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Formulation and in vitro evaluation for sun protection factor of Lutein ester extracted from *Tagetes erecta* Linn flower (Family-Asteraceae) sunscreen creams

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ABSTRACT

The effectiveness of Sunscreens is determined by sun protection factors (SPF), which is supposed to indicate the level of protection from UV radiation. The present study was designed to study the sunscreen activity of herbal formulation containing lutein ester extracted from Tagetes erecta L. flowers (Family Asteraceae). This study investigates its in vitro sun protection factor (SPF) by COLIPA method of Lutein ester in a cream formulation. The Sun Protection Factor of lutein ester cream exhibited less activity (SPF= 1.08±0.02) with Boot Star Rating 4 which approaches to good sunscreen activity.

Key words: lutein ester, Tagetes erecta, sunscreen, Sun Protection Factor, Boot Star Rating.

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INTRODUCTION

Extraterrestrial sunlight includes x-ray, ionizing, ultraviolet, visible, and infrared radiation, and radiowaves. The solar spectrum at the earth's surface (sea-level) consists of wavelengths of electromagnetic energy only between 290 and 3000 nm, while the spectrum implicated in human skin reactions involves wavelengths up to 1800 nm. Ultraviolet (UV) radiation is arbitrarily subdivided into three bands, UVA (320-400 nm), UVB (290-320 nm) and UVC (200-290 nm). The total flux of UVA at the earth's surface vastly exceeds that of UVB, with all the UVC being completed absorbed by stratospheric ozone. The terrestrial spectrum of solar UV radiation consists of 1–5% of UVB radiation and 95–99% of UVA radiation depending on the latitude, the time of the day and the season of the year. As a barrier and immunological organ in the human, the skin especially epidermis, is particularly subjected to external effects UVB radiation is fully absorbed by the stratum corneum and the top layers of the epidermis, whereas up to 50% of incident UVA radiation penetrates skin deep into the dermis. Ultraviolet irradiation is involved in the pathogenesis of skin cancers, causes premature aging of the skin and photoimmunosuppresion. It also plays a role in the pathogenesis of photosensitive diseases such as chronic actinic dermatitis, polymorphous light eruption, actinic prurigo, hydroa vacciniforme, and photoallergic or phototoxic drug reactions. Both UVB and UVA radiation may affect the biomolecules of the skin [1, 3, 4].

After attention has been given to the harmful effects of the sunrays, to avoid unwanted skin effects of the sun, the use of sunscreen preparations became absolutely necessary. Efficacy of sunscreen is defined as the ability to protect the skin against ultraviolet-induced burning, with the level of performance indicated by the sun protection factor (SPF)[2,3]. The efficacy of sunscreens is characterized by the sun protection factor (SPF). The SPF is a numerical rating system to indicate the degree of protection provided by a sun care products like sunscreen [5]. SPF is defined as the ratio of the minimal erythema dose (MED) of solar radiation measured in the presence and in the absence of a sunscreen agent [6].

Regulatory agencies like the US-FDA and COLIPA (The Comité de Liaison de la Parfumerie in Europe) has recommended in vivo testing on human volunteers using an erythemal endpoint to determine the SPF of topical sunscreens [7]. Although it is a recommended and recognized method by COLIPA, it has several disadvantages like being expensive, time-consuming and is potentially hazardous to human clinical subjects. Having said this, there are still many questions left unanswered about both the scientific accuracy and reproducibility of in vivo measurements of SPF, whereas, an in vitro measurement has the advantage of not exposing human subjects to harmful UV radiation, is cost-effective and provides us with statistically significant data which helps us to develop an effective sunscreen product. Thus, for economical, practical and ethical considerations a suitable method for in vitro determination of SPF is used more often [8]. SPF is primarily a measure of UVB protection, as UVB is 1000 times more erythemogenic than UVA. These products don't necessarily offer adequate UVA protection. Protection against UVA is becoming a major concern since UVA damage is now implicated in photocarcinogenesis, photoaging and immunosuppression. An in

vitro method based on determination of critical wavelength which is obtained using spectrophotometry. Critical wavelength is the wavelength where the integral of the spectral absorbance curve reaches 90% of the integral from 290 nm to 400 nm. It measures a sunscreen's extinction capacity in the UVA range in relation to its overall extinction between 290 nm and 400 nm. The critical wavelength determination does not promote the false notion of UVB and UVA as separate entities but rather as part of continuous electromagnetic spectrum. As the critical wavelength increases, so too must the protection against UVA. A complete description of a products photoprotective characteristics results when critical wavelength is used in conjunction with SPF.

Sunscreen creams incorporate a wide variety of chemicals like derivatives of 3-benzylidenecamphor, 4-aminobenzoic acid, cinnamic acid, salicylic acid, benzophenone and 2-phenylbenzimidazole, Avobenzone and Zinc oxide [9] which have particular absorbance and are effective over various areas of UV spectrum. In order to get a broad spectrum UV protection, more than one active sunscreen ingredients are added in the sunscreen product [8]. The EU has regularly listed 27 different organic and inorganic sunscreen ingredients since two decades, which are approved by Australian Government- Department of Health and Ageing, Therapeutic Goods Administration (TGA) for use in Australia whereas only 16 ingredients are listed in US-FDA monograph, out of which Avobenzone and Zinc oxide are used frequently since 1978[10]. The inorganic materials like Titanium dioxide incorporated in formulation as sunscreen reflect and scatter ultraviolet and visible radiation from a film of inert metal particle which forms an opaque barrier, they are photo stable, do not react with organic sunscreens and due to their light scattering properties there is less variability in the photo-protective effect of inorganic agents as compared to organic agents. However, inorganic sunscreens are cosmetically unacceptable because of their opaque quality and occlusiveness. The higher refractive index of Titanium dioxide explains its whiter appearance and thus lower cosmetic acceptability [11]. Also, these sunscreen ingredients have been increasingly reported for allergic and contact dermatitis, phototoxic and photo-allergic reactions, contact urticaria and even solitary cases of severe anaphylactic reactions [12]. Therefore, the researchers have turned their attention towards developing herbal sunscreen agents which are effective with less or no side effects.

Flowers of Tagetes erecta L. (Asteraceae) is commonly known as French marigold flower (Engl.), Hajai (Arabic), sthulapushpa (Sans.)[13]. In India plant is cultivated in Andhra Pradesh , Tamil Nadu ,West Bengal , Karnataka, Uttar Pradesh etc. Flowers are in various forms, sizes and colors.

Lutein is an oxycarotenoid, or xanthophyll, containing 2 cyclic end groups (one beta and one alpha-ionone ring) and the basic C-40 isoprenoid structure common to all carotenoids. It is one of the major constituents and the main pigment of Marigold flowers. Although the polyene chain double bonds present in lutein could exist in a cis or trans conformation, giving rise to a large number of possible mono-cis and poly-cis isomers, the vast majority of carotenoids are in the all-trans configurations [14]. Lutein being xanthophyll carotenoids with potent antioxidant properties protecting the skin from acute photo-damage.

Therefore, the study was aimed at isolating and identifying the carotenoid (lutein ester) followed by developing a validated and effective topical dosage form.

MATERIALS AND METHODS

Plant material:

Flowers of Tagetes erecta L. (Asteraceae) was collected from farms of village kasabe-Sukene, Taluka Niphad, District-Nashik, Maharashtra state, India. It was authenticated Taxonomically with the courtesy from Botanical Survey of India, Koregaon Park, Pune. The herbarium was deposited with Reference No. (BSI**/WRC/Tech./2011**). Voucher no. **SNEBTA4.**

Preparation of lutein ester from dried flowers of Tagetes erecta L:

Dried powder material of petals of Tagetes erecta L. (50 g) was extracted with 250 volumes of petroleum ether for 5-6 days at temperature of 60±2 °C. The extract was filtered, petroleum ether was distilled off. Remaining extract was concentrated and dried to obtain Lutein esters [15].

Formulation of Sunscreen cream:

The Sunscreen cream was prepared by following procedure, the formulation of the cream is specified in Table No.1

Table No 1: Formulation of lutein ester sunscreen cream

Sr. No.	Ingredients	Components (%w/w)
1.	Cetostearyl alcohol	5
2.	Stearic acid	4
3.	Petroleum Jelly	1
4.	Glycerin	5
5.	Potassium hydroxide	1
6.	Water	85
7.	Methyl paraben sodium	0.20
8.	Propyl paraben sodium	0.05
9	Lutein ester	1

Step 1- Aqueous phase preparation: Potassium hydroxide (1%w/w) was dissolved in deionised water (85%w/w), followed by addition of glycerin (5%w/w), sodium methyl paraben (0.2%w/w). The resulting mixture was then heated up to 80°C.

Step 2- Oil phase preparation: Sodium propyl paraben (0.05%w/w), stearic acid (4%w/w), cetostearyl alcohol (5%w/w) petroleum jelly (1%w/w) and lutein ester extracted from flowers of Tagetes erecta L. (1%w/w) were added and heated at 80°C.

Step 3- Mixing phase: Oil phase was added to aqueous phase at 80°C with continuous stirring for 20-25 mins and then it was homogenized at 8000 rpm till uniform emulsion was obtained. The emulsion was then poured into wide mouthed container and stored at temperature not exceeding 37°C.

Determination of physical parameters of cream:

Preparation of herbal cream has always been a challenging task and the cream is accepted only if it is tested appropriately for various physical parameters like ease of spreadability, appearance, pH, viscosity and pleasant feeling as specified in Table No.2.

Table No.2: Physical Parameters of lutein ester sunscreen cream

Sr. No.	Parameters	Observations
1.	Color	faint orange
2.	Odor	characteristic
3.	Spreadability	Good and uniform
4.	pH	6.8
5.	Viscosity	28000cP
6.	Total microbial count	Nil
7.	Patch test for irritancy	No irritation reaction persists

Determination of in vitro SPF:

This study was performed by Transmittance measurement of the lutein ester extracted from flowers of Tagetes erecta L. (Asteraceae) cream. The Optometrics Model SPF-290 Analyzer measures the sun protection factor of the cream over a wavelength range from 290nm-400nm. Approximately 110mg of sample was applied and spread on 56cm^2 area of Transpore tape to obtain a sample film thickness of 2µl/cm^2 (to get an even film) as suggested in the operational manual of Optometrics LLC for the sample application technique. The samples thus prepared were exposed to Xenon arc lamp for determining the SPF and Boots Star Rating.

WIN SPF has used the following equation for calculating SPF value.

$$SPF_{SCAN} = \frac{\sum_{290}^{400} E\lambda\, B\lambda}{\sum_{290}^{400} \frac{E\lambda\, B\lambda}{MPF\lambda}}$$

Where,
MPFλ = scan MPF value
Eλ = spectral irradiance of terrestrial sunlight under controlled conditions
Bλ = erythemal effectiveness

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RESULT

The topical formulation of lutein ester extracted from flowers of Tagetes erecta L. (Asteraceae) was studied for all parameters of cream and in vitro SPF determination. The results of cream and SPF are mentioned in Table nos. 2 and 3. The parameters of cream complies with official acceptance criteria and SPF of this cream is found to be 1.08±0.02 with Boots Star Rating 4 indicating that the cream formulated can be considered as an efficient validated topical product.

Table No.3: Results of SPF and other Parameters of lutein ester sunscreen cream

Sr no.	Parameter	Scan I	Scan II	Scan III	Average value
I	SPF	1.05	1.09	1.10	1.08
II	Standard Deviation	0.01	0.02	0.03	0.02
III	UVA/UVB Ratio	0.836	0.843	0.643	0.774
IV	Critical Wavelength	389.9	389.2	387.9	389.0
V	Boots Star Rating	4	4	3	4

Figure No. 1: SPF-290 Graph Report of lutein ester Sunscreen Cream (Scan I)

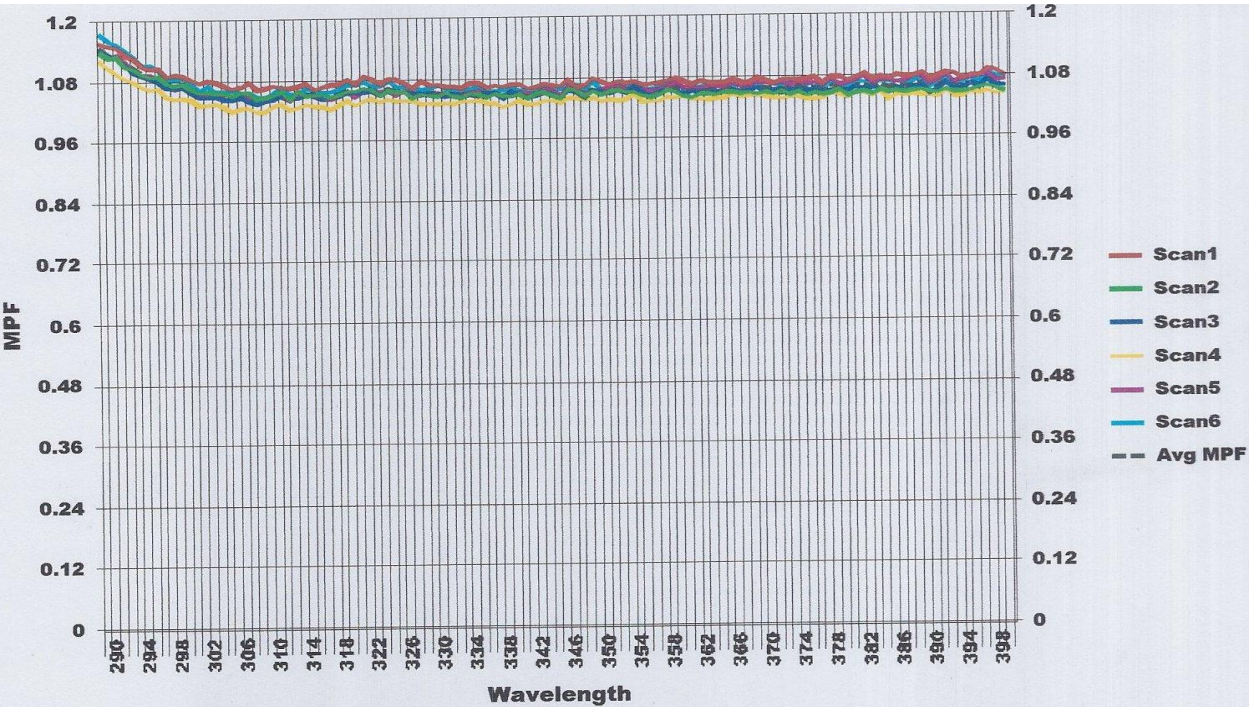

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Figure No.2: SPF-290 Graph Report of lutein ester Sunscreen Cream (Scan II)

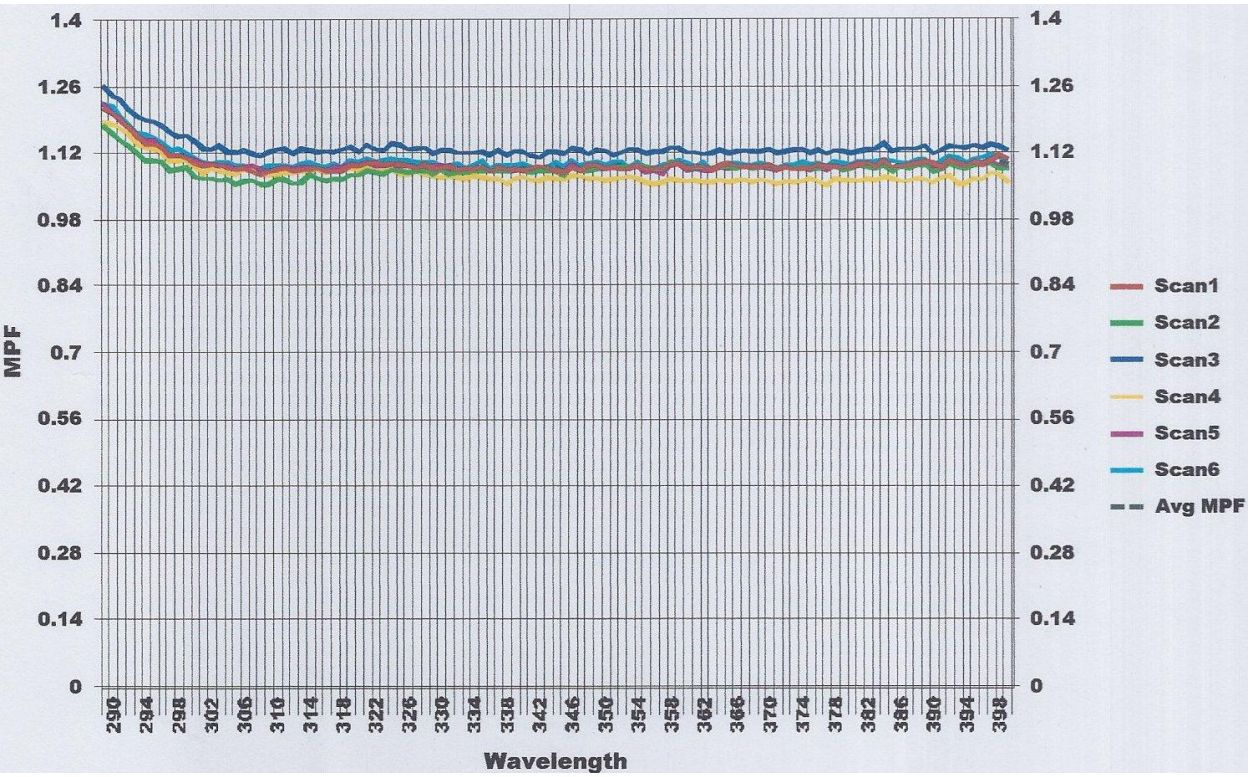
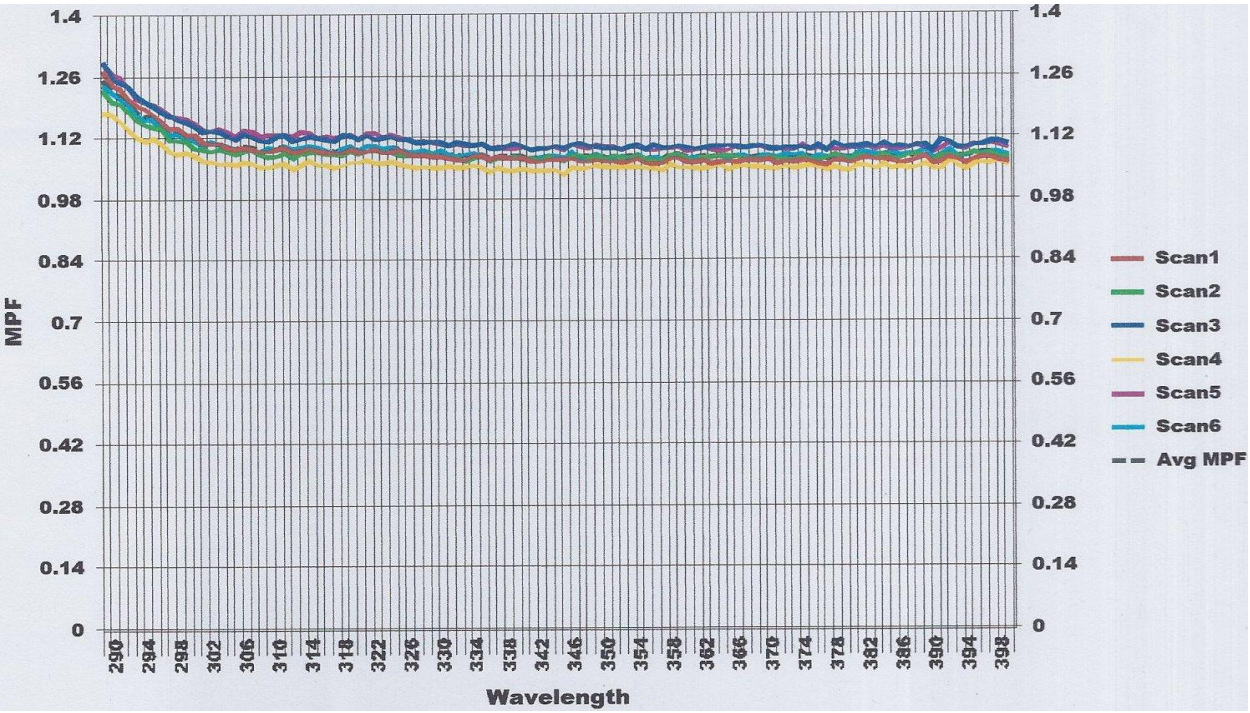


Figure No.3: SPF-290 Graph Report of lutein ester Sunscreen Cream (Scan III)



DISCUSSION

The Optometrics Model SPF-290 Analyzer is a computer controlled instrument that is designed to measure the sun protection factor of sunscreen preparations. For US-FDA standards the protection factor is calculated over the wavelength range from 290-400nm. To initiate an analysis a reference scan was done with the blank substrate (which consists of data from 23 wavelengths) in the incident beam. The sample was then applied to the substrate and the first sample scan was made. Data was collected in the same manner as the reference data, ratioed to the reference and plotted as a MPF (Monochromatic protection factor). Ratioing the sample signal to the reference signal negates any effect of wavelength dependent variables in the optical system (source, monochromator and detector). Up to 6 sample scans were made to compensate for variables in the substrate and sample application.

The SPF 290 software uses Trapezoidal Approx calculation technique to approximate the integral for SPF and Erythemal UVA protection factor. These include UVA/UVB ratio, critical wavelength and cumulative absorbance. The Average Absorbance method is used for calculating average protection factor; this method averages and computes the standard deviation based on the absorbance scan data. This method of calculation gives a better average value assuming that sample thickness is the largest variable in performing a protection factor measurement.

For the calculation of standard deviation, Diffey's method is used, based on B. L. Diffey's paper[16] on using Transpore Tape® as the substrate for SPF measurements. Diffey's equation applies weighing by recognizing that the MPF measurements for a set of scans have some distribution. Therefore, the standard deviations of the MPF measurements at each wavelength are factored in to the Diffey SPF standard deviation calculation.

CONCLUSION

The described in vitro method, though, presents some limits; it has spared the exposure of human subjects to harmful ultraviolet radiations that can pose potential risks of skin cancer, hence, it is still preferred and is undoubtedly beneficial as it gives accurate and reproducible results. This method has thus helped to determine the SPF value of a novel drug like Tagetes erecta L. (Asteraceae) and stating that it has good sunscreen activity and can be considered as active sunscreen agent or can be incorporated into other sunscreen formulations as an additive to enhance the activity.

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