RESEARCH ARTICLE

Antioxidant activity and sun screening effects of Bacterial melanin

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ABSTRACT
The present work was delineated to formulate and evaluate antioxidant and photoprotective properties of melanin produced by *Pseudomonas mosselii* strain STSGRDS1 against the purchased fungal melanin STSGRDM1 as standard. Initially, antioxidant assay was examined for the isolated bacterial melanin STSGRDS1 against the standard melanin STSGRDM1 that already have substantiated to have the antioxidant ability by DPPH and ABTS, following with the detailed screening of radical scavenging activity were performed which includes the valuation of peroxides, hydroxyl radicals, reduction potential, and phosphomolybdenum method. This report depicts the antioxidant potential of the extracted melanin that could be further used as a potent antioxidant on scientific basis, as the percentage of free radical scavenging increased with increasing concentration of melanin. The study also signifies that bacterial melanin formulated creams had the potency to protect against ultraviolet (UV) rays with sun protection factor (SPF) enhancement ranging from 1.95 to 26.06 and transmission spectroscopy revealed that the formulations have satisfied protection against UV A and UV B rays with good average UV A protection factor indicating sunscreen activity of the pigment. As a result, the isolated bacterial strain has immense photoprotection potential and radical scavenging activity that can be resorted to in cosmetic formulations, UV protection devices, etc.

Key words: Melanin, Pseudomonas sp, antioxidant, SPF, Transmission spectroscopy

Introduction

Melanins are pigments that are negatively charged, hydrophobic, and ubiquitous in nature (Butler *et al.*, 1998). Organisms synthesize melanins by hydroxylation and polymerization of organic compounds. Production of melanin is observed in all taxa from both Prokaryota and Eukaryota (Smith et al., 2006). Melanins are grouped into three divisions namely eumelanin, brown to black pigment which springs by oxidative polymerization of tyrosine, dihydroxyphenylalanine (DOPA), dopamine and tyramine; pheomelanin, yellow to red pigment which has similar biosynthetic pathway as eumelanin; allomelanins, brown to black pigment formed by the polymerization of di or tetrahydrofolate via pentaketide pathway (Nicolaus, 1968). Fungal strains that produce melanin include *Cryptococcus neoformans*, *Sporothrix schenckii*, *Sepia officinalis*, *Aspergillus niger*, *Penicillium marneffei*, *Paracoccidioides brasiliensis*, *Histoplasma capsulatum*, *C.neoformans* (Youngchim et al., 2004) and therefore the bacterial strains are known to turn out melanin include *Aeromonas salmonicida*, *Azotobacter* sp., *Mycobacterium* sp., *Micrococcus* sp., *Bacillus* sp., *Legionella* sp., *Streptomyces* sp., *Rhizobium* sp., *Vibrio* sp., *Proteus* sp., *Azospirillum* sp., *Pseudomonas aeruginosa*, *Hypomonas* sp., *Burkholderia cepacia*, *E.coli*, *Bordetella pertusis*, *Campylobacter jejuni*, *Yersinia pestis* etc (Geng et al., 2010; Coyne et al., 1992) . Microbial melanins have applications in various fields such as agriculture, cosmetic and pharmaceutical industries (Dastager et al., 2006).

Oxidative stress due to UV radiation had diminution in the antioxidant defense system which correlates with melanogenesis (Hu et al., 2009). Free radicals are known to be highly unstable, reactive species that contain one or more unpaired electrons. The reactive oxygen species (ROS) includes oxygen radicals like superoxide (O^-_2), hydroxyl (OH^-), peroxyl (RO2), hydroperoxyl (HO_2.) and some non-radical oxidizing agents like hydrogen peroxide (H_2O_2), hypochlorous acid (HCIO) and ozone (Babior, 2004). ROS are involved in the etiology of many diseases such as aging, cancer, neurodegenerative disorders, coronary heart disease, Alzheimer's disease and inflammation as indicated by the

manifestation of oxidative stress (Kar et al., 2012). Antioxidants play an acute role in acting as physical barriers that prevent ROS generation or ROS access to important biological sites, scavenge ROS, neutralize catalytic systems or ROS diversion, prevention of generation of ROS by binding/inactivation of metal ions and antioxidants that break the chain which plays an important role in scavenging destroying ROS (Karadag et al., 2009). The incompetent natural defense of organisms against the free radicals steers the development of synthetic antioxidants for scavenging purposes. Whereas, the adverse carcinogenicity of synthetic antioxidants, making it a need to search for effective natural antioxidants. Fungal melanins such as *Aspergillus fumigatus* (Raman et al., 2015), *Lachnum singerianum* (Ye et al., 2012), *Cryptococcus neoformans* (Casadevall et al., 2012) has been validating to be an effective natural antioxidant.

The most commercial application in the field of cosmetics includes sunscreen lotions, which performs as photoprotective component due to its ability to protect against the UV damage and free radical scavenging capacity (Riley, 1997). Major factor in the etiology of unwanted changes in skin appearance is due to the exposure to sunlight. The harmful effects of solar radiation is mainly caused by the ultraviolet (UV) region of electromagnetic spectrum that is categorized into 3 regions: UV A from 320nm to 400nm; UV B from 290nm to 320nm; UV C from 200nm to 290nm. UV C radiations get filtered by atmosphere before reaching earth. UV B radiations are partially filtered that in turn is responsible for damages in skin due to sun burn. UV A radiations penetrates to deep layers of epidermis and dermis, which induces premature aging of the skin. UV radiations are the causative factor for skin cancer (Dutra *et al.*, 2004). Melanin acts as a reliable UV protective agent in bioinsecticide preparation like *Bacillus thuringenesis* (Bt) insecticidal crystals (Wan et al., 2007, Zhang et al., 2007).

Due to profused application possibilities, the applications of melanin are unrestrained to one particular field and henceforth the demand of the hour. In this study, the melanin-producing *Pseudomonas* sp STSGRDS1 was screened and various biological properties of antioxidant and cosmetological importance were explored.

Materials and Methods

Screening of melanin producing bacteria

The garden soil samples were collected from Tirupur, Tamilnadu, India. 0.1ml of diluted samples in Nutrient broth (from 10^{-7} dilution) was individually spread on to tyrosine basal agar plates with pH 7.0. The media and glasswares were autoclaved at 15 psi (121˚C) for 20 minutes prior to the experiment; these agar plates with media and inoculum were incubated at 37˚C for 48h. Microorganisms producing melanin were identified by the presence of pigmented colonies with zone formation. Colonies were selected out for subculturing and characterization. The isolate STSGRDS1 was identified hinged on morphological and biochemical characterization which is predicted on Bergey's Manual of Systematic Bacteriology and 16S rRNA gene sequencing were done using primers 27f (5'- AGA GTT TGA TCC TGG CTC AG-3') and reverse primer 1492r (5'- TAC CTT GTT ACG ACT-3'). The strain STSGRDS1 was sequenced and it had been deposited in the National Centre for Biotechnology Information (NCBI) database under the Gene Bank accession no.MN967075. The strain STSGRDS1 16S rRNA gene sequence was searched in nucleotide sequence databases of the National Centre for Biotechnology Information (NCBI) by running the BLASTN program (Altschul et al., 1990).

Production, extraction and purification of melanin

Tyrosine basal broth (Yabuuchi & Ohyama, 1972) containing 0.2% tyrosine was used for the production of melanin. 5ml of culture suspension were taken as primary inoculum for 50ml of production medium and kept in an environmental shaker at 140rpm at 37˚± 2˚C for 180h. After 180h of incubation, the cell-free supernatant was further acidified to pH 2 using 1N HCL. Visualization of black precipitation of melanin was observed at the bottom of the flask at lower pH. Further treatment with acid, water and ethanol helped to render purified melanin (Sajjan et al., 2013).

Antioxidant assays (Raman et al., 2013)

DPPH radical scavenging assay

The radical scavenging activity by melanin (i.e) its antioxidant property was investigated by using DPPH assay. 1mg/ml of the melanin extract was added to 2 ml of DPPH solution and absorbance was read spectrophotometrically using colorimeter at 500 nm before and after incubation at 37˚C for 30 mins. DPPH inhibition percentage had been calculated using the formula.

DPPH inhibition (%) = [Control absorbance – Test absorbance] x 100

ABTS radical scavenging assay

The solution containing 2-2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS),(2.45mM ammonium persulfate in 7mM ABTS solution) was kept in dark for 12-16h at room temperature. 50µl of STSGRDM1 and STSGRDS1 were added to 300µl ABTS solution and therefore the final volume was made up to 1ml using ethanol. After 5 minutes the absorbance was read at 745nm.

Hydroxyl radical scavenging assay

The reaction mixture contained 100µl each of 2.8mM deoxyribose, 0.1mM EDTA, 1.0M H2O2, 0.1mM ascorbate and 20mM KH_2PO_4-KOH buffer of pH 7.4. 50µl of

STSGRDM1 and STSGRDS1 were respectively added such that the overall volume was made up to 1ml. The reaction mixture was incubated for an hour at 37˚C after which 500µl each of 70% ethanol and 1% thiobarbituric acid was added. The mixture was kept in a boiling water bath for 20min and cooled. The pink color developed was measured at 535nm.

Nitric oxide method

The reaction mixture of 300µl of 100mM sodium nitroprusside, 20µl of STSGRDM1, STSGRDS1 was added to PBS (pH 7.2) and made it up to 1ml. This was further incubated at 25˚C for 150 min. After incubation 500µl of the reaction mixture was taken and added to equal volume of Griess reagent (1% sulphanilamide, 2% orthophosphoric acid, 0.1% N-(1-naphthyl)-ethylene diamine hydrochloride). The absorbance was checked at 546nm.

Phosphomolybdenum method

The tubes containing 50µl of STSGRDM1 & STSGRDM1 melanin at different concentrations and 2ml each of reagent (0.6M H_2SO_4, 28mM Na_2PO_4 and, 4mM ammonium molybdate) and distilled water were incubated at 95˚C for 90 min. After the mixture is cooled to room temperature, the absorbance of each solution was measured at 695nm against blank and the scavenging activity was determined.

Reduction potential

Different concentrations of the sample of 100µl (STSGRDM1 and STSGRDS1) was mixed with 2.5ml of 1% potassium ferricyanide and 2.5ml of phosphate buffer (pH 6.6). The mixture was made up to 6ml by using distilled water and incubated at 50˚C for 20min. After incubation, 2.5ml of 10% trichloroacetic acid was added and mixed thoroughly. To 2.5ml of this reaction mixture equal amount of water was added and 0.5ml of 0.1% ferric chloride was added to it. The absorbance of the colour was measured spectrophotometrically at 700nm.

Statistical analysis

All the statistical analysis such as the mean and standard deviation of the experiments were done by Microsoft Excel.

Sun Protection Factor (SPF)

Determination of SPF (Suryawanshi et al., 2015; Sayre et al., 1979; Mansur et al., 1986)

The *in-vitro* determination of SPF was estimated according to the modified protocol. 0.5ml and1ml of bacterial melanin of 5% and 10% formulated cream respectively was weighed and transferred to a 50ml volumetric flask which is kept as a stock solution. The working stock was prepared by adding 10mg in 10ml ethanol to give 1000µg/ml and further serially diluted to other tubes to give different concentrations such as 5ml from the working stock was transferred to 5ml of ethanol to give 500µg/ml, 250µg/ml,125µg/ml. The absorption of each concentration prepared was determined from 290-320 nm, taking ethanol as a blank. The absorption data were obtained in the range of 290 to 320, every 5 nm followed by the application of the Mansur equation.

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

Where, CF is correction factor (=10); EE (λ) - erythemal effect of radiation with wavelength λ; I(λ) -solar intensity spectrum; Abs(λ)-Absorbance of a sunscreen product. The values of EE (λ) ×I (λ) are constant. The obtained absorbance values Abs (λ) were multiplied with the respective EE (λ) ×I (λ) values and then summation was taken and multiplied with the correction factor 10.

SPF determination by transmission spectrum (More et al., 2013)

Sunscreen activity was evaluated by the *in-vitro* method through recording the transmission spectrum of formulations in the range 290-400nm. The sample was applied on polyvinyl chloride (PVC) sheet strip, spread uniformly with the help of capillary to form a thin film. The strip was then placed inside UV-Vis cuvette in such a way that the formulation touches the transparent side of the cuvette. It was allowed to equilibrate for 15 mins to ensure levelling of the formulation between PVC and the wall of the cuvette. The cuvette was placed inside a UV spectrophotometer (Shimadzu) and a transmission spectrum was recorded from 290-400 nm, using air as reference. The data was appropriately processed to calculate UVA and UVB protection factors using the following formulas:

1) Average transmittance spectrum of sunscreen in either region is averaged in sequence to produce one value, which describes UV A, or UVB blocking.

$$\textbf{T (UVA) avg} = \frac{{}^{400}\Sigma_{320}\, T\,\lambda\, X\,\Delta}{{}^{400}\Sigma_{320}\,\Delta\lambda}$$

$$\textbf{T (UVB) avg} = \frac{{}^{320}\Sigma_{290}\, T\,\lambda\, X\,\Delta\lambda}{{}^{320}\Sigma_{290}\,\Delta\lambda}$$

100-T(UVA) or T(UVB) gives % blocking or % protection against the UVA or UVB.

2) Average UVA protection factor (PF)

Arithmetic mean of monochromatic protection factor (MPF) calculated between 320-400nm.

$$\textbf{PF Am} = \frac{{}^{400}\Sigma_{320\ MPF}\ \lambda\, X\,\Delta\lambda}{{}^{400}\Sigma_{320}\,\Delta\lambda}$$

Where, A(λ) is absorbance at λ T(λ) is transmittance at λ

MPF is monochromatic protection factor i.e 1/T ΔA is measured wavelength interval (5nm).

Results and Discussion

Strain characterization

The melanin-producing strain STSGRDS1 was isolated from garden soil and observed on tyrosine basal agar medium. As shown in Figure 1, the isolate showed dark-colored colonies as L-tyrosine is sparingly soluble in the medium.

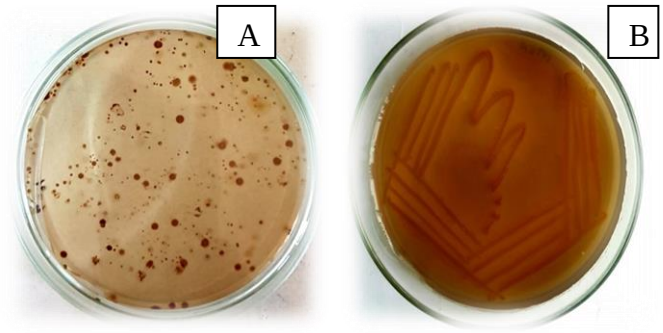


Figure 1. *Primary screening for melanin producing bacteria showing formation of colonies of very dark brown colour (a) and Melanin producing isolate streaked in tyrosine basal agar medium showing utilization of tyrosine (b).*

In earlier reports, the dark color colonies showing dark pigment-producing strains were picked and characterized as melanin producers. (Santhanalakshmi et al., 2017) isolated organisms producing melanin from garden soil that showed the formation of pigmented colonies by *Streptomyces sp*. in starch casein agar. (Korumilli et al., 2014) isolated *Pseudomonas sp.* from seawater samples in marine agar showing dominant dark black colour in agar plates. (Hiren et al., 2017) isolated 25 bacterial isolates from the rice field, sugarcane industry, and garden soil producing dark brown melanin pigment in tyrosine casein agar medium. (Shoumita et al., 2018) isolated two melanin-producing isolates from marine water on the L-tyrosine agar medium. (Kshitija et al., 2016) isolated bacterium producing melanin from a borewell water sample using nutrient agar medium enriched with L-tyrosine amino acid. (Santhanalakshmi et al., 2018) isolated from soil producing melanin pigment in tyrosine medium identified as *Pseudomonas sp.* bacteria producing melanin showing dark pigment colonies on tyrosine basal agar.

As shown in Figure 2, the isolate selected after primary screening were further checked for their ability to produce melanin in tyrosine basal broth.

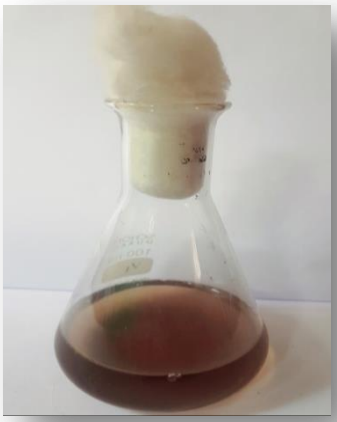


Figure 2. *Secondary screening of STSGRDS1*.

Comparing with earlier reports (Turick et al., 2002; Surwase et al., 2013; Kiran et al., 2014) indicates that the melanin production could be increased with optimization of culture conditions. The white colored medium turned from white to dark brown in color after complete melanin production. The brown pigment-producing strain was identified to be *Pseudomonas* sp. STSGRDS1, based on the morphological characterization, molecular characterization such as 16S rDNA sequence and biochemical characteristics as shown in Table 1.

Table 1. *Pseudomonas sp. STSGRDS1 morphological and biochemical characterization studies*

Characteristic	Result
Colony morphology	circular, entire, raised, slimy, opaque
Gram's reaction	Rods
Biochemical tests	catalase, oxidase, citrate, nitrate
	Positive

The strain showed 100% homology with *Pseudomonas mosselii*. (Altschul et al., 1990) reported that the 16S rRNA gene was amplified using specific primers and amplified products were visualized by agarose gel electrophoresis. The amplicons were sequenced using 16S rDNA sequencing and the identity of the sequences was determined using NCBI blast where the sequences were searched against the GenBank database. The obtained sequences were further submitted to obtain accession numbers.

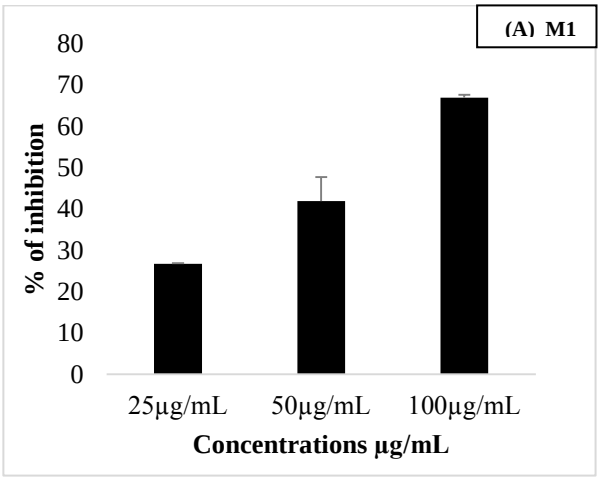
Antioxidant assays

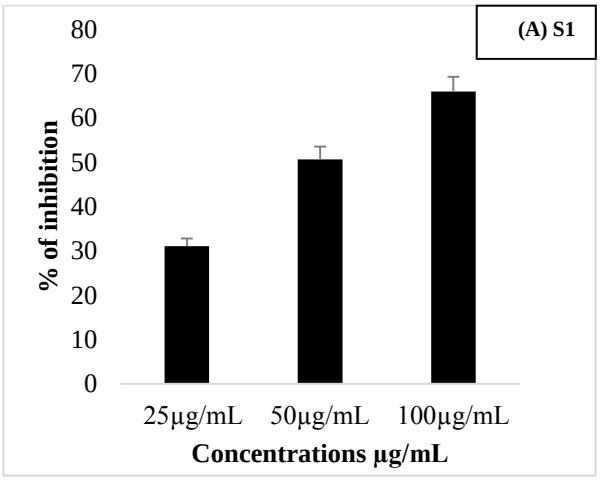
In this study, radical scavenging activity of the purified melanin STSGRDS1 was compared with the standard purchased fungal melanin (Mykoteck, Goa, India). As shown in Figure 3, melanins showed immense radical scavenging activity compared to the standard fungal melanin. (Sajjan et

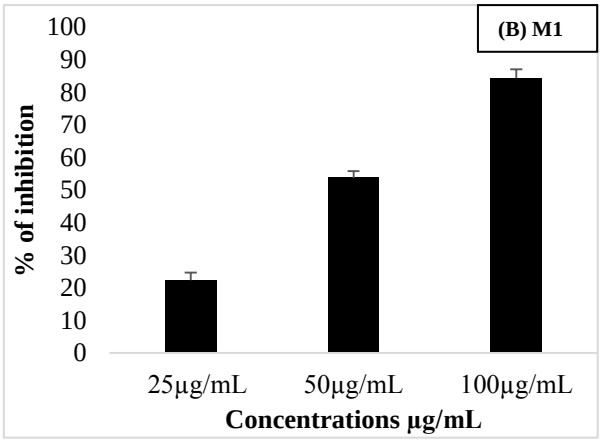
al., 2013) showed melanin produced from *Klebesiella sp.* showed 70% radical scavenging activity.

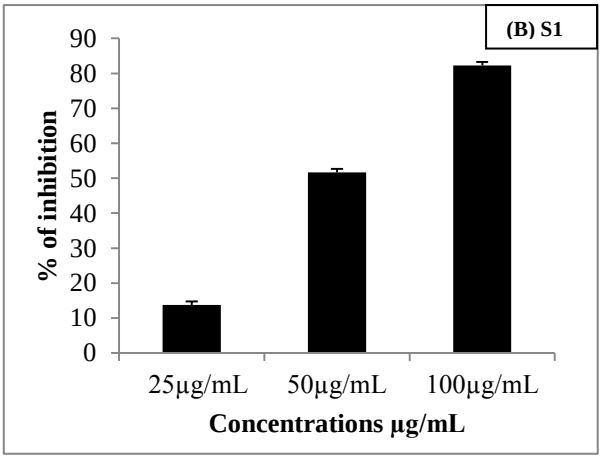
Manivasagan et al., 2013 reported 90% free radical scavenging activity for melanin 3.5 mg/ml from *Actinoalloteichus sp.* Wang et al., 2007 reported the significant decrease in singlet oxygen production observed in the presence of eumelanin extracted from pig retinal pigment epithelium cells.

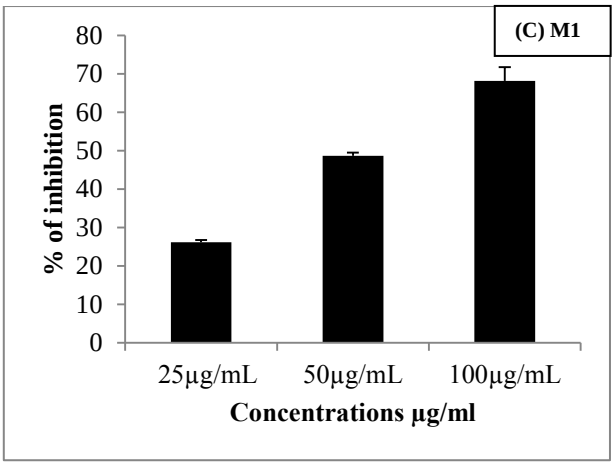
Tarangini et al., 2014 showed effective radical scavenging activity of a model DPPH radical. El-Naggar et al., 2017 showed melanin pigment of *Streptomyces glaucescens* NEAE-H proved good antioxidant activity of a model ABTS. Tu et al., 2009 reported that melanin extracted from the muscle of silky fowl add the same ability of scavenging superoxide anion with the same effectiveness. Many reports had indicated the superior ability of melanin to scavenge free radicals and our experimental reports indicated the same.

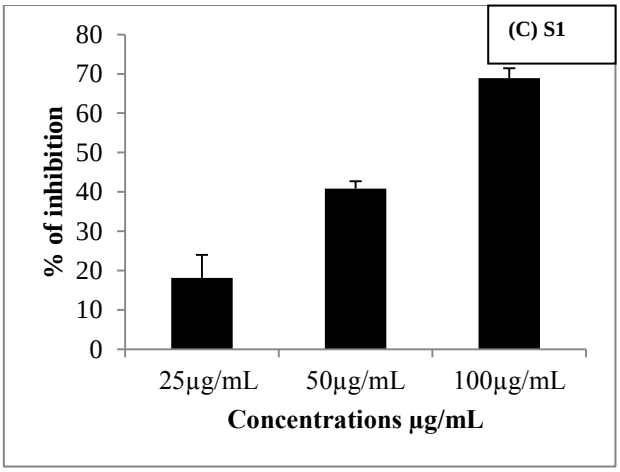












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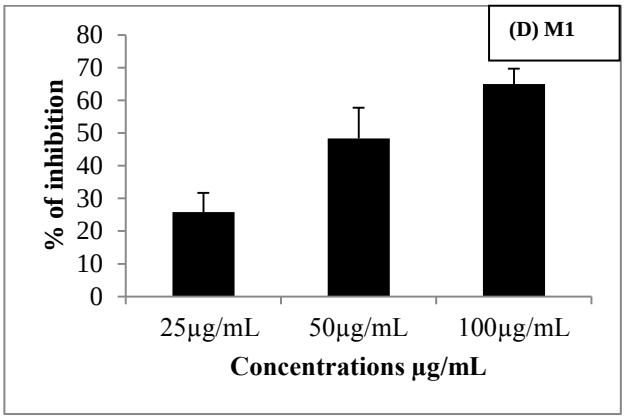

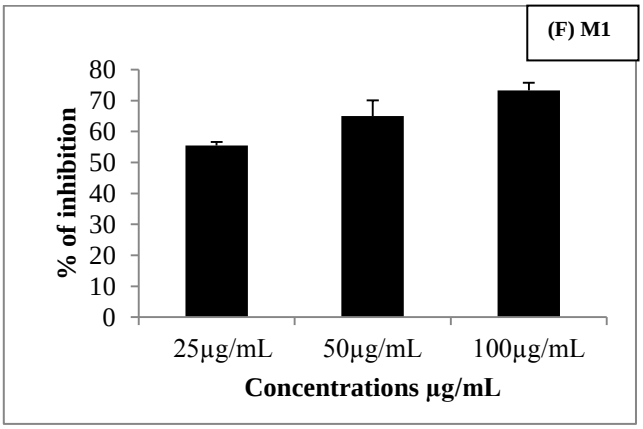

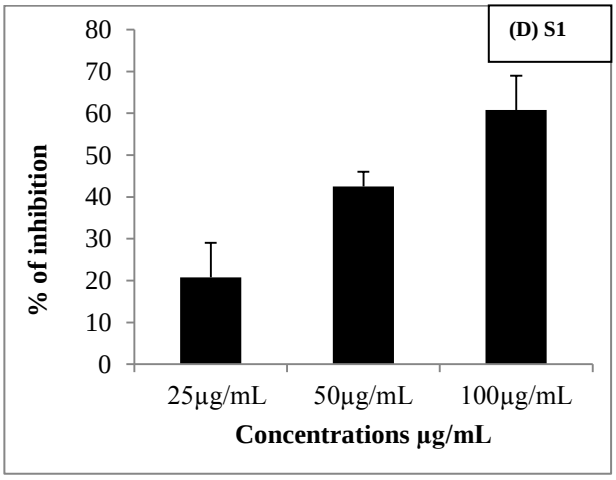

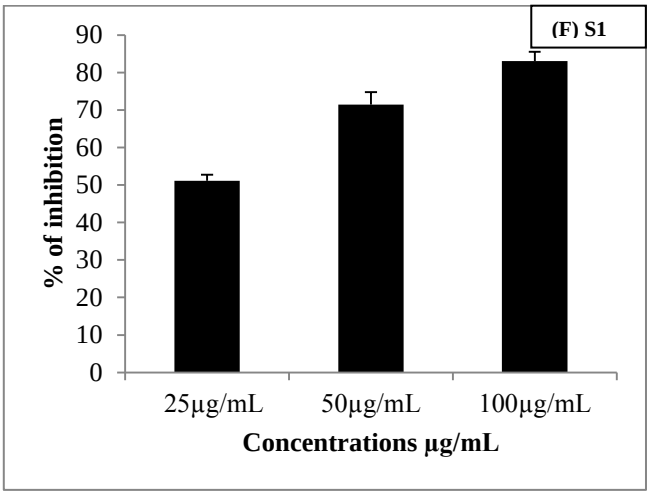

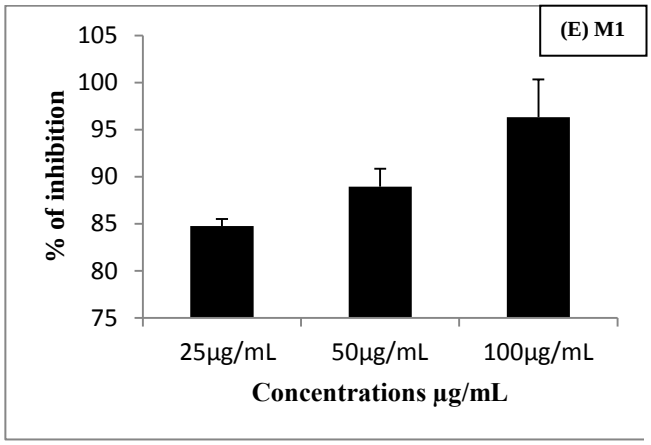

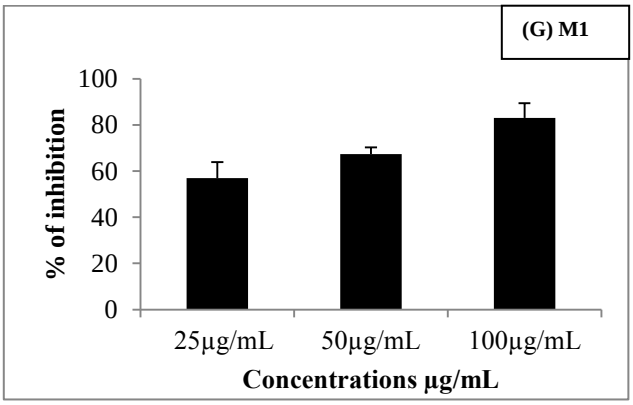

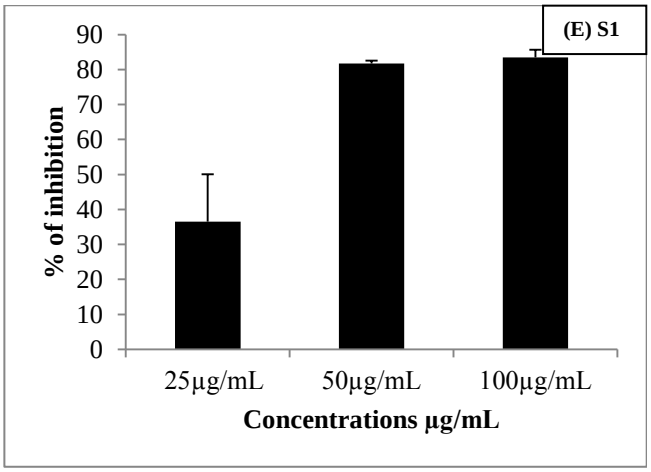

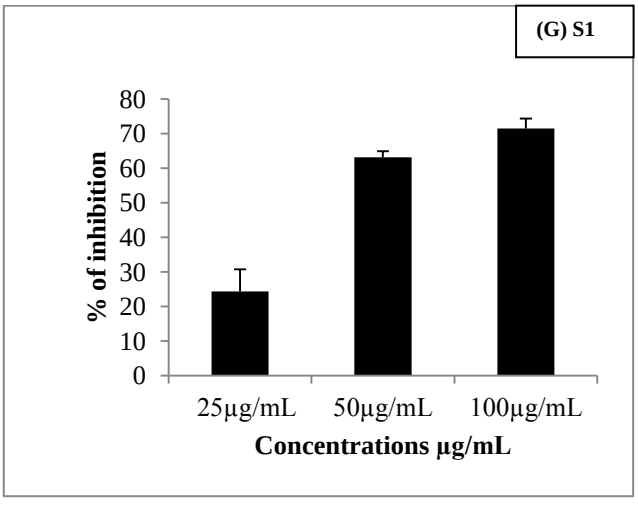

Figure 3. *A, B, C, D, E, F, G, denote the graphical representations of DPPH, ABTS, Hydrogen peroxide radical scavenging and Hydroxyl radical scavenging assay,Nitric oxide scavenging, Reducing Power Assesment, Phosphomolybdenum method, Reduction potential method respectively*

Table 2. *Antioxidant activity of standard melanin STSGRDM1 and sample melanin STSGRDS1.*

S.No	Antioxidant activity	Concentrations	% of inhibition	
			M1	S1
1.	DPPH	25µg/mL	26.72 ± 0.17	31.04 ± 1.74
		50µg/mL	41.89 ± 5.80	50.61 ± 2.91
		100µg/mL	66.96 ± 0.66	65.97 ± 3.32
2.	ABTS	25µg/mL	22.35 ± 2.35	13.77 ± 2.01
		50µg/mL	53.75 ± 2.05	51.7 ± 4.94
		100µg/mL	84.375 ± 2.67	82.3 ± 1.69
3.	Hydrogen peroxide radical scavenging	25µg/mL	26.13 ± 0.60	18.17 ± 5.78
		50µg/mL	48.62 ± 0.82	40.81 ± 1.93
		100µg/mL	68.15 ± 3.60	68.94 ± 2.48
4.	Hydroxyl radical scavenging assay	25µg/mL	25.8 ± 5.93	20.8 ± 8.20
		50µg/mL	48.3 ± 9.47	42.5 ± 3.53
		100µg/mL	64.96 ± 4.75	60.8 ± 8.20
5.	Nitric oxide scavenging	25µg/mL	84.75 ± 0.77	36.5 ± 13.57
		50µg/mL	88.95 ± 1.90	81.725 ± 0.81
		100µg/mL	96.305 ± 4.02	83.45 ± 2.19
6.	Phosphomolybdenum method	25µg/mL	55.47 ± 1.16	51.15 ± 1.62
		50µg/mL	64.95 ± 5.16	71.45 ± 3.32
		100µg/mL	73.25 ± 2.47	83.05 ± 2.47
7.	Reduction potential	25µg/mL	56.98 ± 6.95	24.35 ± 6.43
		50µg/mL	67.34 ± 2.91	63.15 ± 1.76
		100µg/mL	83.09 ± 6.37	71.45 ± 2.89

**All values were reported in the mean of two readings* ± S.D

Cosmetic formulations

Determination of sun protection factor

The formulated cream that is treated with the bacterial melanin was checked by absorption spectroscopy by the Mansur equation method. Formulations starting from the range of 290nm - 400nm were depicted as shown in Table 3. The bacterial melanin used in the study was observed to enhance the SPF values as shown in Table 3, thereby providing more protection against the harmful radiations such as UV radiation. Brenner et al., 2008 reported the efficacy of melanin as sunscreen was assumed to be about 1.5 to 2 Sun Protection Factor (SPF) possibly as high as 4 SPF, implying that melanin absorbs 50% to 75% of UV rays and SPF of 2 means doubling the protection of the skin against sunburn. Huang et al., 2012 reported the sun protection effect of melanin from *Cinnamomum burmannii* and *Osmanthus fragrans.* Tarangini et al., 2014 reported enhancement of SPF value by melanin produced by *Bacillus safensis.* Priyanka et al., 2017 reported the SPF values ranging from 0.4 to 23.5 from plant extracts *Datura metal*, *Loranthus,* and *Eucalyptus*. These results collaborating previous reports and prove that bacterial melanins also enhance SPF values and are effective sunscreens, therefore makes it an essential ingredient in cosmetic formulations. More *in vivo* and clinical trials were required to confirm its utility.

Table 3. *Normalized product function used in SPF calculation.*

Wavelength ((λ nm)	EE(λ) x I(λ) (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	**1**

Table 3(a). *Determination of SPF in standard STSGRDM1.*

Formulated cream %	Concentr-ation (µg/ml)	Wavelength	290	295	300	305	310	315	320	SPF
		EF X I	**0.015**	**0.081**	**0.287**	**0.327**	**0.1864**	**0.083**	**0.018**	
5%	125µg/ml	A	0.236	0.197	0.196	0.200	0.201	0.169	0.162	
		EF x I x A	0.003	0.015	0.056	0.065	0.037	0.014	0.002	**1.95**
	250µg/ml	A	0.443	0.337	0.423	0.370	0.343	0.341	0.339	
		EF x I x A	0.006	0.027	0.121	0.120	0.063	0.028	0.006	**3.70**
	500µg/ml	A	0.692	0.523	0.552	0.569	0.544	0.600	0.633	
		EF x I x A	0.010	0.042	0.158	0.186	0.101	0.029	0.011	**5.30**
	1000µg/ml	A	0.801	0.745	0.759	0.789	0.751	0.800	0.745	
		EF x I x A	0.012	0.061	0.217	0.258	0.139	0.066	0.013	**7.66**
10%	125µg/ml	A	0.423	0.364	0.355	0.439	0.359	0.379	0.341	
		EF x I x A	0.006	0.029	1.101	0.143	0.066	0.031	0.006	**3.82**
	250µg/ml	A	1.722	0.578	1.884	1.563	1.571	1.577	1.600	
		EF x I x A	0.025	0.046	0.540	0.511	0.292	0.130	0.028	**15.72**
	500µg/ml	A	1.756	0.731	2.117	1.694	1.599	1.626	1.714	
		EF x I x A	0.026	0.059	0.607	0.553	0.298	0.134	0.030	**17.07**
	1000µg/ml	A	1.810	0.890	2.468	1.873	1.643	1.814	1.810	
		EF x I x A	0.027	0.072	0.708	0.612	0.300	0.150	0.032	**19.07**

Table 3(b). *Determination of SPF in standard STSGRDS1.*

Formulated cream %	Concentr-ation (µg/ml)	Wavelength	290	295	300	305	310	315	320	SPF
		EF X I	**0.015**	**0.081**	**0.287**	**0.327**	**0.1864**	**0.083**	**0.018**	
5%	125µg/ml	A	0.463	0.417	0.422	0.427	0.368	0.379	0.360	4.05
		EF x I x A	0.007	0.033	0.121	0.139	0.068	0.031	0.006	
	250µg/ml	A	0.856	0.718	0.739	0.744	0.659	0.735	0.767	7.22
		EF x I x A	0.013	0.058	0.212	0.243	0.122	0.061	0.013	
	500µg/ml	A	1.832	1.539	1.564	1.531	1.406	1.591	1.475	15.19
		EF x I x A	0.027	0.124	0.448	0.500	0.262	0.132	0.026	
	1000µg/ml	A	2.869	2.323	2.259	2.256	2.127	2.161	2.042	22.27
		EF x I x A	0.043	0.188	0.648	0.737	0.396	0.179	0.036	
10%	125µg/ml	A	0.467	0.420	0.427	0.498	0.397	0.385	0.374	4.36
		EF x I x A	0.007	0.034	0.122	0.162	0.074	0.031	0.006	
	250µg/ml	A	0.952	0.793	0.804	0.889	0.811	0.833	0.786	8.32
		EF x I x A	0.014	0.064	0.230	0.290	0.151	0.069	0.014	

500µg/ml	A	1.949	1.862	1.631	1.763	1.656	1.600	1.533	16.9
	EF x I x A	0.029	0.150	0.468	0.576	0.308	0.132	0.027	
1000µg/ml	A	3.206	2.670	2.599	2.692	2.519	2.430	2.623	26.06
	EF x I x A	0.048	0.216	0.745	0.880	0.469	0.201	0.047	

Table 4 (a). *In-Vitro determination of 5% and 10% cream by Transmission spectroscopy- Sample STSGRDM1.*

5% Cream STSGRDM1			**10% Cream STSGRDM1**		
Wavelength	**%T ± S.D**	**MPF**	**Wavelength**	**%T ± S.D**	**MPF**
290	7.7±1.060	14.2	290	4.1±0.141	25
295	8.2±0.530	13.3	295	4.55±0.353	23.2
300	8.7±0.742	12.1	300	4.9±0.424	21.7
305	9.1±0.622	11.5	305	5.15±0.63	21.2
310	9.4±0.544	11.1	310	5.5±0.42	19.2
315	9.9±0.353	10.3	315	5.7±0.565	18.8
320	10.2±0.353	10	320	6 ±0.65	17.8
325	11.3±0.403	9	325	6.65±0.919	16.6
330	13±0.141	7.7	330	7.55±077	14.2
335	13.7±0.282	7.4	335	10.35 ±0.212	9.8
340	14.1±0.282	7.1	340	10.75±0.2	9.4
345	14.5±0.494	7	345	11.05±0.49	9.3
350	15.9±0.424	6.4	350	11.5 ±0.42	12.5
355	16.2±0.424	6.3	355	11.85± 0.49	11.7
360	16.4±0.424	6.2	360	12.7±0.424	11.6
365	16.6±0.424	6	365	13±0.46	11.6
370	16.8±0.636	6.1	370	13.45±0.35	11.6
375	17.1±0.636	5.9	375	14±0.56	11.6
380	17.3±0.777	5.9	380	14.45±0.35	11.6
385	17.6±1.060	5.9	385	14.85 ±0.353	11.5
390	19.2±0.777	5.4	390	15.5± 0.49	11.5
395	19.6±0.707	5.3	395	15.6±0.42	10
400	19.9±0.565	5.2	400	15.85±0.35	9.3

Table 4 (b). *In-Vitro determination of 5% and 10% cream by Transmission spectroscopy- Sample STSGRDS1.*

5% Cream (STSGRDS1)			10% Cream (STSGRDS1)		
Wavelength	**%T ± S.D**	**MPF**	**Wavelength**	**%T ± S.D**	**MPF**
290	10.4±0.494	9.9	290	10.15±0.21	10
295	11.45±0.353	8.9	295	10.45± 0.353	9.8
300	11.6±0.424	8.8	300	10.65±0.355	9.6
305	12.75±0.212	7.9	305	11±0.424	9.3
310	13.3±0.282	7.6	310	11.6±0.141	8.6
315	14.4±0.28	7	315	12.2±0.282	8.3
320	21.25±0.49	4.7	320	12.55±0.35	4.6
325	38.9±0.494	2.5	325	13.85±0.49	3.7
330	44.9±0.424	2.2	330	14.25±0.494	3.5
335	47.55±0.49	2.1	335	14.8±0.426	3.5
340	47.9±0.422	2.1	340	15.1±0.424	3.5
345	49.95±0.77	2	345	15.5±0.426	3.4
350	50.4±0.56	2	350	16 ±0.565	3.1
355	50.8±0.565	2	355	16.4±0.707	3.1
360	51.05±0.63	1.9	360	16.9±0.568	3.1
365	51.5±0.56	1.9	365	17.35±0.355	3.1
370	52.25±0.77	1.9	370	17.95±0.353	2.6
375	52.6 ± 0.848	1.9	375	18.55±0.212	2.2
380	52.95 ±0.919	1.9	380	19.1±0.424	2.2
385	53.3±0.84	1.9	385	19.65±494	2.1
390	53.45±0.919	1.9	390	20±0.464	2
395	53.8 ±1.272	1.9	395	20.5±0.426	2
400	54.1 ±1.29	1.8	400	21.05± 0.777	2

Table 5. *Determination of percent protection of formulated cream against UV rays and UVA analysis*

Sample	Formulation	% protection against UV A	% protection against UV B	Average UVA protection factor
STSGRDM1		84.65	91.13	15.84
STSGRDS1	5%	54.51	87.68	25.68
STSGRDM1		87.93	95.01	12.06
STSGRDS1	10%	82.97	88.9	17.02

In-vitro sunscreen activity by transmission spectroscopy

The in-vitro activity of the cream formulated with the bacterial melanin was checked by the transmission spectroscopy. By UV-vis spectrometer from the ranges 290nm to 400nm, the transmission spectrum of the formulated cream was obtained as shown in Table 4.The values of transmission were further calculated to find out the percentage of protection against the UV-A and UV-B, further to calculate the average UVA protection factor shown in Table 5. More et al., 2013 formulated creams that have the potency to protect UV rays containing the leaves extract (0.5%, 1%, 1.5%) of *Butea monosperma* determined by transmission spectroscopy method showed the concentration of extract in formulation increases, UV rays protection and average UVA protection factor also increases.

Conclusion

The present study concluded that the isolate belongs to *Pseudomonas* sp.STSGRDS1 which observed to have dark brown pigment-producing ability. Further, the biological application of the strain was confirmed by antioxidant assay and Sun protection factor makes it potential strain to be used in the field of therapeutics and cosmetics. More *in vivo* and clinical trials were required to confirm its utility.

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