Research Article

Formulation and *in–vitro* Evaluation of Sun Protection Factor of Flavonoids Extracted from *Tagetes erecta* (L.) Flowers, *Cucumis sativus* Fruits and Essential Oil of *Ocimum sanctum* Leaves Sunscreen Cream

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ABSTRACT

The present study was designed to study the sunscreen activity of herbal cream formulation containing flavonoids extracted from *Tagetes erecta* Linn flowers (Family-Asteraceae), *Cucumis sativus* fruits (Family-Cucurbitaceae) and essential oil extracted from leaves of *Ocimum sanctum* (Family-Labiatae). The parameters of creams complies with official acceptance criteria and SPF calculated by COLIPA method. Sun protection factor is a laboratory measure of the effectiveness of sunscreen; the higher the SPF, the more protection a sunscreen offers against the ultraviolet radiations causing sunburn. Flavonoids extracted from *Tagetes erecta L.*flowers, *Cucumis sativus* fruits extract and essential oil extracted from leaves of *Ocimum sanctum* and in combination sunscreen cream was formulated have SPF of 1.50 with boot star rating 4 indicating that cream formulated can be considered as an efficient validated topical product. From the result obtained in the study, we can positively conclude that ethanolic extract of *Tagetes erecta* flowers, aqueous extract of *Cucumis sativus* fruits and essential oil extracted from leaves of *Ocimum sanctum* formulated sunscreens will enhance and significantly contribute to the UV absorbing properties of conventional sunscreen formulation. It will also help in broadening the UV protection ability of the sunscreen along with the greatest advantage of avoiding the adverse and undesired effects of synthetic sunscreen compounds.

Keywords: *Tagetes erecta, Cucumis sativus, Ocimum sanctum*, SPF, Boot star rating.

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).[1] Exposure to solar radiation is recognized to have negative effects on the human skin. It has been known for decades that sunscreens are capable of protecting man from harmful effects of solar radiation such as premature aging or cutaneous cancer, basal cell carcinoma, sunburns, malignant melanomas.[2-5] UVA rays can be divided into two key wavelengths: short-wave UVA (320 - 340 nm) and long wave UVA (340 - 400 nm).[6-7] Ultraviolet radiation A and B (UVA, UVB) are harmful by various mechanisms.

UVB and UVA penetrate the ozone layer and reach the earth's surface but the atmosphere filters more UVA than UVB[8]. Fortunately, UVA is not quite so powerful in its effects as UVB as it has an additive (cumulative) effect with UVB on the skin. UVA and UVB are the only wavelengths that need to be screened out as we still have an ozone layer over most of the earth.[9]

Sun protection products, including sunscreen creams, are designed to absorb or reflect the sun UV radiation to protect the skin from damage. The rating system for sunscreens specifies Sun Protection Factor (SPF) value, which can defined as a time factor for the protection of skin compared to exposure without any protection[10-12]. An individual's response to UV radiation and melanin production is dependent on skin color and other genetic factors. Even if an individual has dark skin, or whose skin readily produces melanin when exposes to UV radiation, may still experience sunburn as a result of high intensity of UV radiation and an extended length of exposure. Sunscreens are cosmetic formulations that block UV rays. Sunscreens are assigned sun protection factors, or SPF, ratings that are supposed to indicate the level of protection from UV radiation. This is important for human well-being because exposure to ultraviolet radiation (principally UV-B radiation) has a number of effects on health including sunburn, skin cancer, immune suppression and damage to the eye.[12]

Tagetes erecta Linn. (Fam. Compositae) locally known as Gendaphul is a stout, branching herb. Different parts of this plant including flower are used in folk medicine to cure various diseases. Leaves are used as an antiseptic and in kidney troubles, muscular pain, piles and applied to boils. The flower is useful in fever, epileptic fits (Ayurveda), astringent, carminative, stomachic and scabies and in liver complaints and also said to purify blood and flower juice is given as remedy for bleeding piles and also in rheumatism, cold and bronchitis.[13-15]

Cucumis sativus Linn.(Fam. Cucurbitaceae) locally known as cucumber even though long, dark green, smooth-skinned garden cucumbers are familiar vegetables in the produce sections of most groceries, cucumbers actually

come in a wide variety of colors, sizes, shapes and textures. You'll find white, yellow, and even orange-colored cucumbers, and they may be short, slightly oval, or even round in shape. Their skins can be smooth and thin, or thick and rough. It is found wildly in the Himalayan regions and also cultivated throughout India. The fruits contain an enzyme, erepsin, Vitamin B_1 and C, ascorbic acid, proteolytic enzyme, rutin, oxidase, succinic and maleic dehydrogenases and so on. The seeds contain α- and β-amyrin, sitosterols and cucurbitasides, whereas, the leaves contain free cucurbitasides B and C and ferredoxin. Phytonutrient list for cucumbers are its cucurbitacins, lignans, and flavonoids. These three types of phytonutrients found in cucumbers provide us with valuable antioxidant, anti-inflammatory, and anti-cancer benefits.[16-17]

Ocimum sanctum Linn (Fam. Labiatae) locally known as sacred basil Indigenous to India and parts of north and eastern Africa, Hainan Island and Taiwan, China. It is cultivated in south-east Asia.[18] An herb or shrub, up to 1m high, often much branched. Leaves green to greenish-brown, 2.5–7.5 cm long, 1–3 cm wide, oblong, ovate or oval-oblong, with acute top, cuneate, obtuse to rounded base, pinnate veins, serrate or entire and undulate margin; thin but fleshy, both surfaces thinly pubescent; petiole cylindrical, 1–2cm long, thinly pubescent.[18] In literature essential oil of basil is reported to have anti inflammatory, anti-microbial,antioxidant and radioprot ective activity. The present work was planned to study the sunscreen activity of herbal sunscreen containing ethanolic extract of flower of *T. erecta*, aqueous extract of fruit of *Cucumis sativus* and essential oil extracted from leaves of *Ocimum sanctum*. It has become very important to study the sunscreen activity of herbal drugs, so as to avoid the various effects of synthetic chemical sunscreens like aminobenzoic acid derivatives, anthranilates, benzophenones, cinnamates, salicylates in organic sunscreens like titanium dioxide and zinc oxide. The therapeutic properties of *T.erecta, Cucumis sativus and Ocimum sanctum* are very well recorded in the text of traditional Indian medicines Siddha and Ayurveda. However the sunscreens activity of both these plants has not been reported till date. This forms the basis for selection of these plants for its sunscreens activity.

Table 1: Formulation of sunscreen cream

S. No	Ingredients	Uses	Quantity (w/w)
1	*Tagetes erecta* (Etha. Extract)	Active ingredient	2
2	*Cucumis sativus* (water extract)	Active ingredient	3
3	*Tulsi oil*	Active ingredient	2 drops
4	Cetosteryl alcohol	Emulsifier	5
5	Steric acid	Emollient, co-emulsifier	2
6	PEG-200	Emulsifier	2
7	Cetyl alcohol	Emollient, co-emulsifier	1
8	Methyl paraben	Preservative	0.3
9	Propyl paraben	Preservative	0.06
10	Carbopol 940	Gelling agent	0.5
11	Disodium EDTA	Chelating agent	0.02
12	Triethanolamine	Surface active agent	0.5
13	Distilled water	Vehicle	q.s.to 100 gm

MATERIALS AND METHODS

Plant Material

The flowers of plant *Tagetes erecta L. (Asteraceae)* was purchased from local flower market of Amravati.

The fresh fruits of plant *Cucumis sativus* L. (*Cucurbitaceae)* was collected from the local market of Amravati.

Tulsi oil (Dr. Urjita Jain) of plant species *Ocimum sanctum (Labiatae) was* purchased from local retailer of Amravati.

Plant species identified and confirmed their authentication by Botanist Dr. Prabha Bhogaonkar, Vidarbh institute of science and humilities, (V.M.V), Amravati.

Extraction of plant material

1. Air-dried flowers parts of plant *Tagetes erecta* was defatted with Pet. ether then extracted with 90% ethanol in Soxhelet apparatus, then solution was concentrated on water bath to a thick paste and dried under vacuum.[19]

2. Fresh fruits of *C. sativus* were cleaned, cut into small pieces, and macerated with water. The extract was filtered and distilled in a water bath. The extract was solidified under reduced pressure in a rotary evaporator.[20]

Reagents

Ethanol (Merck) analytical grade was used.

Instruments

UV Spectrophotometer: Optometric LLC SPF-290S

Brookfield Viscometer: Brookfield Engineering Labs Inc.

PH meter: (Digital model - 111E) Environmental and Scientific Institute and company

Micro centrifuge: REMI RM-12 C

Formulation of Sunscreen cream

Preparation of Herbal Sunscreen cream was formulated and their composition and ingredient was given in Table 1.

Step I:

Water phase was prepared by collecting deionised water (72 %) and then 5 % water was removed aside from this for final volume makeup. Water soluble components Disodium EDTA (0.02%), Sodium Methyl Paraben (0.3%) and Triethanolamine (0.5%) were dissolved in deionised water, meanwhile, carbopol (0.5%) was allowed to swell using an homogenizer and heated up to 80 0C.To this weight quantity of T. erecta and Cucumis sativus extract added and mix it.

Step II:

Oil phase was prepared by heating Sodium Propyl Paraben (0.06%), Stearic acid(2%), Cetyl alcohol (1%),PEG200, cetosteryl alcohol and Tulsi oil added and heated upto 80^0C.

Step III:

Oil phase was added in water phase at 80 ^{O}C with continuous stirring for 20-25 min and then it was homogenized till uniform emulsion is formed. The finished product has dark yellow color and semisolid cream-like consistency. It was then poured into the wide mouth 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 2.

Determination of *In-Vitro* SPF of sunscreen cream

This study was performed by Transmittance measurement of the three creams. The Optometric Model SPF-290 Analyzer measures the sun protection factor of the cream over a wavelength range from 290nm-400nm. Approximately 100 mg of the investigational sample was applied and spread on 50 sq.cm area to obtain a sample film thickness of 2µl /cm2 on Transpore surgical tape to get an even film as suggested in the operation manual of Optometric LLC SPF-290S for the sample preparation and application technique. The samples thus prepared were exposed to Xenon arc lamp with UV range 290nm to 400 nm for determining the SPF.

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

Where,

MPFλ = scan MPF value

Eλ = spectral irradiance of terrestrial sunlight under controlled conditions

Bλ = erythemal effectiveness.

The SPF-290 software used Trapezoidal Approximately calculating technique to approximate the integral for SPF and Erythemal UVA protection factor. These include UVA/UVB ratio, critical wavelength, cumulative absorbance, etc. The Average Absorbance method is used for calculating average protection factor. For calculation of standard deviation, Diffey's method is used[21].

RESULTS AND DISCUSSION

The topical formulation of flavonoids extracted from flowers of *Tagetes erecta L. (Asteraceae)* and fruits of *Cucumis sativus* and Tulsi oil was studied for all physical parameters of cream. Table 2 represents all physical parameters observe good consistency, viscosity, uniformity, colour and odor of cream. The in vitro SPF and boot star rating of herbal sunscreen cream was determined. The results of cream SPF and boot star rating of cream are mentioned in Table 3, Table 4 and graph obtained for observed reading are mention in Figure 1, Figure 2, and Figure 3. The graphs are plotted as MPF against wavelength which consists of sample scan for 6 times which calculate average MPF. The parameters of cream complies with official acceptance criteria and SPF of this cream is found to be 1.50±0.11 with Boots Star Rating 4 which is compared with standard boot star rating guideline given in Table 4 and Table5 indicating that the cream formulated can be considered as an efficient validated topical product.

Table 2: Physical parameters of sunscreen cream

S. No.	Parameters	Observation
1	Color	Dark yellow
2	Odor	Characteristic
3	Spredability	Good and uniform
4	pH	6.8
5	Viscosity	28000
6	Patch test for Irritancy	No irritation reaction persist

Table 3: Results of SPF and other parameters of sunscreen creams

S.No	Test Sample	Parameters				Average Values
		Scans	1	2	3	
1.	Sunscreen Sample	**SPF**	1.40	1.58	1.52	**1.50**
		Standard Deviation	0.08	0.16	0.08	0.11
		UVA/UVB Ratio	0.930	0.735	0.773	0.813
		Critical Wavelength	387.5	385.9	386.3	386.6
		Boot Star Rating	5	3	3	4

The above readings are averages of 3 replicate each consisting of 6 scans

Table 4: The boots guideline for star rating

Mean UVA/UVB Ratio	Star Rating Category	Star Rating Designation
0 to 0.2	-	No claim
0.21 to 0.4	*	Minimum
0.41 to 0.6	**	Moderate
0.61 to 0.8	***	Good
0.81 to 0.9	****	Superior
0.91 and above	*****	Ultra

The study yielded a Sun Protection Factor (SPF) and Boot star rating as presented below following the application of the test samples on transpore Tape.

Table 5: SPF and boot star rating of sunscreen cream

Test Samples	SPF	Boots Star Rating
Sunscreen Sample	1.50	4-superior

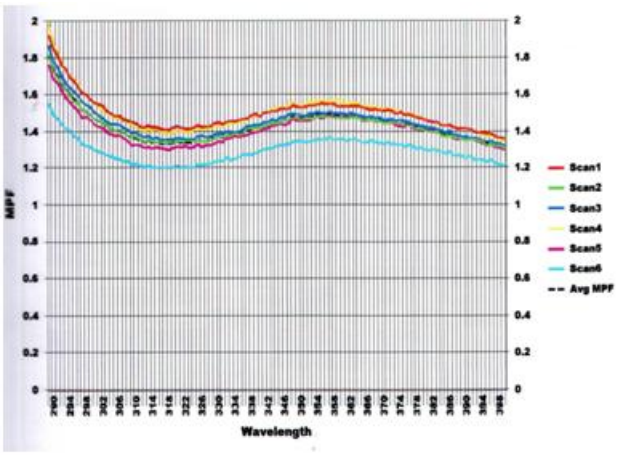

Figure 1: SPF-290 Graph Report of flavonoids Sunscreen Cream (scan I)

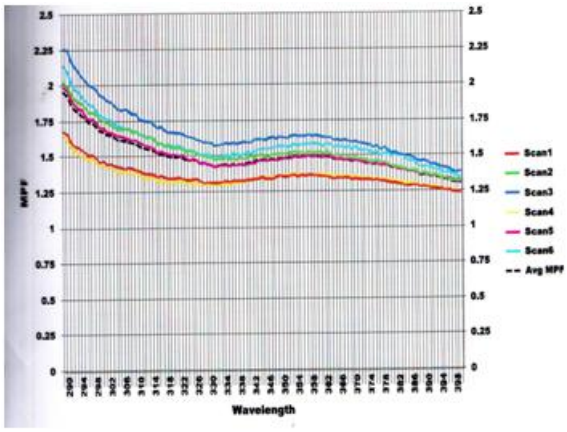

Figure 2: SPF-290 graph report of flavonoids sunscreen cream (scan II)

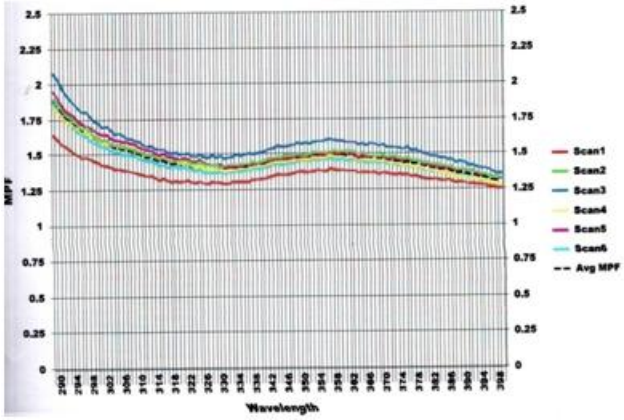

Figure 3: SPF-290 graph report of flavonoids sunscreen cream (scan III)

CONCLUSION

The SPF is quantitative measurement of the effectiveness of the sunscreen formulations. The SPF value of sunscreen cream was found to be 1.50 with ultra boot star rating 4. SPF value for sunscreen above 2 is considered as having good sunscreen activity. It indicates that formulated sunscreen cream was found near the range of good sunscreen activity and all these three may be considered as good candidate for sunscreen or cosmoceutical purpose. 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 thus helped to determine the SPF value of a novel drug like *Tagetes erecta L. (Asteraceae), Tulsi oil* and *Cucumis sativus* 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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