

## EVALUATION OF IN VITRO SUNSCREEN ACTIVITY OF HERBAL CREAM CONTAINING EXTRACT OF *CURCUMA LONGA* AND *BUTEA MONOSPERMA*

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### ABSTRACT

The aim of the present study was to evaluate the *in vitro* sunscreen activity of Herbal cream containing rhizome extract of *Curcuma longa* Linn. (Fam. Zingiberaceae) and flower extract of *Butea monosperma* Lam. (Fabaceae). The dried powdered rhizomes and flowers were extracted successively with petroleum ether and ethanol in soxhlet apparatus. Two sunscreen creams were formulated using ethanolic extract and tested for the physicochemical parameters such as colour, odour, spreadability, pH, specific gravity, limit test for lead and viscosity. Stability study

and sensitivity study was done by centrifugation method and patch test for irritancy respectively. The *in vitro* SPF of the formulations was determined according to the UV Spectrophotometric method of Mansur et al. The sunscreen cream containing ethanolic extract of *Curcuma longa* under study produced high absorbance at 290-320 nm wavelength range and SPF obtained was  $12.08 \pm 4.44$ , where as for *Butea monosperma* sunscreen cream it was  $10.21 \pm 1.13$ . From the result obtained in the study, we can positively conclude that *Curcuma longa* sunscreens significantly contribute in the preparation of photoprotective cosmetic formulations which could prevent the skin with harmful effects of ultraviolet radiations. It will also help in broadening the UV protection ability of the sunscreen along with the greatest advantage of avoiding the adverse and undesired effects of synthetic sunscreen compounds.

**KEYWORDS:** *Butea monosperma*, *Curcuma longa*, sun protection factor (SPF).

## INTRODUCTION

The rapid growth of commercially available products containing sunscreens indicates that even though a suntan is still desired people are conscious of the possible dangers of photo-ageing and skin cancer, occurring as a result of sun overexposure. The harmful effects of solar radiation are caused predominantly by the ultraviolet (UV) region of electromagnetic spectrum, which can be divided into three regions: UVA, from 320 nm to 400nm, UVB, from 290 nm to 320 nm and UVC, from 200 nm to 290 nm. UVC radiation is filtered out by the ozone layer and is responsible for the damage due to sunburn.<sup>[1]</sup> UVA radiation reaches the deeper layer of epidermis and dermis and provokes the premature ageing of the skin.<sup>[2]</sup> Ultraviolet radiations have been implicated as a causative factor of skin cancer. The efficacy of sunscreen is usually expressed by the sun protection factor (SPF), which is defined as the UV energy required for producing a minimal erythema dose (MED) on protected skin, divided by the UV energy required for producing a MED on unprotected skin.

$$\text{SPF} = \frac{\text{Minimal erythema dose in sunscreen protected skin}}{\text{Minimal erythema dose in non-sunscreen protected skin}}$$

The minimal erythema dose (MED) is defined as the lowest time interval or dosage of UV light radiation sufficient to produce a minimal, perceptible erythema on unprotected skin.

<sup>[3,4]</sup> Although modern sunscreen containing UV filters are highly efficient to protect the skin from deleterious effects of the sun, but herbal sunscreens are rapidly replacing them due to associated side effects with UV filters. So many herbal sunscreens are available in market in the form of creams, lotions and gels having labeled sun protection factor. Most commonly used herbs are aloe Vera, basil, green tea, almond, olive, jojoba and cucumber etc. incorporated in herbal sunscreens.<sup>[5]</sup> The photoprotection afforded by topical sunscreen against solar ultraviolet radiation exposure can be determined *in vivo* or *in vitro* and it is ideally determined by photo-testing in human volunteers. This type of determination has been used for many years and although useful and precise, is a time consuming process, complex and expensive, particularly when information concerning to the protection against long wavelength (UVA) is required.<sup>[6, 7]</sup> As a consequence, much effort has been devoted to the development of *in vitro* techniques for assessing the photoprotection of sunscreen compounds. The methods *in vitro* are in general of two types. Methods which involve the measurement of absorption or the transmission of UV radiation through sunscreen product film in Quartz plates or Biomembrane and methods in

which the absorption characteristics of the sunscreen agents are determined based on spectrophotometric analysis of dilute solution. <sup>[8]</sup> *Curcuma longa* Linn. (Fam. Zingiberaceae) is commonly known as Haldi in Hindi, is a perennial plant having short stem with large oblong leaves. <sup>[9]</sup> Its oil has great importance in medicines. It contains essential oil (5%), alkaloid, and yellow matter curcumin (5%) a polyphenol which is active substance of turmeric. It also contains turmeric oil (5-8%) cupric acid (1%). *C. longa* is being used in cosmetic herbal formulation viz. Vico turmeric, JCICM-6 and many others. Curcumin, the active compound of turmeric is a polyphenol used in skin care preparations. <sup>[10, 11]</sup> *Butea monosperma* is medium sized deciduous tree commonly known as 'flame of forest' belongs to the (family- fabaceae). It is extensively used in Ayurveda, Unani and Homeopathic medicine and has become a cynosure of modern medicine. The roots are used to night blindness, helminthiasis, piles, ulcers and tumours. The bark is reported to antitumour and anti ulcer activities, the leaves possess antimicrobial property. The *B. Monosperma* contain Kinotannic acid, Gallic acid, pyrocatechin, butrin, palasitrin, alanind, allophonic acid, butolic acid, cynidin, histidine, lupenone, and shelloic acid. Flavonoids are potent antioxidants and have free radical scavenging abilities. <sup>[12, 13]</sup> The present work was planned to study the sunscreen activity of herbal sunscreen containing ethanolic extract of rhizome of *C. Longa* and ethanolic extract of flower of *B. Monosperma*. However the sunscreens activity of these plants has not been reported till date. This forms the basis for selection of these plants for its sunscreens activity.

## MATERIALS AND METHODS

### Plant material

Rhizomes of *C. longa* and flowers of *Butea monosperma* were collected from the local areas of Mahabubnagar district, Andhra Pradesh, India and the plant materials were taxonomically identified and authenticated at Department of Botany, Govt. M.V.S .Degree & PG College, Mahabubnagar, Andhra Pradesh.

### Extraction of plant material

The rhizomes and flowers were cleaned, dried under the shade and pulverized. The coarse powder extracted successively with petroleum ether and ethanol using Soxhlet apparatus. The extracts were dried using rotary vacuum evaporator and stored in desiccators until further use.

### Reagents

Ethanol (Merck) analytical grade was used.

### Instruments

UV Spectrophotometer: UV 1700 Shimadzu, Japan

Brookfield Viscometer: LVDV-I prime, Brookfield Engineering Laboratories Inc. U.S.A,

PH meter: ELICO LI 610

Micro centrifuge: REMI RM-12 C

### Formulation of Sunscreen

**Step I:** Water phase was prepared by collecting deionized water (73%) and then (5%) water was remove aside from this for final volume makeup. Water soluble components disodium EDTA, sodium methyl paraben and triethanolamine were dissolved in deionized water; meanwhile carbopol was allowed to swell using a homogenizer and heated up to 80 °c.

**Step II:** Oil phase was prepared by heating sodium propyl paraben, stearic acid, cetyl alcohol, cetomacrogal-1000, Cetosteryl alcohol and ethanolic extract at 80 °c.

**Step III:** Oil phase was added in water phase at 80°C with continuous stirring for 20-25 min. and then it was homogenized till uniform emulsion is formed. The finished product has yellow color and gel like consistency. It was then poured into the wide mouth container and stored at temperature not exceeding 37°C (Table 1).

**Table 1. Composition of *Curcuma longa* and *Butea monosperma* Sunscreen cream:**

| Sr. No. | Ingredients   | Uses                    | Components (%w/w) |
|---------|---|-------------------------|-------------------|
| 1.      | Cetosteryl alcohol                                  | Emulsifier              | 5                 |
| 2.      | Na Methyl Paraben                                   | Preservative            | 0.3               |
| 3.      | Na Propyl Paraben                                   | Preservative            | 0.06              |
| 4.      | Stearic acid  | Emollient, coemulsifier | 2                 |
| 5.      | Cetomacrogal-1000                                   | Emulsifier              | 2                 |
| 6.      | Carbopol  | Gelling agent           | 0.5               |
| 7.      | Triethanolamine                                     | Surface active agent    | 0.5               |
| 8.      | Disodium EDTA                                       | Chelating agent         | 0.02              |
| 9.      | Cetyl alcohol                                       | Emollient, coemulsifier | 1                 |
| 10.     | <i>C. longa/ Butea monosperma</i> ethanolic extract | Active Ingredients      | 1                 |
| 11.     | Purified water                                      | Vehicle                 | q. s. (upto 100)  |

### Physicochemical Analysis

Physical parameters of cream formulation such as color, odor, spreadability, PH, specific gravity (25°C), limit test for lead of the herbal sunscreen was determined by the standard technique and methods.<sup>[14]</sup> Viscosity of the sunscreen was measured using a Brookfield viscometer at 10-100 rpm, measurement was made at 25°C. Stability of sunscreen was determined by centrifugation. During centrifugation studies the sunscreen was centrifuged at 3500-13500 rpm at the interval of 10 minutes and further observes for phase separation. To ensure sunscreen is free from adverse effects a sensitivity study using path test for irritancy was done.

### Sample preparation

1.0 g of sample was weighed, transferred to 100 ml volumetric flask, diluted to volume with ethanol followed by ultrasonication for 5 min and then filtered through cotton, rejecting the first 10 ml. A 5.0 ml aliquot was transferred to 50 ml volumetric flask and diluted to volume with ethanol. Then 5.0 ml of aliquot was transferred to 25 ml volumetric flask and the volume completed with ethanol. Thereafter, absorbance values of each aliquot prepared were determined from 290-320 nm at 5 nm interval, taking ethanol as a blank. The measurements were taken thrice and the determinations were made at each point, followed by application of Mansur equation.

Mansur et.al (1986) developed a very simple mathematical equation which substitutes the *in vitro* method proposed by Sayre et al. (1979), utilizing UV Spectrophotometry and the following equation.<sup>[8, 15]</sup>

$$SPF_{\text{spectrophotometric}} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Where CF=Correction factor (10), EE (λ) = Erythrogenic effect of radiation with wavelength λ, Abs (λ) = Spectrophotometric absorbance values at wavelength λ. The values of EE×I are constant, they were determined by Sayre et al.

### Sun Protection Factor Determination

The aliquots prepared were scanned between 290-320 nm and the obtained absorbance values were multiplied with the respective EE (λ) values. Then, their summation were taken and multiplied with the correction factor (10). Data was expressed as mean ± S.E.M.

## RESULTS

In this research herbal sunscreen a cream containing ethanolic extract of rhizomes of *C. longa* and flower extract of *B. monosperma* were evaluated by UV spectrophotometry applying Mansur mathematical equation. The results of the physicochemical analysis of tested sunscreen showed in Table 2.

**Table 2. Physical parameters of formulated sunscreens**

| Sr. No. | Parameters               | Observations                     |                                       |
|---------|--------------------------|----------------------------------|---------------------------------------|
|         |                          | <b><i>C. longa</i> sunscreen</b> | <b><i>B. monosperma</i> sunscreen</b> |
| 1       | Color                    | Dark yellow                      | Light yellow                          |
| 2       | Odour                    | Characteristics                  | Characteristics                       |
| 3.      | Spreadability            | Good and uniform                 | Good and uniform                      |
| 4.      | PH                       | 6.3                              | 6.1                                   |
| 5.      | Specific gravity         | 0.88                             | 0.80                                  |
| 6.      | Limit test for lead      | passes                           | Passes                                |
| 7.      | Viscosity (cps)          | 830                              | 821                                   |
| 8.      | Patch test for irritancy | No irritation reaction persists  | No irritation reaction persists       |

Stability results tested by centrifugation method revealed that the phase separation was not observed. This showed the stability of this formulation at high stress condition. During the storage and handling of cosmetic formulation spreadability and viscosity are the prime parameters which affect the formulation acceptability. As the speed of rotation has increased viscosity of tested samples decreased, this behavior of both the formulation revealed the pseudoplastic behavior of products. Formulation with pseudoplastic flow produce a coherent protective film covering the skin surface and this activity is important for adherence on the skin. <sup>[16]</sup> SPF values of samples obtained using the UV spectrophotometric methods are shown in Table 3.

**Table 3. Determination of SPF value of *Curcuma longa* and *B. monosperma* Sunscreen Cream**

| Sr. no.               | Wave length | EE value | <i>C. longa</i> Sunscreen | <i>B. monosperma</i> sunscreen |
|-----------------------|-------------|----------|---------------------------|--------------------------------|
| 1                     | 290         | 0.015    | 0.0252±0.009              | 0.0138±0.03                    |
| 2                     | 295         | 0.0817   | 0.1494±0.0068             | 0.0592±0.02                    |
| 3                     | 300         | 0.2874   | 0.2864±0.021              | 0.2303±0.01                    |
| 4                     | 305         | 0.3278   | 0.2875±0.381              | 0.3176±0.19                    |
| 5                     | 310         | 0.1864   | 0.2907±0.022              | 0.2516±0.03                    |
| 6                     | 315         | 0.0837   | 0.1525±0.001              | 0.1324±0.07                    |
| 7                     | 320         | 0.018    | 0.0163±0.001              | 0.0072±0.01                    |
| Sun Protection Factor |             |          | <b>12.08 ± 4.44</b>       | <b>10.21±1.13</b>              |

We can see that high absorbance values were obtained at 290-320 nm wavelength range. The value of SPF obtained for *C. longa* sunscreen cream was  $12.35 \pm 4.44$  where as for *Butea monosperma* sunscreens cream it was  $10.21 \pm 1.13$ . *C. longa* sunscreen cream showed significant sunscreen activity.

However there are many factors affecting the determination of SPF values as for example, the use of different solvents in which the sunscreens are dissolved; the combination and concentration of the sunscreen; the type of emulsion, the effect and interactions of vehicle components, such as esters, emollients and emulsifiers used in the formulations; the interaction of vehicle with the skin; the PH system and emulsion rheological properties, among other factors, which can increase or decrease the UV absorption of each sunscreen. Excipients and other active ingredients can also produce UV absorption bands, thus interfering with those of UVA and UVB sunscreens. This effect is reflected in a finished formulation especially for lotions with an SPF greater than 15.<sup>[17, 18]</sup> Therefore, to develop sunscreens with better safety and high SPF, the formulator must understand the physicochemical principle, not only the UV absorbance of the actives, but also vehicle components, such as esters, emollients and emulsifiers used in the formulations, since sunscreens can interact with other components of the vehicles, and these interactions can affect sunscreens efficacy.

## DISCUSSION

The SPF is the quantitative measurement of the effectiveness of a sunscreen formulation. To be effective in preventing sunburn and other skin damage, a sunscreen product should have a wide range of absorbance between 290nm to 400nm. The in vitro SPF is useful for screening test during product development as a supplement of the in vivo SPF measure. The proposed UV spectrophotometric method is simple, rapid, uses low cost reagents and can be used for *in vitro* determination of SPF values in many cosmetic formulations. Extracts of many plants, citrus fruits and leafy vegetables as source of ascorbic acid, vitamin E and phenolics compounds and enzymes possess the ability to reduce the oxidative damage.<sup>[19]</sup> These oxidative damages ultimately reduce the protective enzyme level and imbalances the level of total protein and ascorbic acid and other antioxidant level in cells. The creams comprises such extracts could be utilized for the protection of photo-induced intrinsic oxidative stress as well as structural alteration in skin. Natural substances extracted from plants like green tea polyphenols, *aloe barbadensis* extract and aromatic



compounds isolated from lichens have been considered as potential sunscreen resources on similar grounds.<sup>[20, 21]</sup> Sunscreens contain a wide variety of chemicals that have specific absorbance in some parts of the UV spectrum. There is few single chemical substance that has absorbance over the full range of UV. This property is needed for a product to be considered as a proper wide spectrum sunscreens. The *Prunella vulgaris* extract demonstrated a concentration dependent photoprotection.<sup>[22]</sup> Extract of *Krameria triandra* root has shown protective effects against UV-B induced photodamage in human keratinocyte cells.<sup>[23]</sup> Several studies have shown the flavonoids to act as scavengers of superoxide anions, singlet oxygen, hydroxyl radicals, and lipid peroxy radicals. Phenolics are believed to be capable of acting in redoxsensitive signalling cascades to inhibit DNA damage. Many flavonoids such as quercetin, luteolin and catechins are better antioxidants than the nutrients vitamine C, vitamine E and  $\beta$ -carotene. Therefore, the phenolics may be beneficial in preventing UV-induced oxygen free radical generation and lipid peroxidation, i.e. events involved in pathological status such as photoageing and skin cancer.<sup>[24]</sup>

The phytochemical investigation of *C. longa* revealed the presence of alkaloids, glycosides, flavonoids, tannins, phenolics, phytosterols, essential oils etc.<sup>[25]</sup> The phytochemical investigation of *B. monosperma* flower revealed the presence of flavonoids include Butin, Butein, paltitrin, Flavone Glycosides Coreopsin, tannins, phenolics, flavonols, saponins etc.<sup>[13]</sup> Absorption of UV radiation is the main characteristics for identification of flavonoids in natural sources. The result showed strong-to-moderate absorption of UV radiation along the whole range and this ability may be due to the presence of flavonoids. The result obtained were showed the ability of *C. longa* sunscreen cream and *B. monosperma* sunscreen cream to absorb UV radiation and hence proved its UV protection ability.

## CONCLUSION

From the result obtained in the study we can positively conclude that *C. longa* sunscreens have significant UV absorbing property. This will be a better, cheaper and safe alternative to harmful chemical sunscreens that used now days in the industry.

## REFERENCES

1. Dutra EA, Oliveira DAGC, Kedor-Hackmann ERM, Santoro MIRM., Determination of sun protection factor (SPF) of sunscreens by ultraviolet spectrophotometry,



- Brazilian journal of Pharmaceutical sciences, 2004; 40 (3): 381-385.
2. COLIPA method for the *in vitro* determination of UVA protection provided by sunscreen products Guidelines a, 2007; 2-4.
  3. Wood C, Murphy E, Sunscreen efficacy, Glob. Cosmet. Ind, 2000, 167: 38-44.
  4. Wolf R, Wolf D, Morganti P, Ruocco V, Sunscreens, Clinic. Dermatol. New York, 2001; 19: 452-459.
  5. Hogade G, Patil B, Dhumal P, Comparative Sun protection factor determination of fresh fruits extract of Cucumber vs marketed cosmetic formulation, Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2010; 1(3): 55-59.
  6. Gasparro FP, Mitchnick M, Nash JFA, Review of Sunscreens safety and efficacy, Photochem. Photobiol. Oxford, 1998; 68: 243-256.
  7. Azevedo JS, Viana Junior NS, Soares CD, UVA/UVB Sunscreens determination by second order derivative ultraviolet spectrophotometry, *Farmaco Pavia*, 1999; 54: 573-578.
  8. Mansur JS, Breder MNR, Mansur MCA, Azulay RD, Determination of sun protection factor by Spectrophotometry, An. Bras. Dermatol, 1986; 61: 121-124.
  9. Kokate CK, Purohit AP, Gokhale SB, Textbook of Pharmacognosy, 41<sup>th</sup> Edn., Pune; Nirali Prakashan: 2008, pp. 11.109-11.111.
  10. Pandey G, Active principles and Median lethal dose of *Curcuma longa* linn., International Research journal of Pharmacy, 2011; 2(5): 239-241.
  11. Neha S, Ranvir GD, Jangade CR, Analgesic and antipyretic activities of *Curcuma longa* rhizome extracts in Wister Rats, Veterinary World, 2009; 2(8): 304-306.
  12. Singh BP, Sahu S, Antimicrobial Activity and Spectral Characterization of Flower of *Butea monosperma*, Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2012; 3(2): 598-606.
  13. Sathish R, Sravan P, Natarajan K, Sridhar N, Hepatoprotective and Anti Pyretic Activities of Methanolic Extract of *Butea Monosperma* Lam Stem Bark In Wister Rats, Asian J. Pharm. Res., 2011; 1(4): 130-133.
  14. Henry M D, Analysis of Creams and Lotions, In: Baired Edn. Manual of Cosmetic analysis, USA: 1997, pp. 32-33.
  15. Sayre RM, Agin PP, Levee GJ, Mariowe E, Comparison of *in vivo* and *in vitro* testing of sunscreens formulas, Photochem. Photobiol, 1979; 29: 559-566.

16. Butler H, Quality Stability and Safety Assurance, In: Poucher's Perfumes, Cosmetics and Soap, Netherlands; Kluwer Academic Publisher Dordrecht 2000, 507-621.
17. Riegelman S, Penna RP, Effect of vehicle components on the absorption characteristics of sunscreen compounds, J. Soc. Cosmet. Chem., 1960; 11: 280-291.
18. Agrapidis-Paloympis LE, Nash RB, Shaath NA, The effect of solvents on the Ultraviolet absorbance of sunscreens, J. Soc. Cosmet. Chem, 1987; 38: 209-221.
19. Atrooz OM, The anti-oxidant activity and polyphenolic contents of different plant seeds extracts, Pak. J. Biol. Sci., 2009; 12: 1063-1068.
20. Wang ZY, Agrawal R, Bickers DR, Mukhtar H, Protection against ultraviolet B radiation-induced photocarcinogenesis in hairless mice by green tea polyphenols, Carcinogenesis, 1991; 12: 1527-1532.
21. Rancan F, Rosand S, Boehm K, Fernandez E, Hidalgo ME, Quihot W, Protection against UV-B irradiation by natural filters extracted from lichens, J. Photochem, Photobiol, 2002; B: 68: 133-139.
22. Psotova J, Svobodova A, Kolarova H, Walterova D, Photoprotective properties of *Prunella vulgaris* and rosmarinic acid on human keratinocytes, J. Photochem. Photobiol, 2006; B: 84: 167-174.
23. Khazaeli P, Mehrabani M, Screening of Sun protective activity of the ethyl acetate extracts of some medicinal plants, Iranian Journal of Pharmaceutical Research, 2008; 7(1): 5-9.
24. Svobodova A, Psotova J, Walterova D, Natural phenolic in the prevention of UV-induced skin damage, a review, Biomed. Papers, 2003; 147: 137-145.
25. Khan MGU, Nahar K, Rahman M, Hasan M, Rashid M, Phytochemical and Biological Investigation of *Curcuma longa*, Dhaka University Journal of Pharmaceutical Sciences 2008; 8 (1): 39-45.