ORIGINAL ARTICLE

In vitro screening of the sunscreen potential of hydroalcoholic *Erythrina variegata* bark extract

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Abstract

Aim: To screen the sunscreen activity of antioxidant *Erythrina variegata* bark extract. **Materials and Methods:** Methodology included procurement, standardization and extraction of the *E. variegata* bark extract, preliminary phytochemical screening, quantification of the total phenolic and flavonoid content, and further screening its sunscreen activity in comparison with the standard sunscreens as well as marketed formulations by determining the effective absorption spectra using ultraviolet (UV) spectrophotometer. **Results and Discussion:** The results revealed that the bark extract showed the presence of various phytoconstituents such as phenols, tannins, flavonoids, alkaloids, etc. The extent of phenolic constituents was found to be higher than that of flavonoids. Further the extract showed a greater absorption of UV light mainly in the UVA and UVB region as compared to standard para-aminobenzoic acid and benzophenone. The marketed sunscreen products containing chemical sunscreens showed the absorption curves in UVA as well as UVB regions hence the herbal extract matched the requirements of the absorption curves of the chemical sunscreens. **Conclusions:** Based on the preliminary research conducted, it can be concluded that the *E. variegata* bark extract possess a potential sunscreen activity by exhibiting effective absorption spectra in the harmful UV radiation range. Thus, the chemical sunscreen agents could be replaced with the herbal extracts to formulate highly effective sunscreen preparations which will enhance and effectively contribute to the UV absorbing properties thereby overcoming the discomfort and unwanted reactions associated with chemical sunscreens.

Key words: *Erythrina variegata* bark, hydroalcoholic extract, photo-absorption, sunscreen, ultraviolet protection

INTRODUCTION

Exposure of human skin to ultraviolet radiations (UVRs) is etiologic in production of reactive oxygen species, oxidizing tissue lipids, and proteins to per-oxy radicals. These highly reactive species are sequestered by antioxidants. The skin membranes endogenously synthesizes some of the antioxidants like glutathione, however an external supply of molecules is required to combat the UV-induced oxidative stress and skin damage. Synthetic antioxidants have been reported for their adverse effects. Phytoantioxidant sunscreens thus could be advantageous. The previous studies revealed high antioxidant activity of *Erythrina variegata* bark extract against free radicals.[1] Thus, the present research aims to evaluate whether the test extract possess the ability to absorb the harmful UVRs, and thereby be revealed as a herbal UV absorber sunscreen.

MATERIALS AND METHODS

Collection and Authentication of the Plant Specimen

Fresh bark of *E. variegata* plant was collected from Boisar area, District Palghar, Maharashtra, India. The plant was further identified and duly authenticated by a botanist.

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Preparation and Extraction

Bark of the plant was de-dusted, chopped, and shade dried at room temperature. Further the plant part was subjected to extraction by maceration technique. Coarsely grinded bark powder was moistened using hydroalcoholic solvent (water and ethanol [Pallav Chemicals, India] mixture, 3:7) and further sufficient solvent was added to allow the complete diffusion of the menstruum. The flask was sonicated using a bath sonicator (Inco, India) and then kept on orbital shaker (Neo Lab, India) for specified time at 80-90 rpm for complete maceration. Macerated slurry was filtered using vacuum. Filtrate, thus obtained was concentrated by evaporation of the solvent to get the dried extract. The liquid extracts were further subjected to the preliminary phytochemical screening using simple chemical tests to determine the presence of various phytoconstituents.

Quantitative Phytochemical Analysis

Estimation of total phenol content

The total phenol content in the hydroalcoholic *E. variegata* bark extract was estimated using Folin–Ciocalteu's reagent (Pallav Chemicals, India) method. A volume of 0.5 ml of the respective hydroalcoholic bark extract obtained by maceration technique (1 mg/ml test extract in hydroalcoholic solvent) and 0.1 ml (0.5 N) Folin–Ciocalteu's reagent were mixed, and the mixture was further incubated at room temperature for about 15 min. As the reaction preceded 2.5 ml volume of the saturated sodium carbonate solution was added and further incubated for 30 min at room temperature. Further the absorbance of the final mixture was measured at 760 nm using UV-Visible Spectrophotometer, Shimadzu UV 1800 double beam instrument. Gallic acid (Sigma Aldrich, USA) a known phenolic compound was employed as a reference standard. The standard curve of the gallic acid was plotted and used in the estimation of the phenols in the test extract as described in Figure 1. The total phenol values were thus expressed in terms of gallic acid equivalent (mg/g of the extracted compounds). The assay was performed in triplicates and the mean values are presented as ± standard deviation (SD)[2] using GraphPad Prism-5 software.

Estimation of total flavonoid content

The concentration of flavonoid content in the hydroalcoholic *E. variegata* bark extract was estimated using aluminum chloride colorimetric method. The reaction mixture constituted 3 ml volume in which the 1 ml volume of hydroalcoholic bark extract obtained by maceration technique as sample (1 mg/ml test extract in hydroalcoholic solvent) was taken and mixed with 0.5 ml volume of (1.2 % w/v) aluminum chloride (Loba Chemicals, India), 0.5 ml volume of (120 mM) potassium acetate (Loba Chemicals, India), and 1 ml volume of distilled water was incubated at room temperature for 30 min. The absorbance of the resultant samples was measured at 415 nm using UV-Visible Spectrophotometer, Shimadzu UV 1800 double beam instrument. Quercetin (SD Fine Chemicals, India) a known flavonoid was used as the standard. The standard curve of the quercetin was plotted and used in the estimation of the flavonoid content in the test extract as described in Figure 2. The total flavonoid content is expressed in terms of quercetin equivalent (mg/g of the extracted compounds). The assay was performed in triplicates and the mean values are presented as ± SD[2] using GraphPad Prism-5 software.

Effective Absorption Spectra Measurements

Dried extract of the bark of *E. variegata* plant obtained using the maceration technique was dissolved in hydroalcoholic mixture at a concentration 5 mg/100 ml-20 mg/100 ml. The solutions were further filtered using Whatman filter paper (GE Healthcare, India) to remove any gross plant debris. Similar concentration solutions using accurately weighed quantity of p-amino benzoic acid (Loba Chemicals, India) and benzophenone (Loba Chemicals, India) were prepared in distilled water both serving as standard drugs. Marketed formulations EKRAN 30® lotion (Wonder Products, India) and SUNKARE 50® Gel (Aurochem Laboratories, India) were also

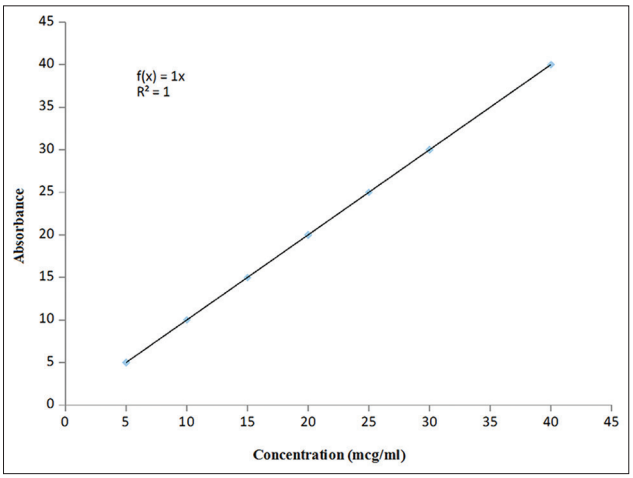

Figure 1: Standard curve of gallic acid

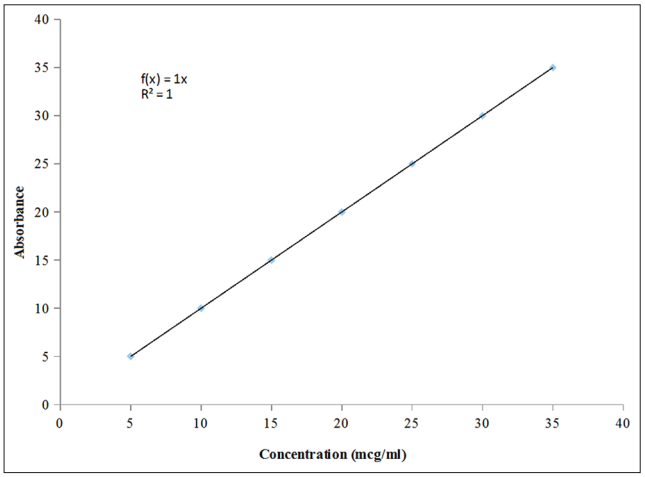

Figure 2: Standard curve of quercetin

used for comparison. Further the spectra of the extracts, standard and marketed formulations were measured using Shimadzu UV 1800 double beam UV-Visible spectrophotometer in the range 200-400 nm employing reference cell containing respective pure solvents. The data thus provided details about the effective absorbing region (sunscreen effective spectrum) and the λ_{max} of the individual test solutions.[3] The observations of the study are described in the Figures 3-7.

RESULTS

The preliminary phytochemical screening of the hydroalcoholic extracts of the barks of *E. variegata* plant revealed the presence of tannins, alkaloids, carbohydrates, amino acids, steroids, and flavonoids as given in Table 1.

The extractive yield was estimated to 24 g extract per 100 g of crude bark powder. Total phenolic content was estimated to 350.6 ± 0.62 mg/g of gallic acid equivalent/g of dry extract. The values obtained using the standard curve of gallic acid (y = 0.013x + 0.448). Further, the total flavonoid content of the bark extract computed to 43.83 ± 0.45 mg/g of quercetin equivalent/g of dry extract. The standard curve of quercetin used was y = 0.018x-0.014. Comparatively hydroalcoholic *E. variegata* bark extract constitutes more total phenolics than the flavonoids.

UV spectrum obtained using hydroalcoholic extract of *E. variegata* bark at a concentration of 20 mg/100 ml exhibited a λ_{max} at 286 nm and 275 nm, with also a moderate absorbance in the range of 290-400 nm which is specifically UVB and UVA region. Para-aminobenzoic acid and benzophenone used as a standard sunscreen agents showed a λ_{max} at 265 and 257 nm, respectively, and the absorption spectra were found to degrade very rapidly showing negligible absorbance over 300 nm. The spectrums of the marketed formulations EKRAN 30® lotion gave λ_{max} at 298nm, 310 nm and SUNKARE 50® Gel gave λ_{maxb} at 292 nm, 308.5 nm wavelength, respectively, further the absorbance in both spectrums was very good up to 350 nm. The figures given below thus represent the representative absorption curves obtained at a concentration of 20 mg/100 ml.

The results thus obtained proved to be beneficial in determining the potential sunscreen agents obtained from herbal source.

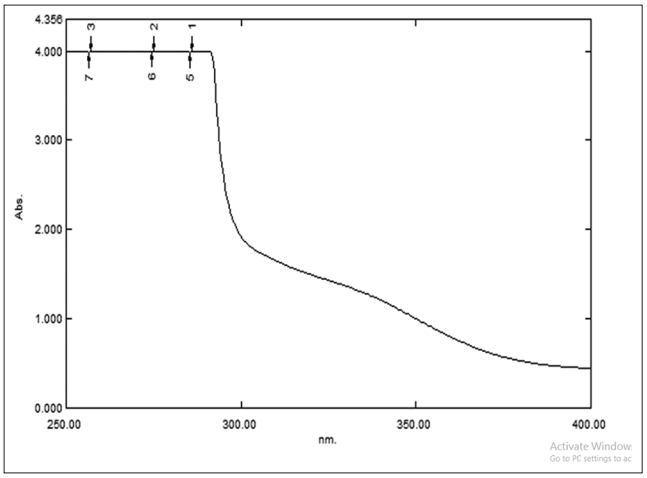

Figure 3: Spectra of *Erythrina variegata* hydroalcoholic bark extract

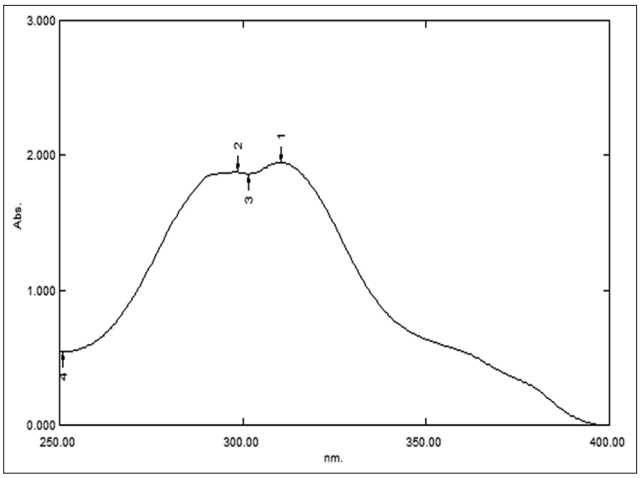

Figure 4: Spectra of EKRAN 30® lotion

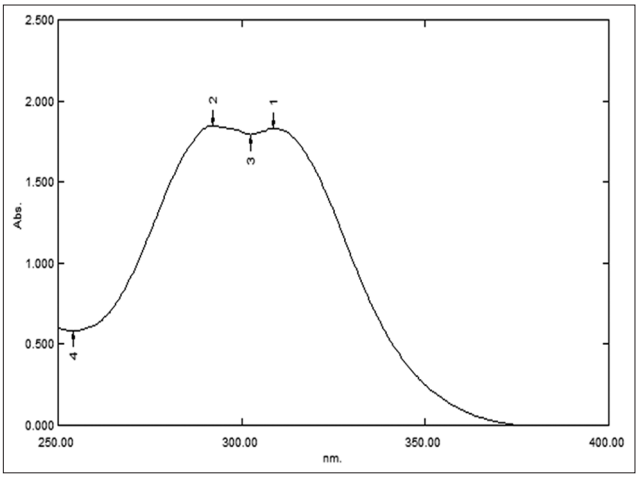

Figure 5: Spectra of SUNKARE 50® Gel

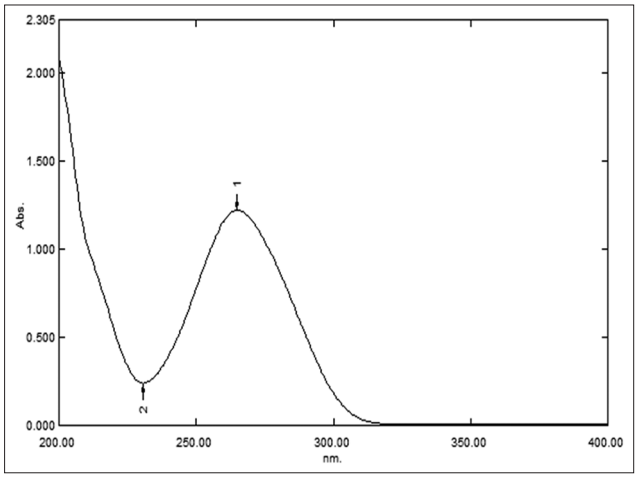

Figure 6: Spectra using standard: Para-amino benzoic acid

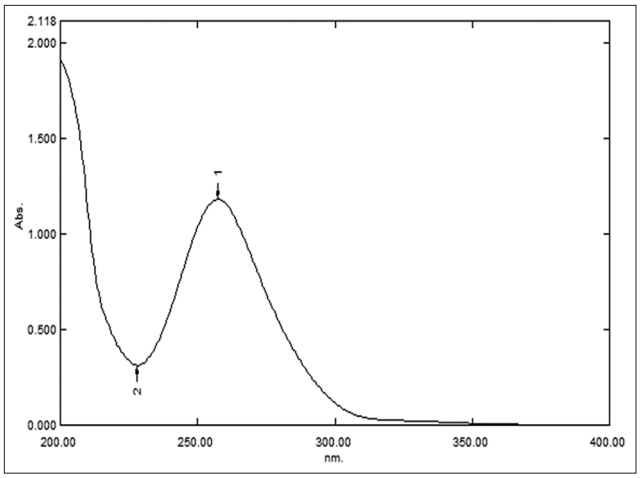

Figure 7: Spectra using standard: Benzophenone

DISCUSSION

UVRs or solar radiations are electromagnetic radiations with wavelength in the range of 200-400 nm. These radiations are classified according to the wavelength as UVA (320-400 nm), UVB (290-320 nm), and UVC (100-290 nm). Prolong and unprotected UVR could exhibit marked short term as well as long term effects on the skin. Sunscreen formulations mostly contain compounds which are highly effective in absorbing UVB and thereby not providing any protection against UVA radiations. Exposure to UVB radiations can produce DNA damage. Although UVA radiations are less energetic and less erythemogenic than UVB rays, UVA rays penetrate the skin more deeply than the UVB rays and are consequently capable of causing oxidative damage to the deeper portions of the skin tissues. UVA rays also magnify the damage caused by UVB rays to the skin tissue. UVA-induced reactive oxygen species generation and further damage caused to the DNA is mediated by formation of a modified guanine nucleotide (8-hydroxyguanine), lysis of single-stranded as well as oxidation of pyrimidine bases.[4,5] Therefore, protection against the UVA rays becomes important as well.

E. variegata also called Erythrina indica is a thorny deciduous tree growing to 60 feet tall. It belongs to the family *Fabaceae* (Legume family). It is also commonly known as Coral tree, Indian coral tree, tiger's-claw (English).[6] Traditionally, it has been used as a nervine sedative, febrifuge, antiasthmatic, and antiepileptic. In some experiments, it has potential effects for treatment of some diseases such as convulsion, fever, bacterial infection, insomnia, helminthiasis, cough, antibacterial[7]/dental caries prevention, cuts and wounds, antioxidant, analgesic and anti-inflammatory, cardiovascular diseases, central nervous system diseases, and antiosteoporotic effects,[8] as a smooth muscle relaxant and in calcium homeostasis.[6]

The selection of the solvent in extraction of the crude *E. variegata* bark powder as hydroalcoholic mixture was

Table 1: Preliminary phytochemical screening of the *Erythrina variegata* bark extract

Constituents	*Erythrina variegate* bark extract
Triterpenoids: Hirshonn reaction	+
Saponins: Foam test	+
Steroids	
Lieberman–Burchard test	+
Lieberman test	–
Salkowski reaction	–
Phenols	+
Flavones	
Shinoda test	+
NaOH	+
Flavanones and coumarins	+
Anthocyanins	–
Carbohydrates	
Molish's test	+
Reducing sugars	+
Benedict's test	
Alkaloids	
Dragendroff's test	+
Wagner's test	+
Mayer's test	+
Hager's test	+
Quinones	+
Tannins	
Ferric chloride test	+
Basic lead acetate	+
Proteins	
Biuret	+
Millon's test	+
Amino acids	
Ninhydrin	+
Tyrosin	–
Gycosides	
Anthraquinones glycosides	
Borntrager's test	+
Modified Borntrager's test	–
Cardiac glycosides	
Legal's test (cardenoloids)	+
Keller–Killiani test (deoxy sugars)	+
Liebermann' test (bufadenoloids)	–
Saponin glycosides	
Foam test	+

based on the literature review and prior reported antioxidant activity. The hydroalcoholic bark extract obtained from *E. variegata* plant exhibited good absorbance in the range 290-400 nm which is specifically UVB and UVA region which is superior as compared to its synthetic counterparts like para-aminobenzoic acid and benzophenone whose curve degrades very rapidly thereby showing negligible absorbance over 300 nm. As compared to marketed preparations also the bark extract have proved a potential UV absorber in UVA and UVB region. The presence of polyphenolics, flavonoids, and tannins responsible for their antioxidant activity through free radical scavenging activity could be the potential actives in the *E. variegata* bark extract. The presence of these phytoconstituents are reported in the literature and also verified from the preliminary phytochemical tests. Isoflavones isolated from the bark extracts mainly constitutes erythrinins A, B and C, osajin, alpinum, styrene, oxyresveratrol, dihydroxystilbene and dihydroxyresveratrol, etc.[6] Flavonoids, like phytoconstituent, are also potential UV protective.[9] The hydroalcoholic bark extract obtained from *E. variegata* thus provides an anti-oxidant mediated sunscreen effect to protect the skin against the photo-induced damage. However, the extract could be further incorporated in a formulation and subjected for quantifying the sunscreen activity by computing the sun protection factor.

CONCLUSIONS

The hydroalcoholic extracts of *E. variegata* bark were found to be good and highly effective absorbents of UV rays in the UVB as well as UVA region. Thus, we can optimistically conclude that the hydroalcoholic bark extract of *E. Variegata* contains photo-absorptive compounds which when incorporated in an herbal formulation can result in an exceedingly effective sunscreen preparation showing a protective action throughout the broad UV region. The extracts may also be used along with established standard drugs, enhancing and effectively contributing to UV absorbing properties of the sunscreen. This will in turn also help in widening the UV protection ability of conventional sunscreen formulations. This replacement with herbal extracts will also help overcome the discomfort and unsolicited reactions, especially in patients suffering from skin cancers. Moreover, the synthetic pathway adopted for the synthesis of chemical sunscreen agents that generates a lot of harmful waste could be minimized, thus, favoring Green Chemistry.

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