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Stability, Antioxidative and Pharmaceutical Potentials of Porphyra-334 Isolated From a Hot-Spring Cyanobacterium *Nostoc* sp. Strain HKAR-2

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ABSTRACT

Ultraviolet radiation has pronounced detrimental effects on all the sun-exposed organisms, including humans, since it is responsible for various types of cellular photodamage. To counteract their adverse effects, cyanobacteria has developed the ability to synthesize mycosporine-like amino acids, natural bioactive photoprotective compounds, having pharmaceutical and biotechnological potentials. The stability and antioxidative potentials of porphyra-334 (p-334), isolated from a hot-spring cyanobacterium *Nostoc* sp. strain HKAR-2 were evaluated. P-334 was also compared with *Aloe vera*, a natural plant extract in terms of its antioxidative potentials, using butylated hydroxytoluene (BHT) as standard control. The antiradical activities of porphyra-334 and *Aloe vera* gel were determined using the free radicals: DPPH and ABTS. In their radical form, 1, 1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$) and 2,2′-azinobis (3-ethylbenzothiazoline 6-sulfonate ($ABTS^{\bullet+}$) has an absorbance maxima at 517 and 734 nm respectively, which disappears upon reduction by an antiradical compound. P-334 had maximum antioxidative potentials in comparison to BHT and *Aloe vera* gel. P-334 may serve as a potential replacement for the commonly used synthetic antioxidants which could otherwise be toxic and carcinogenic. The topical application of P-334 formulation in water on the skin of female albino hairless mice reduced the clinical signs of erythema and edema following UV-B irradiation. The formulation was also found to be effective in lowering the generation of ROS and cytotoxic thymine dimer formation. The results suggest that in future these natural bioactive sunscreening compounds may find application in cosmetic industries as a potent cheaper photoprotectant and antioxidant thereby, providing protection against UV-induced photoaging and skin cancer.

INTRODUCTION

A substantial loss in the stratospheric ozone layer caused by anthropogenically released pollutants has aroused tremendous scientific and public concern about the effects of increased solar ultraviolet (UV) radiation, particularly UV-B radiation (280-315 nm) on the Earth's surface, especially in the Arctic, Antarctic, and temperate regions during the past few decades[1-3]. UV-B radiation is a highly active component of the solar radiation that may affect the normal metabolic processes of all organisms including cyanobacteria either by direct effects on DNA[4], proteins and lipid molecules[5] or indirectly by the formation of reactive oxygen species (ROS)[6]. Increased in UV-B radiation has aroused interest in searching for the natural photoprotectant and antioxidative biomolecules from various organisms inhabiting diverse ecosystems.

Mycosporine-like amino acids (MAAs) are well-known UV-screening/absorbing secondary metabolites produced by a number of cyanobacteria and other taxonomic groups[7,8]. The family of MAAs is currently known to consist of approximately 23 structurally related colorless, water-soluble compounds which are composed of cyclohexenone or a cyclohexenimine chromophore conjugated with the nitrogen substituent of an amino acid or an amino alcohol. Several characteristics of MAAs, such as strong UV absorption maxima between 309 and 362 nm and high molar extinction coefficients (ε = 28,100 - 50,000 M^{-1} cm^{-1}), photostability, without generating reactive intermediates such as radicals or other oxidizing species under radiation, which might cause phototoxic effects on living organisms[9,10], suggest their role as natural sunscreen filters for photoprotection. The amount of MAAs in some algae depends on the depths for their habitation and, moreover, ultraviolet irradiation induces the production of MAAs in the frond[8]. On the other hand, the amounts of MAAs are also sensitive to spawning seasons in some invertebrates[11], suggesting that specific functions are latent other than for UV absorption. A possible role of MAAs as an osmolyte is also suggested since MAAs show high solubility to water and high content *in vivo*[12].

MAAs may protect the cell against UV-B radiation not only by dissipating the absorbed energy as heat but also by scavenging reactive oxygen species such as singlet oxygen, superoxide anions, hydroperoxyl radicals, hydroxyl radicals and inhibiting lipid peroxidation[13,14] resulting

from UV-induced production of ROS[15,16]. Suh et al.[15]showed that mycosporine-glycine can effectively quench singlet oxygen.

Nowadays, antioxidants have gained more importance because of their positive involvement as health promoters in conditions such as cardiovascular problems, atherosclerosis, treatment of many forms of cancer, and the ageing process[17]. The use of synthetic antioxidants, such as 2- and 3-tert-butyl-4-methoxyphenol (butylated hydroxyanisole, BHA), butylated hydroxytoulene (BHT) and tert-butylhydroquinone (TBHQ) in food, cosmetics and pharmaceuticals have been questioned because of carcinogenic and toxic issues, and thus, in the recent years there is a growing interest for the search of naturally occurring antioxidants which would serve as a replacement for the synthetic antioxidants[18-20.]

The present work aimed to investigate the stability of porphyra-334 under varying temperature regimes and pH conditions and also to compare its antioxidative potential with another natural product, *Aloe vera* gel to augment their use as a potent natural antioxidant. We have also made an attempt to analyze the adverse effects of UV-B radiation (1.0 Wm^{-2}) on skin of mice and assessed the photoprotective potentials of porphyra-334.

MATERIALS AND METHODS

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azinobis (3-ethylbenzothiazoline 6-sulfonate) and Butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich, Mo, USA. The standard marketed *Aloe vera gel* was purchased from Anjilika Ayurvedic Centre, Varanasi, India.

Culture Maintenance

The *Nostoc* sp. strain HKAR-2 isolated from a hot-spring, Rajgir, India[21] was acclimatized to grow at 28 ± 2 ^{0}C in a culture room under axenic conditions in autoclaved BGA (without nitrogen) medium[22] under continuous fluorescent white light of 12 Wm^{-2}. The cultures were hand shaken 5 times daily to avoid shelf shading and clumping. For induction of MAAs, the cyanobacterial samples were exposed to artificial UV-B radiation (1 Wm^{-2}) in a UV-chamber in open glass Petri dishes covered with 295 nm cut-off filter foils (Ultraphan; Digefra, Munich, Germany) to avoid any UV-C radiation.

Extraction of MAAs

Cells were harvested by centrifugation and MAAs were extracted in 2 mL of 100 % methanol (HPLC-grade) by overnight incubation at 4 ^{0}C. After extraction, the aliquots were centrifuged (10,000 g for 5 min) and supernatants (methanolic extracts) were evaporated to dryness at 45 ^{0}C and redissolved in 1 mL of double-distilled water. Thereafter, samples were filtered through 0.2 µm pore-sized microcentrifuge filters and subsequently analyzed with the HPLC system.

HPLC Analysis

The filtered samples of MAAs were analyzed with a HPLC (Waters 2998, Photodiode Array, pump L-7100, USA) equipped with a Licrospher RP 18 column and guard 5 µm packing; 250 mm × 4 mm inside diameter). The samples (100 µL) were injected into the HPLC column through a Waters 717 Plus Autosampler. Acetic acid (0.02 %, v/v) in double-distilled water was used as a mobile phase, which was isocratically run at a flow rate of 1 mL·min^{-1}. MAAs were detected at the wavelength of 330 nm, and absorption spectra were recorded each second between 250 and 400 nm directly on the HPLC-separated peaks. The sharp peak with retention time of approximately 3.1 min (λ_{max}-334 nm), identified as porphyra-334 was eluted with the help of a fraction collector and lyophilized.

Stability of P-334 under varying temperature regimes, storage periods and pH conditions

Stability studies of isolated fraction of porphyra-334 were performed at varying temperature regimes (4, 15, -20 and 45 °C) for different time intervals (upto 3 months), and also under different pH conditions (1.0, 3.0, 6.0 and 12.0). The absorbance of the samples was recorded at desired time intervals using a double beam spectrophotometer (UV-VIS 2900, Hitachi, Japan).

Radical Scavenging Activity

Radical scavenging capacity of P-334 was measured using DPPH and ABTS free radical scavenging assays.

(a) DPPH

The free radical scavenging activity of P-334 was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method as proposed by Blois [23]. A 100 µl of sample at different concentration (0.20–1.0 mg/ml) was added to 3 ml of DPPH solution (0.1 mM) in 80 % methanol. Subsequently, the

mixture (samples + DPPH) in glass vials were gently shaken and incubated in the dark at room temperature for 30 min and the decolorization reaction was visually monitored and absorbance of the reaction mixture was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity, and *vice versa*. The EC_{50} value, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50 %, was calculated from the results and used for comparison. The capability to scavenge DPPH radical was calculated using the following equation:

DPPH Scavenging Activity (%) = [(A1- A2)/A1] x 100

Where A1 is the absorbance of the control reaction and A2 is the absorbance in the presence of the sample. Butylated hydroxytoluene (BHT) was used as control. Antioxidant capacity for each concentration of test samples was expressed as percentage activity in terms of radical scavenging.

(b) ABTS

The method used for measuring the total antioxidant activity of P-334 in aqueous solution is based on the decrease in the absorbance (decolorization) of the radical cation of 2,2′-azinobis (3-ethylbenzothiazoline 6-sulfonate; $ABTS^{\bullet+}$) generated by oxidation of ABTS with potassium persulfate[24]. The cation has characteristic absorption peaks at 413, 660, 734 and 820 nm, and the addition of antioxidants to the pre-formed radical determines its reduction and can be followed by a decrease in absorbance. ABTS radical cation was produced by reacting 7 mM ABTS stock solution (in distilled water) with 2.45 mM potassium persulfate (final concentration). The mixture was maintained in darkness at room temperature for 12–16 h before use to guarantee the complete formation and stability of the radical. The photometric assay was conducted with 0.9 ml of $ABTS^{\bullet+}$ solution and 0.1 ml of different concentration of purified P-334 were mixed for 45 s and the absorbance was measured at 734 nm after 1 min. Dose-dependent responses were analyzed. BHT was used as control[25,26]. Absorbance measurements were taken after 1 min since the decolorization reaction provoked by the reference antioxidant (BHT) was practically completed by this time[24]. EC_{50} or IC_{50} was also calculated. IC_{50} is the concentration required to provoke absorbance inhibition of 50 %. The antioxidative ability of the samples was calculated by determining the decrease in absorbance by using the following equation:

$$E\% = ((Ac-At)/Ac) \times 100,$$

Where At and Ac are the absorbance of test samples and $ABTS^{\bullet+}$ respectively.

Experimental Animal

Female albino hairless mice, aged 8–10 weeks housed under pathogen-free conditions, in controlled temperature (22 °C), humidity (60-70 %) and visible light (12 h light/12 h dark) were used. They were supplied with water and commercial mouse diet. The experiments were performed with the approval of the Ethics Committee, Banaras Hindu University, Varanasi, India.

MAA Formulation and UV Treatment

A water emulsion of MAA formulation containing porphyra-334 was prepared to access their photoprotective role on mice-skin cells. Mice were anesthetized by intraperitoneal injection of the combination of ketamine and medetomidine prior to UV-treatment. The MAAs formulation at a concentration of 0.01 µg ml^{-1} was topically applied on the dorsal skin surface of mice 20 min before irradiation. Untreated and exposed animals (NTI), as well as untreated and unexposed animals were used as controls. UV-B radiation at a dose of 1.0 Wm^{-2} was used for irradiation. Skin samples were collected after 0, 4, 8, 12 16 and 24 h of exposure. The samples were subjected for photoprotective and antioxidative activity analysis in terms of free radical generation.

Histopathological, Antioxidative and Thymine Dimer Analysis

The formation of erythema and edema following UV-B exposure were assessed visually and evaluated by using the Draize scoring system[27] ranging from 0 (no erythema assigned to color of non-treated and non-irradiated skin) to 4 (highest redness corresponding to non-treated and irradiated skin). Edema values were 0: no edema, 1: slight edema (well-defined raising), 2: moderate edema (raised approximately 1 mm) and 3: severe edema (raised more than 1 mm). Production of H_2O_2 following UV-B exposure was accessed by absorption spectroscopy analysis at 260 nm. Genomic DNA was extracted from the skin samples after 24 h of UV-B exposure and thymine dimers were detected using the method as described earlier by Sinha et al.[28]. Briefly, the blot paper (GB002, ROTH, Karlsruhe, Germany) and positively-charged nylon membrane (Roti-® Nylon plus, K058.1, ROTH, Karlsruhe, Germany) were placed on a dot blot manifold

(Minifold I, Schleicher and Schuell) between the upper and middle blocks. Equal amount of DNA (1000 ng) were dissolved in TE buffer (Tris-HCl 10mM, EDTA 1mM; pH 8.0) to make a volume of 500 µl and were transferred to the nylon membrane through the circular holes at the top or upper block. The DNA samples dissolved in TE-buffer was allowed to settle on the nylon membrane by means of a vacuum applied to the outlet at the bottom. Nylon membrane bound to DNA was washed once with TE buffer in a sterile Petri plate. Thereafter, membrane was air dried and was kept in an oven at 80 ºC for 1 h to immobilize the DNA. Subsequently, the membrane was soaked in PBS-T [0.14 M NaCl, 3.4 mM KCl, 10.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4 + 0.1 % (v/v) Tween 20] with 5 % (w/v) skimmed milk powder (SMP) for 1 h to block the non-specific sites. Afterwards, membrane was incubated with primary antibody (anti-thymine dimmer KTM53, Kamiya Biomedicals, Seattle, USA; diluted 1:100 in PBS-T + 5 % SMP) for 2 h at room temperature and then washed (3 x 10 min) with PBST. After completion of incubation period in primary antibody, the membrane was incubated with the secondary antibody (anti-mouse IgG, Fab specific, peroxydase conjugate, Sigma, Saint Louis, Missouri, USA) diluted (1:100) in PBS-T + 5 % SMP for 1 h at room temperature and washed with PBS-T (4 x 15 min). Finally, the membrane was placed in a detection reagent, a mixture of oxidizing reagent and enhance luminal reagent in equal quantity (Western lightning chemiluminescence reagent plus, NEL104, Perkin Elmer LAS, Inc. Boston). The image was scanned with Kodak Digital Science (Image Station 440 CF, New Haven, CT, USA) and the results were evaluated by using the software provided by the manufacturer and the frequency of thymine dimmers were calculated as described by Sinha et al.[28].

Statistical analysis

The experiments were repeated thrice for accuracy of the results. All results are presented as mean values of three replicates and statistical analysis were done by one-way analysis of variance.

RESULTS

Analysis of Porphyra-334

The absorption spectroscopy and HPLC analysis revealed prominent absorption peak of UV-B absorbing compound (λ_{max}-334 nm, retention time-3.1 min) in *Nostoc* sp. strain HKAR-2. The

detail characterization of the compound by Richa & Sinha[29] revealed its identity as porphyra-334. The 3-D plot the purified fraction of porphyra-334 has been shown in Figure1.

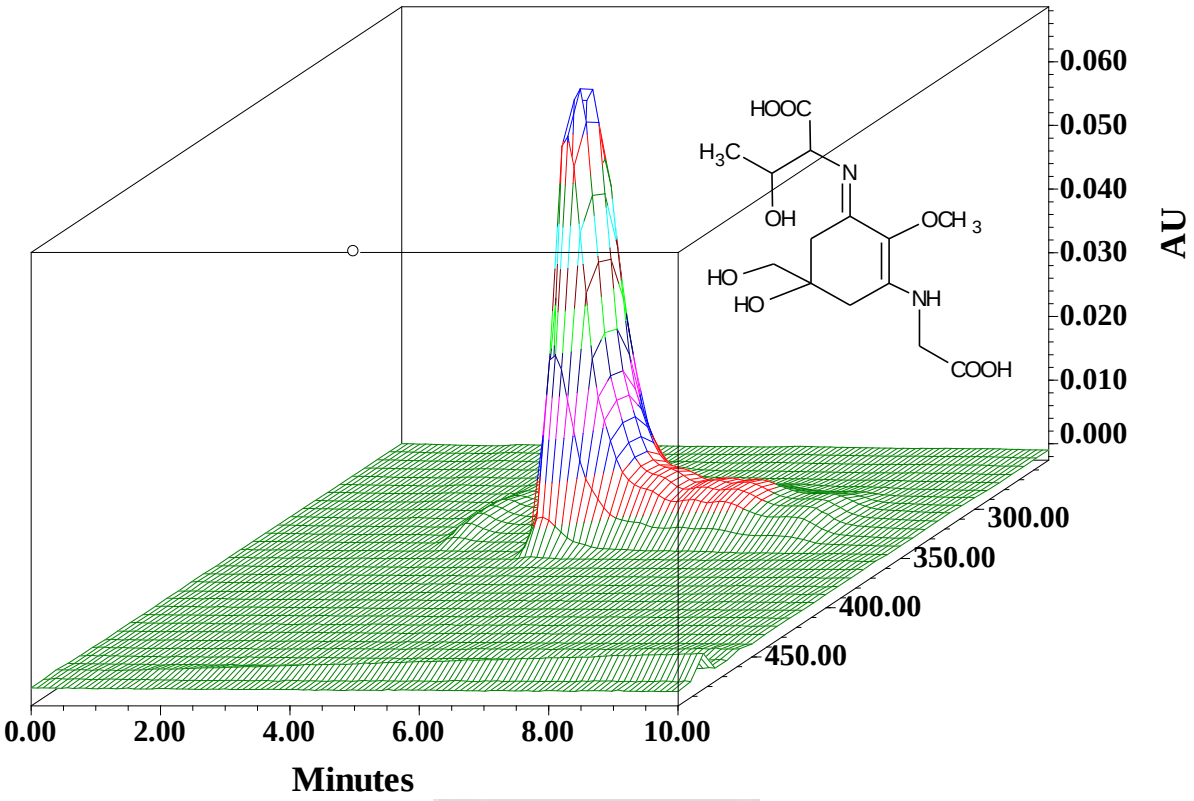

Figure 1: 3-D plot of purified fraction of porphyra-334

Stability

Porphyra-334 when stored under varying temperature conditions of 4, 15 and -20 ºC showed almost constant absorbance (Fig. 2A, 2B and 2C respectively). But, a slight decrease in absorbance was observed when maintained at high temperature (45 ºC) for 3 months (Fig. 2D). Thus, porphyra-334 was found to be stable at varying temperature regimes, thereby, providing an advantage of storing them even at room temperature and reducing the storage cost when used as a sunscreening agent. Porphyra-334 was also found to be stable under both acidic and basic conditions (Fig. 3).

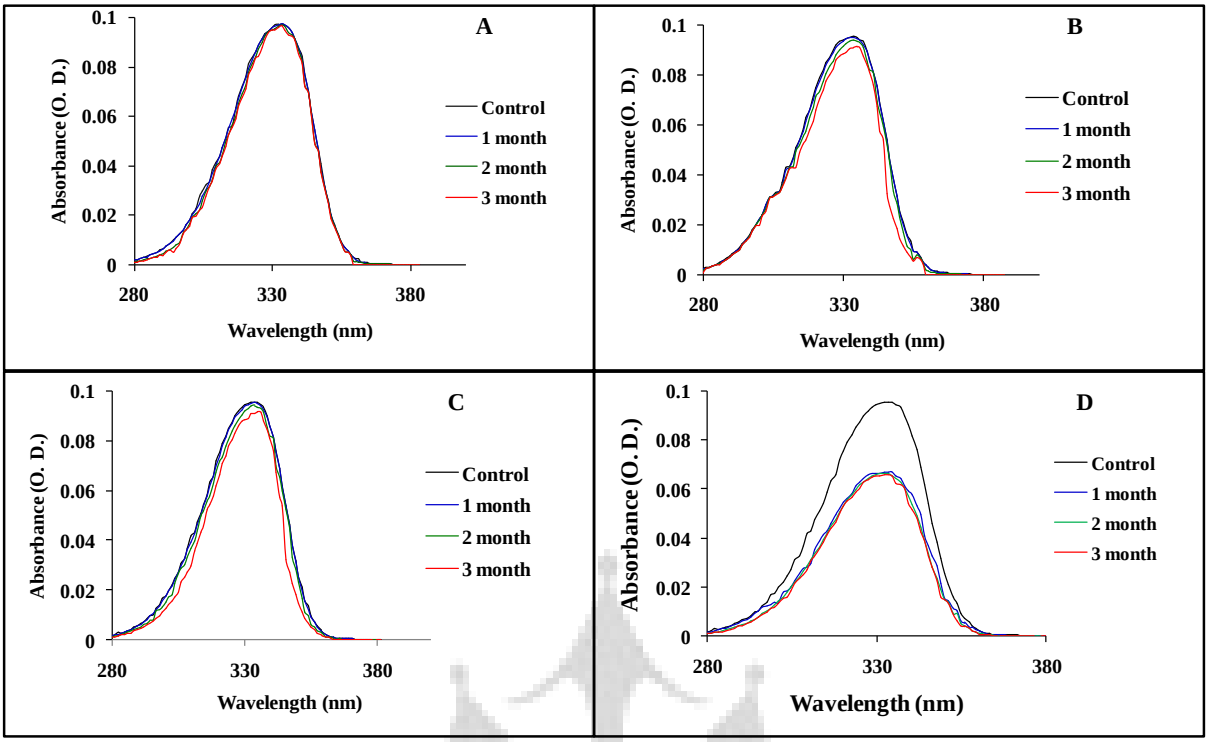

Figure 2: Stability of P-334 under varying temperature conditions for different time intervals. A: 4, B: 15, C: -20 and D: 45 ^{0}C respectively.

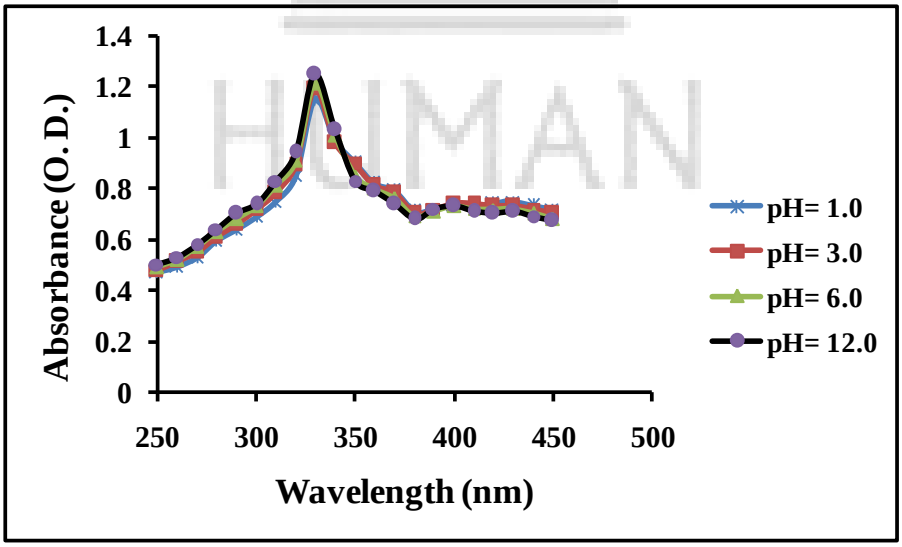

Figure 3: Stability of porphyra-334 under different pH conditions

Free radical scavenging activity

In order to investigate the efficacy of purified P-334 as a potential sunscreen, the percentage of its radical scavenging capacity was measured. Decolorization of DPPH was monitored visually as well as with a spectrophotometer. DPPH$^{\cdot}$ is a stable free radical and accepts electron or hydrogen radical to become a stable diamagnetic molecule[30]. A freshly prepared DPPH$^{\bullet}$ solution exhibits a deep purple colour with an absorption maximum at 517 nm. This purple colour generally fades when an antioxidant is present in the medium. Thus, antioxidant molecules can quench DPPH$^{\bullet}$ (by providing hydrogen atom or by electron donation) and convert it to a colourless product, resulting in a decrease in absorbance at 517 nm[31]. In brief, the reduction capacity of DPPH$^{\bullet}$ was determined by the decrease in its absorbance at 517 nm, which is reduced by antioxidants[32]. Figure 4A illustrates a significant decrease in the concentration of DPPH$^{\bullet}$ due to the scavenging ability of P-334, *Aloe vera* and BHT (as standard). As illustrated in Fig. 4B, all the three (P-334, *Aloe vera* and BHT) showed increased free radical scavenging activity with the increasing concentration. The percentage radical scavenging (antioxidant) activity of P-334 (81.12 %) at a concentration of 1 mg/ml was recorded to be higher than the standard antioxidant BHT (75.46 %) and the other natural *Aloe vera* gel (35.02 %). However, the antioxidant activity of P-334 was comparable with the commonly used synthetic antioxidant, but much higher than the *Aloe vera* gel. The quality of the antioxidants in the extracts was determined by the efficient concentration (EC_{50}) values shown in Table 1. A low EC_{50} value indicates strong antioxidant activity in a sample. The EC_{50} value of the P-334 was 0.1135 mg/ml, being the lowest indicating that this compound exhibited the highest radical scavenging effect. The EC_{50} values of *Aloe vera* was 0.4113 mg/ml, which was about 4-fold higher than P-334. BHT has the EC_{50} value of 0.1357 mg/ml. The scavenging effect on the DPPH radical decreased in the order: P-334 > BHT > *Aloe vera*. The results indicate that P-334 had significant effects on scavenging free radicals.

Concerning ABTS, the antioxidant activity was found to be 36.1 and 73.89 % for *Aloe vera* gel and P-334 respectively. Standard control BHT showed 69.87 % activity (Fig. 5). The scavenging effect on the ABTS•+ radical decreased in the order: P-334 > BHT >*Aloe vera*. The EC_{50} value of the P-334 was 0.1535 mg/ml, being the lowest indicating that this compound exhibited the highest radical scavenging effect. The EC_{50} values of *Aloe vera* was 0.6598 mg/ml, which was about 4.3-fold higher than P-334. BHT had the EC_{50} value of 0.1879 mg/ml (Table 1). The data obtained illustrated that DPPH method showed higher antioxidant activity in comparison to

ABTS method. However, the results obtained clearly demonstrated that P-334 extracted from a hot-spring cyanobacterium *Nostoc* sp. strain HKAR-2 had very high antioxidative potential as compared to the synthetic (BHT) and natural *Aloe vera* gel. Thus, it can be concluded that P-334 could serve as a replacement for the commonly used synthetic antioxidant which otherwise seems to be toxic and carcinogenic.

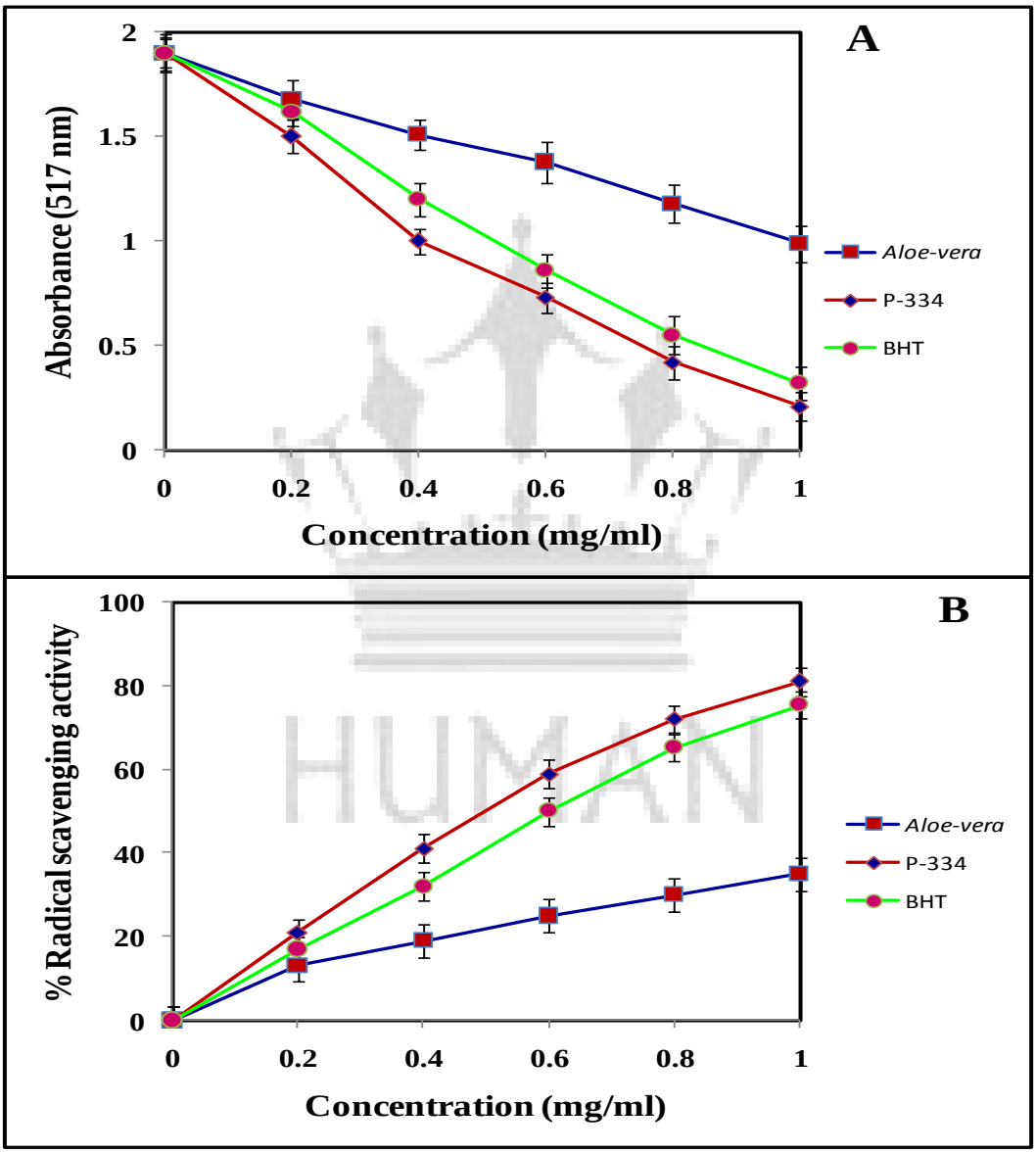

Figure 4: Free radical (A) and percentage radical scavenging activity (B) of P-334, *Aloe vera* and BHT using DPPH assay

Table 1. Efficient concentration (EC_{50}) values of BHT, *Aloe vera* and P-334.

Samples	EC_{50} (mg/ml)	
	DPPH	**ABTS**
BHT	0.1357	0.1879
Aloe vera	0.4113	0.6598
P-334	0.1135	0.1535

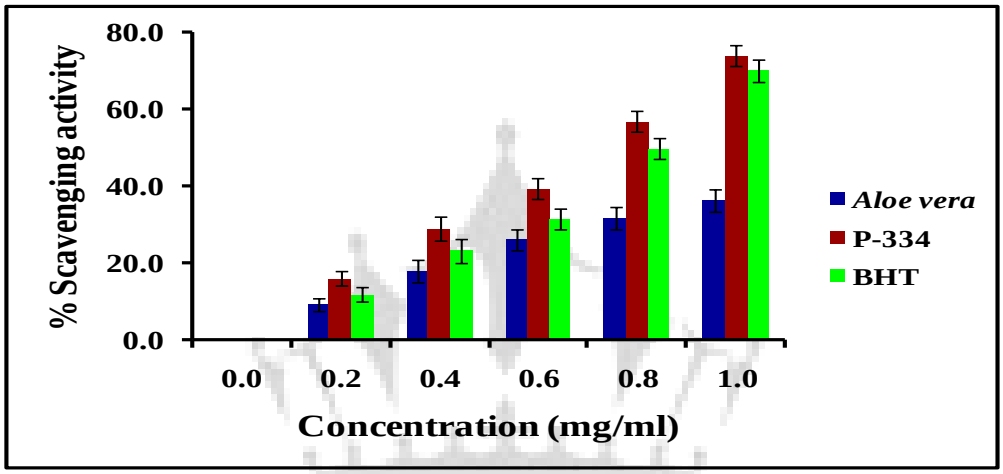

Figure 5: Percentage of free radical scavenging of P-334, *Aloe vera* and BHT using ABTS assay

Photoprotective Role of P-334

The cutaneous photoprotective ability of porphyra-334 (p-334) isolated from the thermophilic cyanobacterium *Nostoc* sp. strain HKAR-2 was evaluated in mouse skin *in vivo*. Clinical signs of sunburn, such as erythema and edema and production of H_2O_2 were measured from the skin biopsies at 8, 16 and 24 h post-radiation. Exposure to a UV-B dose of 1.0 Wm^{-2} induced significant erythema and edema in the p-334 non-treated and UV-B exposed mice (NTI) within 8 h. Mice treated topically with p-334 formulation in water showed a lower redness level. The initial erythemic response in p-334 treatment decreased after 8 h and more than 50 % inhibition was observed after 24 h of treatment (Fig. 6A). Similar results were observed in case of edema also. Signs of edema appeared in the exposed skin surface of mice after 8 h of exposure and remained until the end of experiments. However, after the application of the formulation slight edema was observed (Fig. 6B).

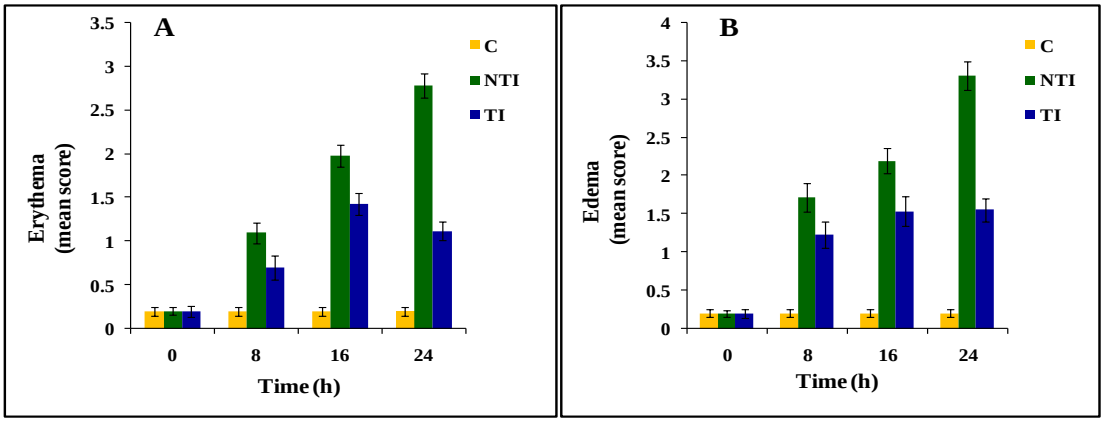

Figure 6: Formation of erythema (A) and edema (B) in mice skin cells after exposure to 1.0 Wm^{-2} UV-B radiation. Erythema formation was assessed visually and evaluated using the Draize scoring system, which ranges from 0 (no erythema assigned as control: non-treated and non-irradiated) to 4 (non-treated and irradiated skin). C: Control; NTI: non-treated and irradiated; TI: treated and irradiated.

ROS Scavenging Potential of P-334

Production of H_2O_2 following UV-B exposure was evaluated in the skin of mice by measuring its OD at 260 nm. A significant increase in the production of H_2O_2 was observed upto 4 h, but thereafter a slight decrease in the H_2O_2 production was observed. Topical application of the formulation was significantly inhibit the H_2O_2 production by almost 50 % after 12 h (Fig. 7).

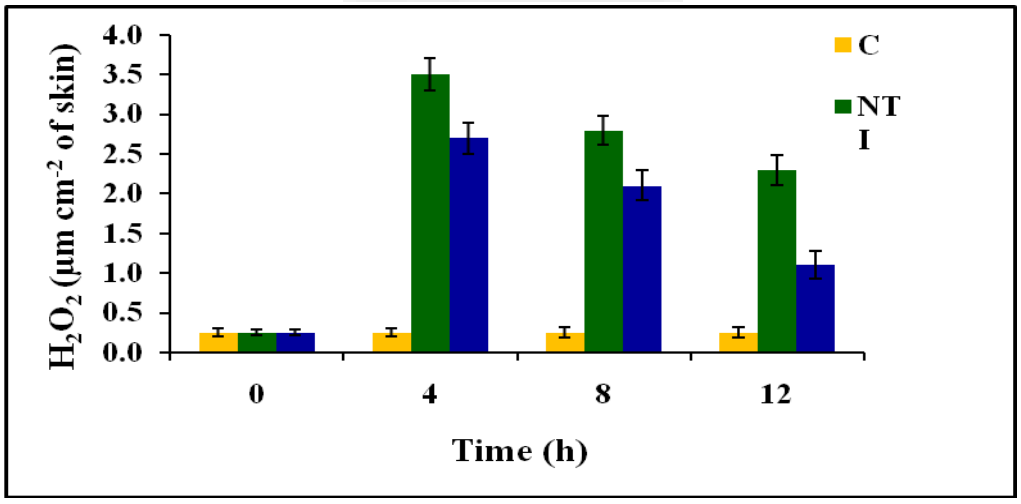

Figure 7: Porphyra-334 mediated inhibition of UV-B induced H_2O_2 production in the skin of hairless albino mice. C: Control; NTI: non-treated and irradiated; TI: treated with the P-334 formulation and then irradiated with UV-B.

Role of P-334 in Reducing Thymine Dimer Formation

MAA formulation also reduced the frequency of thymine dimer formation in the skin of female albino hairless mice following UV-B exposure, in comparison to the MAA non-treated and UV-B irradiated skin cells (Fig. 8).

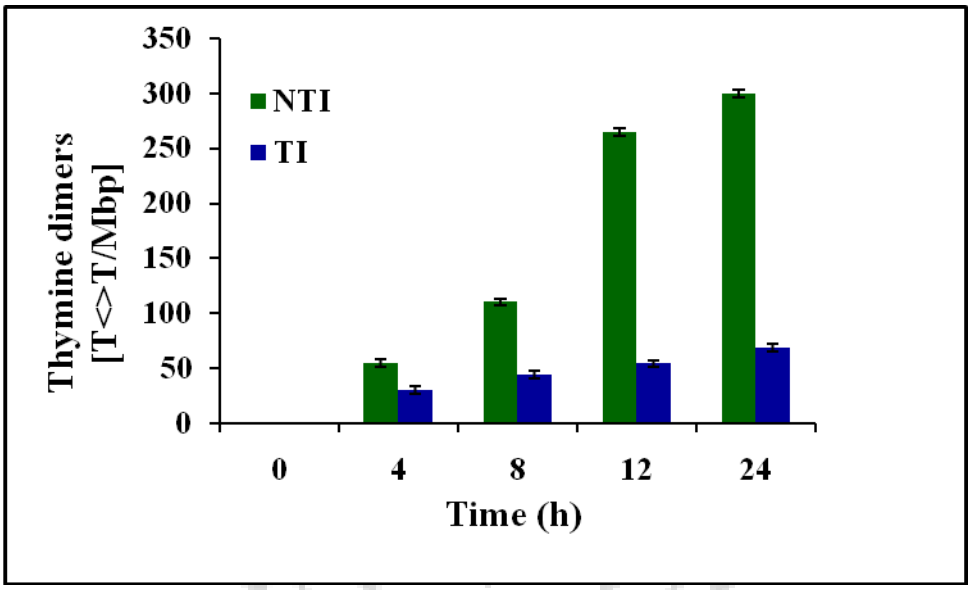

Figure 8: Formation of thymine dimers in P-334 (MAA) treated and untreated cells of hairless albino mice skin following exposure to UV-B radiation. NTI: non-treated and irradiated; TI: treated with the P-334 formulation and then irradiated with UV-B.

DISCUSSION

The role of certain MAAs such as p-334 and shinorine in maintenance of the antioxidant defence system of the skin has been elaborated by Coba et. al.[33]. Recently, the *in vitro* antioxidative activity of p-334+shinorine from *Porphyra* as well as other MAAs from different red algae and a marine lichen has been found out. Coba et al.[13,14,33] demonstrated the scavenging of superoxide anions and inhibition of lipid peroxidation by MAAs in a water-soluble medium, confirming their high antioxidant activity. The MAAs glycine and usujilene have also been reported to inhibit lipid peroxidation in aqueous extracts of marine organisms and were able to scavenge singlet oxygen generated from certain endogenous photosensitizers [8,15,34]. The antioxidative activity of certain MAAs such as porphyra-334, shinorine, asterina- 330 and palythine in terms of scavenging of hydrosoluble radicals was found to be dose-dependent and it increased with the alkalinity of the medium (pH 6 to 8.5)[33]. Yakovleva et al.[35] illustrated the antioxidative role of

mycosporine-glycine (MG) in the thermal-stress susceptibility of two scleractinian corals, *Platygyra ryukyuensis* and *Stylophora pistillata*. Their findings strongly suggest that MG is functioning as a biological antioxidant in the coral tissue and zooxanthellae and reveals its importance in the survival of reef building corals under thermal stress. The MAAs porphyra-334 + shinorine and shinorine alone isolated from the red algae *Porphyra rosengurttii* and *A. devoniensis* respectively, showed dose-dependent antioxidant activity in terms of scavenging of hydrosoluble radicals[33]. It has been reported that a suncream containing 0.005 % MAAs (porphyra-334 + shinorine) can neutralize UV-A effects as efficiently as a suncream with 1 % synthetic UV-A filters and 4 % UV-B filters[36]. By using DPPH free radical scavenging assay[37] demonstrated the antioxidative action of two MAAs (λ_{max}-322 and 324 nm) from a green alga *Tetraspora* sp. CU2551. Rastogi & Incharoensakdi[38] also reported the dose-dependent antioxidative activity of MAAs (shinorine + M-307) isolated from *Gloeocapsa* sp. CU-2556.

UV-B radiation has been reported to induce a variety of DNA damages. Sinha et al.[28] have demonstrated that exposure to UV-B radiation could induce the formation of thymine dimers in three rice-field cyanobacteria e.g., *Nostoc* sp., *Anabaena* sp. and *Scytonema* sp. By using dot-blot and chemiluminescence techniques these workers successfully developed a relatively quick, simple and efficient method for the quantitative analysis of thymine dimers in aquatic primary producers including cyanobacteria. It was also found that the frequency of thymine dimers increased with the increase in UVR exposure time, and after 120 min of exposure it reached upto 35–40 T^T/Mbp in all three studied cyanobacteria. UV radiation has been reported to cause single- and/or double-stranded breaks in the native DNA molecules in various cyanobacterial species[39]. Besides efficient repair mechanisms, cyanobacteria protect themselves from photodamage by production of certain UV-absorbing/screening compounds such as mycosporine-like amino acids (MAAs)[40,41], however, they are unable to avoid UV radiation completely from reaching DNA in superficial tissues. Since, MAAs is UV-absorbing compounds, it inhibits the UV-B radiation to reach the DNA molecule so that least amount of thymine dimers are formed in the cells and protect them to undergo lethal mutations. MAAs can dissipate absorbed radiation as heat without producing ROS[42] and can also block the production of both 6–4 photoproduct (6–4PPs) and cyclobutane pyrimidine dimer (CPD) formation[43].

The *in vitro* test performed using a formulation of natural bioactive P-334 in water showed to confer a broad spectrum of protection against UV-B radiation in the skin of female albino hairless mice. The high molar extinction coefficients and photostability favours MAAs as a potent natural sunscreen for photoprotection[44]. Reactive oxygen species, such as H_2O_2, superoxide anion and singlet oxygen are thought to be involved in UV damage[45,46]. Fortunately, the skin possesses a wide range of interlinked antioxidant defence mechanisms, e.g., catalase and SOD enzymes, to protect itself from damage by UV-induced ROS. In the present investigation, we have demonstrated that topical application of MAA formulation is capable of reducing the generation of H_2O_2.

CONCLUSION

Mycosporine-like amino acid i.e., porphyra-334 can be considered as effective natural sunscreen substance, preventing the deleterious effects induced by ultraviolet radiation. The results suggest that in future these natural bioactive compounds may find application in cosmetic industries as a potent photoprotectant. Future research must be focused on the analysis, biosynthesis and mode of action of these natural UV-absorbing compounds.

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Conflict of Interest

The authors declare no conflict of interest.

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