alcoholic solution (so that it itself is decontaminated [9].

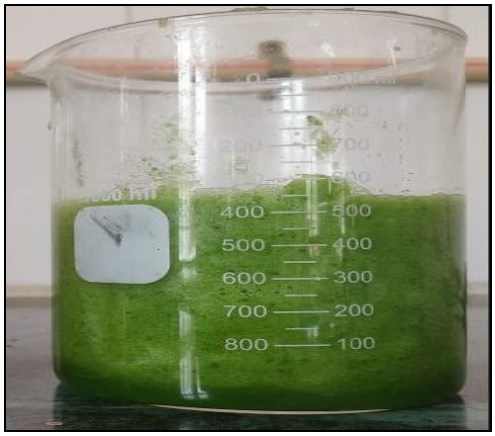

Figure 1: Extraction from Cucumber

Extraction from Tomato (*Solanum Lycopersicon*):

The extract is produced by crushing tomatoes into crude tomato juice that is then separated into serum and pulp. The tomato pulp is then extracted with ethyl acetate. The final product is obtained after solvent removal by evaporationwater bathat 40-60°C [10].

Figure 2: Extraction from Tomato

Extraction from Aloe-vera (*Aloe barbadensis*) :

Aloe vera Leaf Gel Extract: The gel (400 g = 2.5 g dry matter) was homogenized in a Waring blender, then diluted with an equal volume of PBS and homogenized for second time. The extract was kept at 4 ℃ overnight, then filtered through cloth. The clear filtrate was kept at 20 °C [11].

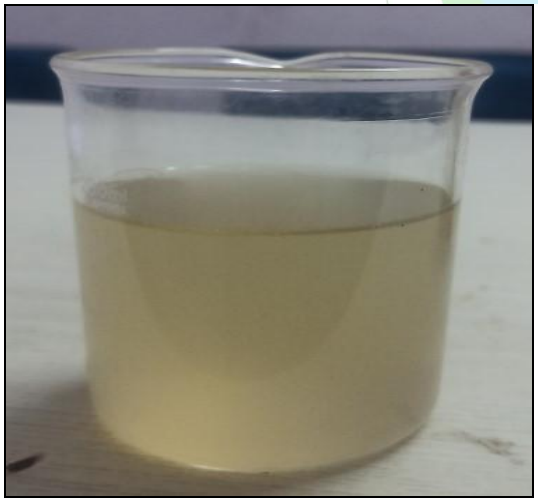

Figure 3: Extraction from Aloe-vera

SUNSCREEN FORMULATION AND EVALUATION

The cream bases were prepared via emulsification process. Briefly, an oil phase containing lipophilic substances and an aqueous phase containing hydrophilic substances were separately heated in a water bath to 80^{o} C. Afterwards, the aqueous phase was gradually added into the oil phase with constantly stirring until the mixture was congealed at the room temperature [12]. The ingredients of sunscreen were decided after evaluating multiple research article of sunscreen and their excipients table No. 1[13].

Table 1: Role of Ingredients for herbal sunscreen [13]

Ingredients	Role of Ingredients in formulation
Extract of Cucumis sativus	UV protector
Extract of Solanum	UV protector
Extract of Aloe barbadensis	UV protector
Cetostearyl alcohol	Emulsion stabilizer/ Surfactant
Stearic acid	Thickener
PEG-200	Emollients
Cetyl alcohol	Thickening agent
Methyl paraben	Preservative
Propyl paraben	Preservative
Carbopol 940	To provide high viscosity to formulation
Disodium EDTA	Chelating agent
Triethanolamine	Reduce the acidity or alkalinity of the formulation

Development of Formulation

As compared to lotion or any other dosage form, creams are more efficient due to good stability, spread ability, occlusivity, penetration power and cost effectiveness. Long contact time and hydrophobic active drug solubility in oil phase keeps cream dosage forms always a choice of manufacturers. Cream formulations of varying Phyto chemicals composition were developed. All studied concentrations were in the legislated range.

Step I: Aqueous Phase Preparation: Disodium EDTA, Sodium Methyl Paraben and Triethanolamine weighed accurately and dissolved in De ionized Water; meanwhile, Carbopol was added to swell using a homogenizer and heated up to 80°C.

Step II: Oil Phase Preparation: Sodium propyl paraben, Stearic acid, Cetyl alcohol, Polyethylene glycol, Cetostearyl alcohol and respective quantities of Cucumber (*Cucumis sativus*), Tomato (*Solanum Lycopersicon*), and Aloe-vera (*Aloe barbadensis*) weighed accurately and mixed and heated at 80°C.

Step III: Mixing Phase: Oil phase was added to aqueous phase at 80°C with continuous stirring for 20-25 min and then it was homogenized till uniform emulsion formed. It was then poured into the wide mouth container and stored at temperature not exceeding 37°C [13].

Evaluation of Formulation

Physical Parameters:

Appearance, color, and homogeneity were determined [14,15].

Subjective Properties:

Consistency, feel on application and irritation parameters are determined.

Spread ability:

Two glass slides of standard dimensions (20 × 5 cm) were selected. The formulation was on one of the slides. The

other slide placed on the top of the cream such a manner that the formulation sandwiched between the two slides in an area occupied by 7.5 cm, alongside 100 gm weight was placed uniformly to form a thin layer. The weight was removed and the excess of cream adhering to the slides was scrapped off. The two slides in a position were fixed to stand (45° angle) without slightest disturbance and in such a way that only the lower slide held firmly by the opposite fangs of the clamps allowing the upper slide to slip off freely by the force of weight tied to it. 60 gm of weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 5 cm and separate away from the lower slide under the direction of weight was noted. The experiment repeated for 3 times and the mean taken for three such dimensions was calculated [16,17]. The results were recorded. The Spread ability is calculated by using formula:

S = M*L/T

Where,

S= Spread ability, L= Length of glass slide, M= Weight tied to the upper slide and T= Time.

In present experiment M= 60 gm and L= 7.5 cm.

Extrudability:

The extrudability of herbal sunscreens was determined in this study by calculating the percentage of formulation extruded from the collapsible tube based on the weight in grams necessary to extrude at least 0.5 cm of gel ribbon in 10 seconds [16]. After that, the extrudability was estimated using the formula:

$$Extrudability = \frac{Appliedweighttoextrudegelfromtube\ (gm)}{Area\ (cm2)}$$

Percentage	Extrudability
90	Excellent
80	Good
70	Fair
50	Poor

Determination of Viscosity:

The Brookfield viscometer (RVDV-II+PRO) was used to test viscosity, with the proper number of spindle selected (No. 4). A 50 ml beaker was used to hold 50 g of preparation until the spindle groove was dipped and the rpm was set at 6 rotations per minute. Herbal sunscreen viscosity was measured at 5, 10, 20, 50, and 100 rpm. The viscosity was computed using the factor obtained from the reading [18].

Viscosity = Dial Reading × Factor.

For LV-4 at 6 RPM Factor is 1M (1000)

Determination of pH

The pH of herbal sunscreens was determined using a digital pH meter. pH was measured after 1 g of the formulation was dissolved in 100 ml of newly prepared distilled water for 2 hours. The purpose of this study was to guarantee that the pH of the produced herbal sunscreens is like the pH of the skin after 24 hours of use. The results were triple-checked, and reading was recorded [18].

Thermal Stability

The oil separation from herbal sunscreens was evaluated in a humidity chamber at 60-70 % RH and 37±1°C. A 20 mm wide and 5 mm thick stripe of herbal sunscreens was applied to the internal wall of a 100 ml capacity chamber in its whole heights. The beaker was stored in a humidity chamber for 8 hours at 60-70 % relative humidity and 37°C. There should be no oil separation in the herbal sunscreen to pass the test [19].

Determination of SPF

1. A UV-Vis Spectrophotometer (LABMAN Scientific instruments Pvt. Ltd.)was used to examine the in-vitro efficacy of herbal sunscreen.
2. A 0.10 percent solution (w/v) of herbal sunscreen cream in ethanol was made by dissolving 0.040 gram of herbal sunscreen cream in 40 ml of ethanol.
3. Further, it was kept for ultra-sonication for 5 min and filtered through cotton filter, discarded the initial 10 ml.
4. Afterwards 1 ml aliquot was transferred to 10 ml volumetric flask and the volume was adjusted with ethanol.
5. The absorption spectra of samples in solution were obtained in the range of 290-450 nm using 1 cm quartz cell and ethanol as blank.
6. The absorption data obtained in the range of 290-320 nm every 5 nm interval and 3 determinations were made at each point.
7. Marketed Sunscreen Product: 1 Marketed formulation was selected as standard.
8. Sun Protection Factor Determination: SPF of formulated creams and marketed sunscreen product was calculated by the application of following Mansur mathematical equation (Equation 2). Three times each sample was analyzed [21].

$$SPF = CF \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times A(\lambda)$$

Whereas,
CF= Correction factor;
EE= Erythemogenic effect;
I= Intensity of solar light of wavelength;
A= Absorbance

Table 2: Normalized product function used in the calculation of SPF (Sayre et al., 1979)

Wavelength (λ nm)	EE x I (normalized)
290	0.015
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.018
Total	1

EE – erythemal effect spectrum; I – solar intensity spectrum (Both Constant)

RESULTS

As,3 plantsselected for preparation of this sunscreen cream. Different methods were used for extracting. The results of collected yield are specifically mentioned hereunder:

Table 3: Extraction yield

S. No.	Crude drug Taken quantity	Yield Obtained (Grams)
1.	Cucumber (*Cucumis sativus*)	3
2	Tomato (*Solanum Lycopersicon*)	2
3	Aloe-vera (*Aloe barbadensis*)	1.5

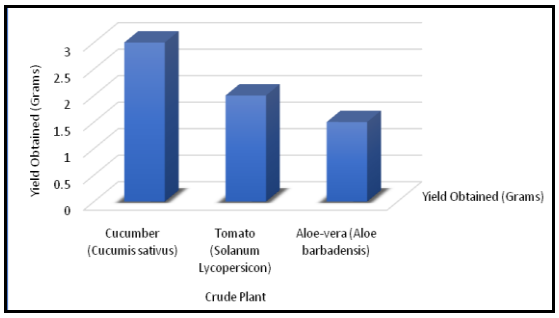

Figure 4: Comparative presentation of extracted yield

Sunscreen Formulation

For the development of sunscreen, I used different quantities of ingredients and extracts. Following formula was developed according to the article reviewed.

Table 4: Composition of extracts and ingredients for herbal sunscreen

Ingredients	Role of Ingredients in formulation	Formulation - 1	Formulation - 2	Formulation - 3
Extract of Cucumis sativus	UV protection	3	4	5
Extract of Solanum Lycopersicon	UV protection	3	4	5
Extract of Aloe barbadensis	UV protection	3	4	5
Cetostearyl alcohol	Emulsion stabilizer/ Surfactant	5	5	5
Stearic acid	Thickener	2	2	2
PEG-200	Emollients	2	2	2
Cetyl alcohol	Thickening agent	1	1	1
Methyl paraben	Preservative	0.3	0.3	0.3
Propyl paraben	Preservative	0.06	0.06	0.06
Carbopol 940	To provide high viscosity to formulation	0.5	0.5	0.5
Disodium EDTA	Chelating agent	q. s.	q. s.	q. s.
Triethanolamine	Reduce the acidity or alkalinity of the formulation	0.5	0.5	0.5
Distilled Water q. s. to 100 gm				

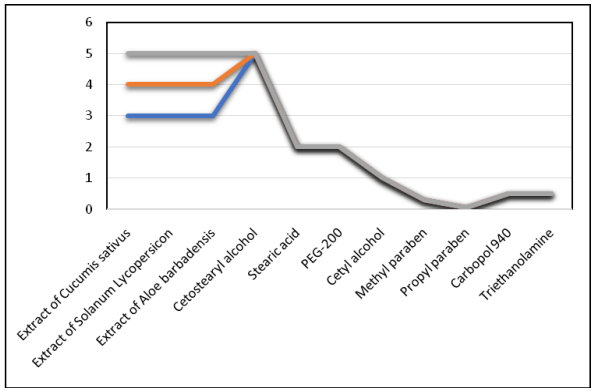

Figure 5: Composition of extracts and ingredients for herbal sunscreen

Figure 6: Prepared sunscreen cream Formulation 3

Evaluations:

Results are tabulated hereunder for General properties, consistency, pH determination by digital pH meter, spreadability, extrudability, irritation study and stability.

Table 5: Composition of extracts and ingredients for herbal sunscreen [9]

S. No.	Characteristics	Formulation 1	Formulation 2	Formulation 3
1	Colour	Greenish Creamy	Greenish Creamy	Greenish Creamy
2	Odour	Characteristic	Characteristic	Characteristic
3	Appearance	Cream like	Cream like	Cream like
4	Consistency	Smooth	Smooth	Smooth
5	pH	6.4	6.8	6.0
6	Spreadability	12	12.50	19.50
7	Extrudability	Good	Good	Excellent
8	Spreadability (gm.cm/seconds)	11	12	16
9	Irritation Study	Non irritant	Non irritant	Non irritant
10	Thermal stability	Stable	Unstable	Stable

Apart from these studies SPF determination was the most important part of this study and it resulted favourable after comparison with marketed formulation.

In-vitro Determination of SPF by UV-spectrophotometer (LABMAN Scientific instruments Pvt. Ltd.)

Despite being a source of life and energy, sunlight causes serious health problems such as sunburn, pigmentation, wrinkles, dermatitis, urticaria, aging, immunological suppression, and a variety of skin malignancies.

SPF is a measure of how effective sunscreens are at preventing sunburn SPF. The absolute protection efficacy of sun care products against erythemal-effective UV light was determined using in vitro transmittance measurements and weighted with the erythema action spectrum and the "standard" output spectrum of a UV solar simulator used for SPF testing [20,22].

The SPF of herbal sunscreens was estimated using the equation. The aliquots were scanned between 290 and 320 nm, and the absorbance values obtained were multiplied by the corresponding EE (λ) and I (λ) values. Then, their summation was taken and multiplied with the correction factor i.e., 10. All herbal sunscreens revealed significant SPF.

Figure 7: UV absorption for SPF determination of prepared formulation

When comparing herbal sunscreens, the F3 formulation showed promising results, with higher SPF ratings than other formulations. This could be because of the presence of various extracts in higher quantity, which has an SPF of roughly 22.08, or because of the synergistic activity of all

herbal compounds utilized in herbal sunscreen formulations, such as *Cucumis sativus*, *Solanum Lycopersicon*, and *Aloe barbadensis*[14,23]. Efficacy of photoprotection found in following order Marketed formulation > F3 > F2 > F1. These results reveal that the prepared F3 herbal sunscreen have good SPF and good sun protection activity.

Table 6: All sunscreen formulation and SPF calculation

Wavelength (λ nm)	EE (λ) x I (λ)	F 1	F 1	F 2	F 2	F 3	F 3	Standard	Standard
		Abs. (λ)	EE (λ) x I (λ) x Abs. (λ)	Abs. (λ)	EE (λ) x I (λ) x Abs. (λ)	Abs. (λ)	EE (λ) x I (λ) x Abs. (λ)	Abs. (λ)	EE (λ) x I (λ) x Abs. (λ)
290	0.015	1.014	0.01521	1.116	0.01674	1.878	0.02817	1.983	0.029745
295	0.0817	1.039	0.08489	1.119	0.09142	2.219	0.18129	2.275	0.1858675
300	0.2874	1.128	0.32419	1.14	0.32764	2.418	0.69493	2.945	0.846393
305	0.3278	1.126	0.3691	1.18	0.3868	2.32	0.7605	2.291	0.7509898
310	0.1864	1.122	0.20914	2.15	0.40076	1.97	0.36721	1.95	0.36348
315	0.0839	1.118	0.0938	1.19	0.09984	1.76	0.14766	1.88	0.157732
320	0.018	1.11	0.01998	1.6	0.0288	1.59	0.02862	1.76	0.03168
Total	1		1.11631		1.352		2.20838		2.3658873
Multiplication with correction factor (10)			**11.1631**		**13.52**		**22.0838**		**23.658873**

In the SPF determination study according to the formula EE (λ) x I (λ) both were constant according to the material and methods. Correction factor was also taken stable for all preparations it was 10. The main difference was the absorbance value to the specific dilutions. It showed for F1, 11.16 SPF, for formulation 2, 13.52 SPF for formulation 3, 22.08, SPF and for standard marketed preparation it was 23.66, SPF. The standard preparation claiming for 30 SPF but I got result showing 23.66 SPF.

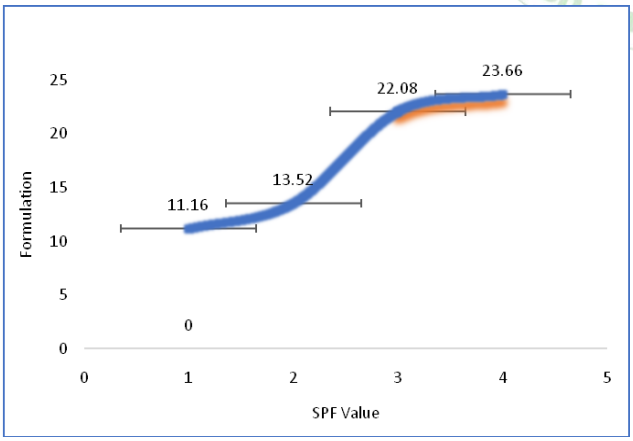

Figure 9: SPF Value comparison Chart

Stability Testing

Phase separation was found for F1 to F2 at maximum rpm (1000), showing that these formulations are unstable under high stress. Water was removed from these formulations during the freeze-thaw research, indicating that they may not be able to withstand various environmental changes during product shipping. In comparison to F3 formulation, which were proven to be stable under stress conditions.

CONCLUSION

Present research work on sun rays protective *Solanum Lycopersicon*, strong antioxidant, photo protective *Aloe barbadensis* and moisturizing as well as cooling *Cucumis sativus* is incorporated together to develop efficient all-in-one sunscreen product. Formulation 3 comprising three formulations found to be promising as sunscreen in all aspects but still this research works has limitations, which are due to various factors like time for study, finance, resource facilities etc. In vivo is a major scope for this study. As the rate of sunscreen use is low, education and awareness about the hazards of sun exposure and the benefits of regularly applying sun screening agents to reduce these effects must be spread. This type of study will also helpful in providing complete solution or all in one product to consumers to protect from harmful effects of sunrays.

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