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FORMULATION AND *IN VITRO* EVALUATION FOR SUN PROTECTION FACTOR FOR OIL FROM RESIN OF COMMIPHORA MUKUL SUNSCREEN CREAM

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ABSTRACT

Evaluation of sunscreen activity is an important aspect in the cosmetic industry, as exposure to sunlight is a recognized as a major factor in the etiology of the progressive unwanted changes in the skin appearance and physiology due to UV rays present in the sunlight. In the present study, sunscreen activity of formulated cream containing essential oil from Resin of plant Commiphora mukul Engl family: Burseraceae respectively was determined by absorption spectroscopy and transmission spectroscopy methods. In absorption spectroscopy, the absorption spectrum of dilute test solution of formulation from 290-320nm was studied to determine SPF against UV-B, most risky UV rays while in the transmittance spectroscopy, UVA and UVB protection and average UVA protection factor were calculated by taking transmission of formulations from 290nm-400nm using PVC as substrate. The results of the present study shows that the formulated creams have potency to protect against UV-rays. Result shows that it may absorb the UV radiation and possess good sun protection activity against ultraviolet radiation. These results show that Essential Oil responsible for ultraviolet absorption may be isolated from Resin of plant Commiphora mukul Engl and used in sunscreens preparations for better protection against sun rays.. From the result of the present study, it concludes that cream of essential oil from Resin of plant Commiphora mukul Engl can be used as sunscreen.

KEY WORDS

Sunscreen activity, Absorption and Transmittance spectroscopy,Commiphora mukul

INTRODUCTION

Extraterrestrial sunlight includes x-ray, ionizing, ultraviolet, visible, and infrared radiation, and radiowaves. The solar spectrum at the earth's surface (sea-level) consists of wavelengths of electromagnetic energy only between 290 and 3000 nm, while the spectrum implicated in human skin reactions involves wavelengths up to 1800 nm. Ultraviolet (UV) radiation is arbitrarily subdivided into three bands, UVA (320-400 nm), UVB (290-320 nm) and UVC (200-290 nm). The total flux of UVA at the earth's surface vastly exceeds that of UVB, with all the UVC being completed absorbed by stratospheric ozone. The terrestrial spectrum of solar UV radiation consists of 1–5% of UVB radiation and 95–99% of UVA radiation depending on the latitude, the time of the day and the season of the year. As a barrier and immunological organ in the human, the skin especially epidermis, is particularly subjected to external effects UVB radiation is fully absorbed by the stratum corneum and the top layers of the epidermis, whereas up to 50% of incident UVA radiation penetrates skin deep into the dermis. Ultraviolet irradiation is involved in the pathogenesis of skin cancers, causes premature aging of the skin and photoimmunosuppresion. It also plays a role in the pathogenesis of photosensitive diseases such as chronic actinic dermatitis, polymorphous light

eruption, actinic prurigo, hydroa vacciniforme, and photoallergic or phototoxic drug reactions. Both UVB and UVA radiation may affect the biomolecules of the skin [1, 3, 4].

After attention has been given to the harmful effects of the sunrays, to avoid unwanted skin effects of the sun, the use of sunscreen preparations became absolutely necessary. Efficacy of sunscreen is defined as the ability to protect the skin against ultraviolet-induced burning, with the level of performance indicated by the sun protection factor (SPF)[2,3]. 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 [5]. 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 [6].

Regulatory agencies like the US-FDA and COLIPA (The Comité de Liaison de la Parfumerie in Europe) has recommended in vivo testing on human volunteers using an erythemal endpoint to determine the SPF of topical sunscreens [7]. 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 [8]. SPF is primarily a measure of UVB protection, as UVB is 1000 times more erythemogenic than UVA. These products don't necessarily offer adequate UVA protection. Protection against UVA is becoming a major concern since UVA damage is now implicated in photocarcinogenesis, photoaging and immunosuppression. An invitro method based on determination of critical wavelength which is obtained using spectrophotometry. Critical wavelength is the wavelength where the integral of the spectral absorbance curve reaches 90% of the integral from 290 nm to 400 nm. It measures a sunscreen's extinction capacity in the UVA range in relation to its overall extinction between 290 nm and 400 nm. The critical wavelength determination does not promote the false notion of UVB and UVA as separate entities but rather as part of continuous electromagnetic spectrum. As the critical wavelength increases, so too must the protection against UVA. A complete description of a products photoprotective characteristics results when critical wavelength is used in conjunction with SPF.

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 [9]. 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 [8]. 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[10]. 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 [11]. 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 [12]. Therefore, the researchers have turned their attention towards developing herbal sunscreen agents which are

effective with less or no side effects. Flowers of Tagetes erecta L. (Asteraceae) is commonly known as French marigold flower (Engl.), Hajai (Arabic), sthulapushpa (Sans.)[13] . In India plant is cultivated in Andhra Pradesh , Tamil Nadu ,West Bengal , Karnataka, Uttar Pradesh etc. Flowers are in various forms, sizes and colors. Lutein is an oxycarotenoid, or xanthophyll, containing 2 cyclic end groups (one beta and one alpha-ionone ring) and the basic C-40 isoprenoid structure common to all carotenoids. It is one of the major constituents and the main pigment of Marigold flowers. Although the polyene chain double bonds present in lutein could exist in a cis or trans conformation, giving rise to a large number of possible mono-cis and poly-cis isomers, the vast majority of carotenoids are in the all-trans configurations [14]. Lutein being xanthophyll carotenoids with potent antioxidant properties protecting the skin from acute photo-damage. Therefore, the study was aimed at isolating and identifying the carotenoid (lutein ester) followed by developing a validated and effective topical dosage form.

MATERIALS AND METHODS

Plant material:

The Plant material (Resin) was purchased from local market of Mumbai. From this resin Guggul oil-I was distilled out and Guggul oil-II was procured from the market.

The Plant Material (Resin) of plant *Commiphora mukul* Engl family: Burseraceae. Was authenticated from Ayurved Sevasang, Nasik (MS) India, and after the authentication of resins was used for further studies.

Extraction essential oil from resin of *commiphora mukul* engl :

The Resins of plant *Commiphora mukul* Engl. were subjected for maceration for 7 days with organic solvent alcohol, after the maceration the resins were steam distilled by using the live steam distillation method. The oil globules with water were collected in 250 ml of Erlenmeyer flask. After completion of the live steam distillation process the mixture of oil globules and water were treated with organic solvent ether and they were continuously shaken in 500 ml of separating funnel for 3-4 minutes to separate oil globules from water, then separating funnel along with the mixture was kept aside for 10-15 minutes for the separation of two layers that is organic layer with oil globules at upper side of separating funnel and, aqueous layer at lower side of separating funnel.

The mixture was separated by using separating funnel and then the organic layer along with the oil globules were passed over the calcium carbonate to remove or absorb the remaining water molecules. Then the mixture of oil globules and organic solvent was again taken into 500 ml of Round bottom flask for the separation of oil.

The oil was subjected for distillation process, and then it was collected in 100 ml of closed Erlenmeyer flask as oil was of volatile type. Then the weight of oil was taken for the determination of practical yield of oil, the practical yield of extracted oil was 4.0%. Then it was stored in umber coloured container for the further analysis and processes, in a cool place.

Formulation of sunscreen cream:

Components used in the formulation of cream is mentioned in **Table 1**

Procedure

Step-I: Water phase was prepared by collecting purified water (72 %) and then 5 % water was removed aside from this for final volume makeup. Water was heated to 80^{O}C and in it Sorbitol, Disodium EDTA, Methyl Paraben and Propylene glycol was added.

Step-II: Oil phase was prepared by heating propyl paraben, stearic acid, cetyl alcohol, cetomacrogal-1000, arlacel and glyceryl monostearate, along with light liquid paraffin at 80 ^{O}C.

Step-III: Oil phase was added in water phase at 80 ^{O}C with continuous stirring for 20-25 min and then it was homogenized for 10 min. cooling was started upto 60 ^{O}C, Guggul oil was added in the formed emulsion. $_{P}$H was adjusted by adding Sodium Hydroxide. Final volume was made upto 100 % with the purified water.

Determination of physical parameters of cream: Preparation of herbal cream has always been a challenging task and the cream is accepted only if it is tested appropriately for various physical parameters like ease of spreadability, appearance, pH, viscosity and pleasant feeling as specified in **Table 2**. Creame is

also tested for its thermal stability as per ISO guideline (IS 6608:2004) specifies in **Table 3**.

Determination of in vitro spf:

This study was performed by Transmittance measurement of the Essential oil extracted from Resin of *Commiphora mukul* Engl cream. The Optometrics 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 Transpore tape to obtain a sample film thickness of 2µl/cm2 (to get an even film) as suggested in the operational manual of Optometrics 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.

WinSPF has used the following equation for calculating SPF:

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

Where, MPFλ = scan MPF value, Eλ = Spectral Irradiance of terrestrial sunlight under controlled conditions, and Bλ = Erythemal Effectiveness.

Table 1: Composition of Guggul oil Sunscreen Cream (O/W):

Sr. No.	Ingredients	Components (%w/w)
1.	Stearic acid (Emollient, Coemulsifier)	02.50
2.	Cetyl alcohol(Emollient, Coemulsifier)	01.25
3.	Cetomacrogal-1000 (Emulsifier)	02.25
4.	Arlacel(Emulsifier)	01.50
4.	Glyceryl Monostearate (Coemulsifier)	02.00
5.	Light liquid paraffin (Mineral oil)	09.00
6.	Propylene glycol (Humectant)	03.00
7.	Sorbitol (Humectant)	02.00
8.	Disodium EDTA (Chelating Agents)	00.10
9.	Methyl Paraben (Preservative)	00.20
10.	Propyl Paraben (Preservative)	0.02
11.	Purified Water (Vehicle)	70.98
12.	Sodium Hydroxide (PH Adjustment)	00.20
13.	Guggul oil (w/w)	05.00

Table 2: Analysis Report of Guggul oil Sunscreen Cream

Sr. No.	Test	Observation
1.	Colour	Light Yellow
2.	Odour	Sweet Herbaceous
3.	Appearance	Viscous Cream
4.	Specific gravity by Pycnometer	0.97
5.	Viscosity 3 RPM	22000
6.	Viscosity 6 RPM	15000
7.	Viscosity 12 RPM	11500

Table 3: Thermal Stability of the sample as per ISO 6608: 2004

Sr. No.	Analysis	Result	Acceptance Criteria
1.	Thermal Stability	Passes	To pass the test

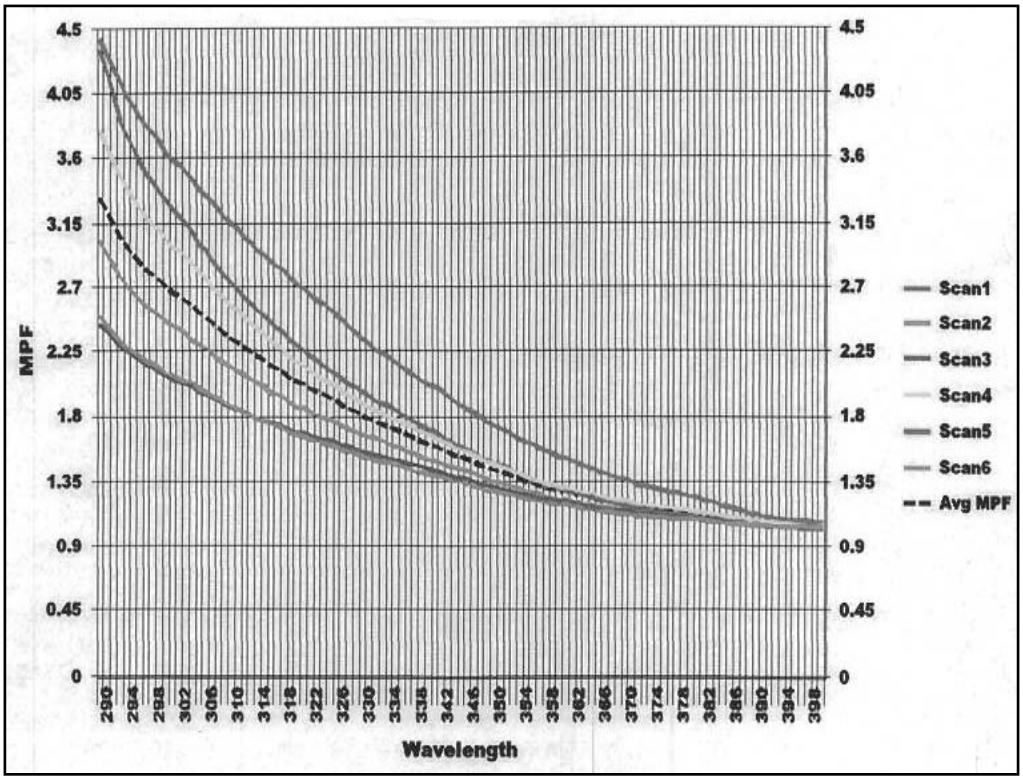
RESULT

The topical formulation of Essential oil extracted from Resin of *Commiphora mukul* Engl was studied for all physical parameters of cream that mentioned in **Table 3** and in vitro SPF determination. The results of cream and SPF are mentioned in **Table 4**. The parameters of cream complies with official acceptance criteria and SPF of this cream is found to be 2.23±0.48 with Boots Star Rating 1 indicating that the cream formulated can be considered as an efficient validated topical product.

Table 4: Determination of Sun Protection Factor of Guggul oil cream

Sr.No	Test Sample	Parameters	Values
		SPF	**2.23**
		SD	**0.48**
1	**Guggul Oil Cream**	**UVA/UVB RATIO**	**0.321**
		Critical Wavelength	**359**
		Boot Star Rating	*****

Figure 1: SPF-290 Graph Report of Guggule oil Sunscreen cream



DISCUSSION

The Optometrics 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, rationed 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 [16] 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.

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