Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Issue 4, 2011

Research Article

FORMULATION AND IN VITRO EVALUATION FOR SUN PROTECTION FACTOR OF MORINGA OLEIFERA LAM (FAMILY-MORINGACEAE) OIL SUNSCREEN CREAM

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Received: 7 Aug 2011, Revised and Accepted: 15 Sep 2011

ABSTRACT

The present study was designed to study the sunscreen activity of herbal formulation. There is no evidence regarding the sun protection factor studies on seed oil of *Moringa oleifera L*, (Moringaceae). This study investigates its in vitro sun protection factor (SPF) by COLIPA method of oil in a cream formulation. The sun protection factor was analyzed by using Optometric LLC, SPF- 290S is a recording ultraviolet – visible (UVVIS) spectrophotometer using samples exposed to Xenon arc lamp. The sun protection factor of Moringa fixed oil cream exhibited less activity (SPF= 2.04±0.02) suggesting it can be used to maintain the natural pigmentation of the skin or can be used as an adjuvant in other formulations to enhance the activity. SPF of formulation was found 1.46 with ultra boot star rating 1 which approaches toward sunscreen activity.

Keywords: *Moringa oleifera* (Moringaceae), SPF, Sunscreen activity.

INTRODUCTION

The skin is the most superficial part of the body. The signs of ageing are most visible in the skin. Although, ageing skin is not a threat to a person, it can have a detrimental effect on the psychology of a person. A look into the causes of skin ageing, the available treatments and preventive measures for this inevitable change is important to help both the already aged, as well as, the youth. The sun protection factor of a sunscreen is a laboratory measure of the effectiveness of sunscreen — the higher the SPF, the more protection a sunscreen offers against UV-B (the ultraviolet radiation that causes sunburn)[1-3] .The SPF is the amount of UV radiation required to cause sunburn on skin with the sunscreen on, relative to the amount required without the sunscreen[4].

As a barrier and immunological organ in the human, the skin especially epidermis, is particularly subjected to external effects. Light is the major environmental component to which skin is exposed daily and this light comprises of UV radiations which have been reported for damaging effects to the skin[5]. There are three types of UV rays; UV-A (320-400 nm), UV-B (280-320 nm), UV-C (200-280 nm) [6]. Exposure to UV-A radiation results in damage to the elastic and collagen fibers of connective tissue of skin, which causes premature ageing (photo-ageing), while UV-B radiation bring about acute inflammation (sun burn) and intensification of photo-ageing[7]. In addition to these changes, UV-B radiations are also reported to induce immune-suppression which reduces normal immunological defense mechanisms of the skin, therefore chances of development of malignant tumor increases [8-10]. The application of sunscreens is an efficient method of protecting skin against UV radiations. Thus, it has become a necessity to develop a validated topical sunscreen product which will provide protection against both UV-radiations and hence, topical formulations like sunscreen cream, lotion, spray, gel are prepared.

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[11]. 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[12].

Regulatory agencies like the US-FDA and COLIPA (*The Comité de Liaison de la Parfumerie in Europe*) has made *in vivo* testing on human volunteers using an erythemal endpoint to determine the SPF of topical cream mandatory[13]. 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[14].

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 [5], 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[12].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 [13]. 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[14]. 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[15]. Therefore, the researchers have turned their attention towards developing herbal sunscreen agents which are effective with less or no side effects.

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[16]. 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[17].

The Moringaceae is a single-genus family with 14 known species of these, *M. oleifera* Lam. (syn. *M. pterygosperma* Gaertn.) is the most widely known and utilized species[18, 19]. It is a small or medium-sized tree, about 10 m high, cultivated throughout India. *M. oleifera* is referred to as the "drumstick tree" or the "horseradish tree," whereas in others it is known as the "kelor tree" [20]. In literature seed extract of *Moringa* species reported to have anti-inflammatory, purgative, tonic [21-24], analgesic [23], potential antitumor [25], anti-fungal [26],antispasmodic, anti-inflammatory and diuretic activity[27]. The % composition of mixed fatty acids in seed oil as Palmitic- 11.04, Stearic- 3.58, Arachidic- 3.44, Behenic -7.09, Palmitoleic- 2.38, Linoleic- 1.83 % respectively 17,18. Characteristics of seed oil Density (mg mL-1) 240 C- 0.8660, Refractive Index at 400 C- 1.4648, Saponification Value-179, Iodine Value (g of I/100 g of oil)-67.00, Unsaponifiable Matters 0.78[28,29].

The study was designed with an objective to determine the *In vitro* Sun Protection Factor (SPF). In present study we have made an attempt to formulate the topical cream from seed oil of *Moringa oleifera L* and evaluation of the same.

MATERIAL AND METHODS

Plant Material

The plant specimens for the proposed study were collected from in-house garden in Nashik; district Nashik, Maharashtra, India. The specimens were identified and authenticated by Botanical Survey of India, Pune, Maharashtra (Ref. BSI/ WRC/ TECH/ 2011). The herbarium of the plant was deposited in the BSI against voucher no. MEGA 03. The seed were cleaned, dried under direct sunlight and powdered by a mechanical grinder.

Extraction of dried seeds of *Moringa oleifera L* (Family-Moringaceae)

Air-dried seeds of *M.oleifera L* (Fam. Moringaceae) was separately powdered and extracted with 50 volumes of petroleum ether (60-80°C) using a Soxhlet apparatus. This process of extraction was repeated for 6h, the petroleum ether distilled out by distillation assembly, then concentrated by hot plate drying and air-drying at temperature of 40±2 °C.

Sunscreen cream formulation [30]

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 ^{0}C.

Step II: Oil phase was prepared by heating Sodium Propyl Paraben (0.06%), Stearic acid (2%), Cetyl alcohol (1%), Cetomacrogal-1000 (2%), Cetostearyl alcohol (5%) and *M. concanensis* seed oil (20%) at 80 ^{0}C.

Step III: Oil phase was added in water phase at 80 ^{0}C with continuous stirring for 20-25 min and then it was homogenized till uniform emulsion is formed. To improve the aesthetic value of the product, perfume was added in quantity sufficient. The finished product has white colour and gel-like consistency. It was then poured into the wide mouth container and stored at temperature not exceeding 37^{0}C.

Table 1: Formulation of *Moringa oleifera L* seed oil sunscreen cream

Sr. No.	Ingredients	Use	Components (%w/w)
1	Cetosteryl alcohol	Emulsifier	5
2	Stearic acid	Emollient, Co emulsifier	2
3	Cetomacrogal-1000	Emulsifier	2
4	Cetyl alcohol	Emollient, Co emulsifier	1
5	Carbopol 940	Gelling agent	0.5
6	Disodium EDTA	Chelating Agents	0.02
7	Na Methyl Paraben	Preservative	0.3
8	Na Propyl Paraben	Preservative	0.06
9	Triethanolamine	Surface active agent	0.5
10	Purified Water	Vehicle	62
11	*M. oleifera L* seed oil	Active ingredients	20
12	Perfume	-	q.s.

Determination of physical parameters of cream[30]

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

Determination of *in vitro* SPF

This study was performed by Transmittance measurement of the seeds of *Moringa oleifera L* (Moringaceae) cream. The Optometric 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 Optometric 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

Table 2: Physical Parameters of *Moringa oleifera L* seed oil sunscreen cream

Sr. No.	Parameters	Observations
1.	Color	Faint yellow
2.	Odor	Aromatic
3.	Spreadability	Good and uniform
4.	pH	6.86
5.	Specific gravity	1.14
6.	Viscosity	49500-99000
7.	Total microbial count	Nil
8.	Patch test for irritancy	No irritation reaction persists

RESULTS

The topical formulation of dried seeds of *Moringa oleifera L* (Moringaceae) was studied for all parameters of cream and *in vitro* SPF determination. The results of cream and SPF are mentioned in Table nos. 5 and 6. The parameters of cream complies with official acceptance criteria and SPF of this cream is found to be 1.06 with Boots Star Rating 3 indicating that the cream formulated can be considered as an efficient validated topical product.

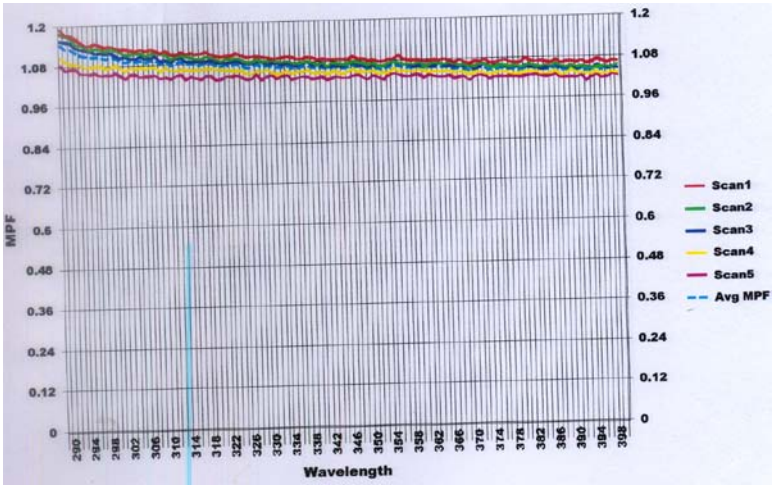

Fig. 1: SPF-290 Graph Report of *Moringa oleifera L* seed oil Sunscreen Cream (Scan 1)

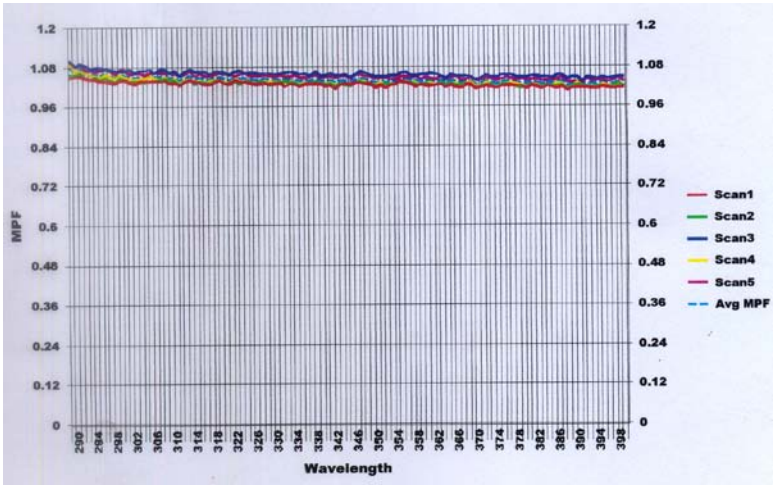

Fig. 2: SPF-290 Graph Report of *Moringa oleifera* L seed oil Sunscreen Cream (Scan 2)

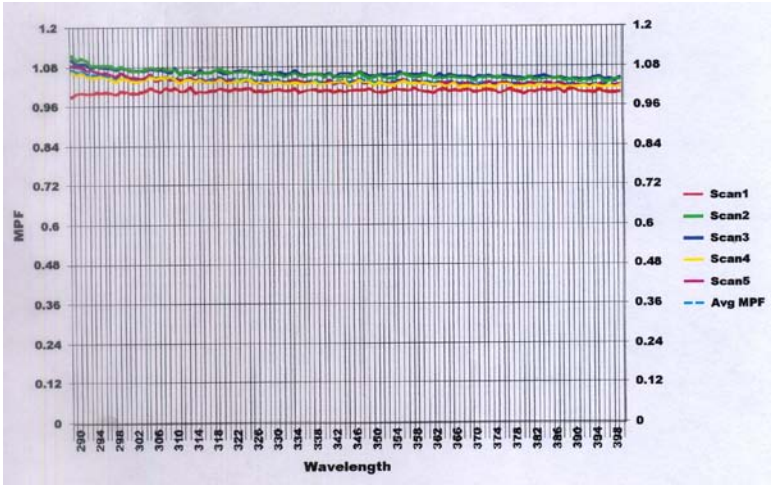

Fig. 3: SPF-290 Graph Report of *Moringa oleifera L* seed oil Sunscreen Cream (Scan 3)

Table 3: Results of SPF and other Parameters of *Moringa oleifera L* seed oil sunscreen cream

Sr. no	Parameter	Scan I	Scan II	Scan III	Average
1	SPF	1.09	1.05	1.05	1.063
2	Standard deviation	0.03	0.01	0.03	0.0231
3	UVA/UVB ratio	0.591	0.657	0.697	0.648
4	Critical wavelength	383.4	384	381.3	382.9
5	Boots star rating	2	3	3	3

DISCUSSION

The Optometric 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, ratio 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 [31] 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 *Moringa oleifera L* (Moringaceae) 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.

ACKNOWLEDGEMENT

We express their sincere gratitude Thanks to Mr. Milind Katariya Sir, Sci Tech Pharma Pvt Ltd.,Sinnar, Nashik for his kind guidance to carry out formulation, Dr. Mrs. Chandrashekhar of Kelkar Education Trust, Mulund (E), Mumbai for their kind help.

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