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RESPONSE SURFACE METHODOLOGY AND SPF DETERMINED THE PHOTO-PROTECTIVE CAPACITY OF *TAGETES PATULA* AGAINST UV-B RADIATIONS

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ABSTRACT

The present study investigates the solar radiation protective competency in *Tagetes patula* extract. The *Tagetes* extract can be used as therapeutic remedy against the harmful erythemal solar-rays and protect soft & sensitive skin from sun tanning and sun poisoning.

Tagetes extract have been treated combinedly with ultrasonication and ethanol followed by filtration. Filtered ethanol extract diluted in a significant quantity of ethanol and undergone for UV-spectrophotometer analysis, where ethanol was used as a reference. Finally, the Mansur equation evaluates the significant sun protection factor (SPF) value of *Tagetes patula* extract.

This study demonstrated the *Tagetes patula* extract have a remarkable tendency to reflect the harmful solar UV-B rays. *Tagetes patula* flower and leaf extract had showed the significant sun protection factor value, reflected as (SPF-30) and (SPF-12), respectively. However, the Response surface methodology significantly establishes the correlation between the reflection and photon energy. The studied erythemal factor have been optimized and evaluated through the optimization plot and shows the significant positive correlation between the reflectance and erythemal response factors. In addition, *Tagetes patula* leaves (NRL) and (NRF) flower extract has application on UV-exposed skin, and help to prevent the skin tissues and cure the sensitive skin from harmful effect of the UV-B rays.

This study concluded that NRF extract of *Tagetes patula* had found greater competency and compatible than NRL extract, in terms of reflectivity and SPF property.

Keywords: Erythemal Response; Sun Protection Factor; *Tagetes patula*, response surface methodology; Photon energy

Introduction

Solar radiation has an adverse effect on the skin tissues, after a continuous exposure of intense sunlight (Modenese *et al*., 2018). An ultimate consequence of harmful solar radiations can eventually provoked an unwanted oxidative stress reaction in mammalian skin tissues and arise numerous skin problems such as sunburn, skin ageing *etc*. (Dunaway *et al*., 2018).

UV radiation emitted from sun has electromagnetic radiation spectrum of 200-400 nm. Ultraviolet (UV) electromagnetic spectra broadly distinguished into the three major spectral bands: UV-A (320-400 nm), UV-B (290 to 320 nm) and UV-C (100-290 nm). UV-A spectra cover entire wavelength ranges that were not completely filtered out by atmospheric ozone layer (Kushwaha *et al*., 2021). In spite of this, it penetrates deeply into the skin epidermis and badly affects the dermal layer tissues, causing an inflammation and thus, leading to a premature skin ageing (Rai and Agrawal, 2017).

UV-B radiation provokes highly damaging effects to the human skin as a result of ozone-layer partial absorption (Melendez-Martínez *et al*., 2019) and eventually had adverse effects on human life-style. At last, UV-C radiation is completely filtered out by earth atmosphere. Thereby, UV-B rays among them had more detrimental effects on skin tissues and is responsible to disturbed the integrity of lipids, proteins and nucleic acids which leads to sun burn, premature ageing and even responsible for causing a skin cancer (Ebrahimzadeh *et al*., 2014). Thus the, UV protective bio-cosmetic products development is a crucial step to prevent the sensitive skin from the detrimental effect of UV radiations, thereby, a dermatologist, prescribed to use regularly sunscreen products (Azarafshan *et al*., 2020; Kushwaha *et al*., 2021). However, many synthetically synthesized sunscreens are already present in a cosmetic market in order to, protect the dermal layer of skin from a detrimental effects of UV-radiations (Gamoudi and Srasra, 2018) but these sunscreen were actually formulated chemically by harnessing the property of nanoparticles like ZnO, TiO_2 (Sargent, and Travers, 2016; Sharma *et al*., 2020). These unwanted side effects in skin arises on account of a continuous exposure of UV radiations (Rai *et al*., 2012), which trigger the activation of number of protein kinases

(Begaj and Schaal, 2018) families belonging enzymes like epidermal receptor kinase (ERK) and Janus kinase (JNK) thereby; inducing a continuous accumulation of reactive oxidative radicals in a skin. Recent report discussed the harmful effect of UV-B in human keratinocytes and only responsible initiators of oxidative stress reactions which are actually regulated by JNK Pathways instead of ERK pathway (Gunaseelan *et al*., 2017). Although, ERK pathway operate when the skin tissues are in a continuous contact to visible light (Ascenso *et al*., 2016). Moreover, transcriptional factor namely C-Jun act on the N-terminal end of Janus-kinase (JNK) & regulate the JNK pathway (Wang *et al*., 2019). Actually, C-Jun, a transcriptional factor controls indirectly the mitogen-activated protein kinase (MAPK) signaling pathway. However, a key component of the JNK pathway are organized in signaling complex with the help of regulatory scaffold proteins such as JNK interacting proteins (JIPs) (Courtial *et al*., 2017) but these regulatory proteins have auto-phosphorylation property, which initiates Rho-associated kinase (ROK) and trigger apoptotic signaling mechanism to ultimate cellular death (Lazovich *et al*., 2011). However, ROK inhibition reduce the activity of cell-survival associated events, including apoptosis, cell proliferations and also reduced inflammatory responses in skin tissues (Morrison *et al*.,2003). This same mechanism come into an existence when the *Tagetes* extract have been applied on skin. In addition to this, studies relevant to UV protection & skin finishing properties of *Tagetes* extract were carried out to explore antibacterial, antifungal and antioxidant like biological activities (Kushwaha *et al*., 2020).

Sun Protection Factor (SPF)

SPF defined as the extent of solar energy required to maintain the ratio of minimal erythemal dose (MED) in sunscreen protected and unprotected skin. To date, newly synthesized sunscreen products efficacies against the harmful UV radiations were determined on the basis of SPF-index value. However, it is very necessary to ensure that efficient sunscreen product is used in the cosmetic formulations (Srinivasan *et al*., 2019).

The minimal erythemal dosage (MED) of impose UV radiation onto skin tissue for a lesser degree time-interval (Fang *et al*., 2015). The MED factor determines the amount of skin tissues affected or straightforwardly determined the level of erythemal responses in unprotected skin [25]. However, this MED factor value actually calibrate the SPF-index efficacy value and established a direct relationship with SPF value (Zouboulis *et al*., 2015), it show as the UV-MED values will increase the SPF index of sunscreen product will also raised as shown in (Equation.1). More elaborately, higher SPF value sunscreens product indicate the skin protectiveness nature than the lower SPF value containing sunscreen products and effectively reduce the deleterious effect of UV-B radiations (Hayden *et al* 2016). Actually, SPF values define the sunscreen products effectiveness against the solar radiations and it can be formulated as per given equation (1):

$$\text{SPF} = \frac{\text{Minimal erythemal dose of sunscreen} - \text{protected skin}}{\text{Minimal erythemal dose of non-sunscreen protected skin}} \quad (1)$$

Nevertheless, it is crucial to determine SPF value of commercially available cosmetic products *via* through *in-vitro* methods.

Nowadays, various chemically synthesized sunscreen products are available in the cosmetic markets, providing protection to the skin from harmful UV rays[26]. But these chemically synthesized cosmetic products were usually not so much effective and safe against UV rays (Kurniawan *et al*., 2019). Now, so many scientist and researchers were focusing extensively on naturally synthesized sunscreen products without having any side-effects (Saewan and Jimtaisong, 2013) otherwise, it may not be recommended to use regularly due to harmful health hazardous associated problems (Rodino *et al*., 2019).

Thus, newly synthesized herbal sunscreen products were spotted to be a good candidate with having a higher degree of UV protective characteristics owing to the presence of UV absorbing bioactive compounds such as carotenoids compounds like lutein and lutein ester (Salehi *et al*., 2018) and it also imparts a coloring property to the *Tagetes patula*. Despite of these advantages, so many bioactive compounds were found in *Tagetes sp*. thereby, it become a valuable resource for medication as well (Kushwaha and Verma, 2017). These advantageous possibilities creates an innovative biological pathways based on the rationalization to develop a natural resources based sunscreen in order to, avoid unexpected side effects in skin as found in a chemically synthesized sunscreen products (Katiyar *et al*., 2011). Hence, the *Tagetes patula* regarded as multipurpose plant with having an ability to provide an excellent photoprotection against UV rays (Park *et al*., 2017) and plays a significant role to develop a novel sunscreen cosmetic products in a cost effective manner (Wang *et al*., 2010).

As per the previously reported scientific reports, the well-known bioactive components were present in a *Tagetes patula* such as phenol, flavonoids and tannins *etc* (Kushwaha and Verma, 2017). *Tagetes* phytoconstituents like flavonoids, a polyphenolic component actually responsible to provide UV-protection to the skin (Kushwaha *et al*., 2021). Moreover, *Tagetes patula* have UV-photoprotection property on account of the presence of flavonoid and its sub components such as Equol (4,7-isoflavandiol), Silymarin (Irrera *et al*., 2017) and Genistein (4,5,7-trihydroxyisoflavone) (Wang *et al*., 2010) *etc*. However, the UV-photo protective property in *Tagetes sp.* on account of flavonoid presence while in this category, legumes are also included owing to isoflavones and its precursors components presence such as daidzein and genistein (Ahn-Jarvis *et al*., 2019). Equol (4,7-isoflavandiol), a flavonoid subcomponent have immunomodulatory characteristics (Oak *et al*.,2017) actually induced by a putative epidermal mediator *i.e.*, *cis*-urocanic acid indicating its potential protective action against the dangerous UV radiations (Liu *et al*., 2015) . A view point of the current study distinguished the *Tagetes* sp. from other plants with having flavonoids and their subcomponents especially play a vital role in protecting skin from harmful UV-B rays and majorly helpful to develop the sunscreen products.

The aim of the current study is to confirm that *Tagetes* sp. extracts have excellent SPF bearing property, and is a good natural resource for the formulation of bio-cosmetics products such as sunscreen against UV-B rays protective action.

Materials and Methods

Current research studies were conducted at the Biochemical Engineering, Research lab, SHUATS, Prayagraj. India

Materials

Tagetes sp. were procured from the agriculture field of SHUATS, Prayagraj. An analytical grade ethanol was purchased from Merck India.

Apparatus

Current study analyzed an optimum range of SPF value of herbal sunscreen products *via* using Systronic-2202-UV-Spectrophotometer model.

Methods

Sample preparation

One gram *T. patula* leaves and flowers extracts quantity were used to ultrasonicate at 20Hz frequency for a 5 minutes (mins). *Tagetes* extract was mixed thoroughly in 100 milliliter (ml) quantity of ethanol and then filtered to discard out the remaining 10ml aliquots. A 5.0 ml quantity of filtered aliquot was diluted with 50ml quantity of ethanol. Diluted aliquot was further diluted with 25ml quantity of ethanol. An observed absorbance were measured in a triplicate manner at different UV-wavelength range (290-320nm) and tested regularly after at 5mins time interval differences against the ethanol blank. Mansur equation (2) computes the exact SPF value (Kushwaha *et al.*, 2021).

$$\text{SPF (spectrometry)} = \text{CF} \times \sum_{290}^{320} \text{EE}(\lambda) \times \text{I}(\lambda) \times \text{abs}(\lambda) \quad (2)$$

Where, CF = correction factor (= 10), EE (λ) = Erythemal effect spectrum; I(λ) = sunlight intensity at a wavelength; abs (λ) = sample absorbance at a particular wavelength (λ).

Both the standardized metric values of EE (λ) and I (λ) calibrate the actual SPF value and each value is shown in Table 1. Sayre *et al.* (1979) recorded and establish the Erythemogenic and UV-radiation intensity relationship. Known variable namely, radiation intensity would be treat as constant in this study.

Table 1: Relationship between the Erythemogenic effect (EE) and radiation intensity (I) as per the wavelength (λ) range

λ (nm)	EE x I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

Normalized sunscreen product formulation compute the SPF index value

Statistical analysis

All the Statistical analysis performed in a triplicate manner and recorded the significant SPF value at the 95% confidence level by using the Minitab17 version software.

Results and Discussion

An effective sunscreen products formulation can be quantitatively determined on SPF basis. An effective sunscreen product should have a wide range of absorbance between 290 and 400 nm against the UV-skin injuries. Evaluations of efficacy of a sunscreen formulation have been assessed through *in-vivo* survey trial test in humans. *In-vivo* test have some drawbacks like time-consuming methodology with a certain degree of variability, not mention in the ethical problems of testing with human. *In-vitro* SPF test screen out UV-protective and efficient sunscreen products, as a supplement of the *in-vivo* SPS measures.

In this study, *Tagetes* leaf and flower extract SPF values were determined through the UV-spectrophotometry and mathematically computes its value as reflected in table 2. SPF value is measured by applying the Mansur equation. Commercially available sunscreen products should have a wide range of reflectance between 290-320nm, as depicted in Figure.1.

Table 2 : Represented the SPF calculated values

Sample	Calculated SPF value
Normal Flowers (NRF)	30± 0.163
Normal Leaves (NRL)	12±0.63

All the experimental have been observed in a triplicate manner (Mean ±SD) at 95% confidence interval.

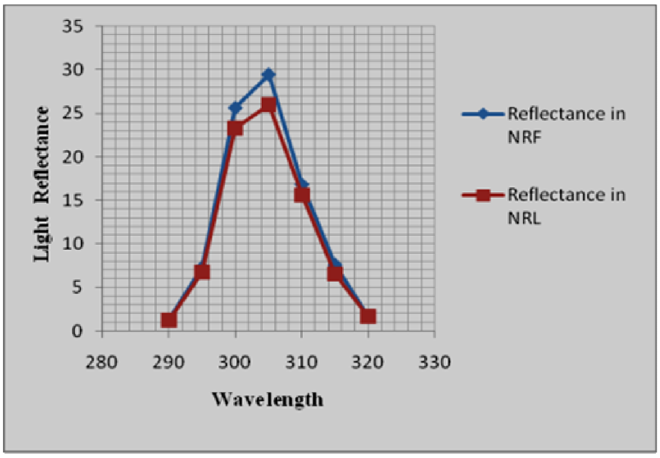

Fig. 1 : UV radiation reflection of flowers and leaves at the wavelength range of 290-320nm. The reflection of NRF and NRL was evaluated significantly at P≤0.05 level.

Figure. 1 present the higher reflectance power of Tagetes extracts against the UV rays than absorption at the range of 300-305 nm. (NRF) flower extract had more reflectance capacity against UV than leaves. Thus, the application of *T. patula* flowers on skin had showed a lesser tendency to show an erythemal effect as compared to leaves. Reason being, a more chromophore deposition had occurred in a skin on application of *Tagetes* flower extract. From this experimental work, it was noted that the maximum UV index and sunlight intensity was observed in summer (during April–June,) and at mid-hours. Thus the higher SPF index value sunscreen can protect the skin from detrimental effect of UV-B rays. *T. patula* flower extract with measured 30+SPF index value may be utilized to manufacture the sunscreen products and significantly effective than the *T.patula* leaf extract against the UV-B radiations.

Response surface methodology of (NRF) flowers

An effective reflectance pattern was shown in a flower (*Tagetes patula*) extract as compared to leaf, and it show reversible pattern in absorbance. Figure (4 & 6) describe the maximum burning effect (erythema) at 290-300nm wavelength range after the absorption of photon-energy in the skin. *T. patula* extracts had sunlight reflecting property, a comparative study of *Tagetes* flower and leaf extract and it was observed that *Tagetes* flowers had greater UV-B light reflectance property than leaves.

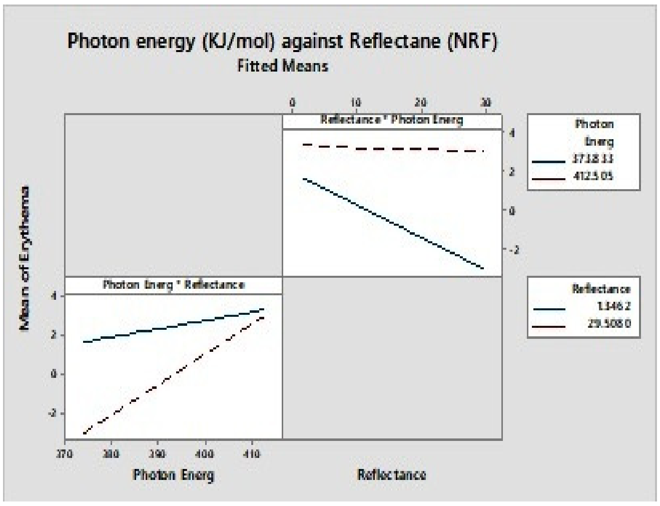

Fig. 2 : Relate the photon energy and reflectance of NRF extract and express erythemal response *via* Response surface methodology

Figure 2 depicted the full matrix of NRF factorial plot and it represents the interaction between the UV-B light reflectance and incident photon energy, as these were expressed in Kilo joule mole^{-1} (KJ/mol).

At the upper right corner of 2D figure 2 graph represent the relation between the UV-B light reflectance capacity and incident photon energy. At upper right corner, UV-B light reflectance capacity remains constant while photon energy responded to an erythemal response (negatively affect have been shown). While lower left corner graph showed the greater light reflectance capacity and established the positive correlation effect in between the response erythema and reflectance. An optimization plot of skin erythemal prediction was presented in Table.3 but it showed a slightly different behavior compare to NRL plot.

Table 3 : Optimization plot for Erythemal prediction in NRF

Response	*Fit	SE Fit	95% CI	95% PI
Erythema	-0.154317	0.242880	(-0.927269, 0.618635)	(-1.44715, 1.13851)

*At 95% confidence level the significant fit value (-0.154317) of the Erythemal response lie down in between the (1.44715, 1.13851) values.

Regression equation showed the erythemal effect in *T. patula* leaf extract (NRL):

Erythema = -11.98 + 0.0371 Photon Energy (KJ/mol) - 1.686 Reflectance + 0.00406 Photon Energy (KJ/mol)* Reflectance

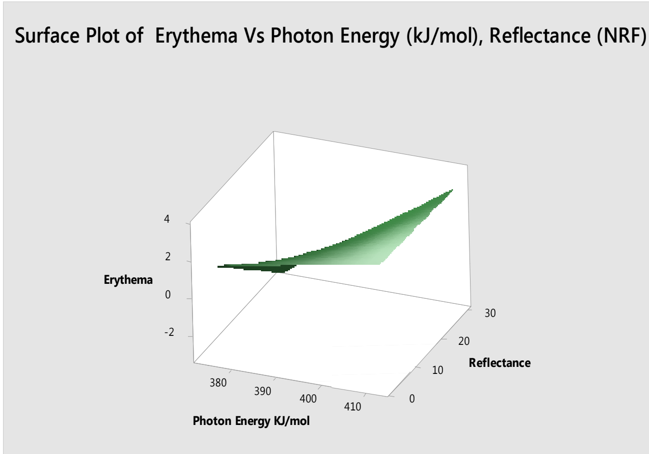

Fig. 3

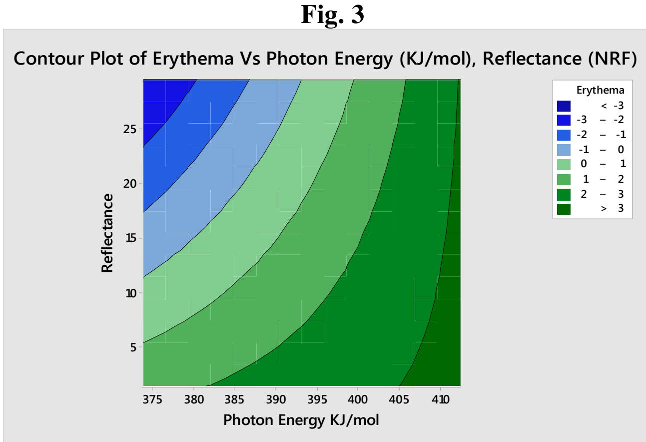

Fig. 4

Fig. 3 & 4 : Showed the surface and counter plot of NRF and established a strong relationship between erythemal response *vs* photon energy & reflectance. Predicts their relation *via* response surface methodology.

The surface and counter plot of NRF shown the relationship between the erythemal response *vs* photon energy of incident light & UV-B light reflectance. Darker green region of the counter plot indicates the maximal erythemal responses. The blue color region represents the lowest erythemal response at the middle upper region to reflectance. But it showed a negative effect (figure 4), it means as the erythemal response decreases, the UV-B light reflectance capacity have been increased. Hence, the most of the portion of 2D graph shifted towards the upper right corner to the lower left region of photon energy.

Response surface methodology of *Tagetes patula* leaves

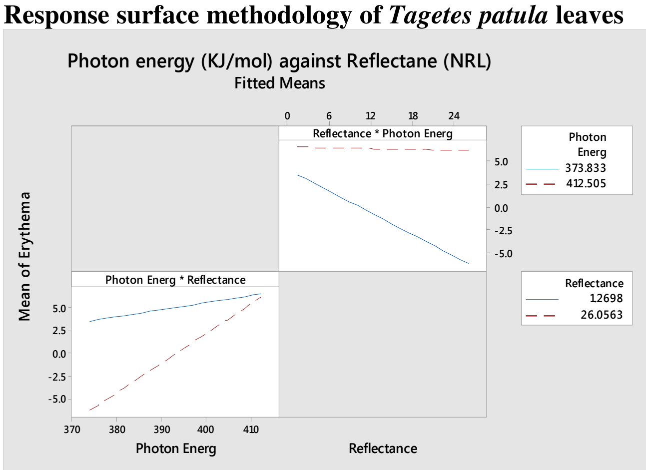

Fig. 5 : Relationship between photon energy and reflectance of NRL extract describe erythemal responses

The full matrix of NRL factorial plot shows the interaction between the reflectance and photon energy (KJ/mol) at upper right corner, wherein; reflectance variable found constant while photon energy tend to be deflected and show the negative effect with the erythemal response. And a lower left corner of graph illustrates the reflectance variable and it shows the more predominant nature than the photon energy (positive effect). An optimization plot constructed for erythemal prediction was shown in table.4.

Table 4 : Optimization plot for erythemal prediction in *T.patula* leaves extract (NRL)

Response	*Fit	SE Fit	95% CI	95% PI
Erythema	-0.356038	0.532367	(-2.05027, 1.33819)	(-3.22426, 2.51218)

*At 95% confidence level significant fit value (-0.356038) of Erythemal response lie in the range of (-3.22426, 2.51218).

Regression equation applied to explain erythemal effect in skin:

Erythema = -20.0 + 0.0645 Photon Energy (KJ/mol) - 4.07 Reflectance + 0.00983 Photon Energy (KJ/mol)* Reflectance

NRL counter (figure 6) and surface plot (figure.7) established the relationship between Erythemal response *vs* Photon-energy parameters for the reflectance. Dark green zone of counter plot as shown in fig.6 reflects the maximum erythemal responses.

Figure 6 counter plot depict the negative effect on account of increment in the erythemal response as more UV-B light is incident on the skin. Graphically, it is marked at the upper left corner of reflectance axis.

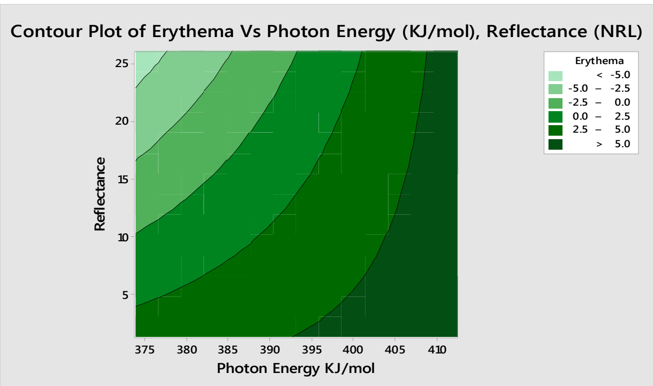

Fig. 6

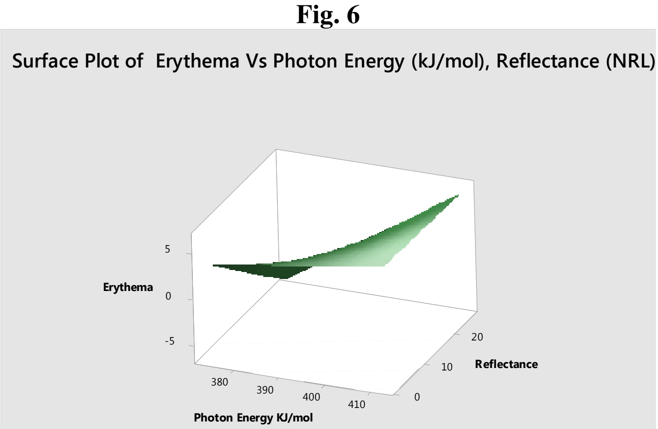

Fig. 7

Figs. 6 & 7 : Represent the counter and surface plot of NRL. This figure establish the erythemal response *vs* incident photon energy of sunlight and reflectance relation *via* through the response surface methodology.

Tested extract with a higher UV-B rays reflectance power had higher SPF index value. In this context, NRF found better and effective one as compared to NRL. A significant SPF value of both flower and leaf extract of *Tagetes* is correspond to 30.16, 12.63 at (P≥0.05) level. Both the significant SPF-12 and SPF-30$^+$ were counted as boundary layer and act as a protective skin barrier against the UV-B radiation. In addition, SPF-12 had found >93% of UV-protection capacity while SPF-30$^+$ blocks efficiently 97% of the total incident UV-radiations. Thus the SPF-12$^+$ and SPF-30$^+$ factor containing sunscreen products could be an effective herbal remedies against the detrimental effect of UV-B light, hence correctly synthesized SPF-12$^+$product serve as a good competent and protective herbal product for the UV sensitive skin (Korac *et al*.,2011). In addition, effective sunscreens products are not only depending on the SPF factor, but it also depend on the quantity and number of times of herbal extract applied on the skin.

Tagetes plant extract with 12$^+$SPF was found an effective and UV-protective compare to SPF30$^+$, if it is used properly. Additionally, the sunscreen products with 12$^+$SPF were safer even for 2-4years age group children and professionals who are directly facing to sunlight viz., fishermen, building workers, street sellers, policeman and lifeguards (Mbanga *et al*., 2014; Kushwaha *et al*., 2021).

In the context of UV finishing properties, a lot of studies have been carried out till date on application of natural extract onto the UV-sensitive skin and protect from harmful effect of solar radiations (Smaoui *et al*., 2017). Otherwise, direct constant exposure of UV-light causes skin problems like Actinic keratosis, (Heurung *et al*., 2014; Jallad, 2017)' solar elastosis (Mancebo *et al*., 2014) and UV-insensitivities.

Genetically, an immune suppression causes alteration in gene pattern due to mutation, a big reason for causing skin cancer in humans. Thus prescribed precaution should be taken, in order to, avoid the serious consequences of genetic mutation in skin caused by the direct exposure of solar radiation (Radice *et al*., 2016).

Recently developed phyto-cosmetic sun screen emulsions notably contain a numerous organic UV filters like polymethyl-methacrylate (PMMA), ethyl-hexyl-methoxycinnamate (EHM) or octinoxate or octyl methoxycinnamate (OMC) (Bom *et al*., 2019) but they all show their negative effects including eczematous dermatitis, burning sensation and increased risk of skin cancer (Lim *et al*., 2018). Therefore, sunscreen manufactures should minimized or avoided the use of these harmful organic compounds, so as to protect the skin from unwanted side effects.

For instances, inorganic blockers like Titanium dioxide (TiO_2) and Zinc oxide (ZnO), carbonates, phosphates usage at the molecular or nano-scale level or their combination ideally improves the SPF index value and has a photostability efficacy of developed sunscreen products (Tomazelli *et al*., 2018). Furthermore, some lesser toxic and biocompatible hybrid materials have been utilized as an active ingredients in cosmetics products and it provides an effective protection grade of UV-B values (Neha *et al*., 2019) as well as improves the skin texture on account of minimizing the reactive oxygen species (ROS) production and prevent it there from negative side effects (Kushwaha *et al*., 2020).

To overcome the toxicity and oxidant effect caused due to constant incidence of UV radiations (Rebaya *et al*., 2015). To date, so many antioxidant compounds have been discovered and are known to extract free radicals from skin tissues exposed to UV light (Nunes *et al*., 2018) *eg.,* Cinnamic acid and rutin. Actually, these compounds by nature are secondary metabolites; as they are formed during phenolics synthesis in plants (Kushwah and Verma, 2017) that has UV-photo-protective property. These metabolites are act as actual precursor of various synthetic derivatives like ethyl-hexyl-methoxycinnamate (EHM) or octyl methoxycinnamate (OMC) (Ghate *et al*., 2016).

However, the presence of nanomaterials (TiO_2 and ZnO) in a sunscreen formulations imparts additional property of cytotoxicity, genotoxicity (Kyu-Bong *et al*., 2017) and potential photo-carcinogenecity to the skin tissues because it does not contain any organic ingredients (Ruszkiewicz *et al*., 2017). Application of organic ingredients enhanced an UV-adsorption property and reduces the health hazardous effect caused due to oxidative stress by free radicals production (Pathak *et al*., 2017). Thus, the ecofriendly wastes of *Tagetes patula* flower petals have multifunctional and valuable phyto-constituents components like lutein and reutein which can usually protects the skin from a complete range of UV wavelength without having a harmful side effects. This study finds that the presence of these phyto-constituents in *Tagetes* plants, which are actually improves the SPF index value, thereby; the *Tagetes patula* extract is a good source of bio-cosmetics product generation.

Conclusion

This study concluded that ample evidence is exist to validate the acute and chronic skin changes caused by solar radiations particularly, UV-B radiation, and benefited the sunscreens affords in the reductions in and even prevention of such physiological changes. Furthermore, the photoprotective efficacy is determined through Sun protection factor (SPF) and against the protection grade of ultraviolet radiations (UV-R). The SPF represents the effectiveness of a sunscreen formulation against the solar radiations.

The *Tagetes* extract reflects the UV-B radiation. Thereby; *Tagetes patula* extract is consider as better alternative option for developing a natural cosmetic with no side effects as compared to synthetic cosmetic with respect to skin protection from UV radiation. For this purpose, the employed method of study is simple, fast, inexpensive and easy-to-use.

UV-light reflection was well analyzed with the help of response surface methodology and found that the NRF and NRL *Tagetes patula* extracts had significantly showed the fruitful results.

The NRF extract has minimum erythemal response as compared to NRL extract. In addition, RSM is carried out to correlate the relation between the photon energy and reflectance and the erythemal response and photon energy. In this context, the photon energy-reflectance relation show negative effect and erythemal response and photon energy relation revealed the positive effect which reflects the UV-light incident to a greater extent causing a more detrimental effect to the skin tissues. Particularly, recently developed herbal sunscreens also possess the outstanding capacity to address the demand of consumers to protect the skin from harmful UV-light and discovering such novel components and provoke to use lighter ointments are expected to continue in the future.

Conflict of Interest

All the authors of the study declare no conflict of interest.

Author's Contribution

This research work was carried out in collaboration between all authors, read and approved the final manuscript. Dr. Deepshikha planned the study design and elaborates the main concept. Prof. Yashodhara as mentor, suggested a valuable comment and principally responsible for writing of the manuscript and Dr. Prashant suggest and helped to elucidate the research data.

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