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Determination of Sun Protection Effect of Herbal Sunscreen Gel

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ABSTRACT

Exposure to sunlight can trigger various biological responses ranging from sunburn, erythema to skin cancer. Synthetic sunscreen formulations available in market pose a variety of adverse effects. Therefore, formulation of the herbal sunscreen formulation and evaluation of its sun protection activity is an important aspect in the cosmetic industry. The *Butea monosperma* flowers extract has reported good sun-protecting activity and ellagic acid is sun-protecting factor boosting agent. In the present study, sunscreen activity of 5% formulated gel containing the *Butea monosperma* flowers extract and ellagic acid was determined by absorption spectroscopy, transmission spectroscopy and by COLIPA standard method for sun protection factor determination. In absorption spectroscopy, the absorbance of dilute test solution of formulation from 290-320 nm was studied to determine SPF against UV-B while in the transmittance spectroscopy, UVA and UVB protection and average UVA protection factors were calculated by taking transmission of formulations from 290 nm-400 nm using PVC as substrate also samples were submitted to Kelkar Cosmetology lab, Mulund for the determination of sun protection factor by COLIPA standard method using UV-200S Transmittance analyzer. In this method sun protection factor is determined by taking transmission of formulation from 290nm- 450nm using transpore tape. The present study shows that there is increase in the sunscreen activity with increase in the percentage of actives in the formulation and having good potency to protect against UVA and B rays.

INTRODUCTION

Skin is the outermost & largest organ of the body when the mammalian skin is exposed long-term to U.V radiation; it induces oxidative stress by generating the reactive oxygen species which trigger the development of sunburn, erythema, edema, immunosuppressant, photoaging, skin cancer etc.[1] Exposure to UVA radiation results in damage to the elastic and collagen fibres of connective tissue of skin, which causes premature ageing (photo-aging).while UV-B radiation bring about acute inflammation(sun burn) and intensification of photo-aging. UVC radiation is filtered by the atmosphere before reaching earth. UVB radiation is not completely filtered out by the ozone layer and is responsible for the skin damage due to sunburn. UVA radiation reaches the deeper layer of the epidermis and dermis and provokes the premature aging of the skin.[2] The efficacy of sunscreen is usually expressed by the sun protection factor (SPF), which is defined as the UV energy required for producing a minimal erythema dose (MED) on protected skin, divided by the UV energy required for producing a MED on unprotected skin.

$$\text{SPF} = \frac{\text{Minimal erythema dose in sunscreen protected skin}}{\text{Minium erythema dose in non-sunscreen protected skin}}$$

The minimal erythema dose (MED) is defined as the lowest time interval or dosage of UV light radiation sufficient to produce minimal, perceptible erythema on unprotected skin.[3] Although synthetic sunscreens available in market are highly efficient to protect the skin from deleterious effects of the sun, but herbal sunscreens are rapidly replacing them due to no or less side effects.[4] The photoprotection afforded by topical sunscreen against solar ultraviolet radiation exposure can be determined in-vivo or in-vitro and it is ideally determined by photo-testing in human volunteers. This type of determination has been used for many years and although useful and precise, is a time-consuming process, complex and expensive, particularly when information concerning to the protection against long wavelength (UVA) is required. As a consequence, much effort has been devoted to the development of in-vitro techniques for assessing the photoprotection of sunscreen compounds.[5] The methods in-vitro are in general of two types. Methods that involve the measurement of absorption or the transmission of UV radiation through sunscreen product film in Quartz plates or Biomembranes and methods in which the absorption characteristics

of the sunscreen agents are determined based on spectrophotometric analysis of the dilute solution.[2] COLIPA standard method is the most recently updated scientific method for evaluation of SPF of sunscreen. It has been developed by COLIPA internationals. It uses UV- 200S transmittance analyzer, which is dedicated instrument for SPF determination. It determines the protection performance of suncare product against erythema inducing radiations i.e. 290nm-450nm using transpore tape.[6] Transpore tape is having the uneven topography that distributed the sunscreen similar to the human skin.[7] *Butea monosperma* is commonly known as flame offorest, belonging to the family (Family: Febaceae).[10]

The ethanolic extract of *Butea monosperma* flower has 91% UV-absorbing properties. It is reported that *Butea monosperma* contains flavonoids like butein, butin, butrin, isobutrine, coreopsis, ioscoreopsin, monospermoside that have excellent antioxidant and photoprotective activity. It also helps to prevent wrinkles, premature skin aging, and skin cancer. Ellagic acid is a powerful free radical scavenger. It also has the ability to boost the sun protection factor.*Butea monosperma* has reported antioxidant, anticarcinogenic, antimicrobial, and anti-inflammatory activity. The combination of these plant extracts and ellagic acid in the formulation may exert high sunscreen property which may be more effective than marketed synthetic sunscreen formulation. Investigation on this line of thinking can assign a new role to an existing plant and increase its commercial use.

MATERIALS AND METHODS

Collection of Plant Materials

The fresh flowers of the plant (Butea monosperma) were collected in March 2019, from placeBodwad village, Tal: - Muktainagar, Dist:-Jalgaon. The collected specimen of Butea monosperma was authenticated by Prof. Vidya A. Patil (Research Officer-Botany), TES, P.O. Nahata college science and commerce for Research in Ayurveda and Siddha Bhusawal, Maharashtra-425201, species were deposited at herbarium. The flowers of *Butea Monoasperma* dried in direct sunlight. The dried flowers of *Butea Monoasperma* were crushed into coarse powder by using a grinder. The both powders were stored in a vessel for future use.

Preparation of plant extracts

Preparation of ethanolic extract of *Butea monosperma (*Palash*)*

The flowers were dried in the shade and then ground to produce a coarse powder. The flowers were defatted by using petroleum ether and then extracted with ethanol for 72 hours using a soxhlet apparatus. The extract was filtered using muslin cloth and then concentrated in a water bath at 600C. The percent yield was found to be 10% w/w. The extract was kept in a sterile bottle and stored under refrigerated conditions for further analysis.

Chemicals and Materials

All the materials used in formulation and evaluation of formulation were of analytical grade.

Formulation of Sunscreen Cream

1% of Carbopol 934 was dispersed in a sufficient quantity of distilled water with continuous stirring. Then, 0.5% methyl paraben and 0.2% propyl paraben were dissolved in a sufficient quantity of water and added to the above dispersion. The solution was cooled, and 5% propylene glycol was added. Further Butea monosperma flower extract was mixed with the obtained solution, and the volume was made up to 100ml by adding the remaining distilled water with continuous stirring and the additionof ellagic acid. Triethanolamine was added drop-wise to the formulation for adjustmentof the required skin pH (6.8-7) and to obtain the gel at the required consistency.

The method described above for sunscreen gel was formulated by using *Butea monosperma* extract in the gel with 5% combinations with 0.1% ellagic acid.

Determination of Sun Protection Factor by using Mansur equation[2]

The efficacy of sunscreen is expressed by the Sun Protection Factor (SPF). An in vitro method of determining SPF of the sunscreens is by using the Mansur equation.

Procedure

1 gm and of 0.5 gm of 5% formulated sunscreen gel respectively was weighted and transferred to a 50 ml volumetric flask, diluted to volume with ethanol, and then filter throughWhatman filter paper, to give 1000 µg/ml solution. Rejecting the first 10 ml, a 2.5 ml

aliquot was transferred to 50 ml volumetric flask and diluted to volume with ethanol to produce 50 µg/ml solutions. Then 5 ml aliquot was transferred to 50 ml volumetric flask and the volume completed with ethanol (100 µg/ml solutions) also 200 µg/ml solutions was prepared by diluting 5 ml of aliquot with 25 ml of ethanol in the volumetric flask. The absorption of each aliquot prepared was determined from 290-320 nm, taking ethanol as a blank. The absorption data were obtained in the range of 290 to 320, every 5 nm, and 2 determinations were made at each point, followed by the application of Mansur equation.

$$\text{SPF (spectrophotometric)} = \text{CF} \times \sum_{290}^{320} \text{EE}(\lambda) \text{x I}(\lambda) \text{ x Abs}(\lambda)$$

Where, CF is correction factor (=10); EE (λ) - erythemal effect of radiation with wavelength λ; I(λ) -solar intensity spectrum; Abs(λ)-Absorbance of sunscreen product. The values of EE (λ) ×I (λ) are constant as given in Tables 1 and 2. The obtained absorbance values Abs (λ) weremultiplied with the respective EE (λ) ×I (λ) values and then summation was taken and multiplied with the correction factor 10.

SPF Determination by transmission spectroscopy[2]

Sunscreen activity was evaluated by in-vitro method through recording transmission spectrum of formulations in the range 290-400nm.The analysis was carried out in duplicate and the values are recorded as mean values of two readings.

Procedure

Sample was applied on polyvinyl chloride (PVC) sheet strip, and spread uniformly with help of capillary to form a thin film. The strip was then placed inside the UV-Vis cuvette in such that the formulation touches transparent side of cuvette. It was allowed to equilibrate for 15 mins to ensure levelling of the formulation between PVC and wall of the cuvette. The cuvette was placed inside a UV spectrophotometer (Shimadzu) and a transmission spectrum was recorded from 290-400 nm, using air as a reference. The data was appropriately processed to calculate UVA and UVB protection factors using the following formulas.

1) The average transmittance spectrum of sunscreen in either region is averaged in order toproduce one value, which describes UV A, or UVB blocking.

$$T(UVA)avg = \frac{\sum_{320}^{400} T\lambda \times \Delta\lambda}{\sum_{320}^{400} \Delta\lambda}$$

$$T(UVB)avg = \frac{\sum_{290}^{320} T\lambda \times \Delta\lambda}{\sum_{290}^{320} \Delta\lambda}$$

100-T (UVA) or T (UVB) gives % blocking or % protection against UVA or UVB.

2) Average UVA protection factor (PF)

The arithmetic mean of monochromatic protection factor (MPF) calculated between 320-400 nm.

$$PFAm = \frac{\sum_{320}^{400} MPF\lambda \times \Delta\lambda}{\sum_{320}^{400} \Delta\lambda}$$

A (λ) is absorbance at λ

T (λ) is transmittance at λ MPF is monochromatic protection factor i.e.1/T Δ λ is measured wavelength interval (5nm)

Sun protection factor determination by COLIPA Standard method

5% formulated sunscreen cream samples were submitted to Kelkar Cosmetology lab; Mulund for the determination of sun protection factor by COLIPA standard method. This method uses UV-2000S ultraviolet transmittance analyzer for determination of Sun protectionfactor. Its principle is based on the sample transmittance measurement.

Procedures

Approximately 110 mg of the prepared investigational sample was applied and spread on 56 cm^2 area to obtain a sample film thickness of 2mg/cm^2 on Transpore tape to get an even film. The sample thus prepared was exposed to Xenon flash lamp for determining Sun Protection

Factor.

The UV-2000S ultraviolet transmittance analyzer software uses a trapezoidal approx. calculation technique to approximate the integral for SPF and erythemal UVA protection factor. These include mean SPF, standard deviation of SPF, mean UVA/UVB ratio, critical wavelength etc.

UV-2000 calculates the SPF characteristic according to the ratio:

$$SPF = \frac{\int_{290}^{400} E\lambda S\lambda d\lambda}{\int_{290}^{400} E\lambda S\lambda T\lambda d\lambda}$$

Where, E (λ) is the erythema action spectrum, S (λ) is the solar spectral irradiance T (λ) is the spectral transmittance of the sample with the integral is calculate across the 290-400 nm wavelength limits.

RESULTS

Determination of Sun Protection Factor by using Mansur equation

SPF of 5% sunscreen gel containing *Butea monosperma* and ellagic acid (5:1) extract in combination was checked by absorption spectroscopy using Mansur equation method. Absorbances obtained in spectrum was considered for SPF calculations and depictedin Table 1.

SPF of 5% formulated sunscreen gel was found to be 1.562, 7.294, and 25.09 for 200µg/ml, 1000µg/ml, 10000 µg/ml respectively.

Table 1: Determination of sun protection factor values of 5% Formulated sunscreen gel

Conc.	Wavelength	290	295	300	305	310	315	320	SPF
µg/ml	EF×I	**0.015**	**0.081**	**0.287**	**0.327**	**0.1864**	**0.083**	**0.018**	
200	A	0.186	0.155	0.156	0.158	0.157	0.153	0.143	1.562
	EF×I×A	0.0027	0.0126	0.0448	0.0517	0.0292	0.0128	0.0026	
1000	A	0.794	0.730	0.729	0.736	0.729	0.710	0.669	7.294
	EF×I×A	0.0119	0.0596	0.2095	0.2412	0.1358	0.0594	0.0120	
10000	A	2.738	2.488	2.486	2.521	2.518	2.525	2.421	25.09
	EF×I×A	0.0410	0.2032	0.7144	0.8263	0.4693	0.213	0.0435	

SPF Determination by transmission spectroscopy

In this method transmission values of all formulated sunscreen gel were obtained by UV- vis spectrophotometer from 290nm-400nm and shown in Table 2.

The transmission values further calculated to find out % protection against UV-A and UV-B and to calculate average UVA protection factor and depicted in Table-3. Percent blocking of UVA rays of increasing concentration of gel containing extract was found to be 87.22%and UVB rays was found 91.15% to be respectively. Average UVA protection factor was found to be 7.86 for increasing percentage of gel.

Table 2: In-vitro SPF Determination of 5% formulated sunscreen gel by transmission spectroscopy

5% Sunscreen cream		
Wavelength	**%T**	**MPF**
290	0.7	125
295	6	16.12
300	9.6	9.17
305	10.7	9.17
310	10.8	9
315	11.2	8.77
320	11.4	8.54
325	11.9	8.19
330	12.4	7.93
335	13.3	7.46
340	13.7	7.29
345	13.7	7.29
350	13.2	7.57
355	12.5	8
360	11.8	8.4
365	11.5	8.69
370	11.2	8.92
375	11.2	8.92
380	11.6	8.62
385	12.2	8.26
390	13.1	7.63
395	14.4	6.94
400	16.2	6.17

Table 3: Determination of percent protection of 5% Formulated sunscreen gel.

Formulation	% Protection against UVB	% Protection against UVA	Average UVA protection factor
5% cream	91.15%	87.22%	7.86

Sun protection factor determination by COLIPA Standard method

In this method, the SPF value of 5% formulated sunscreen gel was found to be 2.18 and 5.78 respectively, with ultra boost star rating 4, boost star rating three is consider as having good sunscreen activity.

Table 4: SPF and other parameters for 5% formulated sunscreen gel by COLIPA standard method

Sr. no	Parameter	Scan 1	Scan 2	Scan 3	Average value
1.	SPF	1.95	1.91	1.9	1.92
2.	Standard deviation	0.15	0.16	0.14	0.15
3.	UVA/UVB ratio	0.99	0.99	1	0.99
4.	Critical wavelength	389.4	389.4	389.6	389.46
5.	Boost star rating	****	****	****	****

DISCUSSION

The SPF is the quantitative measurement of the effectiveness of a sunscreen formulation. To be effective in preventing sunburn and other skin damage, a sunscreen product should have a wide range of absorbance between 290nm to 400nm. The in vitro SPF is useful for screening tests during product development as a supplement of the in vivo SPF measure. The proposed UV spectrophotometric method is simple, rapid, uses low-cost reagents and can be used for in vitro determination of SPF values in many cosmetic formulations.[5] Several people with sensitive skin, such as those suffering from skin hypersensitivity don't want to use chemical sunscreens due to concern about skin exposure to unknown chemicals. Although a variety of hypoallergenic cosmetic products have been introduced for customers with sensitive skin, there are still limited options in sunscreen agents. Now, however, researchers have claimed that cosmetics having herbal components are more suitable for hyperallergic skin because they are less irritant and more easily adjustable to skin. Topical cosmetic formulations are the most preferred treatments asked by patients and are also often most prescribed by family physicians and dermatologists for sunburn. Patients feel more comfortable using topical therapies because they have milder side effects, are easier to use, are generally less expensive and are more readily available. Herbal cosmetics must have one or more active sunscreening agent with antioxidant properties in order to achieve good photoprotection effect. The concept of complementary or alternative medicine is increasingly becoming more widely accepted and there is a corresponding rising interest in herbal remedies [2]. In this study topical formulations containing *Butea Monoasperma* flowers extract and ellagic acid (5:1) were evaluated for sunscreen activity by three in-vitro methods absorbance spectroscopy, transmission spectroscopy and by COLIPA standard method for SPF determination. Though, in-vitro methods present some limits; it gives accurate and precise result and avoid the

exposure of human subjects to harmful ultraviolet radiation. Excipients and other active ingredients can also produce UV absorption bands, thus interfering with those of UVA and UVB sunscreen. This effect is reflected in a finished formulation. There was no indication of an influence of excipients on the absorbance of the product however very low effect was realized at high concentration. The proof of sunscreen products efficacy is of high importance for the protection of public health as the UV-B fraction of solar radiation is the main contributor to skin sunburn, immunosuppressant and skin cancer. The UVB causes most of the skin problems related to sun exposure: like aging, wrinkles and cancer. The potential of UV radiation to cause skin damage rises exponentially with decreasing wavelength. UV light at 280 nm is 1000 times more damaging than light at 340 nm, therefore, a sunscreen's ability to block UV-B is more important to prevent the negative effects of sun exposure.

Hence in the present study, the spectroscopic method emphasized on protection against UVB by considering the absorbance in the UVB range i.e. from 290nm-320nm. From the result of the present study, it demonstrated that cream shows protection against UVB radiation and indicated as SPF 7.86 for 5% sunscreen gel. Different concentration of formulation can be applicable for different skin type also SPF was measured by transmission spectroscopy to determined amount of UVA and UVB protection and average SPF against UVB and UVB by taking transmittance spectrum of sunscreen in either region. COLIPA standard method was found to be more reliable and reproducible as its uses dedicated instrument for SPF determination. The result shows that as the percentage of extract in formulation increase, Sun protection factor is also increase.

CONCLUSION

Thus, the results of the present study conclude that the formulated sunscreen gel with combination of 5% Butea monosperma extract and 0.1% ellagic acid has good sunscreen activity. Because formulated sunscreen gel is made from natural ingredients, it has fewer side effects than synthetic gels on the market. It also imparts some additional beneficial effects like Anti-wrinkle, Anti-microbial, Anti-cancer and Anti-oxidant activity as well the formulations produced by incorporating different percentages of extracts can be applicable for different type of Skin respectively as per SPF value. This will be better and safe alternative toharmful chemical sunscreens that used now-days in the industry.

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