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A Randomized Study to Determine the Sun Protection Factor of Natural Pterostilbene from *Pterocarpus Marsupium*

Muhammed Majeed [1,2,3,4], Shaheen Majeed [1,2,3,4], Renuka Jain [1,4,*], Lakshmi Mundkur [1], H. R. Rajalakshmi [4], Prachi Lad [4] and Prakriti Neupane [4]

1 Sami Labs Limited, 19/1 and 19/2, First Main, Second Phase, Peenya Industrial Area, Bangalore, Karnataka 560058, India; mail1@samilabs.com (M.M.); shaheen@sabinsa.com (S.M.); lakshmi@samilabs.com (L.M.)
2 Sabinsa Corporation, 20 Lake Dr, East Windsor, NJ 08520, USA
3 Sabinsa Corporation, 750 Innovation Cir, Payson, UT 84651, USA
4 ClinWorld Private Limited, 19/1 and 19/2, First Main, Second Phase, Peenya Industrial Area, Bengaluru, Karnataka 560058, India; rajalakshmi@clinworld.org (H.R.R.); prachi@clinworld.org (P.L.); prakriti@clinworld.org (P.N.)
* Correspondence: renuka@samilabs.com

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Abstract: Ultraviolet (UV) rays and its harmful effects have always been a concern to skin health. Sunscreen and sunblock prevent the harmful effects of UV radiation on the skin. Sun Protection Factor (SPF) is an indication of the sun-protective capacity of an ingredient. There is an ever increasing interest in the cosmetic industry for developing novel functional ingredients from natural sources. The purpose of this study was to determine in-vitro and in vivo SPF of natural 90% pterostilbene extracted from the dried heartwood of *Pterocarpus marsupium* (Indian Kino). The SPF of purified pterostilbene and a formulation containing 0.4% pterostilbene was determined In Vitro using a UV spectrophotometer. Pterostilbene had an SPF of 21.73 ± 0.06, while the cream formulation had an SPF of 8.84 ± 0.01. The in vivo SPF of the 0.4% pterostilbene cream in humans was found to be 6.2 ± 1.30. Primary skin irritation tests in human subjects showed the formulation was safe and had no irritation potential. Pterostilbene was also found to have significant antioxidant activity as determined by free radical scavenging assays in vitro. These results suggest that natural pterostilbene is an antioxidant and shows SPF value both in-vitro and in the human clinical study and thus could be used as an ingredient in topical sun-protective formulations.

Keywords: Pterostilbene; PteroWhite®; sun protection factor; SPF; sunscreen application; sun UV protection; in vitro; in vivo; ISO 24444 method; minimal erythema dose

1. Introduction

The rise in solar ultraviolet (UV) radiations entering the earth's surface has resulted in a dramatic increase in skin-related disorders in humans in the last few decades [1,2]. Sunlight is predominantly composed of 40% visible light, 50% infrared radiations, and 10% ultraviolet (UV), of which 9.5% is Ultraviolet-AUVA and 0.5% Ultraviolet-B UVB radiation. The UV radiation consists of distinct regions depending on the wavelengths (i.e., UVA between the wavelengths 320 to 400 nm, UVB between the wavelengths of 280 to 320 nm, and UVC with shorter wavelengths from 200 to 280 nm) [3]. UVA penetrates deep into the dermis layer and generates reactive oxygen species, inducing photoaging and skin damage [4,5]. Although UVB is a minor constituent of UV radiation, it is known as "burning rays" as it has a thousand times higher capacity to burn skin and causes adverse effects on the epidermal layer [6,7], whereas UVC gets filtered off by the atmosphere [8].

Repeated exposure to the sun results in inflammation or reddening of the skin, also known as erythema or sunburn. Erythema is followed by the activation of melanocytes, which results in the skin being tanned [8]. Long term effects of sunburn can result in irreversible loss of skin elasticity, premature aging, dark spots, and cancer. Ultraviolet radiation is considered an environmental human carcinogen as it has tumor initiator and promoter properties [9,10].

The skin has natural mechanism of photo protection by producing melanin from melanocytes. The photoprotective role of melanin supported by epidemiological data shows an inverse correlation between skin pigments and the incidence of cancer. Melanin is a physical barrier that scatters the UV radiation and also acts as an absorbent filter that reduces the penetration of the radiation through the epidermis [11,12].

Sunscreens—tropical formulations to protect skin from the harmful UV rays—have shown beneficial effects in reducing the symptoms associated with sun exposure. Physical sunscreen reflects radiation, while chemical sunscreens have different mechanisms of action. Inorganic sunscreens like metal oxides protect the skin by reflecting and scattering UV radiation, while organic sunscreens like p-aminobenzoic acid (PABA) and cinnamates absorb the radiation [13]. The active ingredients in sunscreens are known to have side effects, including disruption of the endocrine system and alteration of the hypothalamic-pituitary-thyroid (HPT) axis, and can also affect reproductive homeostasis and development parameters when used long-term [14,15]. Some sunscreens may have ecotoxicological effects and can have an impact on the ecosystem [16,17].

An ideal sunscreen should be safe, inert, non-toxic, photostable, and should be able to protect the skin from both UVA and UVB radiations [18]. Some of the natural photo protectants include aloe vera, pomegranate, tomato, green tea extracts, as well as the oils from soybean, olive, coconut, almond, and jojoba [19] and other natural protectants include the mycosporine-like amino acids (MAA) palythine, porphyra-334, shinorine, tanshinone, and licochalcone [20–24]. The most important biological activities in protection against sun include filtering the UVA and UVB radiations, antioxidant activity, anti-mutagenic activity, and anti-inflammatory activity [25].

Pterostilbene (Figure 1A), a stable structural analog of resveratrol, is an antioxidant that has been extensively studied for its benefits in treating several diseases [26]. PteroWhite® is a patented natural 90% pterostilbene, extracted from the dried heartwood of *Pterocarpus marsupium*. Pterostilbene (3,5-dimethoxy-4′-hydroxy-trans-stilbene) was initially isolated from the heartwood of red sandalwood (*Pterocarpus santalinus*), and its presence in grapevines and blueberries was reported later [27–33]. It is a stilbenoid of the phytoalexins group which is produced by plants in response to stress [33]. It has also shown its potential UVB protection activity through the expression of antioxidant enzymes in UVB induced oxidative stress [33].

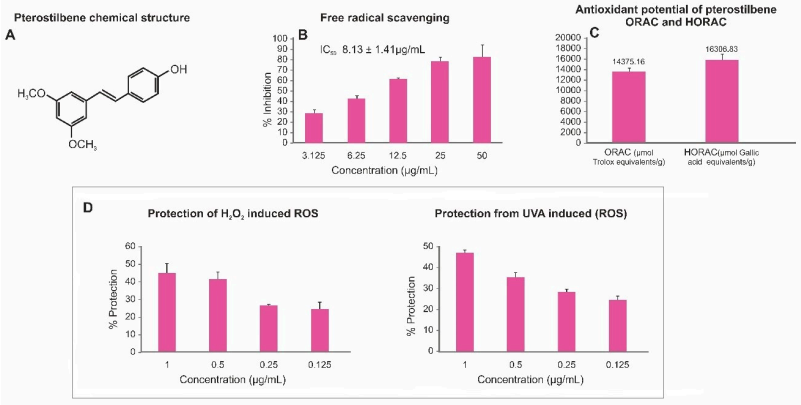

Figure 1. Anti-oxidant activity of pterostilbene. (**A**) Pterostilbene chemical structure; (**B**) free radical scavenging; (**C**) antioxidant potential of pterostilbene Oxygen Radical Absorption Capacity Assay ORAC and Hydroxyl Radical Antioxidant Capacity Assay HORAC; (**D**) protection from reactive oxygen species (ROS).

The sun protection factor (SPF) is the most critical data to quantify the effectiveness of sunscreens, which is universally accepted [34]. The present study aimed to determine the SPF of the 90% natural pterostilbene as an ingredient and in a cream formulation In Vitro and in vivo in human volunteers.

2. Material and Methods

2.1. Test Material

Pterostilbene was extracted and purified from the Indian Kino tree (*Pterocarpus marsupium)* [32]. The extract is standardized to 90% pterostilbene under the trade name PteroWhite®. The other 10% constituents comprise of monomethyl resveratrol, trimethyl resveratrol, polyphenols, tannins coloring matters, ash, and moisture.

The cosmetic cream formulation (CS/118/ML163A) contained 0.4% of natural 90% pterostilbene extract along with neutral SPF base having disodium Ethylenediaminetetraacetic acid (EDTA), cetostearyl alcohol, softemul165, caprylic capric triglyceride, butylated hydroxytoluene, propylene glycol, Xiameter PMX 3031, dow corning 556, sabilize new (thyme oil, monolaurin, magnolol) and amp95 (provided by Sami Labs, Bangalore, India).

2.2. DPPH (2,2-diphenyl-1-picrylhydrazyl) Free Radical Scavenging Activity

Pterostilbene was dissolved in DMSO and diluted in 50% methanol for the assay. Different concentrations of pterostilbene were added to DPPH solution in methanol, and the absorbance was measured at 540 nm using a microplate reader (TECAN Ltd., Männedorf, Switzerland) following 15 min incubation in the dark [35]. The free radical scavenging activity was expressed as a percent of scavenging activity.

2.3. Oxygen Radical Absorption Capacity (ORAC)Assay

The oxygen radical absorption capacity assay (ORAC) for pterostilbene was assessed as per the method described earlier [36]. Different concentrations of the standard (Trolox; T_5 to T_1) or pterostilbene samples (S_3 to S_1), APPH (2, 2′-Azobis (2-amidinopropane dihydrochloride) and disodium fluorescein dye were added to a 96-well dark plate. Fluorescence reading was recorded after every 1 min for 35 min at 485/520 nm (Fluostar Optima Microplate Reader). The final ORAC values were expressed as micromoles of trolox equivalents per liter or per gram of sample (μmol TE/g or μmol TE/L).

2.4. Hydroxyl Radical Antioxidant Capacity (HORAC) Assay

Hydroxyl radical averting capacity was assessed using fluorescein (FL) as the fluorescent probe, as described earlier [37]. The hydroxyl radical was generated by a Co (II)-mediated reaction and the experiment was carried out as described earlier for ORAC. The results were expressed as gallic acid equivalents per gram of test compound.

2.5. Reactive Oxygen Species Scavenging Activity (ROS)

ROS was estimated using dichloro-dihydro-fluorescein diacetate (DCFH-DA) dye. [38] Human dermal fibroblast cells (50,000 cells per well) were pretreated with different concentrations of 90% pterostilbene for one hour, followed by exposure to hydrogen peroxide (125 μM) or UVA radiation (0.6 J/cm^2) for 1 h. Freshly prepared DCFH-DA reagent was added to all the wells (2 μg/well). The plate was incubated at 37 °C for 30 min and fluorescence was recorded at an excitation of 485 nm and emission of 520 nm wavelength using a fluorescence plate reader.

2.6. In Vitro Evaluation of the Sun Protection Factor by UV Spectrophotometry

The SPF was determined In Vitro by spectrophotometer, as described by Yang et al. [34]. Pterostilbene was dissolved at a concentration of 2 mg/mL in ethanol (0.2%) followed by ultrasonication for 5 min and then filtered. A 1 mL aliquot was transferred to a 10 mL volumetric flask and diluted to

volume with ethanol (0.02%). The absorption spectra of the samples were recorded in the range of 290 to 320 nm for every 5 nm using 1 cm quartz cell and ethanol as a blank. A similar procedure was followed for the cream formulation having 0.4% of natural 90% pterostilbene. Three determinations were made at each point, followed by the application of the Mansur equation [39].

$$\text{SPF} = \text{CF} \times \sum \text{EE}(\lambda \times \text{I}(\lambda) \times \text{ABS}\ (\lambda),$$

where: EE (λ) is erythemal effect spectrum; I (λ) is solar intensity spectrum; ABS (λ) is absorbance of Test material; CF is correction factor (–10). As described earlier [40], the values of the constants are shown in Table 1.

Table 1. SPF determination of 90% pterostilbene.

Wavelength λ (nm)	Absorbance (λ)	EE (λ) × I (λ) (Constant)	EE (λ) × I (λ) × ABS (λ)
290	2.047 ± 0.006	0.015	0.030 ± 0.0001
295	2.175 ± 0.012	0.0817	0.177 ± 0.0009
300	2.192 ± 0.009	0.2874	0.629 ± 0.002
305	2.211 ± 0.010	0.3278	0.724 ± 0.003
310	2.126± 0.008	0.1864	0.396 ± 0.001
315	2.096 ± 0.007	0.0839	0.175 ± 0.0006
320	2.138 ± 0.021	0.018	0.038 ± 0.0003
∑ EE(λ) × I (λ) × ABS (λ)		2.173 ± 0.006	
SPF = CF × ∑ EE(λ) × I (λ) × ABS (λ)		21.73 ± 0.06	

EE (λ) = erythemal effect spectrum; I (λ) = solar intensity spectrum; ABS (λ) = absorbance of Test material; CF = correction factor (−10). SPF was determined in three individual experiments in duplicates. The values were found to be 21.80, 21.68, and 21.72. The average SPF value ± standard deviation.

2.7. Ethics

The primary skin irritation potential by a patch test and in vivo SPF of cream formulation of pterostilbene were evaluated at MS Clinical Research Private Limited, Bangalore, India, by following good clinical practice guidelines and were approved by the local ethics committee (Clinicom Ethics Committee). The primary skin irritation study was conducted as per (BIS 4011:2018) guidelines [41] and the SPF study was conducted as per the ISO 24444, cosmetic sun protection factor determination method [42]. The participants in both the studies signed the written consent agreement before enrollment.

2.8. Primary Skin Irritation Test

The primary skin irritation study was conducted in a total of 25 healthy male and female subjects of all skin types (normal, dry, oily, and combination) in the age group of 18 to 55 years. Subjects with Fitzpatrick skin type III to V who were willing to maintain the patch test in position for 24 h, who had not participated in a similar investigation in the past two weeks, who were willing to come for regular follow up visits and were ready to follow instructions during the study period were included in the study. Subjects with infection, skin allergy, corticosteroid treatment, history of excessive sweating, cutaneous disease, a history of underlying medical illness or alcoholism were excluded.

An occlusive chamber capable of holding 40 µL of the test sample was used for the test. The clinical results were assessed using Draize scale scoring. A dermatologist examined the test site for baseline skin condition before the application of the test products. Sodium lauryl sulphate 1% (w/w) was used as a positive control and pterostilbene cream (CS/1118/ML163A) was the test sample. The test materials were pipetted out on the precut filter paper placed inside the allotted Dermaproof® aluminum Finn

chamber prefixed to a Scanpore Tape. Patches of the test formulation were applied onto designated test sites on the back of the subjects between scapula and waist, starting from the bottom of the patch pressing upwards to squeeze out the air. The subjects were instructed to retain the patch in the same position until it was removed after 24 h. The test sites were wiped with a clean tissue to remove any residue before evaluation. Subjects were acclimatized at room temperature for 20–30 min (18 °C to 25 °C, 50% ± 10% relative humidity) before undergoing examination by a dermatologist. The dermatologist visually assessed the skin condition of each test site 20 to 30 min post-patch removal.

The primary skin irritation potential of pterostilbene cream was evaluated using the test method described in IS 4011:2018 methods of test for safety evaluation of cosmetics, third revision (ICS 71.100.40). Positive control as per clause 4.3.1.2.4 and the irritation scoring system is as per clause 4.3.1.3, 4.3.2.6 on the Draize scale for scoring treatment sites. The trial was registered prospectively at clinical trial registry of India with registration number CTRI/2019/01/01/017283.

2.9. In vivo Determination of Sun Protection Factor (SPF)

In the SPF determination study subjects in the age group 18–55 years (both ages inclusive) were included. Participants met the following criteria: Fitzpatrick skin type III and IV; discontinued the use of soaps and cosmetic products in the treatment areas; avoided any exposure of the test area to artificial or natural UV light throughout the study; had not participated in a similar investigation in the past two weeks; came for regular follow up visits; were ready to follow instructions during the study period; had no known abnormal response to sunlight. The exclusion criteria were the same as described for the safety (PIPT) study.

The SPF test method is a laboratory method (ISO 24444:2010) that utilizes a solar simulator of defined and known output to determine the protection provided by sunscreen products on human skin. Bayer's P3 with a standard of a theoretical value of SPF 15 was used. The source of UV radiation was a 16s solar simulator with a dose controller (Model 16S-300-001 V4.0 S/N:24561, Solar Light Company, Inc.Glenside, PA, USA). The light source employed was a small beam 150 W Xenon Arc Solar Simulator having a continuous emission spectrum in the UV range from 290 to 400 nm and compliant with the spectral performance requirements.

On day 1, after subjects were selected, sites were identified on the subject's upper back as being untreated, test areas, and standard areas as shown in Figure 2.

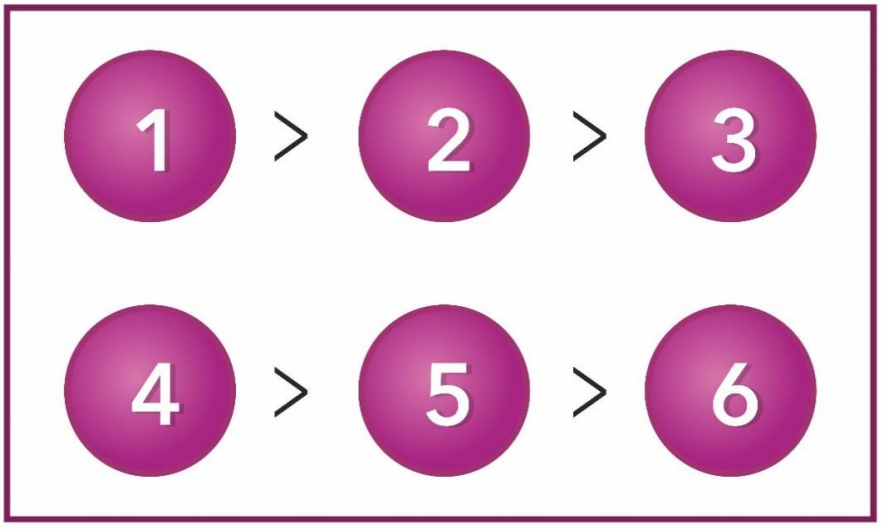

Figure 2. The pattern of UV exposure.

The test site free of pigmentation, pimples, hair, moles, or any dermatological conditions that could interfere with the reading were located below the scapulae and above the waist region of the subjects (upper back). The area of each was 35 cm^2 (7 × 5 cm). The test product, untreated, and

standard were randomized to the site allocation on the subject's back. Post Minimal Erythema Dose MED) μ determination, 2 mg/cm^2 of the test produc, and reference sunscreen formulation were applied to the respective randomized test area. The product was dispensed within the test site using a syringe then spread over the whole test site using light pressure. Exposure of the test sites to a sequence of incremental UV doses started 10 min to 11 min after the application of the product(s). The test and control sites were irradiated with a UV dose as per the individual's MED response and the product's expected SPF value.

Incremental series of delayed erythema responses were induced on five subsites within the test area to determine the sun protection factor. The expected response was maintained as the third dose in the incremental series. These responses were visually assessed for the presence of erythema 16 h to 24 h after UV radiation by the investigator.

2.10. Minimal Erythema Dose (MED) Determination

The minimum dose that produces mild but definite erythema with a clearly defined border when evaluated at 24 h post-exposure was considered as MED. The UV exposure given to the subjects was in the range of 1–10 MED. Six irradiation sites at a 25% incremental dose between two sites were used for the untreated test area, while for the protected test area there were five irradiation sites at 25% incremental doses between two sites, as shown in Figure 3. The MED for all sites was assessed when the erythemal response was optimal (i.e., 22 h (± 2 h) after UV exposure).

2.11. Mean SPF Calculation

The efficacy of a sunscreen compound, expressed by the sun protection factor (SPF), is defined as the energy from UV required producing a minimal erythema dose (MED) on protected skin, divided by the UV energy required to produce a MED on unprotected skin (Equation (1)).

$$SPF = \frac{Minimal\ erythema\ dose\ in\ protected\ skin}{Minimal\ erythema\ dose\ in\ unprotected\ skin} \quad (1)$$

2.12. Statistical Analysis

The arithmetic mean and standard deviation of the total observation was taken for both In Vitro and in vivo SPF determination. All statistical tests used a significance level of $\alpha \leq 0.05$. Two-tailed T-tests were performed for all statistical analyses. All p-values were rounded to four decimal places.

3. Results

3.1. Antioxidant Activity of Pterostilbene

Pterostilbene showed a dose-dependent increase in the free radical scavenging activity and showed 50% (IC_{50}) of free radical scavenging at a concentration of 8.13 μg/mL. (Figure 1B). The oxygen radical absorbing capacity (ORAC) value was found to be 14375.16 μmol trolox equivalent/gram of pterostilbene. While the hydroxy radical absorbing capacity (HORAC) value was found to be 16806.83 μmol gallic acid equivalents/g for pterostilbene (Figure 1C).

3.2. Reactive Oxygen Species Scavenging Capacity of Pterostilbene in Vitro

Reactive oxygen species (ROS) generated due to exogenous and endogenous sources are highly unstable and react with cellular molecules causing cell damage. Pterostilbene showed a dose-dependent increment in the percentage of protection of up to 44.64% ± 5.4% against ROS (Figure 1D). The compound could reduce ROS generated by both UVA radiations and exposure to hydrogen peroxide in vitro, suggesting its potential as a UV protectant (Figure 1D).

3.3. In Vitro SPF of Pterostilbene Cream Formulation of 0.4%Pterostilbene

During the product development process, the In Vitro SPF determination method acts as a screening test and reduces the number of in vivo experiments [43,44]. Pterostilbene as an ingredient had an SPF of 21.73 ± 0.06 in the in-vitro analysis, whereas when formulated in a neutral base pterostilbene had an SPF of 8.84 ± 0.005 (Tables 1 and 2). The base formulation showed negligible activity, suggesting that the SPF value can be attributed to the 0.4% pterostilbene.

During the product development process, the In Vitro SPF determination method acts as a screening test and reduces the number of in vivo experiments [43,44]. Pterostilbene as an ingredient had an SPF of 21.73 ± 0.06 in the in-vitro analysis. The cream formulation containing 0.4% Pterostilbene had an SPF of 8.84 ± 0.005. (Tables 1 and 2). The base formulation showed negligible activity, suggesting that the SPF value can be attributed to the 0.4% pterostilbene.

Table 2. SPF determination of pterostilbene cream formulation in comparison to the base formulation.

Wavelength λ (nm)	**Cream Base**		**0.4% of 90% Pterostilbene Cream Formulation**	
	Absorbance (λ)	**EE(λ) × I (λ) × ABS (λ)**	**Absorbance (λ)**	**EE(λ) × I (λ) × ABS (λ)**
290	0.04 ± 0.005	0.0006	0.744 ± 0.002	0.011 ± 0.005
295	0.011 ± 0.001	0.0009	0.816 ± 0.003	0.067 ± 0.036
300	0.005 ± 0.0001	0.0014	0.873 ± 0.001	0.251 ± 0.144
305	0.002 ± 0.0001	0.0006	0.913 ± 0.002	0.299 ± 0.172
310	0.001 ± 0.0001	0.00018	0.891 ± 0.002	0.166 ± 0.095
315	0.001 ± 0.0001	0.000083	0.886 ± 0.002	0.074 ± 0.042
320	0.002 ± 0.0005	0.000036	0.892 ± 0.002	0.016 ± 0.008
∑ EE(λ) × I (λ) × ABS (λ)	0.003765 ± 0.0001		0.884 ± 0.0005	
SPF = CF × ∑ EE(λ) × I (λ) × ABS (λ)	0.037651 ± 0.001		8.84364 ± 0.0005	

SPF was determined in three individual experiments in duplicates. The values were found to be 8.846, 8.845, and 8.843. The average SPF value ± standard deviation.

3.4. In Vivo Primary Skin Irritation Test of Pterostilbene Cream Formulation

In the primary irritation patch study, 24 out of 25 subjects were enrolled and completed the study. There were no adverse events recorded during the study. One subject was recorded as a loss to follow up after the screening visit. The mean skin irritation score for each tested formulation is shown in Table 3. As per the Draize scale for scoring irritation, the investigational product emerged as a non-irritant product when observed at 0 h, 24 h, and 7 days post patch removal. Positive control (sodium lauryl sulfate) was confirmed to be true to its nature in the study population. The residual reaction was observed in a few subjects at day 7 post patch removal. These subjects were contacted by phone, and the resolution of the reaction was confirmed, which further verifies that pterostilbene cream is safe for topical use.

Table 3. Primary skin irritation assessments.

Investigational Products	Mean Irritation Score-0 Hrs	Irritancy Assessment	Mean Irritation Score-24 Hrs	Irritancy Assessment	Mean Irritation Score-7 Days	Irritancy Assessment
Pterostilbene cream (CS/1118/ML163A)	0.08	Non-Irritant	0	Non-Irritant	0	Non-Irritant
Positive control SLS (sodium lauryl sulphate)	2.29	Irritant	2.33	Irritant	0.5	Non-irritant

3.5. In Vivo Determination of Sun Protection Factor (SPF) of Pterostilbene Cream Formulation

A total of 12 subjects were screened and enrolled, ten subjects had the desired Fitzpatrick skin type III and two with skin type IV. 10 subjects completed the study and two subjects were lost to follow up. There were no adverse effects recorded. The subjects showing SPF value of standard as 15% ± 17% were considered as a valid subject for data consideration, and all the 10 subjects were found to be valid. The mean in vivo SPF of the pterostilbene cream was found to be 6.20 ± 1.30 as shown in Table 4, and individual SPF values ranged from 4.52 to 8.84 from the 10 valid subjects (Figure 3).

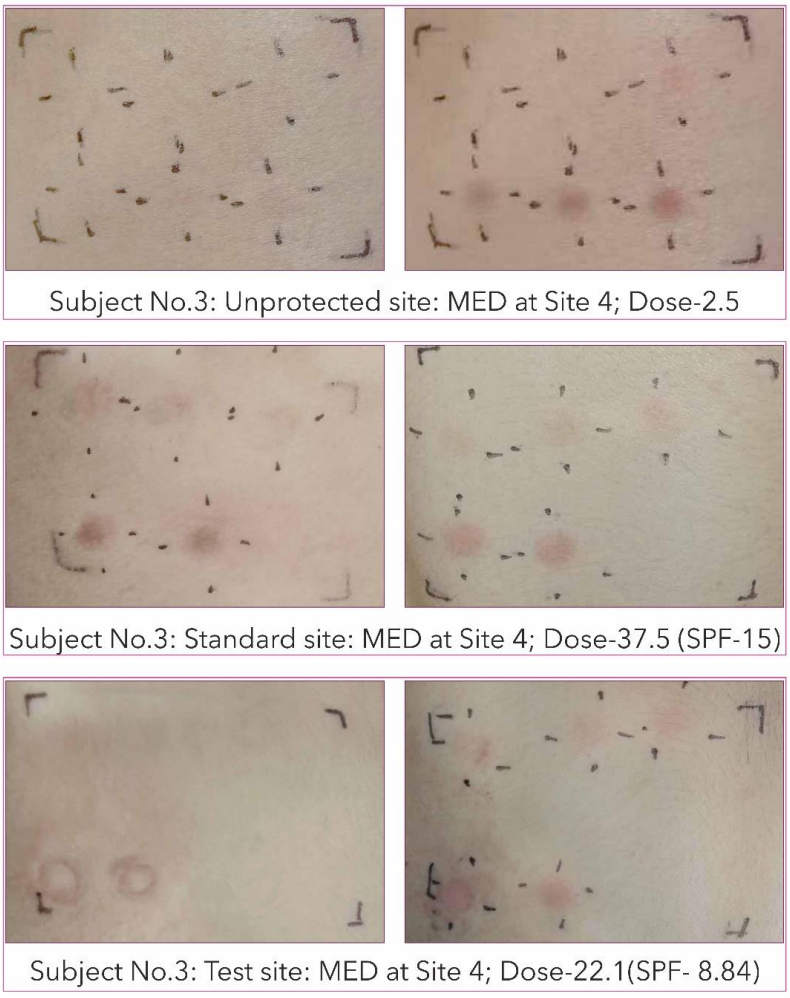

Figure 3. Sample Macro photography of the in vivo SPF determination of pterostilbene.

Table 4. Sun protection factor of pterostilbene cream.

Parameter	Standard Site (Bayer's P3)	Test Product Site Pterostilbene Cream
Mean SPF **(n = 10)**	15.10	6.20
SD	0.81	1.30

4. Discussion

One of the most critical factors affecting skin physiology is the exposure to solar radiation, specifically UV radiation [44]. Sunlight is a mixture of different UV radiations, with each component exerting a distinct effect on the skin [45]. UVB is a potent activator of inflammation and is highly mutagenic and can cause DNA dimers. At the same time, UVA induces oxidative free radical damage to DNA and other macromolecules in the cells [46]. Overexposure to UV radiation causes skin inflammation, oxidative stress, ROS-mediated DNA damage, dysregulation of cellular signaling pathways, and immunosuppression [47]. The primary health risks associated with these changes include aging, cataracts, non-melanoma, melanoma cancer, and immune system damage [48]. The sun protection factor (SPF) is the measure to determine the efficacy of a UV protectant. It indicates the ratio of time needed to produce sunburn on sunscreen-protected skin to the time required to cause sunburn on unprotected skin [31,49]. Pterostilbene is an antioxidant, anti-inflammatory, and anti-carcinogenic agent derived from *Pterocarpus marsupium* and is structurally similar to resveratrol [26,31].

Our result suggests that natural pterostilbene has the potential to be used as an ingredient in sunscreen formulations. UV radiations interact with biological molecules, and one of the significant adverse impacts is the induction of free radicals. The UV protective effect of natural pterostilbene could be attributed to its antioxidant effects. The antioxidant activity is an essential factor in using natural ingredients in sunscreen, as they may additionally offer photoprotective activity along with the sunscreen effect. Increasing concentrations of pterostilbene showed significant inhibition in the free radical scavenging activity from 28–81%. The compound showed radical oxygen absorbance as well as hydroxyl radical absorbing capacity, thus confirming its antioxidant potential in vitro. Skin cells are constantly exposed to reactive oxygen species (ROS) and oxidative stress from exogenous and endogenous sources.

The different types of ROS (i.e., superoxide anion, hydroxyl radical (OH), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$)), are produced during normal physiological conditions due to the partial reduction of molecular oxygen. At low concentrations, ROS is essential for regulating cellular signaling pathways related to cell proliferation and survival [50]. However, ROS is unstable and reacts with other molecules in the cell, causing cellular damage. ROS can damage other cellular molecules and cell structures. Among the most important of these are the actions of free radicals on the fatty acid side chains in cell membranes, especially mitochondrial membranes. Intracellular and extracellular oxidative stress initiated by reactive oxygen species (ROS) induces accelerated skin aging which is characterized by wrinkles and atypical pigmentation [51]. UV induced ROS further interferes with the cell signaling pathways resulting in the breakdown of collagen and reduction in collagen synthesis, leading to wrinkles and sagging of skin [52]. Photoaging is an accelerated aging process induced by sun exposure in skin cells [53–55]. We observed that pterostilbene could reduce the ROS production triggered by hydrogen peroxide as well as UVA radiations in skin fibroblast cells and protect the cells against oxidative damage and photoaging. In our recent clinical study, we report a significant skin brightening and antiaging of pterostilbene in healthy individuals [56].

The In Vitro screening methods offer a high throughput screening assay and reduces the risk related to UV exposure of human subjects [57]. The In Vitro SPF can be measured either by the measurement of absorption or the transmission of UV radiation through sunscreen product films in quartz plates or bio-membranes or by using spectrophotometric analysis of dilute solutions [39,58,59]. The Cosmetics Europe and other standards recommend the SPF determination percent transmission of

a sunscreen lotion sample across the UV spectrum weighted by the erythemal weighting factors at different wavelengths [60]. The SPF calculated by the spectrophotometric method is affected by the solvent used for analysis, type of emulsion, emulsifiers used in the formulation, pH, and other active ingredients, all of which can change the UV absorption of each sunscreen [60].

In the current study, pterostilbene showed an SPF value of 21.73 at 0.02% by the spectrophotometric method. The formulation containing 0.4% of 90% pterostilbene showed an SPF value of 8.84 by In Vitro method and that of 6.2 by in vivo method in human subjects. While this is a low value as per the COLIPA standards, pterostilbene can be used in combination with other ingredients to increase the SPF value and reduce the amount of inorganic chemicals used in sunscreen products. However, the blend with other filters should also be tested for its phototoxicity.

Despite several reports, there are no officially approved natural sun protectants. Earlier reports on SPF of herbal ingredients are on the lower scale [60–62]. Systematic studies evaluating the SPF by In Vitro and in vivo methods for a single herbal ingredient are also very minimal. Thus, pterostilbene with its photoprotective activities along with the SPF value could be highly beneficial in skincare formulations. The patch test observation done using primary skin irritation test showed no adverse events in the subjects, suggesting its potential for safety for use in skin protection creams.

Natural sunscreen is gaining attention in the last decade due to its safety, pleiotropic biological actions, and cost-effectiveness. The bioactive phytoproducts possess a broad spectrum of UV absorption, which cause a protective effect against oxidative stress, inflammation, and cancer, making them highly suitable ingredients for sunscreen formulations. In a recent study, pterostilbene was reported to have anticancer activity associated with the maintenance of skin antioxidant defenses and inhibition of UVB-induced oxidative damage [63]. Pterostilbene was reported to protect against UV-induced photodamage by increasing endogenous defense mechanisms, scavenging UVB-induced ROS, and aiding in DNA repair mechanisms through a PI3K-dependent activation of Nrf2/ARE pathway [64,65]. Our results suggest that pterostilbene, along with other natural ingredients, can be formulated as a natural sunscreen with significant UV protectant activity.

5. Conclusions

UV radiation causes skin damage and there is an urgent need to protect from its harmful effects. Natural ingredients usually have the potential to protect against the toxic effects of UV radiation. Natural pterostilbene has shown its potential as a safe and effective ingredient in topical formulations for sunscreens and UV protection. The product may be used in combination with other ingredients to boost the SPF value of sunscreen formulations. Further research is required in the development of new efficacious and safe plant-based natural ingredients and formulations which are effective in protecting skin from harmful radiations.

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