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In-vitro Antioxidative Property of Water Soluble Bacterial Melanin and Evaluation on Novel Model System

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Abstract: ***Melanin is important because it's the most primitive and universal pigment in living organisms. The present study reports antioxidant activity of melanin pigment from EP83 a Gram negative bacterial strain isolated from garden soil, of Gujarat University Campus, Gujarat, India. The purified melanin fraction showed promising free radical scavenging activity through DPPH, Superoxide Radical Scavenging Activity, Lipid Peroxidation Inhibition Assay, ABTS^{+} scavenging capacity assay methods and also onto its evaluation of fish mince model system. The colored DPPH solution was progressively reduced to half during the course of incubation of 54, 21, 16 hours for 14.9, 29.8 and 44.7µg/mL of melanin concentrations respectively and ABTS+ scavenging capacity of the extract was 26.49 ± 2.34%, 37.24 ± 2.96% and 43.88 ± 2.92% of control for the melanin extract at the concentrations of 10, 15 and 20% (v/v), of melanin respectively. The superoxide anion radical scavenging assayed by both PMS-NADH system and fish mince system reveled dose dependent scavenging activity by the n-butanol extract. Melanin extract also scavenges ABTS+ free radical significantly in a dose as well as time dependent manner.***

Key Words: Melanin, EP 83, Antioxidant Property, Fish Mince Model

I. INTRODUCTION

Melanins are heteropolymers formed by oxi-dative polymerization of tyrosine, dihydroxyphenylala-nine, and catecholamines (stable free radicals) [1], [2]. Melanins readily interact with free radicals and other reactive species due to the presence of unpaired electrons in their molecules.

Exposure to large extent of sunlight may cause erythema or ageing. Ultraviolet (UV) light especially tends to drastically affect skin it also causes cell damage; even worse, it may result in skin cancer, for example, squamous cell carcinoma, basal cell carcinoma and melanoma [3]. UV light also promotes gene mutation and DNA damage [4], produces reactive oxygen species (ROS) and increases oxidative damage [5], [6]. Some research indicates that melanin has a wide absorption spectrum because of its special structure and can protect skin from UV damage [7]. Melanin contains some function groups, such as COOH, OH, SH and NH2 groups; thus, melanin could supply and receive electrons and scavenge ROS produced from organisms or UV to protect from free radicals and lipid peroxidation [8]-[10].

Oxidation is a process that occurs not only when oil is being produced, but also inside our own bodies. Reactions occur continually inside the body, giving rise to the formation of free radicals (peroxidants). As a rule, free radicals do not cause severe damage thanks to the protection provided by antioxidants, which maintain, a balance up to a point. If it is disturbed than, "oxidative stress" occurs, leading to deterioration of normal cell physiology and leads to cell death. Oxidation is a complex, fundamental phenomenon in the progression of cell ageing. Lipid or fat peroxidation tends to be proportional to the number of double bonds in a compound, explaining why oleic acid shows little susceptibility to oxidation.

Melanins are a ubiquitous class of biological pigments; they play an important role in antioxidation. Recent advances in the chemistry of melanins have demonstrated their diversity. The various types of melanin show different physico-chemical properties; their antioxidant properties may not be unique [11]. The study is focused on the antioxidant property of EP83 and its application on to the fish mince model to prove the authentication of results in terms of antioxidant property in addition to production of melanin which could be applied for large purposes. In this study, we investigate the feasibility of extracting melanin from EP83 and evaluating its antioxidant properties.

II. MATERIALS AND METHODS

A. Chemicals

The chemicals including 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt ($ABTS^+$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), were purchased from Sigma-Aldrich (St. Louis, MO, USA). BHA, 2-thiobarbituric acid (TBARS), trichloroacetic acid (TCA), Ammonium thiocyanate, Nitroblue tetrazolium (NBT), phosphate buffer (pH 7.4) and nonenzymatic phenazine methosulfate (PMS) were obtained from SRL, and n-butanol from Burgoyn, Methanol, ethanol, acetone, chloroform, hydrochloric acid were obtained from Rankem, Nicotinamide adenine dinucleotide (PMS-NADH) from Spectrochem, Potassium persulfate from HPLC, Acetic acid, vitamin C, vitamin E and BHA from Merk, $K_3Fe(CN)_6$, $FeCl_3$ and $FeSO_4$ from Nice, Bradford Reagent was prepared freshly, *Labeo rohita* (Rohu) was purchased fresh from local market to prepare Fish mince. All chemicals were of analytical grade with 98-100% purity assay. Deionized water was used wherever required.

B. Preparation of Melanin Extract

Melanin extract was obtained from the bacterial source, *EP83* [12] as a water soluble black-brown pigment. The pigment was extracted and made purified using the previously standardized method [13].

The culture broth was mixed with a four volume of methanol and mixed vigorously. The resulting solution was centrifuged at 10,000 rpm for 10 min. The upper layer was collected and filtered through a 0.22 µpore sized filter paper. The filtrate was concentrated using a rotary evaporator and subsequently extracted with 3.0 M chloroform. The chloroform phase was collected and concentrated to obtain the resultant product, the black-brown pigment.

C. Determination of In-vitro Antioxidative Activities

1) *DPPH Radical Scavenging Assay:* DPPH radical scavenging activity of *EP 83* melanin was performed according to the modified literature method [14]. Primarily, 0.1 mM of DPPH solution was prepared in 95% ethanol before use. To 1 mL of it, different volumes (10, 20 and 30 µL) of melanin suspension of strength 1.55 mg/mL was added along with 30, 20 and 10 µL water. Thus, final concentrations of melanin in melanin mixed DPPH solutions were obtained as 14.9, 29.8 and 44.7µg/mL. The samples were incubated in dark condition at 40˚C throughout the study. Reduction of absorbance at 516 nm supplied with different melanin doses was also carried to plot the graph of % DPPH vs. time course (Fig: 2) required to envisage the scavenging activity. The DPPH radical scavenging activity was evaluated by monitoring the absorbance at 516 nm at various time intervals by keeping the respective melanin strengths dispersed in ethanol as a reference. The percentage of Dose dependent (14.9, 29.8 and 44.7µg/mL) and time dependent (1, 2, 3 days) percent Scavenging activity was measured using following formula:

$$\text{Scavenging activity (\%)} = [(A_0 - A_S)/A_0] \times 100 \ldots\ldots. [15]$$

Where A_0 is the absorbance of the control and A_S is the absorbance of melanin (Sample).

2) Superoxide Radical Scavenging Activity: The superoxide radical scavenging activity was assayed according to the method mentioned in [16]. Superoxide anions were generated in a phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system through the reaction of PMS-NADH and oxygen. It was assayed by the reduction of nitroblue tetrazolium (NBT). All the solutions used in this experiment were prepared in phosphate buffer (pH 7.4). 1ml of NBT (156µM), 1ml of NADH (468µM) and 1ml of extract (0.00-125µg/ml) were mixed. The reaction was started by adding 1ml of PMS (60µM) and the mixture was incubated at 25°C for 5min followed by measurement of absorbance at 560nm spectrophotometrically. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition was calculated using the formula,

$$\text{Inhibition (\%)} = [(A_0 - A_S) / A_0] \times 100 \ldots\ldots\ldots\ldots\ldots. [16]$$

Where A_0 is the absorbance of the control and A_S is the absorbance of the extract.

A percent inhibition versus concentration curve was plotted and the concentration of melanin extract required for 50% inhibition was determined and expressed as IC_{50} value. The lower the IC_{50} value indicates high antioxidant capacity [17].

3) $ABTS^+$ Scavenging Capacity Assay: The ABTS decolorisation assays were carried out as previously described by [18] and it involves the generation of $ABTS^+$ chromophore by oxidation of ABTS with potassium persulfate. The ABTS radical cation (ABTS+) was produced by reacting 7 mM stock solution of ABTS with 2.45 mM potassium persulfate and allowing the mixture to incubate in the dark condition at room temperature for at least 6 hour before use. Absorbance at 734 nm was measured after 10 minutes of incubation after mixing different concentrations of the Melanin extracted (final concentration as 10, 15, 20 %; v/v) with 1 ml of $ABTS^+$ solution. The $ABTS^+$ scavenging capacity of the filtrate was compared with that of vitamin C (50 µM), vitamin E (50 µM) and BHA (0.1 mg/ml).

4) Reducing capacity assay: The reducing power of the extract was determined according to the method standardized by [19]. Different concentrations of the melanin extracts (final concentration 10, 15, 20%; v/v), BHT (0.5 mg/ml) and BHA (0.5 mg/ml) was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1% w/v). The mixture was incubated at 50°C for 20 min. 2.5 ml of trichloroacetic acid (10% w/v) was added to each mixture, which was then centrifuged at 1000g for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1% w/v), and the absorbance was measured at 700 nm in a UV-Vis spectrophotometer. BHA (0.0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4 mg/mL) (Table V) was used as standard for construction of the calibration curve (Fig. 6) and the reducing power was reported as BHT/BHA equivalent per 100 gm of dry sample. Higher absorbance of the reaction mixture indicated greater reducing power**.**

5) Lipid Peroxidation Inhibition Assay: A modified thiobarbituric acid reactive species (TBARS) assay was used to measure Lipid peroxidation. Standard Butylated Hydroxyanisole (BHA) in the concentration range 0- 0.5 mg/Ml (Table VIII) and 0.1ml of extract was mixed in test tubes, the volume was made up to 1ml by adding deionized water. Finally 0.05ml of 0.07M ferrous sulphate ($FeSO_4$) was added to the mixture and incubated for 30min to induce the lipid peroxidation. Further 1.5ml of 20% acetic acid (pH 3.5), 1.5ml of 0.8% TBA and 0.05ml 20% TCA was added, the mixtures were vortexed and was heated in a boiling water bath for 60min. After cooling 5ml of n-butanol was added and centrifuged at 3000rpm for 10min. The absorbance of the organic upper layer was measured at 532nm using a spectrophotometer as calculated by [20]. The percentage inhibition (Dose dependant) of lipid peroxidation was calculated from the formula,

$$\text{Inhibition (\%)} = [(A_0 - A_S) / A_0] \times 100 \ldots\ldots\ldots\ldots\ldots [20]$$

Where A_0 is the absorbance of the control and A_S is the absorbance of the extract/standard.

6) Antioxidative Effects of Melanin in Fish Mince Model System

7) Preparation of Fish Mince: Fish mince was prepared according to the standardized method of [21] with modification. Rohu (*Labeo rohita*) with an average weight of 800 to 1000 g, off-loaded 48 h after capture, was purchased from the local market of Gujarat Fisheries, Ahmedabad. The fish was kept in silica bag (0-4° C) during the transportation. Upon arrival, the fish were washed; filleted and only meat was separated manually with care (Fig. 1) and was finely chopped. The fish mince obtained was divided into four portions (100 g each). One portion, without the addition of Melanin extract, was used as the control and 10 mL of distilled water was added instead. Two portions were mixed with 10 mL of Melanin extract to obtain the final concentrations of 100 and 200 mL/kg mince. Another portion was added with 10 mL BHA to obtain the final concentration of 200 mL/kg mince. The mince was then thoroughly mixed in order to ensure the homogeneous distribution of Melanin and BHA in the mince. Different mince samples were placed in polyethylene bag and kept in ice using a mince/ice ratio of 1:2 (w/w) by keeping the constant temperature to 4˚C. Note that the molten ice was removed every day and the same quantity of ice was replaced. After the designated storage time (0, 3, 6, 9, 12, and 15 days), the samples were taken for analyses of Antioxidant activity viz. DPPH+ Scavenging activity, ABTS scavenging capacity, Reducing capacity and Lipid peroxidation was evaluated.

Fig. 1 Preparation of Fish Mince

8) Statistical analysis

The determination assay for each antioxidant parameter was carried out in thrice. For DPPH Radical Scavenging assay, the obtained concentration was taken as 14.9, 29.8 and 44.7µg/mL of final melanin concentration; and for $ABTS^+$ and Reducing Capacity assay, it was performed using three different melanin concentrations (10, 15, 20 %; v/v) to minimize the percentage of error and achieve consistently significant and consistent data. Data were subjected to analysis of variance (ANOVA). Statistical analysis was performed using the Statistical Package for Social Science (IBM SPSS Statistics 20 for Windows, SPSS Inc., Chicago, IL, USA).

III. RESULTS AND DISCUSSION

A. *DPPH radical scavenging assay results*

Table I: % DPPH Reduction at Regular Interval of Time in Hours

Time (Hours)	% DPPH reduction		
	Melanin Conc.: 14.9 µg/mL	Melanin Conc.: 29.8 µg/mL	Melanin Conc.: 44.7 µg/mL
0	98.64	96.21	99.58
12	70.47	73.49	71.37
24	63.82	64.08	60.80
36	56.91	54.16	58.11
48	49.33	43.93	46.45
60	30.17	33.33	32.94
72	22.35	26.71	23.79
84 (Residual Period)	19.56	16.32	18.06
96	16.86	14.99	14.43

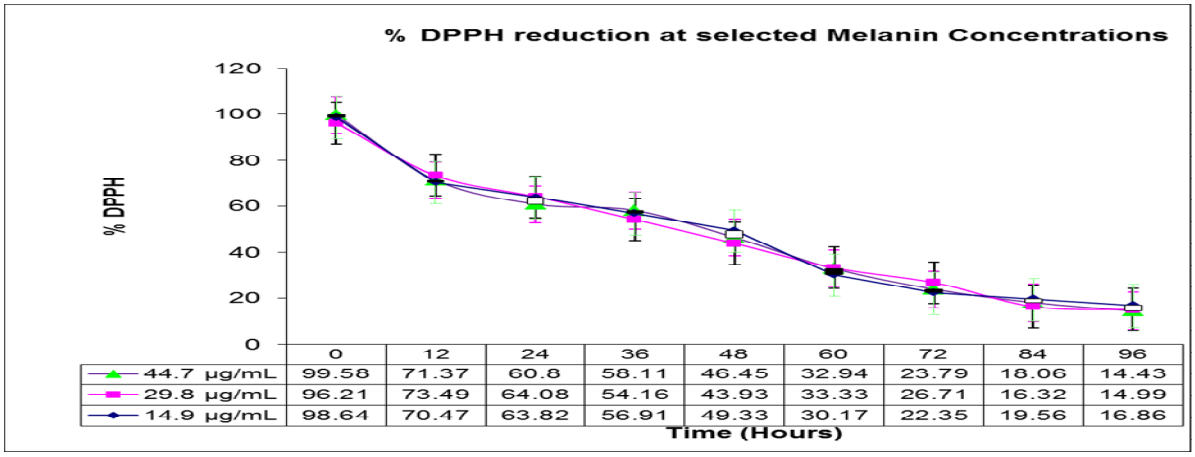


Fig. 2: % DPPH Reduction at Selected Melanin Concentrations

Table II: Dose Dependent Percent Scavenging Activity at Time Interval

Melanin Concentrati (µg/mL)	Time duration (Day)	Scavenging activity (%) $=[(A_0-A_s)/A_0]\times100$
14.9	1st day	35.60%
	2nd day	49.27%
	3rd day	92.11%
29.8	1st day	54.29%
	2nd day	64.86%
	3rd day	96.39%
44.7	1st day	86.88%
	2nd day	91.00%
	3rd day	96.44%

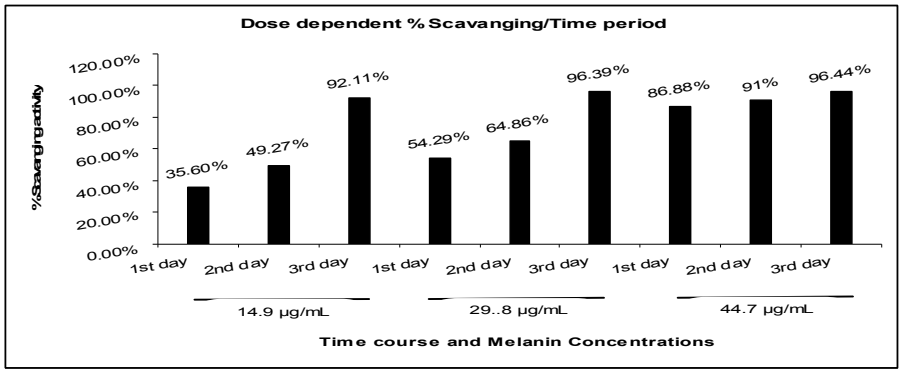


Fig. 3: Dose Dependent % Scavenging Time Period

1) *DPPH assay:* Melanin particle were found to possess antioxidant property in biological systems. It can scavenge free radicals and has the ability to sequester redox active metal Ions [14]. Free radical scavenging activity was evaluated by performing in-vitro DPPH assay. The colored DPPH solution faded reducibly to half during the course of incubation of 54 hours (3rd day), 21 hours (1st day), and 16 hours (1st day) for 14.9, 29.8 and 44.7µg/mL of melanin concentrations respectively (Table II). This may be due to the reduction of the DPPH molecules and electron transfer from melanin suspension. Graph of dose dependant percent scavenging activity at residual period (Fig. 3) also indicates a linear pattern of DPPH reduction for various melanin doses used. The residual period was obtained after 84 hours (Table I) as there was a remarkable decrease in % DPPH contents (Fig. 2).

B. Superoxide Radical Scavenging Activity

Superoxide radicals damage biomolecules directly or indirectly by forming H_2O_2, OH, peroxyl nitrate or singlet oxygen during ageing and pathological events such as ischemic reperfusion injury. Superoxide has also been observed to directly initiate lipid peroxidation (Table III) [22]. The superoxide anion radical scavenging assayed by PMS-NADH system reveled dose dependent scavenging activity of the melanin extract. The results of IC_{50} value as obtained by 50% Inhibition is found to be 42.44 µg/mL of Melanin extract (Fig 4). However the IC_{50} value is much less and thus the significant high antioxidant capacity of melanin extract have been proved [17].

Table III: Superoxide Radical Scavenging Activity

Melanin Concentration (µg/mL)	% Inhibition $= [(A_0 - A_s) / A_0] \times 100$
0	20.24
25	39.16
50	52.84
75	59.37
100	66.00
125	81.88

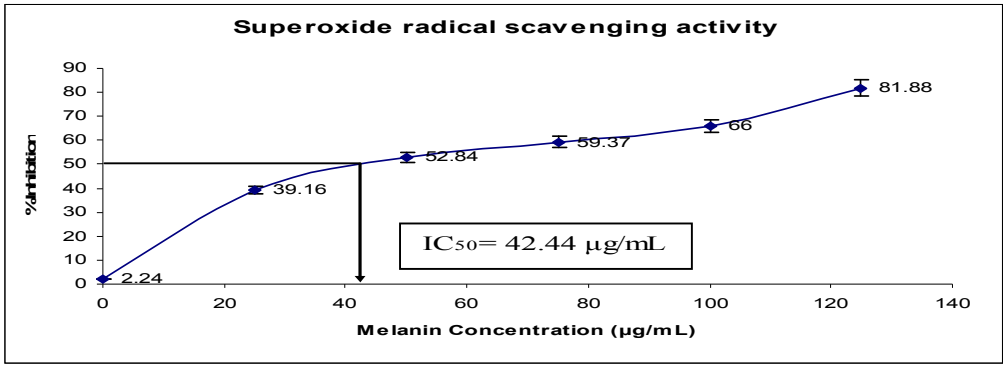


Fig. 4: Superoxide Radical Scavenging Activity

Table IV: $ABTS^+$ Scavenging Capacity Assay Results

Concentration	$ABTS^+$ scavenging capacity*
Melanin Concentration (%; v/v)	
0 %; v/v (Blank)	0.00 ± 0.00
10 %; v/v	26.49 ± 2.34
15 %; v/v	37.24 ± 2.96
20 %; v/v	43.88 ± 2.92
Standards	
vitamin C (50 µM)	81.49 ± 3.54
vitamin E (50 µM)	67.33 ± 2.48
BHA (0.1 mg/ml)	79.99 ± 3.18

Mean ± S.E.*; Sample size: 3

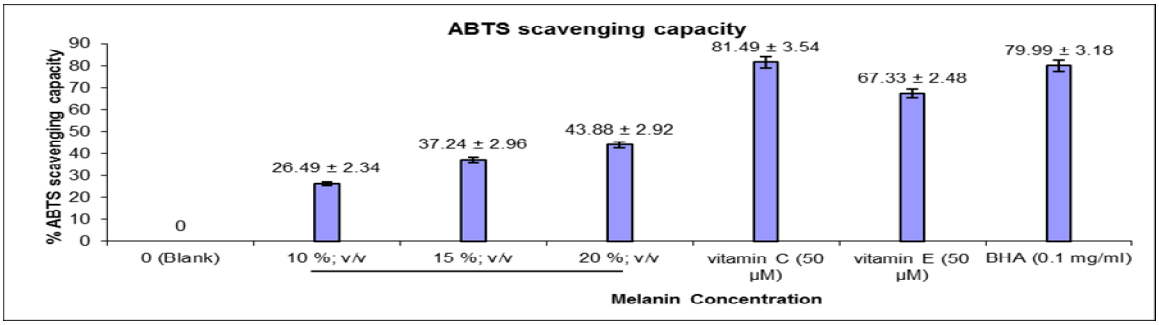


Fig. 5: $ABTS^+$ Scavenging Capacity Assay

The ABTS+ assay was first employed to measure the antioxidant activity of the melanin extract. Different concentrations of the extract (final concentration 10, 15, 20%; v/v), V melanin µM), Vitamin E (50 µM) or BHA (0.1 mg/ml) were incubated with ABTS+ solution, respectively. The ABTS+ scavenging capacity of the extract was 26.49 ± 2.34%, 37.24 ± 2.96% and 43.88 ± 2.92% of control for the melanin extract at the concentration of 10, 15 and 20% (v/v), respectively. Meanwhile, the ABTS+ scavenging capacity of Vitamin C, Vitamin E and BHA was 81.49 ± 3.54%, 67.33 ± 2.48%, 79.99 ± 3.18%, respectively (Fig. 5; Table IV). The results indicated that the extract scavenges ABTS+ free radical significantly in a dose-dependent manner. However, the extract showed lower ABTS+ radical scavenging capacity as compared to standards.

C. Reducing capacity assay results:

Table V: BHA Assay Protocol: Standard Curve

Tube No.	Concentration of BHA (mg/mL)	BHA ml	H_2O ml	Incubate at RT for 30 min	Bradford Reagent ml	Incubate in BWB and Cool	Abs value O.D. at 595 nm
1	0	0	100		3.0		0.000
2	0.05	5	95		3.0		0.060
3	0.1	10	90		3.0		0.101
4	0.15	15	85		3.0		0.119
5	0.2	20	80		3.0		0.137
6	0.25	25	75		3.0		0.158
7	0.3	30	70		3.0		0.232
8	0.35	35	65		3.0		0.262
9	0.4	40	60		3.0		0.324

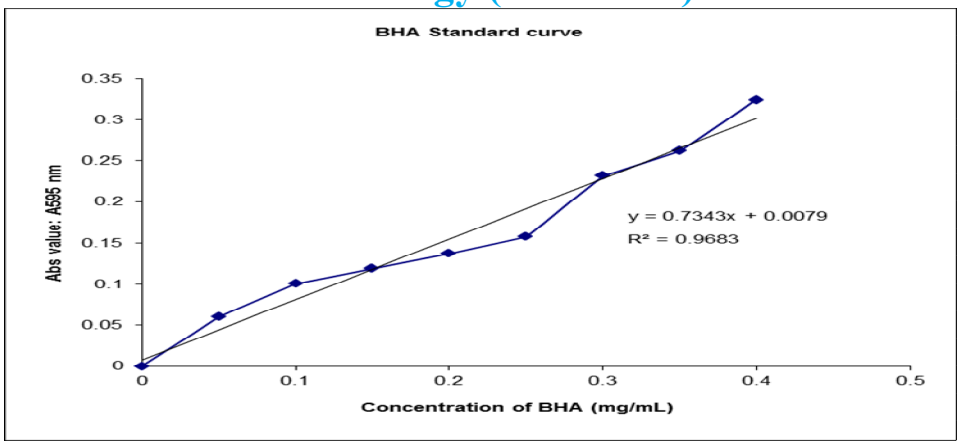


Fig. 6: BHA Standard Curve

Table VI: Result of Reducing Capacity Assay

Concentration	**O.D. at 595 nm**	**Reducing capacity (Absorbance Units at BHA mg/mL conc.)**
0 %; v/v (Blank)	0.001	0.002
10 %; v/v Melanin	0.019	0.020
15 %; v/v Melanin	0.104	0.152
20 %; v/v Melanin	0.110	0.164
BHA (0.5 mg/ml)	0.117	0.199
BHT (0.5 mg/ml)	0.123	0.181

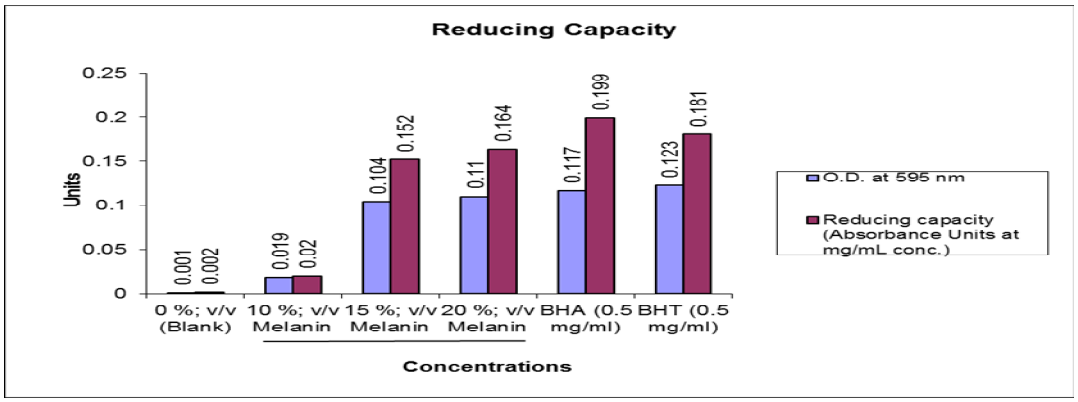


Fig. 7: Reducing Capacity Assay

In the reducing power assay, it was found that the reducing power units of the melanin extract (20%; v/v) was much equivalent as that of 0.5 mg/ml of BHT and 0.5 mg/ml of BHA (Fig. 7; Table VI)

Table VII: Lipid Peroxidation Inhibition Assay Results

Concentration (µgm/mL)	O.D. at 532 nm	Inhibition (%) $= [(A_0 - A_s) / A_0] \times 100$
0 (Control)	1.974	0.00
25	1.613	18.28
50	1.543	21.83
75	1.297	34.29
100	0.328	83.38
125	0.218	88.95

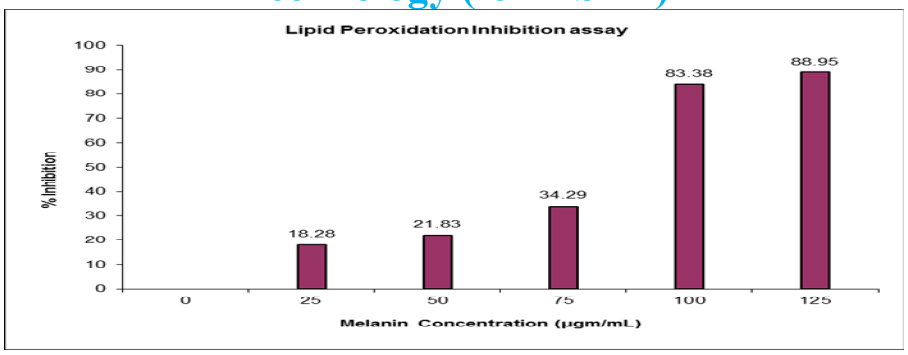


Fig. 8: Lipid Peroxidation Inhibition Assay

Thiobarbituric acid reactive species (TBARS) assay as measured for lipid peroxidation with a concentration range of 0,25,50,75,100 and 125 µgm/ml melanin extract. Fig. 8 shows that % inhibition for lipid peroxidation value increases spontaneously. At higher concentrations i.e. 100 and 125 µgm/ml respectively percentage inhibition increases to double folds. This reveals that lipid peroxidation inhibition is a dose dependent (melanin concentrations). However with increase in melanin concentration above 100µgm/ml, % inhibition does increase but gradually. 50 % lipid peroxidation inhibition is only achieved with a minimum (100µgm/ml) concentration of melanin extract.

Table VIII: Time Dependent Antioxidant Activity of Melanin Effects on Fish Mince

Storage time (Days)	DPPH+ Scavenging activity (% Inhibition)			ABTS scavenging capacity (% Inhibition)			reducing capacity (Abs. Units at mg/mL BHA)			Lipid Peroxidation (% Inhibition)		
	0.00 mL/kg	100.00 mL/kg	200.00 mL/kg	0.00 L/kg	100.00 mL/kg	200.00 mL/kg	0.00 L/kg	100.00 mL/kg	200.00 mL/kg	0.00 L/kg	100.00 mL/kg	200.00 mL/kg
0th Day	0.0	18.88	46.15	0.00	19.88	15.33	0.00	04.37	14.77	0.00	06.36	17.18
3rd Day	0.0	26.49	52.31	0.00	24.31	19.11	0.00	09.36	22.22	0.00	11.88	25.25
6th Day	0.0	31.08	74.11	0.00	38.19	26.87	0.00	11.00	38.60	0.00	16.54	39.58
9th Day	0.0	39.27	87.16	0.00	23.01	48.34	0.00	16.73	44.13	0.00	20.71	54.31
12th Day	0.0	56.24	91.58	0.00	24.23	64.19	0.00	19.37	58.40	0.00	25.76	61.53
15th Day	0.0	63.17	87.36	0.00	29.17	77.59	0.00	26.88	73.44	0.00	38.49	76.00

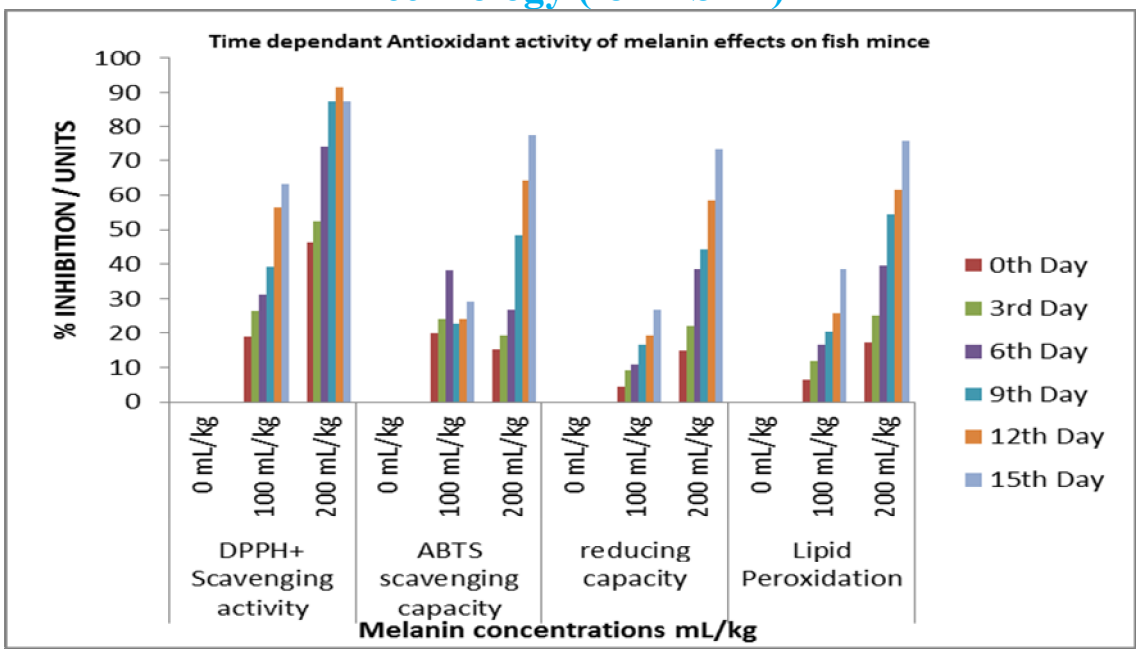


Fig. 9: Time Dependent Antioxidant Activity of Melanin Effects on Fish Mince

The antioxidant property of melanin as produced by *EP 83* has been evaluated to study its effects on in-vitro model system. Fish mince model system was developed according to the method standardized by [21]. Antioxidant property parameters (DPPH+ Scavenging activity, ABTS scavenging capacity, Reducing capacity and Lipid peroxidation), shows both dose dependent and time dependent scavenging % inhibition units (Fig. 9; Table VIII) in the same manner as assayed by PMS-NADH system. Higher concentration of *EP 83* melanin within the system indicates greater antioxidative property.

IV. CONCLUSION

The purified melanin fraction showed promising free radical scavenging activity in DPPH, Superoxide Radical Scavenging Activity, Lipid Peroxidation Inhibition Assay, ABTS+ scavenging capacity assay methods and also onto its evaluation of fish mince model system. Further purification of melanin pigment from strain *EP 83* will lead to a development of pharmaceutically valuable antioxidant. The colored DPPH solution faded reducibly to half during the course of incubation of 54, 21, 16 hours for 14.9, 29.8 and 44.7µg/mL of melanin concentrations respectively and ABTS+ scavenging capacity of the extract was 26.49 ± 2.34%, 37.24 ± 2.96% and 43.88 ± 2.92% of control for the melanin extract at the concentration of 10, 15 and 20% (v/v), of melanin respectively.

The superoxide anion radical scavenging assayed by both PMS-NADH system and fish mince system reveled dose dependent scavenging activity of the n-butanol extract. Melanin extract also scavenges ABTS+ free radical significantly in a dose as well as time dependent manner.

The antioxidative property of melanin could be made used for pharmaceutical potential applications of the targeted natural melanin in approaches including cosmetic elegance and therapeutic approaches.

REFERENCES

[1] V. P. Kurchenko, K. A. Mosse, and A. T. Pikulev, Abstracts of Papers, Vsesoyuznaya konferentsiya po adiobiologicheskim issledvoaniyam avarii na Chernobyl'skoi AES, (All-Union Conf. on Radiobiological Studies of the Chernobyl Power Plant Accident), Minsk: Nauka i Tekhnika, 70-71, 1991.

[2] A. A. Aver'yanov, V. P. Lapikova, G. G. Petelina, and V. G. Dzhavakhiya, Izv. Akad. Nauk SSSR, Ser. Biol., 4: 541-549, 1986.

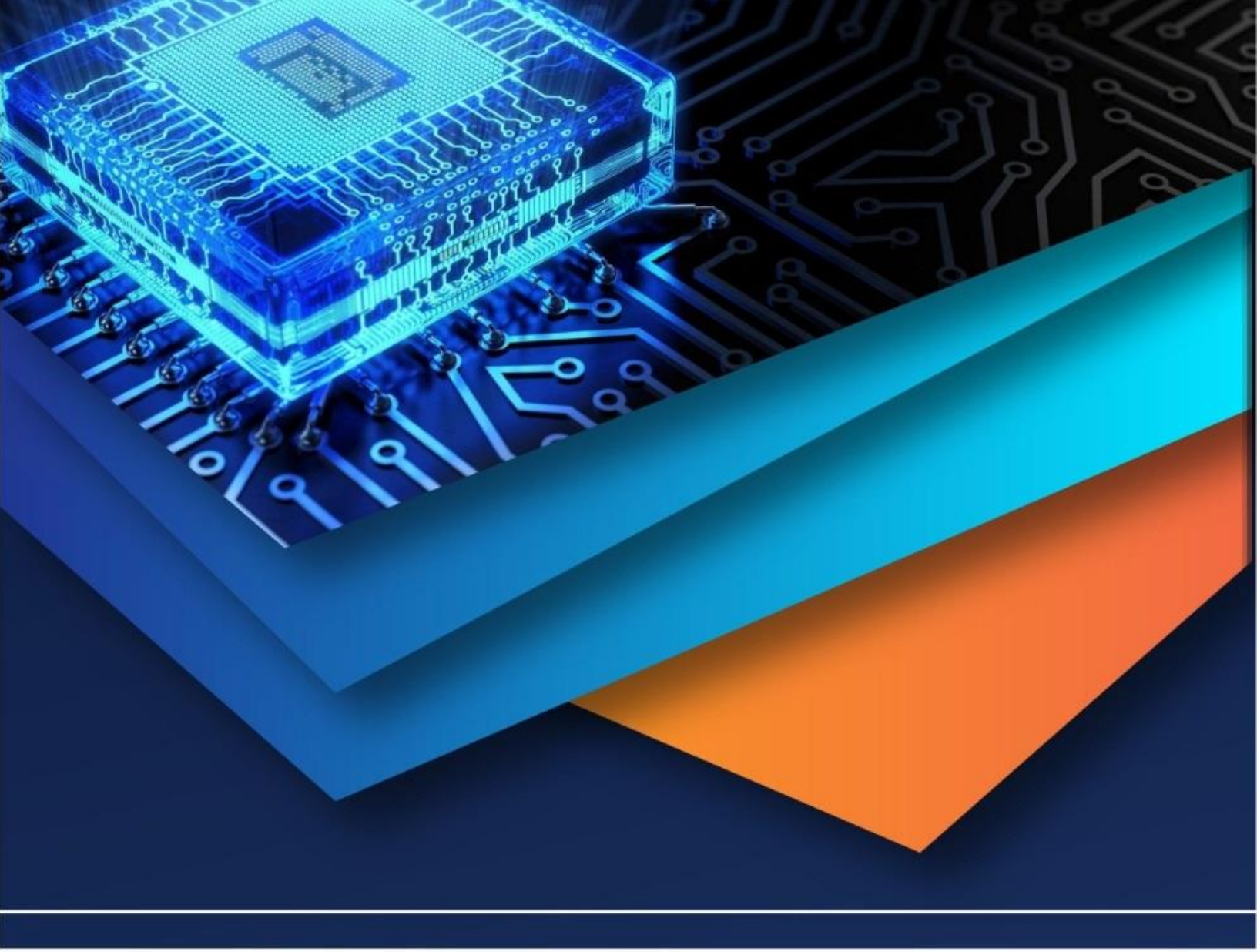
[3] J. Reichrath, Sunlight skin cancer and vitamin D: What are the conclusions of recent findings that protection against solar ultraviolet (UV) radiation causes 25-hydroxyvitamin D deficiency in solid organ-transplant recipients, *xeroderma pigmentosum*, and other risk groups? J. Steroid Biochemi. Mol. Biol., 103, 664-667, 2007.

[4] J. L. Ravanat, Douki, T., & J. Cadet, Direct and indirect effects of UV radiation on DNA and its components. J. Photochem. Photobiol. B, 63, 88-102, 2001.

[5] T. Herrling, K Jung., & J. Fuchs, Measurements of UV-generated free radicals/reactive oxygen species (ROS) in skin. Spectrochim. Acta Part A, 63, 840-845, 2006.

[6] S. Liu, H. Mizu, and H. Yamauchi, Photoinflammatory responses to UV-irradiated ketoprofen mediated by the induction of ROS generation, enhancement of

cyclooxygenase-2 expression, and regulation of multiple signaling pathways. Free Rad. Biol. Med., 48, 772-780, 2010.
[7] M. Brenner & V. J. Hearing, The Protective Role of Melanin Against UV Damage in Human Skin. Photochem. Photobiol., 84, 539–549, 2008.
[8] A. Bilgihan, M. K. Bilgihan, R. F. Akata, A.Aricioglu and A. Hasanreisolu, B. Antioxidative role of ocular melanin pigment in the model of lens induced uveitis. Free Rad. Biol. Med., 19, 883-885, 1995.
[9] K. Bochenek, and E. Gudowska-Nowak, Fundamental building blocks of eumelanins: electronic properties of indolequinone-dimers. Chem. Phys. Lett., 373, 532-538, 2003.
[10] J. Geng, S. Yu, X. Wan, X. Wang, P. Shen, P. Zhou, and X. Chen, Protective action of bacterial melanin against DNA damage in full UV spectrums by a sensitive plasmid-based noncellular system. J. Biochem. Biophys. Methods, 70, 1151-1155, 2008.
[11] J. P. Ortonne, Photoprotective properties of skin melanin. Br. J. Dermatol., 61, 7-10, 2002.
[12] E. A. Pithawala and N. K. Jain, Synthesis and Screening of Melanin from Bacterial Strain *EP83*. International Journal of Scientific Research, Vol. 2(7), 35-36, 2013.
[13] Wei Yu-Hong, Chen. Enhanced production of prodigiosin-like pigment from *Serratia marcescens* SMÄR by medium improvement and oil supplementation strategies. Journal of Bioscience and Bioengineering, 99(6): 616-622, 2005.
[14] K. Y. Ju, Y. Lee, S. Lee, S. B. Park and J. K Lee., Bioinspired polymerization of dopamine to generate melanin like nanoparticles having an excellent free radical scavenging property, Biomacromolecules 12, 625–632, 2011.
[15] S. A. Gautham and R. Onkarappa "In Vitro Antioxidant activity of Metabolite from *Streptomyces fradiae* strain GOS1" Int. J. Drug Dev. & Res., 5(1): 235-244, January-March 2013.
[16] M. Nishimiki, N. A. Rao, K. Yagi, The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun; 46(2): 849- 853, 1972.
[17] M. Raghavendra, R. A. Madhusudhana, R. Y. Pulala, A. Sudharshan, Siva Kumar. Comparative Studies on the In Vitro Antioxidant Properties of Methanolic Leafy Extracts from Six Edible Leafy Vegetables of India. Asian J Pharm Clin Res, Vol 6(3), 96-99, 2013.
[18] Y. Jy, Jh. Koo, S. Yg, K. Kb, Jh. Lee, S. Hs, Bh. Park, J. Ec, P. Jw: Stimulation of melanogenesis by scoparone in B16 melanoma cells. Acta Pharmacol Sin, 27(11):1467–1473, 2006.
[19] C. L. Murrant, M. B. Reid, Detection of reactive oxygen and reactive nitrogen species in skeletal muscle. Microsc Res Tech, 55(4):236–248, 2001.
[20] M. Ohkowa, N. Ohisi, K. Yagi, Assay for lipid peroxides in animal tissues by thio-barbutric acid reaction. Ann Biochem; 95: 351-358, 1979.
[21] JYVA Kamil, Y. J. Jeon, F. Shahidi, Antioxidant activity of chitosan of different viscosity in cooked comminuted flesh of herring (*Clupea jarengus*). Food Chem. 79:69–77, 2002.
[22] G. C. Yen and P.D. Duh, Scavenging effect of methanol extracts of peanuts hulls as free radical and active oxygen species. J Agric Food Chem; 1: 629- 682, 1994.



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