



FORMULATION AND *IN-VITRO* DETERMINATION OF SUN PROTECTION FACTOR OF *OCIMUM BASILICUM*, LINN. LEAF OILS SUNSCREEN CREAM

SHANTANU KALE, AMOL SONAWANE*, AMMAR ANSARI, PRASHANT GHOGE, ASHWINI WAJE

Department of Pharmacognosy, MGV's Pharmacy College, Mumbai-Agra Highway, Panchvati, Nashik 422003

Received: 12 July 2010, Revised and Accepted: 23 August 2010

ABSTRACT

The increasing awareness of the risk of skin cancer with the sun exposure requires that sunscreen products be approximately tested and labeled. Although there exists information on the possible photon-induced reaction such as photoirritation and photosensitization produced by sunscreen creams containing synthetic photo-protective chemicals. Effect of sunscreen cream depends upon sun protection factor (SPF) value and substantivity. In addition due to high cost and time consumption of *in vivo* SPF determination methodologies, *in vitro* SPF determination is gaining more importance. The aim of present study was to formulate a sunscreen cream containing essential oil of *Ocimum basilicum* Linn. as an active ingredient and evaluate experimental method for *in vitro* SPF determination.

Keywords: *Ocimum basilicum*, UV Protection, Sun protection factor, Sunscreens

INTRODUCTION

Sunlight composed of various wavelengths ranging from ultraviolet light through infrared to visible light. Exposure to solar radiation is recognized to have negative effects on the human skin. Among all, ultraviolet light is the most harmful to the skin and causes sunburns, ageing of the skin and over the long term, skin cancer¹. Ultraviolet light is made up of UVA 400-320, UVB- 320-290 and UVC- 290-100 nm range respectively. UVC being the shorter wavelength that is filtered out by the earth's ozone layer. UVB and UVA penetrate the ozone layer and reach the earth's surface but the atmosphere filters more UVA than UVB^{2,3}. Fortunately, UVA is not quite so powerful in its effects as UVB as it has an additive (cumulative) effect with UVB on the skin. UVA and UVB are the only wavelengths that need to be screened out as we still have an ozone layer over most of the earth⁴.

Sunscreens and sunblocks are the two chemicals that absorb or block UV rays of sunlight. The use of skin care products supplemented with several effective agents working through different pathways in conjunction with the use of sunscreens may be an effective approach for reducing UV-B generated ROS mediated photoaging. It has been known for the decades that sunscreens are capable of protecting man from harmful effects of solar radiation such as actinic ageing or cutaneous cancer. All compounds used as sunscreen filter are by their nature, chemicals that are able to absorb UV-A and / or UV-B light. The range of wavelengths which are absorbed by a given compound is termed as absorption spectrum. Basic and applied research concerning sun protection has become a major concern. There exist some information on possible photon induced reaction such as photoirritation, photosensitization and contact dermatitis by sunscreen products containing synthetic photo-protective agents like cinnamates, p- amino benzoic acid and oxybenzone⁵. Although most sunscreen products contain synthetic photo-protective agents of a high sun protection factor (SPF), there is little published data describing use of essential oil in sunscreen products and SPF determination by Optometrics LLC SPF-290S.

One of the key factor in evaluating the potential of sunscreen product is a sun protection factor (SPF) value which can be thought as a time factor for the protection of a skin compared to exposure without any protection^{6,7}. The protection performance of sunscreen product against erythema inducing radiation, calculated from the measured *in vitro* transmittance and weighed with the erythema action spectrum and with a 'standard' output spectrum of a UV solar simulator used for SPF testing^{8,9}. Most recently updated scientific method for evaluating the SPF of sunscreens have been developed by COLIPA internationals.

Ocimum basilicum, Linn. (basil) belongs to the plant family Lamiaceae, sub-family Nepetoideae, and the genus sensulato¹⁰. Plant commonly known as sweet or garden basil (Engl.), Munjariki (Sans.), basilikumkraut (Ger.), Herbe de grand basilic (Fr.), Common basil^{11,12}. Lamiaceae plants are native through the subtropics, especially throughout the Mediterranean region. Basil most commonly found throughout greater part of India. *O. basilicum* Linn. is widely used in India, since the Ayurveda and Unani medicinal systems use it for the treatment of several ailments-

In literature essential oil of basil is reported to have anti-inflammatory¹³, anti-microbial^{14,15}, anti-fungal, antiphagocytic, antioxidant¹⁶, chemomodulatory, insecticidal activity. Alcoholic extract of leaf shows hypolipemic¹⁷, anti HIV-1, larvicidal, cardiac stimulant and aqueous extract shows vasorelaxant and anti-platelet aggregation activity¹⁸. Basil essential oil has been utilized extensively in the food industry as a flavoring agent, and in perfumery and medicinal industry.

Essential oil obtained from the dried leaves of *Ocimum basilicum* Linn. is yellowish green, clear liquid with a strong aromatic odor of terpene fractions. Reported maximum yield value for basil essential oil is 0.38 – 0.65%¹⁹. The essential oil was analysed by GC and GC-MS and had the following major constituents linalool, methyl cinnamate, methyl linalool, methyl eugenol, citral, methyl chavicol, thymol, ρ -cymene, α -pinene²⁰. Characteristics of basil essential oil are as specific gravity at 22^oC 0.9942, optical rotation -11^o, acid Value 11.1, ester Value 178.7, alcohol (as linalool), 4.4%.

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 essential oil of *Ocimum basilicum* Linn. and evaluation of the same.

MATERIALS AND METHODS

Plant material

Plant material of *Ocimum basilicum*, Linn. were collected from local market at Shirdi, Dist Ahmednagar, India. It was authenticated taxonomically and specimen sheet deposited to Botanical Survey of India, Pune. The leaves were cleaned, dried and powdered prior to the isolation of essential oil.

Isolation of essential oil

Dried powdered material of basil leaves, (50 g) macerated with 100 volumes of ethyl alcohol (95%) for 78 hours. After 78 hours ethyl alcohol was decanted, concentrated under vacuum (25ml) and concentrate was subjected to steam distillation (60±2 °C). Further

hydrodistillate was extracted with solvent ether and extract was concentrated under vacuum to yield basil oil. Collected oil was then dried over anhydrous sodium sulphate and stored in sealed vial.

Sunscreen cream formulation

Step I: Aqueous phase preparation: Disodium EDTA (0.02% w/w), Sodium methyl paraben (0.3% w/w) and Triethanolamine (0.5% w/w) were dissolved in deionised water, meanwhile, carbopol (0.5% w/w) was added to swell using a homogenizer (8000 rpm) and heated up to 80 °C.

Step II: Oil phase preparation: Sodium propyl paraben (0.06% w/w), Stearic acid (2% w/w), Cetyl alcohol (1% w/w), Cetomacrogol-1000 (2%w/w), Cetostearyl alcohol (5% w/w) and basil essential oil (5% w/w) mixed and heated at 80 °C.

Step III: Mixing phase: Oil phase was added to water phase at 80 °C with continuous stirring for 20-25 min and then it was homogenized (8000 rpm) till uniform emulsion was formed. It was then poured into the wide mouthed container and stored at temperature not exceeding 37°C.

Determination of in-vitro SPF of sunscreen cream

Approximately 100 mg of the investigational sample was applied and spread on 50 sq.cm area to obtain a sample film thickness of 2 µl /cm² on Transpore surgical tape to get an even film as suggested in the operation manual of Optometrics LLC SPF-290S for the sample preparation and application technique. The sample thus prepared was exposed to Xenon arc lamp with UV range 290nm to 400nm for determining the SPF.

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 = Erythral effectiveness.

The SPF-290 software used Trapezoidal Approx calculating technique to approximate the integral for SPF and Erythral UVA protection factor. These include UVA/UVB ratio, critical wavelength, cumulative absorbance, etc. The Average Absorbance method was used for calculating average protection factor. For calculation of standard deviation, Diffey's method was used.

RESULTS AND DISCUSSION

In the present study essential oil of *Ocimum basilicum*, Linn. was isolated (yield 0.6% w/w) and sunscreen cream was formulated using this oil. The finished product has white colour and gel like consistency. Cream was evaluated for sunscreen activity using *in vitro* SPF method. The SPF is quantitative measurement of the effectiveness of the sunscreen formulations. The SPF value of sunscreen cream was found to be 1.19 with ultra boot star rating 1. SPF value for sunscreen above 2 is considered as having good sunscreen activity. It indicates that formulated sunscreen cream was found near the range of good sunscreen activity and hence *Ocimum basilicum*, Linn. essential oil may be considered as good candidate for sunscreen or cosmeceutical purposes. Further this cream was evaluated for spreadability, viscosity, microbial testing etc.

Table 1: Results of SPF and other parameters of basil oil sunscreen cream

Sr. no.	Parameter	Scan I	Scan II	Scan III	Average value
1	SPF	1.24	1.13	1.21	1.19
2	Standard deviation	0.05	0.04	0.06	0.05
3	UVA/UVB ratio	0.362	0.163	0.306	0.280
4	Critical wavelength	366.8	342.9	359.3	356.3
5	Boot star rating	1	0	1	1

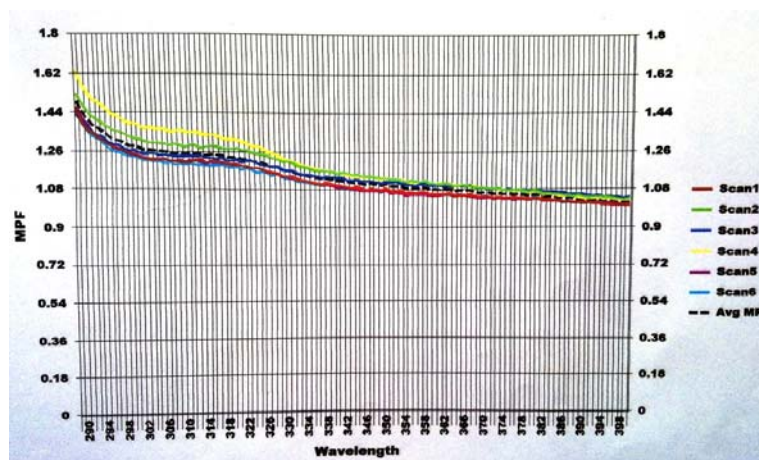


Fig. 1: SPF-290 Graph Report of basil oil sunscreen cream

ACKNOWLEDGEMENT

We express our sincere thanks to Mr. Milind Katariya Sir, Reva Pharma Pvt Ltd., Sinner, Nashik for his kind guidance to carry out formulation, Dr. Mrs. Chandrashekhar and Ms. Priyanka, Kelkar Lab, Mulund for their kind help. My colleagues Harsha, Sonali, Bhagyashree for their helpful support at every time.

REFERENCES

1. P. Treffel, B. Gabard ; Skin Penetration and SPF of UV Filters from Two Vehicles; Pharmaceutical Research, 1996, 13(5), p770-774
2. COLIPA Guidelines: Guidelines for The Colorimetric Determination of a Skin Color Typing and prediction of The Minimal Erythral Dose Without UV Exposure, 2007, P1-9

3. Rai R, Srinivas CR, Photoprotection-A Review, Indian J of Dermatol, Venereol, Leprol 2007; 73; p73-79.
4. Vinayak V. Patil, S. B. Patil, M. S. Kondawar, N. S. kaikwade, C. S. Magdum, Study on Spathodea Campanulata L. Extract as an Anti-Solar, International J. of Green Pharmacy, 2009, p248-249
5. Westerhop W ; The Relation Between Constitutional Skin Color and Photosensitivity Estimated From UV Induced Erythema and Pigmentation Dose-Response Curve, J. Of Invest Dermatol, 1988, 124, p869-871
6. Sheu, MT; Lin, CW; Huang, MC; Shen, CH; Ho, HO, Correlation of In Vivo and In Vitro Measurements of SPF, J. of Food and Drug Analysis, 2003, 11(2), p128-132
7. Art Springsteen, Ramona Yurek, Michelle Frazier and Kevin F. Carr; In Vitro Measurement of SPF by Diffuse Transmittance, Analytica Chemica Acta, 380(2-3), Feb1999, p155-164
8. S L Oliveira, A M Mansanares, E C da Silva, and P R Barja, In Vitro Determination of SPF of Sunscreens Through Photoacoustic Spectroscopy- a New Approach; The European physical J. Jan2008, 153(1), p475-478
9. COLIPA Guidelines- Method For The In Vitro Determination of UV-A Protection Provided by Sunscreen Products, 2007a, p1-20
10. Eva klimankova, Katerina Holadova, Jana Hajslova, Tomas Cajka, Jan Poustka, Martin Koudela; Aroma Profiles of Five Basil Cultivars Grown Under Conventional and Organic Conditions, Food Chemistry, 107(2008), p464-472
11. The Wealth of India, Vol IX, CSIR, New Delhi, 1956, p81-84
12. Norman Grainger Bisset and Max Wichtl, Herbal Drug and Pharmaceuticals- A Handbook for Practice on a Scientific Basis With Reference To German Commission E. Monograph, 2nd Edition, Med pharm scientific publishers, p104-105
13. M.C. Courreges, F. Benencia, In Vitro Anti-phagocytic Effect of Basil Oil on Mouse Macrophages, Fitoterapia, 73(2002), p369-374
14. D. Runyoro, O. Ngassapa, K. Vagionas, N. Aligiannis, K. Graikou, I. Chinou, Chemical Composition and Antimicrobial Activity of the Essential oil of Four Ocimum Species Growing In Tanzania, Food Chemistry, 2009, p1-6
15. Abdullah Ijaz Hussain, Farooq Anwar, Syed Tufail Hussain Sherazi, Roman Przybylski, Chemical Composition, Antioxidant and Antimicrobial Activities of Basil, Food Chemistry, 108(2008), p986-995
16. S. J. Lee, Katumi Umano, Takayuki Shibamoto, Kwang-Geun Lee, Identification of Volatile Components in Basil and Thyme Leaves and Their Antioxidant Activity, Food Chemistry, 91(2005), p131-137
17. Elena Bravo, Souliman Armani, Mohammed Aziz, Hicham Harnafi, Mariarosaria Napolitano *Ocimum basilicum* Ethanolic Extract Decreases Cholesterol Synthesis and Lipid Accumulation in Human Macrophages, Fitoterapia, 79(2008), p515-523
18. Souliman Amrani, Hicham Harnafi, Dounia Gadi, Hassane Mekhfi, Abdelkhalea Legssyer, Mohammed Aziz, Francaise Martin-Nizard, Lisardo Bosca. Vasorelaxant and Anti-platelet Aggregation Effects of Aqueous *Ocimum basilicum* Extract, Journal of Ethnopharmacology, 125(2009), p157-162
19. E. Cassel, R.M.F. Vargas, N. Martinez, D. Lorenzo, E. Dellacassa, Steam Distillation Modeling for Essential oil Extraction Process, Industrial Crops And Products, 29(2009), p171-176
20. Yoshohiro Hasegawa, Katsuhiko Tajima, Nao Toi and Yukio Sugimura, Characteristic Components Found in Essential Oil Of *Ocimum basilicum* L., Flavour And Fragrance Journal, 12(1997), p195-200.