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Research Article

SCREENING METHANOLIC EXTRACTS OF *BETA VULGARIS* ROOTS FOR PHOTOPROTECTIVE ACTIVITY

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ABSTRACT

Herbal cosmetic products incorporated with botanical extracts have become very popular in the market due to their effectiveness and intrinsic satisfaction that even after their routine use they do not lead to any side effects. The present study involved the photoprotective analysis of the gel formulation prepared from the extract of *Beta vulgaris*. The methanolic extract of cortical region of beet root was assessed for the concentration of flavonoids (29.04±1.46 mg/gm), total phenolics (660.5±29.41mg/gm) and free radical scavenging potential (IC_{50} = 8.02mg/ml). Photo-protective activity of the extract was confirmed when addition of beet root extract to paprika containing jelly delayed its discolouration on exposure to UV radiation. The sunscreen efficiency of the herbal gel was then assessed by evaluating SPF and comparing its absorbance with photoprotective cream already being marketed. The investigations clearly indicate its use as a potential sunscreen agent.

INTRODUCTION

Sunscreens have been used since decades to provide shield against UV exposure, specifically UV-A (320-400nm) and UV-B (280-320nm). UV exposure has been implicated to penetrate deep into the layers of epidermis and dermis resulting in generation of ROS[1-4]. This ultimately induces oxidative stress causing sunburns, immune-suppression, premature aging, wrinkles formation and even skin cancer in the worst cases [5-8]. Sunscreen compounds are therefore incorporated in several cosmetic products including creams, shampoos, moisturizers etc. These days the acceptance of herbal cosmetic products has increased among the users due to their less irritant and harmful effects in comparison to chemical based sunscreens. Researchers have revealed the use of natural compounds having antioxidant and photoprotective properties in cosmetic products for sunscreen purposes[8]. Several studies have shown that naturally occurring antioxidants like carotenoids, flavones, flavonoids and phenolics have the ability to break free radical chain reactions imparting photoprotection against UV radiations[8, 10-14].

Beta vulgaris (a member of family Chenopodiaceae) has popularly been used as a colorant in food industry due to presence of betalin pigments. Besides this, researchers have also been studying its free radical scavenging activities and have shown it to be rich in antioxidants by virtue of presence of compounds like carotenoids, folic acids, phenolics, and flavonoids[15-17]. A decrease in the levels of lipid peroxidation has been reported in mice, when their diet is supplemented with 8% freeze dried red beet root leaves[18]. The present study was thus designed to concentrate on the preliminary evaluation of the methanolic extract of beet root for its use in cosmetics. This was done by estimating the photoprotective efficiency of the gel formulation prepared using the extract as well as calculating and comparing its SPF with commercially available sun protecting cream.

MATERIALS AND METHODS

Extraction of plant material

The fresh beet root collected from the local market of Delhi were washed and peeled off. 30gm of the cortical region of the root was homogenized in 60ml of absolute methanol using mortar pestle and incubated overnight at room temperature. The cortex area was chosen based upon the prior analysis done in our lab where cortex was found to be rich in antioxidants in comparison to the pith area[19]. The homogenate was centrifuged at 5000 rpm for 10 minutes and the pellet was discarded. The supernatant was allowed to evaporate at room temperature and the paste obtained was stored at 4°C for further analysis.

Determination of total phenolics content

Total phenolic content was determined by the Folin-Ciocalteau method[20]. 0.5 ml of diluted extract (5 mg/ml) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and incubated for 5 minutes after which aqueous Na_2CO_3 (4 ml, 1 M) was added. The mixture was allowed to stand for 15 minutes and the absorbance was taken at 765 nm. The standard curve was prepared using different dilutions of gallic acid in methanol (0.05 to 0.25 mg/ml) and total phenolic values were expressed in terms of gallic acid equivalent.

Determination of total flavonoid content

Colorimetric Aluminum Chloride method was used for flavonoid determination[11]. Briefly, 1ml of the plant extract (5mg/ml) was mixed with 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water, and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with a double beam UV/Visible spectrophotometer. Quercetin at the concentration of 12.5 to 100 mg/ml in methanol was used as a standard. Total flavonoid contents were expressed as quercetin equivalents.

DPPH radical-scavenging activity

The stable 1, 1-Diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activities of the extracts[21]. 100 µl of different concentrations of beet root extract (5-40mg/ml of the sample) were added to 900 µl of methanolic solution of DPPH (0.1mM). After incubation for 15 minutes at room temperature, the absorbance was recorded at 517 nm. The % inhibition or % DPPH scavenging activity was calculated from the following formulae:

$$\% \text{ DPPH scavenging activity} = [A_{cont} - A_{test} / A_{cont}] \text{ X } 100$$

Where A_{cont} represents the absorbance of control and A_{test} represents the absorbance of test sample. Control was DPPH solution without the plant extract.

On the basis of the % DPPH scavenging activity, the IC_{50} value was also calculated which denotes the concentration of sample required to scavenge 50% of DPPH free radicals.

Preparation of sunscreen gel

Gel formulation containing beet root extract was prepared[22]. For preparation of plain gel, carbopol-934LR (1%) was soaked in distilled water. After 15 minutes, methyl paraben (0.05%), extract (45%), glycerine (5%), and triethanolamine were added with continuous tituration. Based upon the IC_{50} value of the extract, its

concentration in the gel was kept as 10 mg/ml. The formulation was made up to 20 ml using water to get a homogeneous dispersion of the extract in the gel.

Analysis using UV induced Discoloration

The model system, based on UV-induced discoloration of paprika gel (*Capsicum annuum*)[23] was used to check the efficiency of phenolic antioxidants in protecting paprika carotenoids against UV induced discoloration. Gelatin (20 mg/ml) was added to water and heated at 80°C for 15 minutes and then at 50°C for 15 minutes to prepare a jelly like substance. Paprika was then solubilized in acetone and added to jelly (0.5 gm/l). A reference gel was also prepared by adding acetone to jelly in place of paprika. The jelly media were poured into petri dishes with one containing the reference jelly, other containing the extract and next containing same amount of methanol so as to act as control. These petri dishes were incubated overnight in dark and were then exposed to UV radiation in bio-safety cabinet for a period upto 45 minutes. Photographs were taken after regular intervals of 15 minutes.

Estimating Sun Protection Factor

The efficacy of a sunscreen is usually expressed by the Sun Protection Factor (SPF). An *in vitro* method of determining SPF of the sunscreens is by using the following equation[24]

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

Where: EE – erythemal effect spectrum; I –solar intensity spectrum; Abs (l) - absorbance of sunscreen product; CF – correction factor (= 10).

This was used for determining the SPF of the gel formulation prepared. For this, 1 gm of finished preparation was diluted to form different dilutions ranging from 25 to100 µg/ml in absolute ethanol. The absorbance values of the diluted samples were obtained in the range of 250 to 400 nm using 1 cm quartz cell, at every 5 nm interval. Ethanol was used as a blank. The absorbance values from 290 to 320 nm were used for calculating the SPF. The values of the normalized product function ($EE \times I$) documented by Dutra and co-authors[24] were used for calculations. A commercially available herbal sunscreen (denoted as CS) was randomly selected. The absorbance values of 200 µg/ml of CS were also recorded in the similar manner in order to compare with the prepared gel formulation.

RESULT AND DISCUSSION

A number of herbal cosmetic products containing sources of natural antioxidants e.g. green tea, aloe vera and pomegranate are available in the market as sunscreens, anti-wrinkle and moisturizing lotions[8]. Beet roots have also been reported to be rich in antioxidants compounds[16]. Its juice has been found to counteract the xenobiotic-induced oxidative stress in rats by rejuvenating the activity of the majority of antioxidant enzymes in the liver[25]. The estimation of total phenolics and flavonoids in beet root extract supports the previous observations. Table-1 shows the amount of flavonoids and total phenolics present in the extract obtained from fresh samples. The extract has the tendency to scavenge free radicals with an IC_{50} value of 8.02mg/ml.

Table 1: The amount of phenolics and flavonoids in beet root extract

Sample	TPC (mg/g of sample)	FC (mg/g of sample)
Beta vulgaris	660.5±29.41	29.04±1.46

(Results are expressed as mean ± SD of three determinations)

Oral administration of betanin to mouse has been shown to help in preventing UV-B induced skin cancer[26]. This photoprotective activity of beet root extract against UV radiation was tested by observing its effect on UV-induced discoloration of paprika. The natural extracts of rosemary, oregano and green tea have been shown to delay the photo-oxidation of paprika carotenoids preventing its discoloration[23]. The methanolic extracts of beet root also showed similar effects by preventing the discoloration of jelly containing paprika. The colour of gels containing paprika started fading when they were exposed to UV radiation. The gels without beet root extract discolourised much faster in comparison to the gel incorporated with the extract (Figure 1). This indicates beet root extract to have photoprotective potential which delays the degradation of paprika carotenoids on UV exposure.

On the basis of the IC_{50} value of the beet root extract, herbal sunscreen was prepared by incorporating beet root extract at a concentration of 10 mg/ml. The resulting formulation was red in colour and was properly absorbed by the skin after application. This being a preliminary investigation, no preservatives were added to the formulation in order to increase its shelf life.

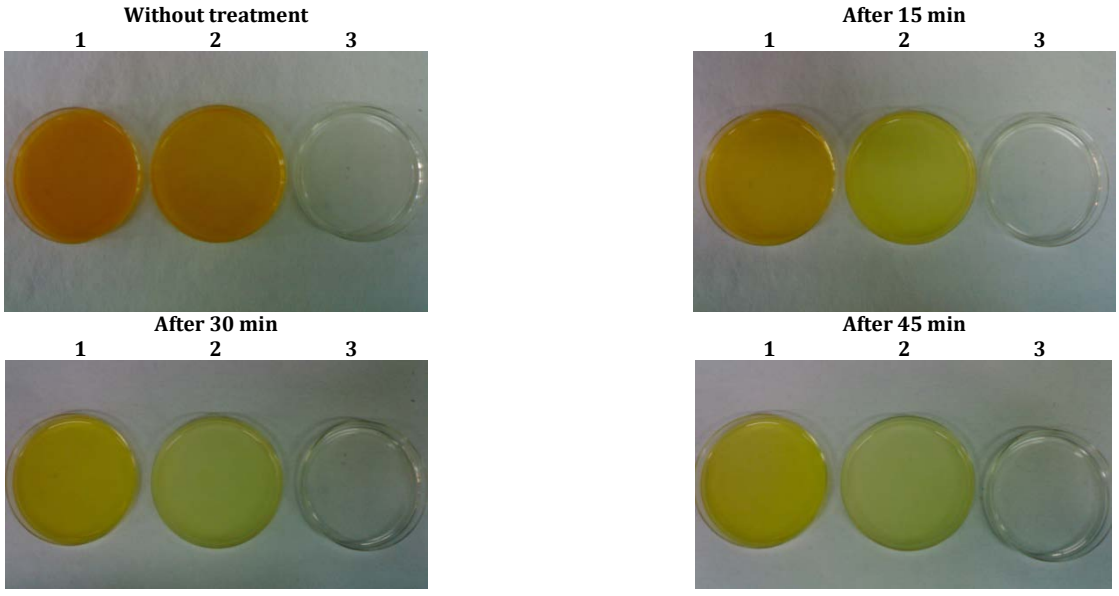

Fig. 1: Pictures showing effect of UV exposure on the gels containing Paprika and extract where:

Gel 1 contains paprika and the beet root extract,

Gel 2 contains only paprika,

Gel 3 is the reference gel without paprika and beetroot extract

SPF determination of the prepared gel formulation was done using UV spectrophotometric method which was calculated to be 1.34. Along with SPF determination, spectrophotometric analysis of gel formulation containing beet root extract was done. For this, the absorbance values of different dilutions of the gel formulation (25-150 µg/ml) were estimated. The results obtained (Figure-2) indicate that 150 µg/ml dilution of the prepared gel formulation had higher absorbance values in comparison to 200 µg/ml dilution of the commercially available sunscreen. A continuous increase in the absorbance values with an increase in the concentration of the formulation under study signifies that the absorbance of the formulation depends on the concentration of beet root extract. So, by varying the concentration of beet root extract added to the gel formulation, it may serve different purposes.

The prepared formulation was stable for around 3-4 weeks both at 4°C and room temperature i.e. during the course of the experiments done in triplicate, after which the gel started degrading. So, addition of preservative compounds to the gel formulation needs to be standardized in order to increase its shelf life. Further the physiochemical analysis of the formulation like its spreadability, viscosity and stability at different conditions has to be evaluated as done in case of creams containing *Nyctanthes arbortristis* and *Tagetes erecta*[27].

The characterization and isolation of the specific antioxidant compounds possessing sunscreen activity from the beet root extract would further help in formulating a more efficient photoprotective formulation.

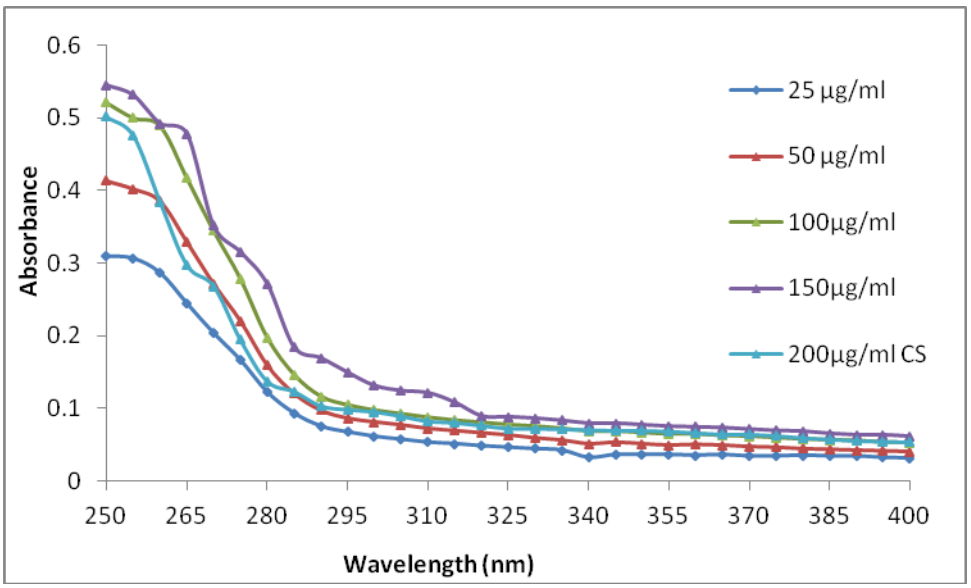

Fig. 2: Ultraviolet absorbance values of beet root gel formulation at different concentrations.

Our results clearly show that incorporation of beet root extract into gel formulation alone or in addition to another photoprotecive agent can be used to prepare different types of sunscreens which can serve a variety of purposes. Such work would hence help in adding new products to meet the growing demand for herbal cosmeceuticals in the market.

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