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)13. 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 sunscreen3.

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 skin4. There are three types of UV rays: UV-A (320–400 nm), UV-B (280–320 nm), UV-C (200–280 nm)5. Exposure to UV-A radiation results in damage to the elastin 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-ageing6. 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 increases8–10. 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 sunscreen11. 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 agent12.

Regulatory agencies like the US-FDA and COLIPA (The Comité de Liaison de la Parfumerie en Europe) has made in vivo testing on human volunteers using an erythemal endpoint to determine the SPF of topical cream mandatory13. Although it is a recommended and recognised 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 often14.

Sunscreens 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 oxide5, 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 product2. 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 197815. The inorganic materials like Titanium dioxide incorporated in formulation as sunscreen reflect and scatter ultraviolet and visible radiation from a film of inorganic 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 acceptability14. 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 reactions15. 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 eye17.

The Moringaceae is a single-genus family with 14 known species of these, M. oleifera Lam. (syn. Pterygosperma Gaertn.) is the most widely known and utilized species18,19. It is a small or medium-sized tree, about 10 m high, cultivated throughout India. M. oleifera is referred to as the “drumstick tree” whereas in others it is known as the “kelor tree”20. In literature seed extract of Moringa species reported to have anti-inflammatory, purgative, tonic21-24, analgesic25, potential antitumor25, anti-fungal26, anti-psoriatic, anti-inflammatory and diuretic activity27. 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 % respectively17,18. Characteristics of seed oil Saponification Value -179, Iodine Value (g of I/100 g of oil) -67.00, Unsaponifiable Matters 0.7828.

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.

Determination of physical parameters of cream

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

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 56cm² area of Transpose tape to obtain a sample film thickness of 2µl/cm² (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.

$$SPF_{color} = \frac{E_{R} \times B_{A} \times D_{A}}{E_{R} \times B_{A} \times D_{A} \times MPF_{A}}$$

Where,
- MPFₐ = scan MPF value
- Eₐ = spectral irradiance of terrestrial sunlight under controlled conditions
- Bₐ = erythemal effectiveness
- Dₐ = dose requirements

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

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Ingredients (Component)</th>
<th>Use</th>
<th>(%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cetostearyl alcohol</td>
<td>Emulsifier</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>Stearic acid</td>
<td>Emollient, Co emulsifier</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Cetomacrogal-1000</td>
<td>Emulsifier</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Cetyl alcohol</td>
<td>Emollient, Co emulsifier</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>Carbopol1940</td>
<td>Gelling agent</td>
<td>0.5</td>
</tr>
<tr>
<td>6</td>
<td>Disodium EDTA</td>
<td>Chelating Agents</td>
<td>0.02</td>
</tr>
<tr>
<td>7</td>
<td>Na Methyl Paraben</td>
<td>Preservative</td>
<td>0.3</td>
</tr>
<tr>
<td>8</td>
<td>Na Propyl Paraben</td>
<td>Preservative</td>
<td>0.06</td>
</tr>
<tr>
<td>9</td>
<td>Triethanolamine</td>
<td>Surface active agent</td>
<td>0.5</td>
</tr>
<tr>
<td>10</td>
<td>Purified Water</td>
<td>Vehicle</td>
<td>62</td>
</tr>
<tr>
<td>11</td>
<td>M. oleifera L seed oil</td>
<td>Active ingredients</td>
<td>20</td>
</tr>
<tr>
<td>12</td>
<td>Perfume</td>
<td>-</td>
<td>q.s.</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameters</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Color</td>
<td>Faint yellow</td>
</tr>
<tr>
<td>2</td>
<td>Odor</td>
<td>Aromatic</td>
</tr>
<tr>
<td>3</td>
<td>Spreadability</td>
<td>Good and uniform</td>
</tr>
<tr>
<td>4</td>
<td>pH</td>
<td>6.86</td>
</tr>
<tr>
<td>5</td>
<td>Specific gravity</td>
<td>1.14</td>
</tr>
<tr>
<td>6</td>
<td>Viscosity</td>
<td>49500-99000</td>
</tr>
<tr>
<td>7</td>
<td>Total microbial count</td>
<td>Nil</td>
</tr>
<tr>
<td>8</td>
<td>Patch test for irritancy</td>
<td>No irritation reaction persists</td>
</tr>
</tbody>
</table>
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 comply 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)
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). Ratiing 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 Erythematous 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 on using Transpore Tape® as the substrate for SPF measurements. Diffey's equation applies weighing variables in the substrate and sample application.

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

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11. COLIPA Guidelines Method For The In Vitro Determination of UVA Protection Provided by Sunscreen Products, 2007a; 1-20

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

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Parameter</th>
<th>Scan I</th>
<th>Scan II</th>
<th>Scan III</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SPF</td>
<td>1.09</td>
<td>1.05</td>
<td>1.05</td>
<td>1.063</td>
</tr>
<tr>
<td>2</td>
<td>Standard deviation</td>
<td>0.03</td>
<td>0.01</td>
<td>0.03</td>
<td>0.0231</td>
</tr>
<tr>
<td>3</td>
<td>UVA/UVB ratio</td>
<td>0.591</td>
<td>0.657</td>
<td>0.697</td>
<td>0.648</td>
</tr>
<tr>
<td>4</td>
<td>Critical wavelength</td>
<td>383.4</td>
<td>384.0</td>
<td>381.3</td>
<td>382.9</td>
</tr>
<tr>
<td>5</td>
<td>Boots star rating</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>


