Broad-spectrum sun-protective action of Porphyra-334 derived from *Porphyra vietnamensis*

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ABSTRACT

There are enormous UV-protective compounds present in the current world market, out of which 98% give protection against UV-B range and the remaining 2% are potent against far UV-A range only. Furthermore, these synthetic compounds have various problems related to photo-stability and cross-stability. There is a vital need of sunscreen agents that will remain stable for prolonged periods and provide broad-spectrum protection against harmful UV range. The Indian Ocean contains large amounts of macro-algae which synthesize varied amount of mycosporine amino acids, "sun-protective compounds" by shikmic acid pathway. In the present study, we have evaluated the sunscreen protection provided by Porphyra-334, a mycosporine amino acid isolated from Indian sp. of *Porphyra.* Furthermore, the isolated compound was detected by high performance thin layer chromatography (HPTLC) fingerprinting, high performance liquid chromatography (HPLC) and ultraviolet (UV), whereas nuclear magnetic resonance (NMR) spectroscopy and infrared spectrometry were used for its structural characterization. Stability studies were performed under different storage and pH conditions. Ultimately a sunscreen formulation was developed and its potential against marketed *Aloe vera* gel was evaluated by *in vitro* sunscreen protection method. It was observed that sunscreen potential of Porphyra-334 was 5.11-fold greater than that of the marketed *Aloe vera* gel preparation.

Key words: *Porphyra*, Porphyra-334, sun protection factor, ultraviolet

INTRODUCTION

Effects of ultraviolet (UV) irradiation on biological matter became an important issue over the past three decades. The term 'UV irradiation' is used to describe the UV region from 280 to 400 nm. Among this range, UV-B irradiation ('UV-B') is defined to occur in the region from 280 to 315 nm; UV-A irradiation ('UV-A'), from 315 to 400 nm;[1-5] and 'UV-C' irradiation, from 200 to 280 nm, which is totally absorbed by the atmosphere and therefore not of biological relevance. Under clear skies at temperate to equatorial latitudes, total UV-B nowadays may be as high as 7 to 8 W mK^2; and UV-A, as high as 45 to 50 W mK^2, respectively.[6-9] Day by day, man-made changes increase the harmful effects of UV radiation, which increases the demands of potent topical sun-screening agents to provide protective screen against these radiations. UV radiations are either reflected or absorbed by sunscreens. Sunscreen application is widely used for prevention of UV-induced sunburn, photo-aging and skin cancer. One of the key factors in evaluating the potential of sunscreens is sun protection factor (SPF), which is assessed by its ability to inhibit erythema 24 hours after exposure of the skin to UV light.[10-16] Longer wavelength is less effective in inducing erythema, i.e., approximately 1000 times more UV-A (315-400 nm) is needed to induce erythema as compared with UV-B (280-315 nm). UV-A generates oxygen and hydroxyl-free radicals, which can cause damage to cellular proteins, lipids and carbohydrates. It can also cause damage to the DNA, disturb the immune system and possibly lead to cancer. Majority of Indian sunscreen products available to consumers provide protection against UV-B and short-wavelength UV-A-II (315-340 nm).[17-24] Only a few synthesized compounds such as titanium dioxide and zinc oxide are broad-spectrum UV-A filters. These compounds are problematic in terms of their stability, i.e., photo-stability and cross-stability; hence the current Indian market requires the most compatible, stable and broad-spectrum compound which can provide a protective screening shield to the human skin.

Porphyra (Bangiales, Rhodophyta), delicious red algae widely consumed in eastern Asia, contains high levels of free amino acids; when exposed to intense radiations, it

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synthesizes UV-absorbing secondary metabolites such as mycosporine-like amino acids (MAAs). MAAs are water-soluble substances characterized by a cyclohexenone chromophore conjugate having absorption maxima ranging from 309 to 360 nm.[25-31] The Indian Ocean comprises of a wide range of macro-algae, which have enormous unexplored therapeutic applications.[32-34] There are almost seven sp. of *Porphyra* identified in India. Among all of these, nowadays *Porphyra vietnamensis* are gaining more attention. Herein, we report the isolation, identification and stability studies of Porphyra-334 from Indian *Porphyra*, and its sun-screening potential was checked against one of the most widely used substances, viz., *Aloe vera* gel.[35,36]

MATERIALS AND METHODS

The absorbing compound was isolated from *Porphyra vietnamensis* (Department of Botany, University of Pune, Maharashtra, India). The standard marketed *Aloe vera gel* was purchased from Brihans Natural Products (BH 045).

EXTRACTION AND ISOLATION PROCEDURE

Ten grams of dried algae was powdered. Extraction of the powder was carried out with a mixture of methanol-water (80:20, v/v), followed by CH_2Cl_2. The aqueous phase was lyophilized till dryness, to yield 5.1 g of a pale yellow powder. An aliquot of the powder was re-dissolved in 80% MeOH and checked for its UV absorbance. A sharp peak with an extinction coefficient of 42,300 $M^{-1}cm^{-1}$ was obtained at 334 nm [Figure 1]. The resulting powder was chromatographed on a silica gel column, using a gradient condition ranging from 20% MeOH and 80% EtOH to 80% MeOH and 20% EtOH, to yield 400 mg of a pale yellow powder. The collected fractions were evaporated and then lyophilized till dryness to yield 50 mg of colorless powder. Purity of the isolated compound was confirmed by HPLC analysis, performed on a Jasco 900 instrument, and 5 μL of isolated fraction was loaded onto ODS (5 μm; Inertsil) column (150 × 4.6 mm). Acetonitrile:Water (45:55) was eluted at a flow rate of 1 mL/min, which showed a single sharp peak with a retention time of 4.9 minutes. The analysis was carried out on highly polar water-soluble compound that absorbs strongly in the UV-A region. Further analysis was done by HPTLC to know the exact position of isolated compound on the chromatogram. The spotting device was Linomat IV Automatic Sampler (Camag, Muttenz, Switzerland), 100 μL syringe (Hamilton Bonaduz, Switzerland). The TLC chamber; glass twin-trough chamber (20 × 10 × 4 cm) (Camag); TLC Scanner 3 linked to WINCATS software (Camag) as densitometer; the HPTLC plates measuring 20 × 10 cm, 0.2 mm thickness, pre-coated with silica gel 60 F254 (E. Merck Kga A, Cat. no. 1.05548, Darmstadt, Germany), were used. Structural characterization was done with the help of NMR spectroscopy and infrared spectrometry. Stability studies of isolated fraction were performed with different storage periods and pH conditions by using calibrated pH meter.

After performing stability studies, the isolated fraction was incorporated into gel and evaluated for sunprotective efficiency by utilizing simple and rapid *in vitro* sun protection determination method.[12] In this step, to 10 mL of water, 0.2 g of carbopol was added, followed by the addition of 50 mg of test sample. The solution formed was heated for 10 minutes at 60°C and kept for 24 hours in a dark place. Only one drop of triethanolamine was added to increase the gelation by cross-linking and pH balance. Marketed *Aloe* vera gel was randomly selected and compared with *Porphyra* gel by making different dilutions (25-100 μL/mL) in methanol. The absorbance of all aliquots was recorded at different values of nm with 5-nm intervals from 250 to 400 nm [Table 1] with UV spectrophotometer (UV-spectrophotometer: Jasco V 530, Jasco, Japan; and high-performance thin-layer chromatography: Jasco, Japan).

The SPF values were determined at wavelengths ranging from 290 to 400 nm. Each determination was made in triplicate (mean ± SD). The observed absorbance values were calculated according to the equation given below.

$$SPF = C.F.230\Sigma^{320} EE(\lambda).\ Abs.(\lambda),$$

where CF = 10 (correction factor), EE (λ) = erythemogenic effect of radiation with wavelength λ, Abs.

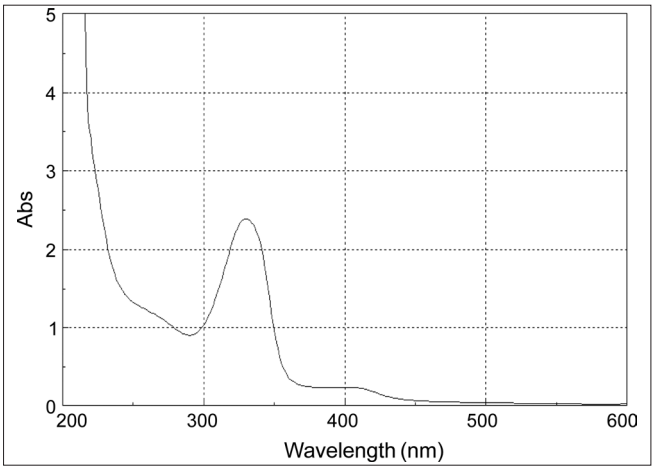

Figure 1: Detection of mycosporine-like amino acid (334 nm) by ultraviolet from the methanolic aliquot of *Porphyra*

Table 1: SPF values using different concentrations of marketed Aloe vera gel formulation and *Porphyra* gel (n = 3, mean ± SD)

Erythemogenic effect	Wavelength	AM (200 μL)	P_1 (100 μL)	P_2 (50 μL)	P_3 (25 μL)
Λ	250	0.650 ± 0.002	0.723 ± 0.002	0.571 ± 0.001	0.290 ± 0.008
Λ	255	0.580 ± 0.007	0.710 ± 0.002	0.523 ± 0.004	0.253 ± 0.004
Λ	260	0.475 ± 0.005	0.610 ± 0.003	0.450 ± 0.003	0.269 ± 0.003
Λ	265	0.392 ± 0.003	0.550 ± 0.001	0.425 ± 0.003	0.265 ± 0.004
Λ	270	0.283 ± 0.004	0.523 ± 0.002	0.410 ± 0.001	0.262 ± 0.001
Λ	275	0.250 ± 0.006	0.471 ± 0.001	0.324 ± 0.001	0.178 ± 0.006
Λ	280	0.216 ± 0.003	0.448 ± 0.004	0.289 ± 0.001	0.155 ± 0.002
Λ	285	0.187 ± 0.006	0.411 ± 0.004	0.262 ± 0.002	0.154 ± 0.002
0.021	290	0.133 ± 0.002	0.339 ± 0.006	0.253 ± 0.001	0.137 ± 0.002
0.137	295	0.107 ± 0.001	0.331 ± 0.005	0.169 ± 0.002	0.124 ± 0.003
0.287	300	0.091 ± 0.001	0.373 ± 0.002	0.155 ± 0.001	0.141 ± 0.001
0.384	305	0.081 ± 0.008	0.394 ± 0.002	0.133 ± 0.001	0.136 ± 0.009
0.19	310	0.069 ± 0.009	0.228 ± 0.006	0.121 ± 0.005	0.122 ± 0.004
0.079	315	0.058 ± 0.004	0.179 ± 0.003	0.091 ± 0.006	0.103 ± 0.004
0.018	320	0.069 ± 0.003	0.164 ± 0.001	0.074 ± 0.005	0.083 ± 0.001
Λ	325	0.056 ± 0.008	0.158 ± 0.007	0.051 ± 0.004	0.078 ± 0.003
Λ	330	0.058 ± 0.001	0.143 ± 0.001	0.068 ± 0.002	0.066 ± 0.001
Λ	335	0.051 ± 0.007	0.146 ± 0.004	0.031 ± 0.005	0.061 ± 0.005
Λ	340	0.057 ± 0.001	0.131 ± 0.007	0.048 ± 0.002	0.062 ± 0.001
Λ	345	0.049 ± 0.006	0.123 ± 0.003	0.037 ± 0.001	0.052 ± 0.004
Λ	350	0.047 ± 0.007	0.110 ± 0.008	0.035 ± 0.001	0.033 ± 0.002
Λ	355	0.043 ± 0.004	0.092 ± 0.002	0.032 ± 0.001	0.021 ± 0.004
Λ	360	0.041 ± 0.005	0.087 ± 0.001	0.035 ± 0.008	0.016 ± 0.001
Λ	365	0.037 ± 0.003	0.077 ± 0.009	0.038 ± 0.003	0.011 ± 0.001
Λ	370	0.034 ± 0.001	0.071 ± 0.003	0.028 ± 0.001	0.024 ± 0.008
Λ	375	0.039 ± 0.009	0.089 ± 0.001	0.024 ± 0.004	0.012 ± 0.005
Λ	380	0.028 ± 0.002	0.053 ± 0.001	0.019 ± 0.007	0.009 ± 0.005
Λ	385	0.026 ± 0.003	0.044 ± 0.004	0.018 ± 0.003	0.017 ± 0.003
Λ	390	0.025 ± 0.003	0.033 ± 0.001	0.020 ± 0.002	0.023 ± 0.001
Λ	395	0.023 ± 0.006	0.020 ± 0.001	0.023 ± 0.002	0.007 ± 0.001
Λ	400	0.021 ± 0.004	0.011 ± 0.001	0.013 ± 0.009	0.019 ± 0.002
	SPF	0.60 ± 0.034	1.23 ± 0.015	0.78 ± 0.045	0.491 ± 0.01

Λ - Not applicable for *in vitro* calculations; SPF - Sun protection factor; EE - Erythemogenic effect; AM - Marketed *Aloe vera* gel (200 μL/mL); *Porphyra* (P_1–P_3) - Freshly prepared gel from concentrations ranging from 25 to 100 μL/mL

(λ) = spectrohotometric absorbance values of a solution at specific wavelength.[12]

RESULTS AND DISCUSSION

In the present study, MAA, known to be present in macro-algae of *Porphyra vietnamensis*, was isolated and detected by UV (334 nm), HPTLC (Rf- 0.43-0.53) fingerprinting and HPLC (R_T 4.9) analysis [Figures 1-3]. In Figure 1, methanolic aliquot showed the sharp peak at 334 nm with an extinction coefficient of 43200 $M^{-1}cm^{-1}$. Melting temperature of Porphyra-334 (164°C-165°C) was studied by DSC (differential scanning calorimeter). Structural characterization was done on the basis of ^{13}C-NMR and ^{1}H-NMR [Table 2], whereas IR studies gave the range of 3100, 1520, 1670, 1330 and 1100 cm^{-1}. Thereafter, stability studies of gel-loaded drug were performed under different pH and storage conditions simultaneously [Figures 4 and 5].

The stability of gel was studied under different pH and storage conditions. Porphyra-334 showed a little decrease in absorbance even after being maintained at room temperature for more than 3 months in low basic and acidic solutions.

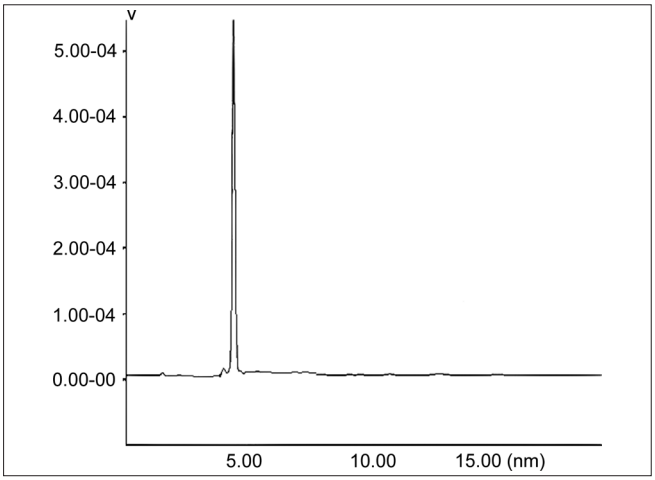

Figure 2: HPLC of Porphyra-334 from *Porphyra vietnamensis*

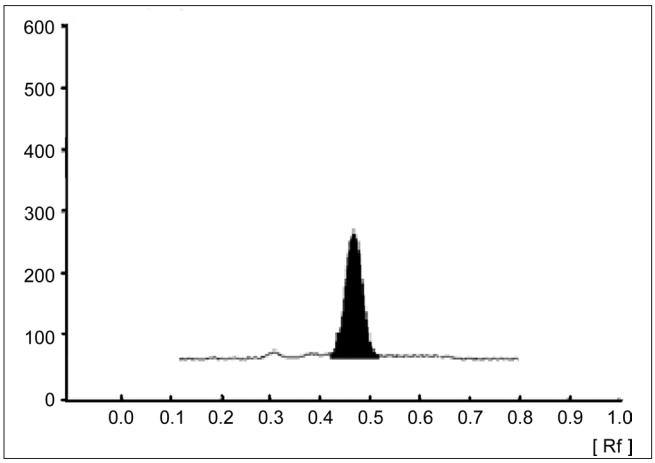

Figure 3: HPTLC of Porphyra-334 from *Porphyra vietnamensis*

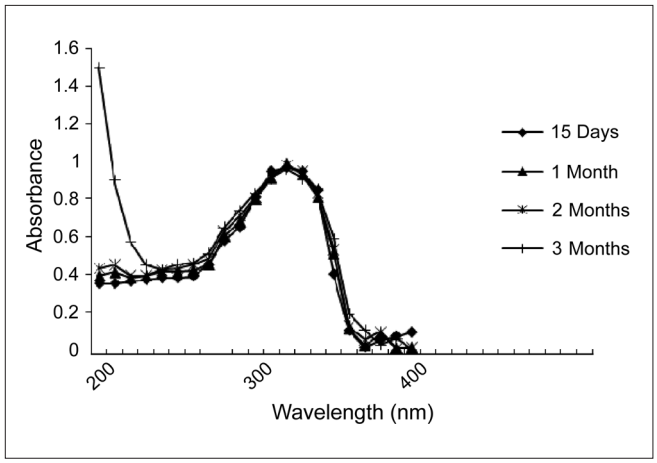

Figure 4: Stability studies of Porphyra-334 with different storage periods

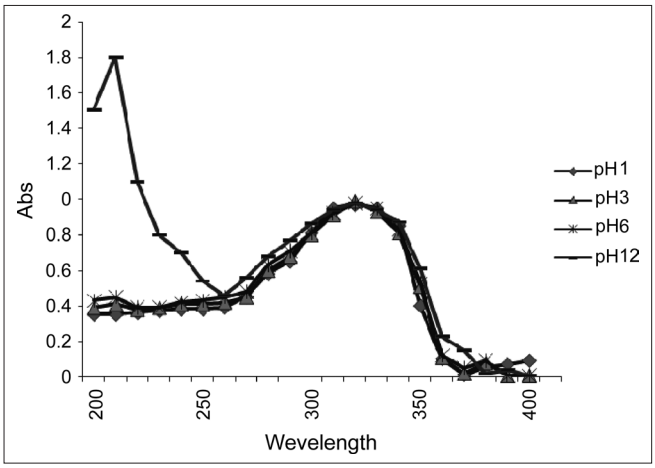

Figure 5: Stability studies of Porphyra-334 under different pH conditions

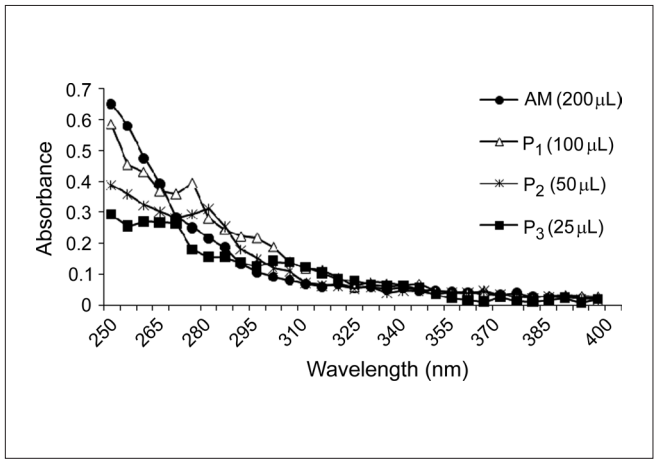

Figure 6: Ultraviolet absorbance values of marketed *Aloe vera* gel and *Porphyra* gel at different concentrations

Table 2: NMR data of Porphyra-334

Position	δ13-C	Δ1-H
1	159.2	-
2	118.1	-
3	167.5	-
4	30.7	3.1
5	66.4	-
6	37.6	2.71
7	71.1	-
8	54.3	2.52
9	51.1	3.52
10	173.4	3.51
11	63.9	3.91
12	176.6	-
13	61.4	4.11
14	18.5	1.11

Among all the MAAs involved in sun protection, Porphyra-334 synthesized in Indian *Porphyra* by shikmic acid pathway was found to be a very potent sunscreen compound. The isolated compound showed concentration (25-100 µL)-dependent increase in absorbances [Figure 6]. The absorbance values of methanolic aliquots of marketed *Aloe vera* gel containing marketed formulation and test product gel were used to calculate the *in vitro* sun protection factor using the erythemogenic effect values (EE values), which are applicable only for the UV-B. The isolated compound was found to have remarkable sun-protective property as compared to marketed formulation for UV range. It was observed that the SPF values of *Porphyra* gel were 5.11-fold greater than those of the marketed formulation. Figure 6 shows that marketed *Aloe vera* gel had low absorption power over broad UV wavelength (250-400 nm) as compared to isolated compound gel, suggesting that Porphyra-334 is more potent. This might be due to π-electron system (UV radiation absorbers) involved in the isolated compounds that are primarily found in conjugated bond structures. π-electron systems are a common theme in the function and characteristics of natural UV-screening molecules.

CONCLUSION

Various studies have proved that Porphyra-334 is widely present in the Indian sp. of *Porphyra*, and its formulation covers a broad UV range with no photo-stability and problems like those faced in case of other marketed formulations. This suggests that this sun-protective formulation constitutes the most prominent and potent amino acid which can in future become a promising molecule in UV-related dermatological ailments.

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