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IN Vitro SUN PROTECTION FACTOR DETERMINATION FROM DRIED LEAVES OF *Elephantopus scaber* L. USING ETHANOLIC EXTRACT

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ABSTRACT

The present study focused on evaluation of photo-protective activity of ethanolic leaf-extract of *Elephantopus scaber* L. The extract was subjected to UV Vis Spectrophotometer for obtaining spectral pattern. The spectral pattern was indicative of presence of such phenolic derivatives in the leaves which are known to have sun-protective property. FT IR result also speaks in the same way i.e. presence of wave number such as 3336 cm^{-1}characteristic phenolic O-H stretched with the aliphatic groups. Presently in pharmaceutical research, sunscreen and sun blocker are given the top most priority to address increasing exposure to UV radiations in the global scenario of ozone depletion. Extensive efforts are on the way to explore, isolate, characterize and market effective sunscreen compounds especially from plant sources. Te efficacy of sunscreen is expressed in terms of SPF value. In the present study leaf extracts of *Elephantopus scaber*, a plant of the family Asteraceae, exhibits SPF value ranging from 4.71-5.29, thus speaking of its ability to effectively block UV B by 75%- 83%. As such, the extract certainly is a very good candidate for getting used by pharmaceuticals in sunscreen preparations.

KEYWORDS : Photoprotective Activity, *Elephantopus scaber*, Phenolic Derivatives, SPF, Sunscreen Preparation

Now a day's Sunscreens with a higher SPF should offer more protection from the sun's harmful ultraviolet (UV) radiation, which is linked to the vast majority of skin cancers, as well as premature skin aging and eye damage. But the answer is not that simple. UV radiation reaches the earth in the form of UVB and UVA rays. The name ultra violet arise from Latin meaning "beyond violet", violet being the color of the shortest wavelengths of visible light. The ultraviolet radiation (UVR) is composed of three different category: ultraviolet A (UV-A, 320- 400 nm), ultraviolet B (UV-B, 280- 320nm) and ultraviolet C (UV-C, 200-280 nm) (Polonini et al., 2011). Various studies show the great influence of solar radiation on skin. Between these UV-A and UV-B are mainly responsible for skin hazards such as sunburn, cutaneous degeneration, cell skin cancer (Balogh et al., 2011). SPF measures sunscreen protection from UVB rays but not measure how well a sunscreen will protect from UVA rays, which are also damaging and dangerous. Dermatologists recommend using a SPF15 or SPF30 sunscreen. Higher SPFs donot give much more protection. UVR responsible to form a complex process associated with morphological and chemical reactions, as DNA is an important macro molecule which absorbs UVR and resulted to cause mutate, what in the future can result in malignant trans-formation of the cell skin cancer The effective protection against UVR is available as preparations for topical use containing solar filters, known as sunscreens. The efficacy of such products is dependent on their capacity to absorb radiant energy. The effectiveness of a sunscreen is measured as a function of their sun protection factor (SPF). Thus, the necessity to provide high SPF and screening efficiency against both ultraviolet A and ultraviolet B wavelengths is evident (Vilela et al., 2011). The SPF of Calendula oil extracted from *Calendula officinalis* in cream formulation possess good activity i.e SPF = 14.84 ± 0.16 (Mishra et al., 2012).

Flavonoids compounds are widely distributed in the plant kingdom and possess a biological action, especially antimicrobial, antioxidant and photo protective activities (Pietta, 2000; Lacombe et al., 2010). The demand for active flavonoid rich extracts has become an important component for the discovery of new molecules active to human photo-protection. That is due to its structural similarity to chemical filters which makes it susceptible to radiation absorption in the ultraviolet region (Agati et al., 2013). Plant extracts rich in flavonoids are capable of absorbing ultraviolet light, usually two maximum peaks of ultraviolet absorption in the UV-B and UV-A regions, what results in the possibility for the use of these extracts in the development of sunscreen formulations (Bobin et al., 1995). *Elephantopus scaber* L. belongs to the family Asteraceae is famous for having several medicinal

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properties (Das and Mukherjee., 2014 and Das and Bandyopadhyay., 2015). The objective of the present study is to preliminary assay of sun protection activity of the plant.

MATERIALS AND METHODS

Plant Material

Fresh leaves of plant specimen i.e *Elephantopus scaber* was collected randomly from their naturally occurring sites e.g Golapbag campus of the University of Burdwan is located at 23.25° N and 87.85° E, it has an average elevation of 40 meters (131 ft) , West Bengal, India.

The plants were identified and the voucher specimens (MD 01 and MD 02) were kept in the department herbarium i.e herbarium of Burdwan University (BURD) for the future references. Mature leaves (of pre flowering and post flowering stages) were washed thoroughly under running tap water, blotted dry and then further dried in hot air oven at 50°C temperature.

Sample Preparation

The dried powdered leaves of these plants (10 g) were extracted in 100 ml of 95% ethanol. The resultant ethanolic extract was filtered through a vacuum filter and the filtrate was defatted using hexane. The ethanolic portions were dried and used for analysis.

Total Phenolic Content

Total phenolic contents of the extracts (three replicates per treatment) were assayed using the Foline Ciocalteu reagent and expressed as mg gallic acid equivalents per gram (mg GAE/g) through the calibration curve with gallic acid. The calibration curve range was 50 to 1000 mg/l (R^2= 0.9907). All samples were performed in triplicates.

Determination of the Maximum Absorption Wavelength and Sun Protection Factor (SPF) in *vitro*

The efficacy of a sunscreen is usually expressed by the sun protection factor (SPF) which is defined as the UV energy required to produce a minimal erythema dose (MED) on protected skin divided by the UV energy required to produce a MED on unprotected skin.

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

The minimal Erythemal dose (MED) is defined as the lowest time interval or dosage of UV light irradiation sufficient to produce a minimal, perceptible erythema on unprotected skin (Aburjai and Natsheh, 2003; Bendova et al., 2007). The higher the SPF, the more effective is the product in preventing sunburn. In vitro screening methods may represent a fast and reasonable tool reducing the number of in vivo experiments and risks related to UV exposure of human subjects, when the technical test parameters are adjusted and optimized.

The in vitro SPFs were determined according to the method described by Mansur et al. (1986).The absorption spectra of samples in solution were obtained in the range of 290 to 320 nm using 1 cm quartz cell, and ethanol as a blank. The absorption data were obtained in the range of 290 to 320, every 5 nm, and 3 determinations were made at each point, followed by the application of Mansur equation (Elizangela et al., 2004; Ashawant et al., 2008) where EE(λ) is the erythemal effect spectrum, I(λ) is the solar intensity spectrum, Abs (λ) is the absorbance of sunscreen product; CF is the correction factor (=10). The value of EE × I are constant, determined by Sayre et al. 1979.

FT-IR Analysis of Sample

Fourier transform infrared spectroscopy (FT-IR) is one of the most powerful approaches to identify and characterize the type of chemical bonds (functional groups) present in compounds. The ethanol extracts of these plants were mixed with KBr salt using a mortar pestle and compressed into thin tablets and IR spectra and peaks were recorded on a Perkin Elmer FT-IR (model RX1) spectrometer between 4000-400 cm. Each analysis was twice done for conirmation.

RESULTS AND DISCUSSION

Phenol Content

The leaf extract of *Elephantopus scaber* extract possess higher phenolic content (39.89 µg/ml) in pre flowering stage whereas phenolic contect degrade in the leaf of post flowering stage (38.19 µg/ml).

Determination of the SPF Value

When SPF content was assayed it was found that SPF content is higher i.e 5.29 in pre flowering stage and

Table 1: Table Showing the Determination of SPF Value of the Ethanolic Leaves Extract of Pre Flowering Stage

Wave length (nm)	EE (λ) X I (λ) Employed	Absorbance (A)	EE (λ) X I (λ) X Absorbance (A)	SPF= Σ EE (λ) X I (λ) X (A) X 10 (Correction Factor)
290	0.0150	0.600±0.003	0.009±0.00005	5.535±0.033
295	0.0817	0.571±0.003	0.04665±0.00025	
300	0.2874	0.551±0.003	0.15836±0.00086	
305	0.3278	0.548±0.003	0.1796±0.00098	
310	0.1864	0.549±0.004	0.1023±0.00075	
315	0.0837	0.562±0.004	0.0470±0.00033	
320	0.0180	0.589±0.004	0.0106±0.00007	
Σ EE (λ) X I (λ) X (A) = 0.55351±0.0033				

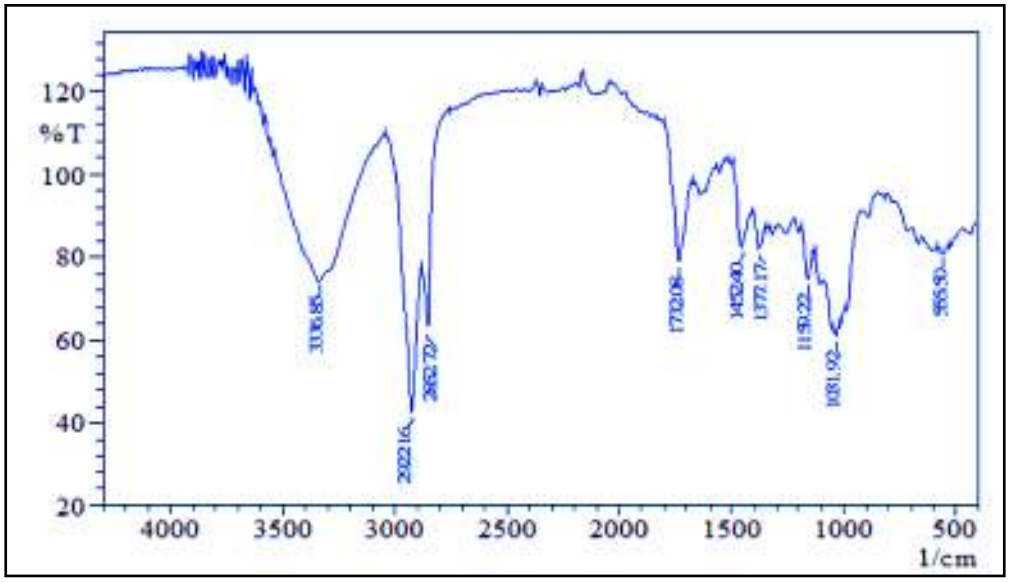

Figure 1 : FT IR Spectrum of Ethanolic Extract of *Elephantopus scaber* Leaves

slightly lower i.e. 4.71 in post flowering stage (Table 1& 2).

Fourier Transform Infrared Spectra of Ethanolic Extracts: The data of FT-IR analysis shows approximately similar pattern with characteristic wave numbers of phenolics antioxidants i.e. Phenolic (O-H) stretch (3336^{-}1 cm), Carboxylic acid (O-H) stretch/ Alkane (C-H) stretch i.e 2922.16^{-}1 cm and 2852.72^{-}1 cm, Carbonyl (C-O) stretch i.e 1732.08^{-}1 cm, Aromatic (C=C) stretch i.e 1452.40^{-}1 cm and Alkane (-C-H) bending i.e 1377.17^{-}1 cm. Multiple peaks of aromatic amine antioxidants that indicate uniformity of spectral properties of ethanolic extracts of across three different phytosources (Figure 1).

CONCLUSION

The outcome of this study speaks the possibility to use this extract as sunscreen in pharmaceutical preparations. FT-IR analysis reveals presence of characteristics phenolics (presence of aliphatic group with characteristic wave number at 3366.85) which are known to play critical role against sunray induced damage. The principal ingredients in sunscreens are usually aromatic molecules conjugated with carbonyl groups (presence of carbonyl group with characteristic wave number at 1732.08). This general structure allows the molecule to absorb high energy UV rays and release the energy as lower

energy rays, thereby preventing the skin-damaging UV rays from reaching the skin. The extracts had sun protection factor (SPF) 4.71 and 5.29. It shows the possibility to use this extract as sunscreen in pharmaceutical preparations. Further research will be conducted to reach the substance responsible for photoprotective activity of extract. Recent trends in pharmaceuticals research shows that the top most priority are given to sunscreen and sun blockers Extensive efforts are underway to explore, isolate, characterize and market effective sunscreen especially from phytosources. The efficacy of sunscreen is expressed in terms of SPF value. SPF actually denotes the number of times longer an individual may stay under the sun before getting skin burn i.e. erythema. The value SPF 15 means that a person who can normally stay in sun for 20 minutes before sun burn can now stay in the sun for 5 hours. It has been reported that SPF 2, 4, 6, 8 and 15 can block UV B with 50%, 75%, 83%, 88% and 93% respectively (URL: http://msykes.com/things/sunscreen/). In this context, our extract with SPF value ranging from 5 to 7 might effectively block UV B by 75% - 83%. Thus the extracts surely hold possibility as effective sun screen.

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