IJPSR (2018), Volume 9, Issue 8 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 09 November, 2017; received in revised form, 25 January, 2018; accepted, 06 February, 2018; published 01 August, 2018

SUN PROTECTIVE FORMULATION FROM CAROTENOIDS EXTRACTED FROM THERMOTOLERANT GENERA *SYNECHOCYSTIS PEVALEKII*

P. A. Tendulkar [1], M. A. Surve [2] and M. A. Deodhar [*2]

Department of Biotechnology [1], Head Cosmetology Division, Scientific Research Centre, Department of Botany [2], Kelkar Education Trust's V. G. Vaze college of Arts, Science and Commerce, Mithagar Road, Mulund (E), Mumbai - 400081, Maharashtra, India.

Keywords:

Zeaxanthin, Freeze- thaw, Sunblockers, Avobenzone, Carotenoids, Sunscreen

Correspondence to Author:
Dr. M. A. Deodhar

Associate Professor, Department of Botany, Kelkar Education Trust's V. G. Vaze College of Arts, Science and Commerce, Mithagar Road, Mulund (E), Mumbai- 400081, Maharashtra, India.

E-mail: drmadeodhar@gmail.com

ABSTRACT: Naturally occurring safe and broad spectrum UV blockers are required now- a- days to protect oneself from the onslaught of UV radiations that are known to cause many skin related ailments. The commercial inorganic sunblockers like Zinc oxide and titanium di oxide and organic sunblockers like Avobenzone and Benzophenone are known to be unstable and also their safety is doubted. Aim of the present communication was to study the secondary carotenoids like Zeaxanthin / Canthaxanthin / β- carotene extracted from thermotolerant genera *Synechocystis pevalekii* is screen for its role in skin protection against UV radiations. Solvents like Petroleum ether, Hexane, acetone, ethyl acetate, ethanol and methanol were used for extraction. Out of all these, freeze – thaw technique in 100% methanol proved to be the best solvent for extraction of carotenoids. The crude extract thus obtained was fractionated by silica gel open column chromatography and the resultant fractions were analyzed by HPLC. The HPLC analysis showed the presence of carotenoids like Zeaxanthin, Canthaxanthin and β- carotene. The next part of the study was to determine the role of zeaxanthin in skin protection against UV radiations. The crude extract and the zeaxanthin fraction were incorporated in a gel based sun protective formulation and analysed for the SPF and boot star rating. It was found that the incorporation of the crude extract as well as the fractions did not result in SPF increase but gave a 5 star boot star rating proving it to be a potential UV A radiation absorber. Also the synergistic effect of the Zeaxanthin fraction with commercial sunblockers like Octyl methyl cinnamate and Avobenzone was studied. It was found that the incorporation of Zeaxanthin not only enhanced the boot star rating, UV A/ UV B ratio but also protected Avobenzone from degradation. Thus Zeaxanthin from *Synechocystis pevalekii* can be used a natural component for sunscreens and as a stabilizing agent for chemical sunblockers like Avobenzone.

INTRODUCTION: UV radiation is a part of electromagnetic radiation ranging from 200 nm to 400 nm. This is further divided into UV C (200 nm to 280 nm), UV B (280 nm to 320 nm) and UV A (320 nm to 400 nm). Both UV A and UV B are known to penetrate the atmosphere and play an important role in premature skin ageing, eye damage and skin cancer.

To combat these UV radiations and to protect skin, there are many marketed products available commercially that include either inorganic or organic sun blockers to protect the skin.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(8).3223-34

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(8).3223-34

However they have their own disadvantages. The inorganic filters (TiO_2, ZnO_2) have a whitening effect [1] in sunscreen formulation whereas the organic sun blockers (Avobenzone, benzophenone) are found to be unstable [2]. Hence it becomes important to find alternative sources of skin protection with herbal / natural alternatives that will synergistically act with commercial sunscreen agents giving them stability.

The carotenoids are known to protect the photosynthetic apparatus from excess light energy. Some xanthophylls are capable of cyclic conversions through de-epoxidation and epoxidation know as xanthophyll cycle [3]. During light stress, violaxanthin is converted to zeaxanthin *via* the intermediate antheraxanthin, which plays direct photoprotective role acting as an anti oxidant and by stimulating non-photochemical quenching within light-harvesting proteins.

The biological properties of carotenoids are manifold. Carotenoids are well known to function as antioxidants and promote oxidative stress resistance. They are also known for their photo oxidant and antioxidant role and its health benefits, its free radical scavenging activity, provitamin A activity and its role in protection against macular degeneration as well as cardiovascular diseases [4, 5].

Carotenoids are also well known for their role in skin protection against ageing and UV radiations. Carotenoids, their derivatives and their precursors are known to be versatile isoprenoids useful in skin care and cosmetics. All the carotenoids are derived from Phytoene and Phytofluene. Carotenoid, phytoene and phytofluene are known to accumulate in human skin as well. Phytoene and phytofluene are known to absorb the UV light. Thus it would be reasonable to expect their role in photoprotection of skin [6].

These colourless carotenoids are good free radical absorbers and thus can be used in stabilizing sunscreening compounds like TiO_2 and Avobenzone which are highly unstable [7].

Skin protection by nutritional interventions is garnering more attention now-a-days. Carotenoids have properties that may interrupt the course of assaults involved with UV radiation that eventually lead to skin damage. UVA (320 - 400 nm) exposure leads to formation of ROS, especially singlet oxygen, causing oxidative stress that can damage cellular components. Carotenoids are potent singlet oxygen quenchers [8].

Nutricosmetics is a new concept; an intersection of two fields *i.e.* cosmecuticals and nutraceuticals that has emerged in the industry. It can be described as consumption of food / oral supplements to produce an appearance benefit. To study the cosmetic effect of carotenoids an experiment was carried where 3 different modes of supplementation of carotenoid were studied *viz*, topical application, oral supplementation and combined oral and topical application. It was found that all the treatment groups showed a significant enhancement in skin elasticity, the effects being more pronounced in combined treatment where it also provided cutaneous hydration along with highest degree of antioxidant protection. This suggests a synergistic effect of oral and topical approaches [9].

Using optical skin surface topography to study the dermal roughness it was found that individuals which had a high carotenoid concentration in skin looked younger for their age as compared to the individual who had low carotenoid concentration in their skin looked older [10].

There are various patents that have been published wherein carotenoids (xanthophylls) have been used in cosmetic products to increase the SPF of the same. In a patent, two compositions, one with lutein and other without lutein were prepared and tested for SPF. The composition with lutein had almost 2 SPF units more than that composition without lutein [11]. Another skin cosmetic with carotenoids that boasts of anti- oxidant enhancing formulation is also reported in a patent [12]. This oil based formulation helps the skin to retain the water and the carotenoids in it protect the skin from oxidative damage. The formulation consists of carotenoids like β- Carotene, α- Carotene, lycopene, and Vitamin E [12].

In the present communication secondary carotenoids like zeaxanthin/ Canthaxanthin / β-carotene extracted from thermotolerant genera *Synechocystis pevalekii* is screened for production of UV protective compounds and its role in skin protection.

MATERIALS AND METHODS

Pigment Extraction and Analysis: 1g of wet biomass was used for extraction of carotenoids in different solvents *viz*, petroleum ether, ethyl acetate, acetone, n- Hexane, Ethanol and 100% methanol. The biomass was soaked in10 mL of 100% methanol and incubated at 4 ºC overnight. After extraction, the mixture was centrifuged for 5 min at 4000 rpm and supernatant (Crude extract) was collected for purification and analysis by HPLC.

Purification of Crude Extract by Silica Gel Open Column Chromatography: 17 g of silica gel (200 - 300 mesh, Alpha chemicals) was suspended in hexane and packed into a glass column (2 × 30 cm) and equilibrated with a mixture of n-hexane. The crude extract obtained from the above procedure was evaporated to dryness with 0.3 g of Silica gel (200 mesh, Alpha chemicals). The dried extract was loaded onto the packed silica gel column and then eluted with a gradient of n-hexane: acetone. The continued elution resulted in the separation of the initial fractions. The subsequent changes in the eluent separated the next fractions. The last coloured bands remaining at the top of the column were removed by 100% methanol. Three different fractions were collected and concentrated to a small volume. Further the dried separated bands were analyzed spectro-photometrically and then by HPLC analysis.

HPLC Analysis of the Fractions: The fractions were analyzed on Agilent 1200 series analytical HPLC equipped with a degasser, autosampler, Dioade array detector (DAD), and an integrator. Mobile phase was first filtered through 0.2 µm filter, sonicated on ultrasonic bath to remove dissolved gas and then used for HPLC. A reversed phase C30 column was used for analysis. Following method parameters were used for analysis.

Column Specifications: Dimensions 250 × 4.6 mm, 5 µm particle size, (Agilent Technologies, USA)

Mobile Phase Solvent A- Methanol: ACN: Water (21: 16.5: 62.5 v/v/v) + 10 mM ammonium acetate

Solvent B- Methanol: ACN: ethyl acetate (50: 20: 30 v/v/v) of the same material was used. The following HPLC conditions were chosen for gradient programme.

TABLE 1: CONDITIONS FOR HPLC SOLVENT GRADIENT

Time (min)	%B	Flow rate (ml/min)
0	20	0.75
10	70	1
40	100	1
60	100	1

Temperature of the column: 20 ºC
Flow rate of mobile phase: 0.75ml/min
Run time: 60 min
Dectector: Variable wavelength Detector (VWD)
Detection wavelength: 450 nm.

Incorporation of Fractions in Cosmetic / Sun Protective Formulation: The basic formulation of sunscreen (gel and cream) having components is shown in **Table 2**. The crude extract and the separated fractions were incorporated in 100 gm formulation at various amounts.

TABLE 2: COMPONENTS FOR GEL AND CREAM BASE PREPARATION

Components	Cream (g/ 100g)	Gel (g/ 100g)
Oil Phase		
CCTG	2.0	-
IPM	4.0	-
Cetosteryl alcohol	2.0	-
Stearic acid	3.0	-
Propyl paraben	0.02	-
Water Phase		
Carbopol 940	-	1.0
Methyl Paraben	-	0.25
EDTA	0.2	0.2
Glycerin	4.0	3.0
Triethanol amine	0.42	0.9
Actives		
Extract	0.025	0.025
Water	84.335	84.335

To study the effectivity of the sunscreen formulation and the protective role of Zeaxanthin, commercial UV protectants / artificial sunblockers like Octyl Methyl Cinnamate and Avobenzone were also incorporated and further studied for its potential role as a sunscreen agent.

SPF Measurement: SPF study was done with the help of UV-2000S Ultraviolet Transmittance Analyzer (Labsphere inc. USA) at V.G. Vaze College, Mumbai and Kelkar Trust's Scientific Research Center, Mulund, Mumbai.

RESULT AND DISCUSSION:

Extraction of Carotenoids: Optimizing the solvent for extraction was an important part of the study. The parameters to be studied were minimum biomass utilization, best extraction solvent, and number of extraction steps involved. To fulfill this aim, various different solvents were tested for the efficient extraction of total carotenoids. Carotenoids are compounds of low polarity and therefore soluble in solvents of low polarity such as hexane. However, they also have a polar part, soluble in polar solvents thus increasing the range of organic solvents useful in the carotenoids extraction [13]. A range of solvents including methanol, ethanol, petroleum ether, ethyl acetate and Hexane were used. The crude extract obtained was studied spectrophotometrically.

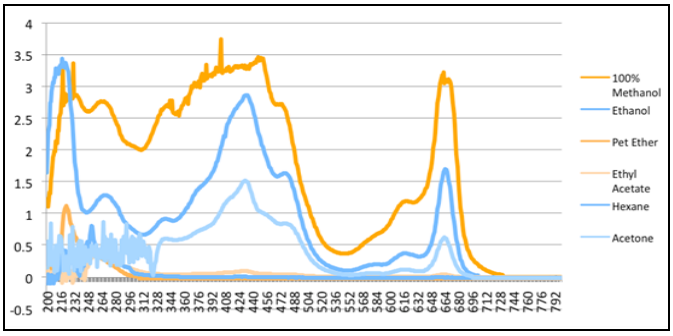

FIG. 1: ABSORPTION PATTERN OF THE CRUDE EXTRACT OF *S. PEVALEKII* OBTAINED IN DIFFERENT SOLVENTS

TABLE 3: MEAN UV A AND UV B ABSORBANCE VALUES OF VARIOUS SOLVENTS

S. no.	Solvents used	Mean UV A Absorbance	Mean UV B Absorbance
1	Petroleum ether	-0.009	0.048
2	Ethyl Acetate	0.058	0.143
3	Hexane	0.017	0.131
4	Acetone	0.629	0.457
5	Ethanol	1.155	0.815
6	Methanol	2.815	2.129

Though the complete light spectrum ranging from 200 nm to 400 nm was scanned for the absorption, the absorbance in the UV region is considered here to study the UV absorbing ability of the extracted carotenoids in the respective solvents. The mean UV A and Mean UV B absorbance values for the various solvents are given in **Table 3**.

Petroleum ether, ethyl acetate and hexane did not show any noticeable absorbance in the UV- A as well as in the UV- B region. The mean UV A and UV B absorbance were not very high thus suggesting that the extract obtained by using these extracts does not absorb UV light. However there was a slight absorbance seen in the UV C region. UV C rays do not reach the earth. Thus absorbing

the UV C rays do not serve any purpose.Ethanol showed a good absorbance in the UV B as well as UV A region. A shoulder peak was observed at 340nm that showed an absorbance of 0.921.

Of the entire solvent range 100% methanol showed the best results for extraction of complete carotenoid content. It showed the highest absorbance in the UV region, the mean absorbance values in the UV B region was 2.129. This mean UV A absorbance increased to 2.815 which was the highest value recorded for any of the solvents. A shoulder peak was also observed in the case of 100% methanol as well. It showed a high absorbance of 2.702.

Thus from the above results 100% methanol gave the highest absorbance in the UV- A as well as UV- B region as compared to other solvents **Table 3**. Also the absorption spectrum was very intense in the wavelength ranging from 400 nm to 800 nm, a range that best represents carotenoids.

Methanol was the solvent of choice for efficient extraction of chlorophyll and total carotenoid along with cell wall disruption techniques from *Nannochloropsis gaditana*, a marine microalga [14]. In another study in *Gracilaria tenuistipitata*, a red seaweed various solvents were screened for efficient extraction of pigments and carotenoids. It was found that methanol serves as the best solvent for extraction of chlorophyll and carotenoids. Then carotenoids detected after HPLC analysis were Zeaxanthin, β- Cryptoxanthin and β- carotene. Apart from being the most efficient solvent, another advantage of methanol is the compatibility with most mobile phases used in the analyses of pigments by HPLC, which minimizes the unfavorable interaction between the solute, the solvent injected and the mobile phase [15].

Separation of Carotenoids by Column Chromatography and Spectrophotometric Determination of the Fractions: The crude pigment extracts were subjected to silica gel open column chromatography with n-hexane: acetone

concentrations being the eluting solvent system, and all fractions were analyzed by HPLC analysis. The fraction separation is elucidated in **Table 4**. The collected fractions were examined spectrophotometrically for the UV absorption. The absorption pattern is depicted in **Fig. 2.**

TABLE 4: FRACTIONATION OF THE CRUDE EXTRACT AT DIFFERENT CONCENTRATIONS OF THE SOLVENT GRADIENT

Eluent	Description
Hexane : Acetone (80:20)	Clearly yellow coloured separated band migrates down the column. About 3 - 4 ml of the fraction is collected. (Yellow fraction)
Hexane : Acetone (60:40)	After the change in the eluent an intense green coloured band migrates down the column. About 2 - 3 ml of the fraction is collected. This band is separated after approximately about 35- 40 min from the start of the column. (Dark Green Fraction)
Methanol (100%)	Methanol wash results into migration of all the remaining bands and get collected together as one fraction. Their migration takes around 30 min. (Methanolic fraction)

The three fractions separated were studied spectrophotometrically. The first and the second fraction i.e. the yellow band and the dark green fraction showed a very high absorbance in the UV-A region (340 nm - 400 nm), where as the methanolic fraction showed a very high absorbance in the UV C (200 nm - 280 nm) and UV-B region (280 nm - 340 nm) **Fig. 2**. These fractions were further analysed by HPLC analysis.

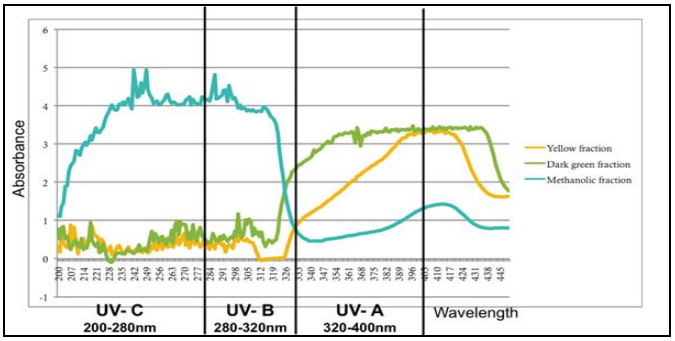

FIG. 2: SPECTROPHOTOMETRIC DETERMINATION OF THE FRACTIONS

HPLC Analysis of Various Fractions: The three fractions separated by column chromatography were further analysed by HPLC. The peaks obtained at various time intervals were compared with authentic samples of carotenoids like Fucoxanthin, Astaxanthin, Zeaxanthin, Lutein, Canthaxanthin and *β*- Carotene. **Fig. 3** gives HPLC chromatogram of the authentic samples of carotenoids. Their concentrations in carotenoid mixture is represented as Area % in **Table 5**.

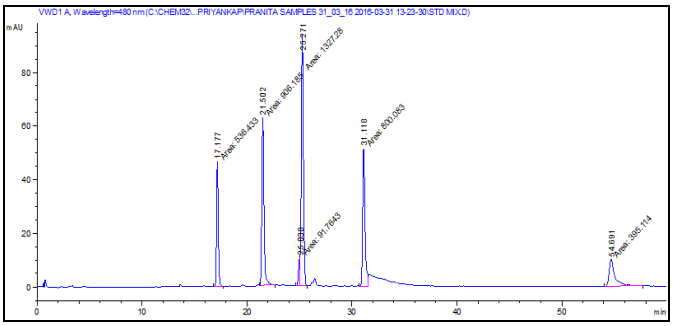

FIG. 3: CHROMATOGRAM OF THE STANDARD CAROTENOID SAMPLES

Standard Mixture of Carotenoid Analysis: In the standard mixture, there were in total 6 different xanthophylls standards used to detect the unknown. Out of these only two were identified. One was *β*-Carotene found in the yellow fraction and other was Zeaxanthin found in the dark green fraction. Canthaxanthin was also detected however in very small quantity.

TABLE 5: INFORMATION SHEET OF THE CAROTENOIDS FOUND IN THE STANDARD MIXTURE

Sr. no.	Carotenoid	Time	Area	Height	Width	Area%	Symmetry
1	Fucoxanthin	17.177	536.4	46.5	0.1922	13.223	0.791
2	Astaxanthin	21.502	906.2	62.7	0.2411	22.337	0.732
3	Zeaxanthin	25.038	91.8	10.7	0.1429	2.262	0
4	Lutein	25.271	1327.3	93.6	0.2364	32.717	0.897
5	Canthaxanthin	31.118	800.1	51.5	0.2591	19.722	0.707
6	β- Carotene	54.691	395.1	10	0.6561	9.739	0.469

Yellow Fraction Analysis: The yellow fraction shows two major peaks- Unknown 1 which does not tally with any of the standard carotenoid sample, at the retention time 43.027 min whose area % is as high as 74.617%. The second peak appears at 55.772 min and is about 15.14%.

It matches with the retention time of β- Carotene. **Fig. 4** shows the HPLC chromatogram of the yellow fraction and the carotenoids in the fraction is represented as Area% in **Table 6**.

TABLE 6: INFORMATION SHEET OF THE CAROTENOIDS FOUND IN THE YELLOW FRACTION

Sr. no.	Carotenoid	Time	Area	Height	Width	Area%	Symmetry
1		36.223	7.9	5.80E-01	0.2268	2.439	0.913
2		41.955	6.2	3.80E-01	0.2694	1.916	1.019
3	Unknown 1	43.027	242	14.2	0.2836	74.617	0.621
4		43.919	2.9	1.40E-01	0.3358	0.879	0.34
5		46.387	16.2	4.50E-01	0.5972	5.008	0.898
6	β- Carotene	55.772	49.1	1.4	0.5974	15.14	0.547

Dark Green Fraction Analysis: The dark green fraction showed six different peaks at different retention times **Fig. 5**. The first major peak eluted at retention time 25.354 min that matches with standard peak of Zeaxanthin (retention time 25.038 min). This component appears to be major component occupying the area% 56.398. The second peak eluted at retention time 30.523 min was coinciding the standard of Canthaxanthin (retention time 31.118 min). But its area% was comparatively smaller (9.693). The other peaks *i.e.* unknown 2 at retention time 41.346 min, area% 13.17 and unknown 3 at retention time 46.897 min and area% 11.705 did not match with any of the above authentic samples.

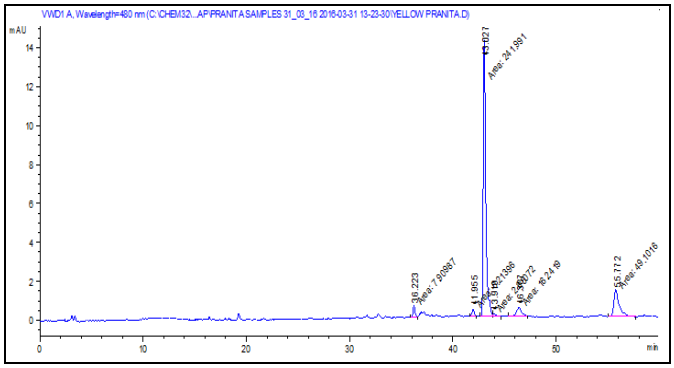

FIG. 4: HPLC CHROMATOGRAM OF THE YELLOW FRACTION

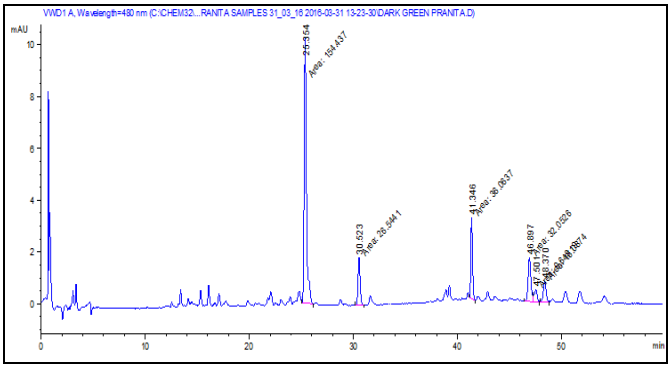

FIG. 5: HPLC CHROMATOGRAM OF THE DARK GREEN FRACTION

TABLE 7: INFORMATION SHEET OF THE CAROTENOIDS FOUND IN THE DARK GREEN FRACTION

Sr. no.	Carotenoid	Time	Area	Height	Width	Area%	Symmetry
1	Zeaxanthin	25.354	154.4	10.2	0.2518	56.398	0.716
2	Canthaxanthin	30.523	26.5	1.9	0.2389	9.693	0.823
3	Unknown 2	41.346	36.1	3.1	0.1921	13.17	0.844
4	Unknown 3	46.897	32.1	1.7	0.3126	11.705	0
5		47.501	9.6	4.80E-01	0.3363	3.524	1.012
6		48.7	15.1	8.10E-01	0.3123	5.51	0.945

Methanolic Fraction Analysis: The major peaks obtained in methanolic fraction **Fig. 6, Table 8** elute at 16.49 min with area% 29.412 (Unknown 4). The other peak (Unknown 5) eluted at 19.086 min was present in high amount 47.348. However both these peaks did not match with any authentic standard carotenoid samples.

TABLE 8: INFORMATION SHEET OF THE COMPONENTS FOUND IN THE METHANOLIC FRACTION

S. no.	Carotenoid	Time	Area	Height	Width	Area%	Symmetry
1		4.735	18.4	3.3	0.0937	4.645	0.763
2		7.709	31	4.3	0.1211	7.845	0.643
3		16.016	42.3	3.3	0.2109	10.713	0.741
4	Unknown 4	16.494	116.2	9.3	0.2091	29.412	0.763
5	Unknown 5	19.086	187.2	12.6	0.2469	47.384	0.706

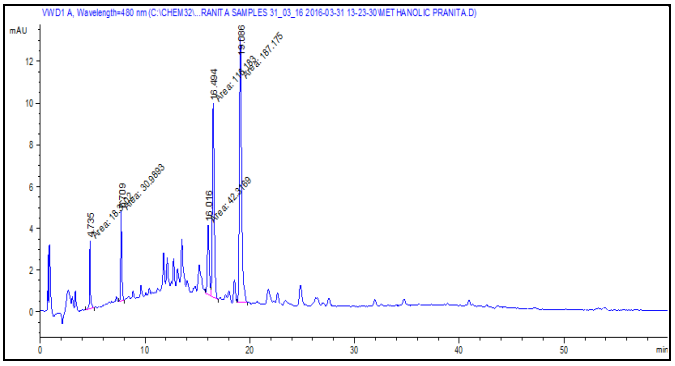

FIG. 6: HPLC CHROMATOGRAM OF THE METHANOLIC FRACTION

Cosmetic Formulation of Crude and Purified Extract of *S. pevalekii*:

Incorporation of the Eluted Fractions into a Cosmetic Formulation: In the present study, as the crude extract as well as the eluted fractions showed a very good ability to absorb various regions of UV light, it was decided to screen the potentiality of extraction in skin protecting cosmetics.

SPF or Sun protective factor is a measure of how well a sun screen will protect skin from UV-B rays. There are various parameters developed to indicate the degree of broadband protection given by sunscreen. Critical wavelength is one such metric being the shortest wavelength at which the sunscreen absorbed 90% of the sun's energy.

If the critical wavelength is 370 nm or greater, the formulation can be described as having broad spectrum protection *i.e.* protecting in both UV A and UV B region. In UK, star rating system was developed that awarded 1-5 stars depending on the ratio of mean UV A/B absorbance. Higher the UVA absorption, higher is the ratio and greater is the number of stars the product earns (Labsphere Inc. UK). Thus more the boot star rating higher is the ability of formulation to absorb in the UV A region.

In initial studies 25 mg of crude extract was incorporated in two types of bases *viz*., Cream and Gel. The formulations were subjected to SPF analysis, Scientific Research Centre of V. G. Vaze College. The results are reported in **Table 9.**

TABLE 9: CRUDE EXTRACT INCORPORATED IN CREAM AND GEL FORMULATIONS

S. no.	Test Samples	Parameters				Average Values
		Scans	1	2	3	
1	Cream without any active	SPF	1.13	1.13	1.12	1.13
		Standard Deviation	0.01	0.01	0.01	0.01
		UV A/UV B Ratio	0.051	0.049	0.046	0.049
		Critical Wavelength	321.00	320.67	320.50	320.72
		Boot star Rating	No Rating	No Rating	No Rating	No Rating
2	Cream containing 25 mg of Crude extract	SPF	1.16	1.15	1.16	1.16
		Standard Deviation	0.02	0.01	0.02	0.01
		UV A/UV B Ratio	0.402	0.408	0.403	0.404
		Critical Wavelength	371.80	371.40	372.20	371.80
		Boot star Rating	No Rating	No Rating	No Rating	No Rating
3	Gel without any active	SPF	0.82	0.82	0.82	0.82
		Standard Deviation	0.01	0.01	0.01	0.01
		UV A/UV B Ratio	0.301	0.298	0.299	0.299
		Critical Wavelength	338.40	337.40	338.20	338.00
		Boot star Rating	No Rating	No Rating	No Rating	No Rating
4	Gel containing 25 mg of Crude extract	SPF	0.96	1.10	1.09	1.10
		Standard Deviation	0.00	0.02	0.01	0.01
		UV A/UV B Ratio	0.925	0.330	0.330	0.330
		Critical Wavelength	385.80	379.20	379.60	379.33
		Boot star Rating	*****	*****	*****	*****

When the crude methanolic extract (25 mg) was incorporated in gel formulation there was negligible increase in SPF which signifies its efficiency in UV B region. The SPF of gel with crude extract was 0.96 whereas control gel without any active, SPF was 0.82. The critical wavelength of the control gel was 338.00 nm whereas when the gel was supplemented with crude extract the critical wavelength was increased to 379.33 nm. If the critical wavelength is greater than 370 nm it indicates the ability of the formulation to absorb in the UV A region which is indicated by boot star

rating 5. The UV A/ UV B ratio is also the indicative of the ability of the formulation to absorb in the UV A region. In control gel the UV A/ UV B ratio was 0.299 where as in the gel containing crude extract the ratio was as high as 0.925 which suggests that the extract has a great potential to absorb the UV A region of light.

But when the extract was incorporated in the cream formulation the SPF value was little higher 1.16. But its UV A/UV B ratio was very low (371.80 nm) and there was no boot star rating. This suggests that the cream formulation was not suitable for incorporation of carotenoids. There are many carotenoid based gels marketed commercially especially for eye protection. Some of them are as listed Eye Promise Zeaxanthin (Zeavision) [16], Icaps Eye Vitamin Lutein and Zeaxanthin Formula (Alcon), Macula Complete (Biosyntrx), Macular Protect Complete (Science Based Health), MaxiVision Ocular Formula (MedOp), Occuguard Plus (TwinLab), Preser Vision (Baush And Lomb) [17].

Effect of Various Concentrations of Crude Methanolic Extract of *S. pevalekii* on Sun Protective Ability: For studying the sun protective ability of crude methanolic extract of *S pevalekii* various concentrations *viz*., 5 mg, 10 mg, 25 mg, 50 mg, 100 mg, 200 mg were incorporated in gel formulations and was further analysed for sun-protective ability. As seen in **Table 10,** all concentrations give very less SPF in the range of 0.94 to 0.96. Similarly all the formulations give a boot star rating of 5 stars which mean all are effective in UV A region.

TABLE 10: DIFFERENT CONCENTRATION OF CRUDE EXTRACT INCORPORATED IN GEL FORMULATION

S. no.	Test Samples	Parameters				Average Values
		Scans	1	2	3	
1	Crude	SPF	0.96	0.96	0.96	0.96
	5 mg	Standard Deviation	0.00	0.00	0.00	0.00
		UV A / UV B Ratio	2.165	2.161	2.162	2.163
		Critical Wavelength	390.17	390.33	390.00	390.17
		Boot star Rating	*****	*****	*****	*****
2	Crude	SPF	0.96	0.96	0.96	0.96
	10 mg	Standard Deviation	0.00	0.01	0.01	0.01
		UV A / UV B Ratio	2.071	2.080	2.084	2.078
		Critical Wavelength	390.33	390.50	390.17	390.33
		Boot star Rating	*****	*****	*****	*****
3	Crude	SPF	0.96	0.97	0.96	0.96
	25 mg	Standard Deviation	0.00	0.00	0.00	0.00
		UV A / UV B Ratio	1.554	1.551	1.552	1.552
		Critical Wavelength	389.00	389.17	389.00	389.06
		Boot star Rating	*****	*****	*****	*****
4	Crude	SPF	0.96	0.96	0.96	0.96
	50 mg	Standard Deviation	0.00	0.00	0.01	0.00
		UV A / UV B Ratio	1.329	1.323	1.329	1.327
		Critical Wavelength	387.17	387.33	387.17	387.22
		Boot star Rating	*****	*****	*****	*****
5	Crude	SPF	0.95	0.95	0.95	0.95
	100 mg	Standard Deviation	0.00	0.00	0.01	0.00
		UV A / UV B Ratio	1.211	1.212	1.216	1.213
		Critical Wavelength	385.33	385.17	385.67	385.39
		Boot star Rating	*****	*****	*****	*****
6	Crude	SPF	0.94	0.94	0.94	0.94
	200 mg	Standard Deviation	0.01	0.00	0.00	0.00
		UV A / UV B Ratio	1.016	1.012	1.017	1.015
		Critical Wavelength	382.83	383.00	383.17	383.00
		Boot star Rating	*****	*****	*****	*****

But UV A/ UV B ratio is the indicator of ability to absorb UV A region, appears to be increasing at lower concentrations of crude extract. At 200 mg the UV A / UV B ratio was 1.016, at 100 mg it increases to 1.213. At 50 mg it becomes 1.327, at 25 mg it is 1.552, at 10 mg it is 2.078 and at the

lowest concentration *i.e.* 5 mg it is the highest *i.e.* 2.163. The results are very promising. The commercial UV absorber, Uvinul A plus when incorporated at 1.5 g / 100 gm formulation UV A/ UV B ratio obtained was 1.638. And when 2 g/g of TiO_2 was incorporated in cream the observed UV A/ UV B ratio was 0.955 [18].

Similarly when 2% TiO_2 was incorporated in formulation the critical wavelength was 387 nm and for Uvinul A plus it was 375.87 nm. The crude methanolic extract at very low concentration of 5 mg (0.005%) exhibits the critical wavelength of 390.17 nm. At 25 mg it is 389.06 nm and at 200 mg it is 383 nm. This suggests the efficacy of the extract in the UV A region.

Incorporation of the Eluted Fractions *viz*, B Carotene and Zeaxanthin and Methanolic Fractions in to Gel Formulation: 25 mg of each *i.e.* β carotene, zeaxanthin and methanolic fraction were incorporated in cosmetic gel formulation and were again tested for SPF and Bootstar rating.

The results of incorporating the eluted fractions in gel base are mentioned in **Table 11**. The SPF in the gel formulation containing Zeaxanthin showed the best SPF (1.21) amongst the other two fractions.

The SPF in the gel with β- Carotene was 1.10 and the methanolic fraction was 1.03. The critical wavelength in the case of gel with β- Carotene as 380.27 nm, for gel with Zeaxanthin it was 386.87 nm and for methanolic was 387.87 nm. There was no bootstar rating obtained for β- Carotene and the methanolic fraction. But zeaxanthin showed a 5 star boot star rating. Thus proving that Zeaxanthin can be one of the potential sunscreening component / sun protectant.

TABLE 11: INCORPORATION OF INDIVIDUAL FRACTIONS IN GEL FORMULATIONS

S. no.	Test Samples	Parameters				Average Values
		Scans	1	2	3	
1	β carotene	SPF	1.10	1.10	1.10	1.10
		Standard Deviation	0.02	0.02	0.02	0.02
		UV A/UV B Ratio	0.422	0.419	0.418	0.420
		Critical Wavelength	380.60	380.00	380.20	380.27
		Boot star Rating	No Rating	No Rating	No Rating	No Rating
2	Zeaxanthin	SPF	1.21	1.21	1.18	1.20
		Standard Deviation	0.02	0.01	0.02	0.02
		UV A/UV B Ratio	0.599	0.627	0.597	0.608
		Critical Wavelength	386.80	387.00	386.80	386.87
		Boot star Rating	*****	*****	*****	*****
3	Methanolic	SPF	1.03	1.03	1.03	1.03
		Standard Deviation	0.00	0.01	0.00	0.00
		UV A/UV B Ratio	0.494	0.491	0.492	0.492
		Critical Wavelength	387.80	387.80	388.00	387.87
		Boot star Rating	No Rating	No Rating	No Rating	No Rating
4	Only Gel	SPF	0.82	0.82	0.82	0.82
		Standard Deviation	0.01	0.01	0.01	0.01
		UV A/UV B Ratio	0.301	0.298	0.299	0.299
		Critical Wavelength	338.40	337.40	338.20	338.00
		Boot star Rating	No Rating	No Rating	No Rating	No Rating

Cumulative Effect of Zeaxanthin Fraction with Known UV B Protectants: The inorganic compounds like TiO_2 and ZnO_2 and Uvinul A plus are a few examples of a broad spectrum sun screening agents. Though TiO_2 and ZnO_2 are potential sunscreens they form a white coloured coating on skin. The organic substances like Octyl methyl cinnamate (OMC) which impart protection in the UV B region; and Benzophenone 3 and Avobenzone give protection only in UV A region.

In the present study our intention was to study the cumulative effect of these UV B protective compounds with Zeaxanthin containing fractions. Hence various concentrations of these compounds like OMC and Avobenzone were incorporated in the gel individually or along with 25 mg of zeaxanthin. The results are interpreted in **Table 12**.

The various concentrations of OMC incorporated were 1g, 200 mg and 40 mg. As the concentration

of OMC increased there was a good increase in the SPF value. At lowest concentration of OMC *i.e.* 40 mg the SPF was 1.32 which was increased to 2.05 at 200 mg OMC and when the concentration was 1% the SPF was the highest 4.51. But at this concentration of OMC the UV A/ UV B ratio was only 0.165 and the critical wavelength was 334 nm which is far less than 370 nm to have a boot star rating. Similarly at 200 mg of OMC SPF was 2.05 but the UV A / UV B was very poor *i.e.* 0.116 and the critical wavelength was 328.07 nm.

When 200 mg of OMC was supplemented with Zeaxanthin there was a little decline in the SPF from 2.05 to 1.09. But the UV A / UV B was increased from 0.116 to 0.486. The critical wavelength became 372.2 nm which is going near to 370 nm to obtain a boot star rating. Still more permutations and combinations are needed to make the combination a broad spectrum.

Avobenzone is a chemical sunscreen known for its instability. The compound is unstable to light exposure and is mainly used along with antioxidants like tocopherols, Vitamin A, Vitamin C and Ubiquinone [19]. Avobenzone (Butyl methoxy dibenzoyl methane) is oil soluble broad spectrum sunscreen. It significantly degrades by UV or normal light. It degrades very fast when combined with physical sunscreens like TiO_2 or ZnO_2. These compounds protect from UV radiation by reflecting light. However this action also generates free radicals. That can subsequently degrade avobenzone. Carotenoids are known to play a very important role in preventing photodegradation of chlorophylls [20].

Hence another aspect of the study was to determine the protective role of zeaxanthin towards Avobenzone, to prevent its degradation. The concentration of Avobenzone used were 200 mg, and 40 mg.

When 200 mg of Avobenzone was used the SPF was 1.25. When 200 mg of Avobenzone was supplemented with 25 mg of Zeaxanthin the SPF remained almost the same *i.e.* 1.22. But there was substantial improvement in UV A / UV B ratio. For 200 mg of Avobenzone UV A / UV B ratio was 0.365 which was improved to 0.409. The critical wavelength increased from 351.4 nm to 359.2 nm. When Avobenzone used was 40mg the SPF was 1.07. The UV A / UV B ratio was very poor *i.e.* as low as 0.047 and the critical wavelength was 320.33 nm.

When 40 mg of Avobenzone was supplemented with 25 mg of Zeaxanthin there was little improvement in SPF observed, it increased from 1.07 to 1.11. The UV A / UV B ratio improved from 0.047 to 0.370. And there was also an increase seen in the critical wavelength from 320.33 nm to 370 nm.

When the same amount (40 mg) of Avobenzone was supplemented with 36 mg of zeaxanthin there was little decline in SPF to 0.95. But UV A / UV B ratio was increased to 0.725 and the critical wavelength was 379.20 nm. Hence the right ratio of Avobenzone and carotenoids not only improves its stability but also makes Avobenzone a broad spectrum UV protectant.

TABLE 12: INCORPORATION OF FRACTIONS WITH KNOWN UV-B PROTECTANTS

S. no.	Test Samples	Parameters				Average Values
		Scans	1	2	3	
1	OMC 1 g	SPF	4.49	4.46	4.57	4.51
		Standard Deviation	0.07	0.06	0.02	0.05
		UV A/UV B Ratio	0.163	0.166	0.165	0.165
		Critical Wavelength	334.00	334.00	334.00	334.00
		Boot star Rating	No Rating	No Rating	No Rating	No Rating
2	OMC 200 mg	SPF	2.06	2.06	2.02	2.05
		Standard Deviation	0.10	0.02	0.05	0.06
		UV A/UV B Ratio	0.114	0.115	0.118	0.116
		Critical Wavelength	328.00	328.00	328.20	328.07
		Boot star Rating	No Rating	No Rating	No Rating	No Rating
3	OMC 40 mg	SPF	1.32	1.29	1.28	1.30
		Standard Deviation	0.05	0.07	0.06	0.06
		UV A/UV B Ratio	0.120	0.121	0.120	0.120
		Critical Wavelength	329.00	329.00	328.60	328.87
		Boot star Rating	No Rating	No Rating	No Rating	No Rating
4	Zeaxanthin 25 mg +	SPF	1.10	1.08	1.09	1.09
	OMC 200 mg	Standard Deviation	0.01	0.02	0.03	0.02

		UV A/UV B Ratio	0.487	0.477	0.495	0.486
		Critical Wavelength	374.40	369.20	373.00	372.20
		Boot star Rating	No Rating	No Rating	No Rating	No Rating
5	Zeaxanthin 25 mg + OMC 40 mg	SPF	1.09	1.09	1.08	1.09
		Standard Deviation	0.01	0.01	0.01	0.01
		UV A/UV B Ratio	0.115	0.115	0.118	0.116
		Critical Wavelength	331.00	330.60	335.40	332.33
		Boot star Rating	No Rating	No Rating	No Rating	No Rating
6	Avobenzone 200 mg	SPF	1.25	1.27	1.24	1.25
		Standard Deviation	0.01	0.02	0.01	0.01
		UV A/UV B Ratio	0.361	0.363	0.371	0.365
		Critical Wavelength	351.00	351.00	352.20	351.40
		Boot star Rating	No Rating	No Rating	No Rating	No Rating
7	Avobenzone 40 mg	SPF	1.07	1.07	1.07	1.07
		Standard Deviation	0.01	0.01	0.01	0.01
		UV A/UV B Ratio	0.040	0.049	0.051	0.047
		Critical Wavelength	319.60	320.60	320.80	320.33
		Boot star Rating	No Rating	No Rating	No Rating	No Rating
8	Zeaxanthin 25 mg + Avobenzone 200 mg	SPF	1.22	1.22	1.22	1.22
		Standard Deviation	0.02	0.01	0.02	0.02
		UV A/UV B Ratio	0.409	0.408	0.409	0.409
		Critical Wavelength	359.20	359.80	359.20	359.40
		Boot star Rating	No Rating	No Rating	No Rating	No Rating
9	Zeaxanthin 25 mg + Avobenzone 40 mg	SPF	1.12	1.09	1.13	1.11
		Standard Deviation	0.01	0.01	0.02	0.01
		UV A/UV B Ratio	0.371	0.373	0.368	0.371
		Critical Wavelength	370.20	369.20	371.20	370.20
		Boot star Rating	No Rating	No Rating	No Rating	No Rating
10	Zeaxanthin 36 mg + Avobenzone 40 mg	SPF	0.95	0.95	0.94	0.95
		Standard Deviation	0.00	0.00	0.00	0.00
		UV A/UV B Ratio	0.726	0.720	0.728	0.725
		Critical Wavelength	378.60	379.20	379.80	379.20
		Boot star Rating	***	***	***	***

Stabilization of Avobenzone by free radical quenching and photostabilization is therefore needed to maintain optimum protective ability of sunscreen. A number of stabilizers for Avobenzone are claimed [19]. Bezalel *et al*., IBR Ltd., have extensively studied the hydroxyl radical quenching ability of colourless carotenoids Phytoene and Phytofluene. To study the ability of Phytoene and Phytofluene, Avobenzone 2% was dissolved in oil (polydecene) and exposed for 10 min to sunlight. The same concentration of Avobenzone was mixed with increasing concentrations of phytoene and phytofluene (0.0037%, 0.0075% and 0.015%) in polydecene solution and exposed for 10 min to sunlight. Level of Avobenzone was quantified using UV visible spectrophotometer. Following 10mins of exposure to sunlight Avobenzone levels were reduced 36%. In the presence of 0.015% of phytoene and phytofluene photodecomposition of Avobenzone was only 17% retaining 50% more Avobenzone than without stabilizer. These observations support the potential increase in stability, safety and effectiveness of sunscreens due to presence of phytoene and phytofluene carotenoids.

CONCLUSION: The crude extract gets efficiently extracted in 100% methanol solvent. The fractionation of the crude extract by Silica gel open column chromatography was successful and gave three different fractions containing *β*- Carotene, Canthaxanthin and (majorly) Zeaxanthin. The activity of zeaxanthin was studied with respect to its property of skin protection. It was found that the incorporation of the crude extract as well as the fraction containing Zeaxanthin did not result in an increase in the SPF value but always showed a 5 star boot star rating.

Also the other parameters like UV A/ UV B ratio and the Critical wavelength were such that it proved the formulation to be a potential sunscreening agent. Avobenzone is a commercial UV blocker that is unstable individually in any cosmetic formulation and requires various stabilizers to stabilize it and maintain its activity. Zeaxanthin was found to be a potential stabilizer of Avobenzone and also work synergistically with it to improve the quality of sun screen formulation.

ACKNOWLEDGEMENT: We extend our heartfelt gratitude to Dr. Reena Pandit, Centre of Energy Biosciences ICT, Mumbai, for providing us the facility of HPLC analysis of the carotenoid samples. We are thankful to Kelkar Education Trust's Scientific Research Centre, Mulund, Mumbai for providing help to analyse SPF of sunscreen formulations. We would like to thank Department of Cosmetology and Perfumery V. G. Vaze College for providing lab facilities. We also appreciate the help provided by Dr. B. B. Sharma (Principal, V. G. Vaze college of Arts, Science and Commerce, Mulund, Mumbai) for providing facilities and his constant support.

CONFLICT OF INTREST: There is no conflict of interest.

REFERENCES:

1. Wiechers S, Biehl P, Luven C, Maier M, Meyer J, Münzenberg, Schulze-Isfort C and Albers P: Titanium Dioxide Particle Size *vs.* Sun Protection Performance; Cosmetics and Toileteries Magazine 2013; 128(5): 2013.
2. Bhatia S, Sharma K, Namdeo AG, Chaugule BB, Kavale M and Nanda S: Broad protectiove sun protective action of Porphyra-334 derived from Porphyra vietnamemsis, Phcog Res 2010; 2: 45-9.
3. Kuczynska P, Rzeminska M and Strzalka K: Characterisation of Carotenoids Involved in the Xanthophyll Cycle, Intech, http://dx.doi.org/10.5772/ 67786.
4. Ishida B and Bartley G: Carotenoids: Chemistry, sources, and physiology. In B. Caballero, L. Allen, and A. Prentice (Eds.), Encyclopedia of human nutrition Oxford: Elsevier Academic Press 2005; 2: 330-338.
5. Namitha K and Negi P: Chemistry and Biotechnology of Carotenoids, Critical Reviews in Food Science and Nutrition 2010; 50(8): 728-760.
6. Martinez AJ, Brahm P, Gonzalez A and Stinco C: A comprehensive review on colorless carotenoids phytoene and phytofluene, Archives of Biochemistry and Biophysics 2015; 572: 188-200.
7. Bezalel L, Aliluiko A, Kachko I and Fishbein D: Phytoene and Phytofluene the Colorless Carotenoids – Anti Aging and Photo Protection – a New Way of Stabilizing Sunscreens, IBR Ltd report.
8. Engelmann NJ, Clinton SK and Erdman JW: Nutritional aspect of Phytoene and Phytofluene, Carotenoid precursors to Lycopene, Advances in Nutrition 2011; 2: 51-61.
9. Anunciato TP and Filho P: Carotenoids and polyphenols in nutricosmetics, nutraceuticals and cosmetics, Journal of Cosmetic dermatology 2011; 11: 51-54.
10. Darvin M, Sterry W, Lademann J and Vergou T: The role of Carotenoids on Human skin, Molecules 2011; 16: 10491- 10506.
11. Dueve O and SaNogueira J: Sunscreen compositions with SPF enhancers, Patent US 2006/0008427 A1 2006.
12. Vuong T: Skin anti-oxidant enhancing formulation and associated method, US 2013/0309183 A1 2013.
13. Mezzomo N and Ferreira S: Carotenoids Functionality, Sources, and Processing by Supercritical Technology: A Review, Journal of Chemistry 2016, Article ID 3164312, 16 pages
14. Henriques M, Silva A and Rocha J: Extraction and quantification of pigments from a marine microalga: A simple and reproducible method, Communicating Current Research and Educational Topics and Trends in Applied Microbiology 2007.
15. Torres P, Chow F, Furlan C and Mandelli F: Standardization of a protocol to extraxt and analyze chlorophyll and carotenoids in *Gracilaria tenuistipitata* VAR liui. Zhang and Xia (Rhodophyta), Brazilian Journal of Oceanography 2014; 62(1): 57-63.
16. Sajilata M, Singhal R, and Kam M: The Carotenoid Pigment Zeaxanthin - A Review, Comprehensive reviews in food science and food safety 2008; 7: 29-49
17. Heiting G: Lutein and Zeaxanthin: Eye and vision benefits, http://www.allaboutvision.com/nutrition/lutein.htm
18. Dike M and Deodhar M: Biotechnological approaches: For understanding reproductive behaviour of *Garciania indica* (choisy) and exploring its fruit extracts for UV protective ability 2016, Thesis submitted to Mumbai University.
19. Afonso S, Horita K, Sousa e Silva J, Almeida I, Amaral M, Lobão P, Costa P, Miranda M, Silva J and Lobo J: Photodegradation of avobenzone: Stabilization effect of antioxidants 2014, Journal of Photochemistry and Photobiology B: Biology 2014; 140: 36-40.
20. Jaswir I, Noviendri D, Hasrini R and Octavianti F: Carotenoids: Sources, medicinal properties, and their application in food and nutraceutical industry; Journal of medicinal plants research 2011; 5(33): 7119-7131.

How to cite this article:
Tendulkar PA, Surve MA and Deodhar MA: Sun protective formulation from carotenoids extracted from thermotolerant genera *Synechocystis pevalekii*. Int J Pharm Sci Res 2018; 9(8): 3223-34. doi: 10.13040/IJPSR.0975-8232.9(8).3223-34.