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## Analysis of antioxidant, antityrosinase and antiglycating properties of a blue-green algae based skin cream

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### Abstract

This study provided a thorough evaluation of a *Spirulina platensis* extract-infused cream, assessing its physical properties, stability, microbial safety, spreadability, irritancy, moisture retention, and bioactive potential. The cream exhibited highly desirable attributes, making it a promising candidate for cosmetics, meeting industry standards. It displayed excellent spreadability, low irritancy, and minimal moisture absorption, indicating stability. Additionally, the cream demonstrated potent antioxidant properties and concentration-dependent antityrosinase activity, suggesting it could address pigmentation concerns. Enzyme kinetics revealed complex interactions, potentially influenced by rich biochemical composition, including phycocyanin. Crucially, the cream also demonstrated the ability to prevent methylglyoxal (MG)-induced glycation in proteins by 58.98% and in pBR322 plasmid by 30.48%. These findings underscore potential of *S. platensis* as a versatile cosmetic ingredient, offering physical excellence, stability, bioactive properties, and glycation inhibition capabilities. This study lays the foundation for multifaceted utility of the cyanobacterial extract in cosmetics and skincare products.

### 1. Introduction

The cosmetics industry is currently undergoing significant transformations, driven by the increasing demand for products that not only enhance beauty but also align with the growing preference for natural and sustainable solutions (Ragusa *et al.*, 2021). In this evolving landscape, *Spirulina platensis*, a cyanobacterium, has emerged as a compelling candidate for elevating the efficacy and appeal of skincare products (Asghari *et al.*, 2016; Pannindriya *et al.*, 2021). This study embarks on the formulation and comprehensive physiological evaluation of an innovative skin cream enriched with *S. platensis* aqueous extract. Through systematic analysis of the antioxidant properties of cream, its ability to inhibit tyrosinase (an enzyme contributing to skin pigmentation), and its capacity to mitigate oxidative stress (linked with skin ageing), the study seeks to uncover the often-overlooked potential of this natural extract.

The integration of *S. platensis* extract into cosmetics holds great promise due to its rich repository of bioactive components, including phycocyanin, carotenoids, vitamins, and essential fatty acids (Paramanya *et al.*, 2023a). Collectively, these constituents offer a range of effects, from potent antioxidant actions countering oxidative stress to anti-inflammatory responses that soothe and protect the skin. Additionally, they stimulate collagen synthesis, enhancing skin suppleness and elasticity (Chu *et al.*, 2010; Wu *et al.*, 2011). However,

the aspect of glycation, which is also crucial in skin ageing, is often under-addressed in cosmetics (Gasser *et al.*, 2011).

This study aims to seamlessly infuse *S. platensis* aqueous extract into an innovative skin cream, with the primary objective of harnessing its attributes to elevate overall skin health and appearance. It is important to note that glycation, a process that contributes to skin ageing, is a key concern in cosmetics (Gasser *et al.*, 2011). The cream, as evaluated in this study, demonstrates its effectiveness in preventing MG-induced glycation in proteins and offers significant potential in addressing glycation-related skin issues, marking a significant advancement in cosmetic formulations. To validate the efficacy of the formulated cream, an extensive array of physiological tests was conducted, with a primary focus on its antioxidant capacity. Oxidative stress, a key contributor to premature skin ageing and diminished skin vitality (Paramanya *et al.*, 2023a; Wu *et al.*, 2011), was closely examined. Additionally, the cream's antityrosinase property, vital for managing hyperpigmentation and achieving a radiant (Panzella and Napolitano, 2019), even skin tone (Paramanya *et al.*, 2023a), underwent rigorous assessment.

This paper provides a comprehensive overview of the formulation process and offers a detailed analysis of the physiological assessments performed. These examinations shed light on the antioxidant, antityrosinase, and antiglycating attributes inherent to the *S. platensis* aqueous extract-infused skin cream. The insights gained from this study have the potential to reshape the cosmetics landscape by offering profound possibilities through the incorporation of *S. platensis* extract into advanced cosmetic formulations, addressing not only oxidative stress but also glycation-related skin concerns.

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## 2. Materials and Methods

### 2.1 Materials

An axenic culture of *S. platensis* PCC 7345 was generously provided by Dr. Vani from Birla Institute of Technology and Science, Pilani, Rajasthan, India. The BG11 medium used in the study was sourced from HiMedia, India. All other chemicals utilized were of high purity grade.

### 2.2 Culture growth and extract preparation

*S. platensis* PCC 7345 was cultivated in BG-11 liquid medium at room temperature (RT), under consistent illumination, maintaining a neutral pH environment. The culture growth was aseptically transferred to centrifuge tubes, and centrifugation was conducted at 7000 rpm for 10 min at 10°C. The resulting pellet was subjected to three washes with distilled water (D/W). For the extraction process, two distinct solvents were employed. One portion of the pellet was suspended in D/W, while another portion was dissolved in a phosphate buffer (0.01 M, pH 7.4), both solutions having a concentration of 100 mg/ml. Subsequently, the solutions were sonicated at 37°C for 10 min with 15 pulses per min. After sonication, the samples underwent centrifugation, and the resulting supernatant was collected. This supernatant was used as an extract (Paramanya *et al.*, 2023b).

### 2.3 Preparation of skin cream

The skin cream formulation followed a methodical approach involving key stages. Initially, stearic acid, lanolin, and mineral oil were precisely measured at ratios of 10%, 10%, and 75%, respectively, based on the cream's total weight target. These ingredients were meticulously combined in a sterile beaker. Subsequently, the amalgamated mixture underwent gentle heating until complete dissolution and amalgamation. A water phase was concurrently heated to a temperature range of 70-75°C, utilizing distilled water to enhance quality. The heated water was gradually introduced into the melted oil phase, with continuous stirring to establish a stable emulsion. The cooling phase, accompanied by constant stirring, facilitated the emulsification process. Simultaneously during the emulsification process, *S. platensis* extract was added to the cream. Finally, as the mixture cooled and reached room temperature, stirring continued intermittently before aseptically transferring the cream to sterilized containers (Mawazi *et al.*, 2022).

### 2.4 Evaluation of cream

#### 2.4.1 Physical properties

The initial assessment involved the observation of the color, odor, and overall appearance of the cream formulation (D'browska *et al.*, 2016).

#### 2.4.2 Test for thermal stability

To evaluate the thermal stability of the formulated cream and lotion, the mixture was carefully dispensed into glass bottles using a spatula. The contents were gently tapped to facilitate settling at the bottom, ensuring that the bottles were filled up to approximately two-thirds of their capacity. Subsequently, the caps were securely tightened, and the filled bottles were positioned upright within an incubator. The incubation was maintained at a constant temperature of 45°C ± 1°C for duration of 48 h (Sharma *et al.*, 2019).

#### 2.4.3 Test for microbial growth in formulated cream

The formulated creams underwent microbial assessment through the implementation of the spread plate method. This entailed inoculating the creams onto st. nutrient agar plates, with a parallel preparation of a control sample. The inoculated plates were then placed within an incubator at 37°C for a period of 24 h. Following the incubation period, the plates were carefully removed, and the presence of microbial growth was scrutinized in comparison to the control (Biswas *et al.*, 2016).

#### 2.4.4 Spreadability test

The spreadability of the cream and lotion formulations was quantitatively determined using an established methodology by Singh and Nanda (2014). Two sets of standardized glass slides were employed in this evaluation. The formulation was sandwiched between the slides under specified load conditions. The duration taken for the upper slide to separate from the lower slide, with the formulation in between, was meticulously recorded. The spreadability was subsequently calculated using the formula: Spreadability =  $(m \times l)/t$ , where *m* represented the weight on the upper slide (30 g), *l* denoted the length of the slide (7 cm), and *t* indicated the time in seconds.

#### 2.4.5 Irritancy test

An irritancy test was conducted, involving the application of the cream to a pre-marked area on the dorsal surface of the left hand. The application duration was noted, and subsequent assessments were carried out at 24 h (Miettinen *et al.*, 2006).

#### 2.4.6 pH measurement

The pH values of the various cream formulations were ascertained using a digital pH meter. Approximately 1 g of cream or lotion was weighed and dissolved in 100 ml of distilled water. The resultant solution was allowed to stand for approximately 2 h. pH measurements were performed in triplicate for each sample, and the average values were calculated (Miettinen *et al.*, 2006).

#### 2.4.7 Moisture absorption studies

For moisture absorption studies, around 50 mg of the cream or lotion formulation was placed within a Petri plate. Simultaneously, water was introduced into a desiccator without any absorbents, allowing it to achieve saturation. The Petri plate containing the formulation was then positioned within the desiccator and maintained undisturbed for a period of 24 h. Subsequent observations were recorded (Singh and Nanda, 2014)

### 2.5 Antioxidant activity of the cream

The free radical scavenging capacity of the cream was evaluated using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. Its ability to neutralize nitric oxide radicals was tested with freshly prepared Griess reagent. For these assessments, different concentrations of the cream (20 mg/ml to 100 mg/ml) were mixed with a pre-prepared solution of 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS<sup>•+</sup>) and incubated for 30 min at 734 nm (Balyan and Ali, 2022; Paramanya *et al.*, 2023b). The percentage inhibition for all assays was calculated using the formula:

$$\% \text{ Inhibition} = [(\text{Abs Control} - \text{Abs Cream}) / \text{Abs Control}] \times 100.$$

## 2.6 Evaluation of anti-inflammatory activity

The reaction mixture was composed of 0.2 ml of freshly extracted egg albumin, 2.8 ml of phosphate-buffered saline at pH 6.4, and 2 ml of cream suspension. Subsequently, these mixtures were subjected to incubation at  $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$  in a biological oxygen demand incubator for duration of 15 min, followed by a heat treatment at  $70^{\circ}\text{C}$  for 5 min. Upon cooling, the absorbance of the samples was determined at a wavelength of 660 nm. Diclofenac was employed as a positive standard. The percentage of inhibition of protein denaturation was computed using the above-mentioned formula (Banerjee *et al.*, 2014).

## 2.7 Determination of antityrosinase activity

A tyrosinase enzyme solution, prepared at 15 units/ml in 0.1 M phosphate buffer with a pH of 6.8, was employed. The mixture of 1.8 ml of phosphate buffer and 0.1 ml of tyrosinase enzyme was incubated for 30 min at room temperature (RT). Concurrently, 0.1 ml of the cream formulation dissolved in dimethyl sulfoxide (DMSO), ranging from 20 to 100 mg/ml, was introduced to the mixture. After incubation, 1 ml of 1.5 M L-tyrosine substrate prompted a 7 min reaction at RT. Absorbance measurements were taken at 475 nm (Di Petrillo *et al.*, 2016). Kojic acid served as the positive control in this assay, facilitating the assessment of the cream's tyrosinase inhibition potential. The per cent inhibition formula was implied to determine the reduction in tyrosinase activity.

## 2.8 Determination of the type of inhibition of tyrosinase

Enzymatic activity of tyrosinase and its kinetics were studied with slight modifications in protocols described by Di Petrillo *et al.* (2016). Furthermore, a systematic study of the enzyme kinetics was conducted, involving various substrate concentrations to understand the relationship between substrate concentration and reaction velocity (Ashraf *et al.*, 2015). The reaction rate ( $v$ ) was computed using the subsequent equation:

$$v = \Delta[P]/\Delta t$$

where

$\Delta[P]$ : Change in concentration of the resulting product over a specific time interval ( $\Delta[P] = [P]_{\text{final}} - [P]_{\text{initial}}$ )

$\Delta t$ : Change in time interval

The kinetic attributes, namely the Michaelis-Menten constant ( $K_m$ ) and the maximal reaction velocity ( $V_{\text{max}}$ ), were determined employing the Lineweaver-Burk plot. Subsequently, the graph generated via plotting  $1/V$  against  $1/[S]$  underwent further analysis. The linear equation ascertained through trend lines was juxtaposed with the Lineweaver-Burk plot equation:

$$1/v_0 = (K_m/V_{\text{max}}) * (1/[S]) + 1/V_{\text{max}}$$

## 2.9 Correlation coefficient calculation

The correlation analysis aimed to explore potential relationships between the cream's antioxidant and antityrosinase activities. Correlation coefficients were calculated using established statistical methods. The Pearson correlation coefficient was employed to quantify the strength and direction of linear relationships between the different assays. The resulting correlation coefficients were interpreted to determine the degree of correlation between the various assays (Balyan and Ali, 2022).

## 2.10 Protection against MG-induced glycation of BSA

BSA (10 mg/ml) was exposed to 10 mM of MG within a phosphate buffer (0.1 M, pH 7.4) containing 0.02% sodium azide, and this mixture was incubated at  $37^{\circ}\text{C}$  for a period of four weeks. In order to assess the prevention of advanced glycation end products (AGEs) formation, cream was solubilized in DMSO (100 mg/ml) and subsequently introduced into the aforementioned mixture. Aminoguanidine (AG) was used as a positive control at a concentration of 1 mM. The evaluation of fluorescent AGEs produced during the glycation of albumin is typically conducted by monitoring their fluorescence at excitation/emission wavelengths of 370 nm and 440 nm, respectively, utilizing the Agilent Cary Eclipse spectrofluorometer (Agilent Technologies, Victoria, Australia) (Balyan *et al.*, 2022).

The percentage of inhibition of glycation was calculated using the following formula: % Inhibition of glycation = (Fluorescence of glycated BSA - Fluorescence of native or cream-coated BSA) / Fluorescence of glycated BSA  $\times$  100.

## 2.11 Protection against glycoxidative DNA damage

In a controlled in vitro study, pBR322 plasmid DNA (0.25  $\mu\text{g}$ ) was suspended in 100 mM potassium phosphate buffer at pH 7.4. Glycation was initiated by adding lysine (20 mM), methylglyoxal (MG, 20 mM), and iron chloride ( $\text{FeCl}_3$ , 100  $\mu\text{M}$ ) to the solution, with and without the cream. The reaction mixtures were incubated at  $37^{\circ}\text{C}$  for 2 h. A control setup included pBR322 DNA in buffer without glycation inducers and DNA + cream as a control. Following incubation, 10  $\mu\text{l}$  of each sample was combined with 2  $\mu\text{l}$  of 6X gel loading dye and loaded onto a 1% agarose gel in TAE buffer. Electrophoresis was conducted at 85 V until migration was approximately two-thirds through the gel. The gel was stained with ethidium bromide (5  $\mu\text{g}/\text{ml}$ ) and visualized using a Gel-Doc system (Kumar and Ali, 2019; Bamne *et al.*, 2023; Paramanya *et al.*, 2023b).

To evaluate changes in band intensity, DNA images from the Gel-Doc were analyzed using ImageJ software (Version 1.53s). The colors in the gel images were inverted to highlight dark bands against a lighter background. A graph showing gray area versus density was generated, and the integrated density (IntDen) was quantified to determine variations in band intensity (Paramanya *et al.*, 2023b).

## 2.12 Statistical analysis

The analysis was conducted using GraphPad Prism version 9 software. Data were evaluated for statistical significance using a two-way analysis of variance (ANOVA). Results are presented as the mean  $\pm$  standard deviation (SD) with a sample size of  $n = 3$ . Tukey's multiple comparisons test was used to determine differences between treatments. Statistical significance was considered at a  $p$ -value of less than 0.05 when compared to the positive control group (Balyan and Ali, 2022).

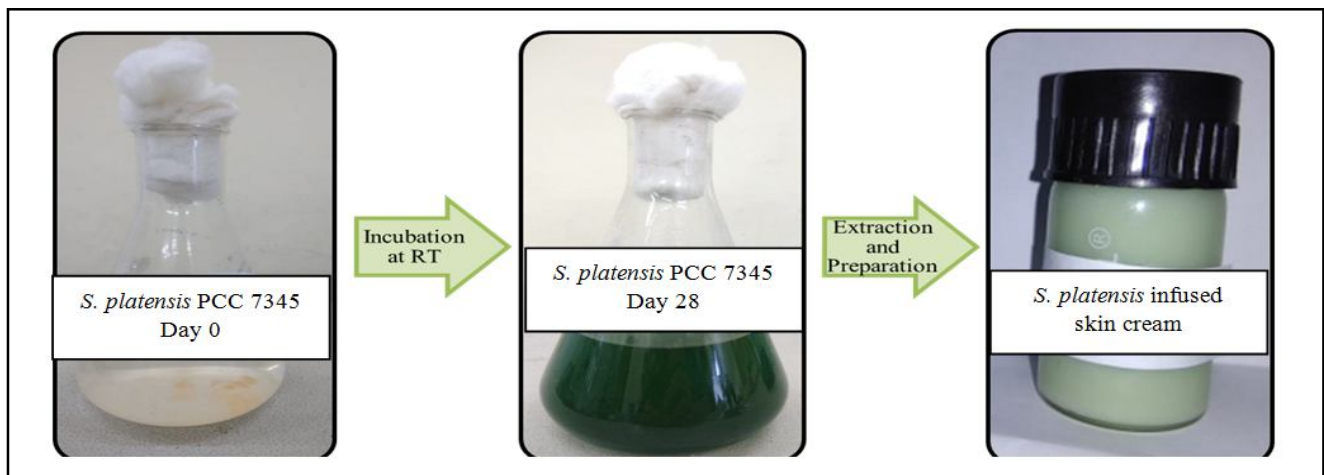
## 3. Results

### 3.1 Physical properties of the cream

The physical and chemical characteristics of the cream formulation were evaluated to determine its suitability for topical application (Table 1).

**Table 1: Physical characteristics of prepared cream**

S. No.	Characteristics	Observation
1.	Colour	Pale green
2.	Texture	Smooth
3.	Odour	Odourless
4.	Consistency	Thick
5.	pH	7.0 ± 0.4
6.	Thermal Stability	No observable changes in colour or texture. No separation or phase separation.
7.	Spreadability	7.23 cm <sup>2</sup> /s
8.	Irritancy	Nonirritant
9.	Moisture Absorbance	Minimal

**Figure 1: Skin cream's formulation prepared using *S. platensis* extract.**

The cream exhibited a pale green color, which is likely due to the presence of *Spirulina platensis* extract. The texture was smooth, which is crucial for enhancing consumer sensory experience and ensuring uniform application (Biswas *et al.*, 2016). The cream was found to be odorless, making it suitable for consumers with a sensitive olfactory system. The pH of the cream was measured at  $7.0 \pm 0.4$ ,

close to the skin's natural pH (typically around 5.5). Thermal stability assessments indicated no observable changes in color or texture, and no phase separation.

### 3.2 Microbial load testing of the cream

The study aimed to evaluate the microbial load of a cream formulated with *S. platensis* extract (Figure 2).

Dilution	Images of plates	Set I	Set II	Set III	Average	(cfu/ml)
10 <sup>-1</sup>		3112	3009	2988	3036.33	3.036 * 10 <sup>4</sup>
10 <sup>-2</sup>		238	266	251	251.67	2.52 * 10 <sup>4</sup>
10 <sup>-3</sup>		121	108	117	115.33	11.53 * 10 <sup>4</sup>

**Figure 2: Microbial load testing of prepared cream.**

(Plates observed after 48 h of incubation at RT. n=3; cfu= colony forming units)

Using dilution factors, the original microbial concentrations in the cream were determined to be approximately  $1.7086 \times 10^6$  cfu/ml. The microbial load values are within the acceptable limits for cosmetic products.

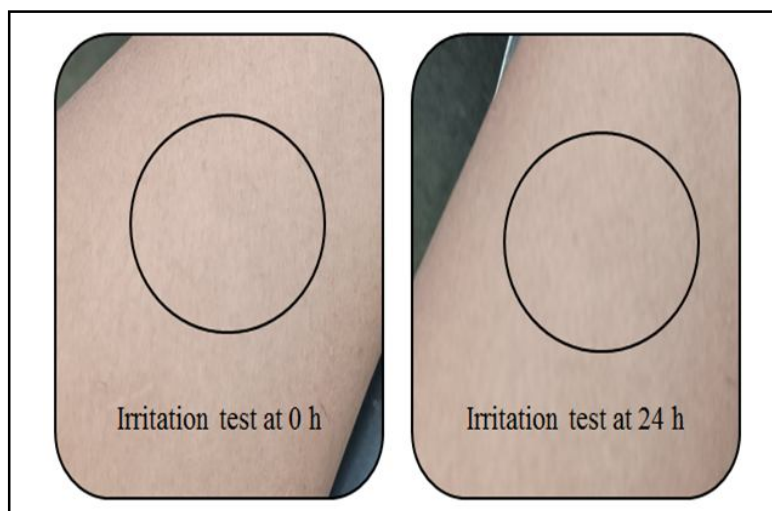
### 3.3 Spreadability test

The spreadability test conducted on the cream yielded a value of  $7.23 \text{ cm}^2/\text{s}$ . This value falls within the desirable range for cosmetic

creams, which is generally considered to be approximately 5 to  $10 \text{ cm}^2/\text{s}$  (Sabale *et al.*, 2011).

### 3.4 Irritancy test

The irritancy test performed on the cream revealed non-irritant effects on the skin at 24 h. The cream was applied to a pre-marked area on the dorsal surface of the left hand, and subsequent assessments were checked after 24 h (Figure 3).



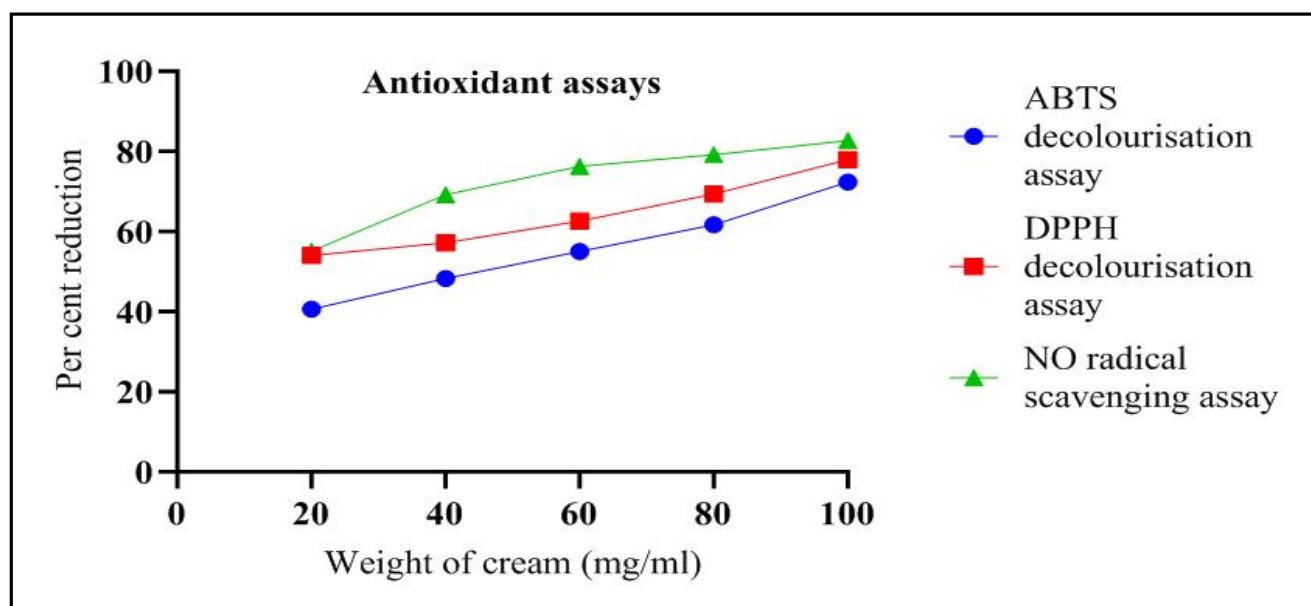
**Figure 3: Irritation test performed on the dorsal surface of skin.**

[No redness was observed indicating non-irritant property of the cream. Irritancy is indicated by redness or rash-like appearance]

Throughout the testing period, no signs of skin irritation or adverse reactions were observed. This indicates that the cream demonstrated a non-irritant profile and can be considered suitable for topical application on the skin (Singh and Nanda, 2014).

### 3.5 Moisture absorption studies

The moisture absorption study conducted on the cream indicated that the amount of moisture absorbed was negligible.



**Figure 4: Antioxidant activity of cream at various concentrations.**

[Results expressed in the percentage of inhibition; mean ( $n = 3$ );

Abbreviations:- ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6 sulfonic acid); DPPH: 2,2-Diphenyl-1-picrylhydrazyl; NO: Nitric oxide]

### 3.6 Antioxidant effect of the cream

The cream exhibited a concentration-dependent trend in its antioxidant and radical scavenging properties across all three assays (Figure 4).

The graph demonstrates the cream's potent antioxidant and radical scavenging potential, as indicated by its effectiveness in counteracting

various radicals in the ABTS, DPPH, and NO assays. The observed concentration-dependent responses suggest significant bioactivity within the cream.

### 3.7 Anti-inflammatory activity of cream

The anti-inflammatory activity of the cream was determined by determination of the protein albumin denaturation (Table 2).

**Table 2: Anti-inflammatory activity of cream**

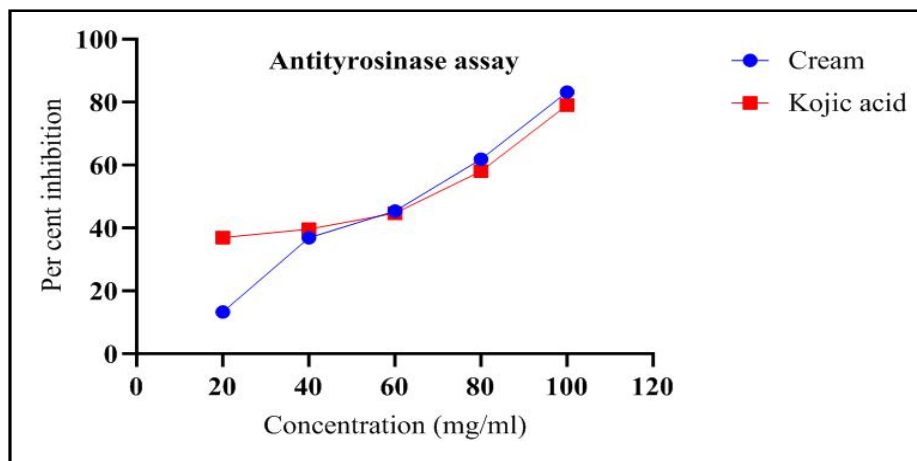
Sample	Concentration	Per cent inhibition
Diclofenac (standard)	2 mg/ml	14.67
<i>S. platensis</i> aqueous extract	0.1 mg/ml	22.67
<i>S. platensis</i> extract-infused cream	2 mg/ml	16

Diclofenac, utilized as the standard reference, exhibited a percentage inhibition of 14.67% at a concentration of 2 mg/ml, which aligns with previous findings reported by Banerjee and colleagues (2014). Remarkably, *S. platensis* aqueous extract, tested at a lower concentration of 0.1 mg/ml, displayed a significantly higher percentage inhibition of 22.67%. This outcome is consistent with observations made by Callela *et al.* (2022) who also noted the enhanced anti-inflammatory properties of *S. platensis*. Furthermore, the *S. platensis* extract-infused cream, evaluated at a concentration of 2 mg/ml, demonstrated a moderate inhibitory effect with a percentage inhibition

of 16%. These collective results indicate that the cream has the potential to effectively inhibit protein denaturation, making it a promising candidate for applications in anti-inflammatory treatments.

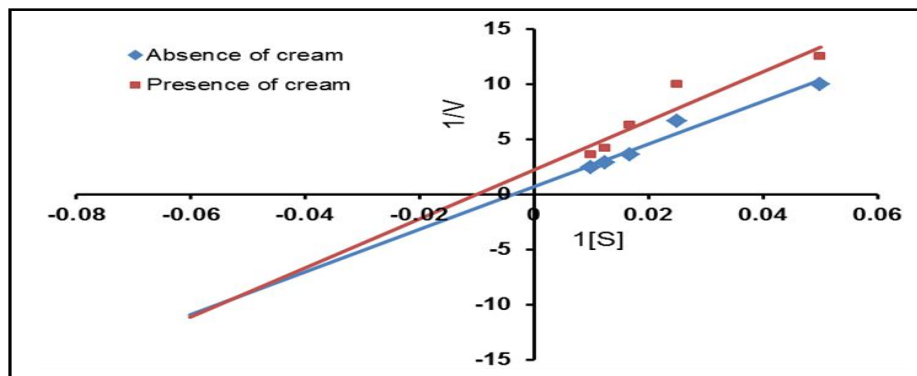
### 3.8 Antityrosinase activity of the cream

The graph shows a concentration-dependent relationship between the cream's concentration and its tyrosinase reduction activity (Figure 5). At 20 and 40 mg/ml, the cream's reduction was lower than that of Kojic acid. However, at 80 and 100 mg/ml, the cream's inhibition approached or exceeded that of Kojic acid.



**Figure 5: Antityrosinase activity of cream as compared to Kojic acid.**

[Results expressed in the percentage of inhibition; mean (n = 3)]



**Figure 6: Lineweaver-Burke plot for inhibition of tyrosinase.**

[V=Velocity, [S]= Concentration of substrate]; Using Figure 6, the following data have been derived.

**Table 3: Kinetics of tyrosinase inhibition**

	Absence of cream	Presence of cream
Equation from graph	$y = 193.17x + 0.696$	$y = 222.21x + 2.2237$
Slope (Km/Vmax)	193.17	222.21
Km	277.64	99.95
Vmax	1.4368	0.4495

### 3.9 Enzyme kinetics

The investigation of tyrosinase activity in the presence of the formulated cream yielded distinct kinetic profiles (Figure 6).

The changes in both Km and Vmax values (Table 3) suggest a significant alteration in the enzyme's behavior when interacting with the cream. The pattern observed indicates mixed inhibition, affecting

both the enzyme's affinity for its substrate (Km) and its maximum catalytic efficiency (Vmax).

### 3.10 Correlation between antityrosinase activity and antioxidant assays

The correlation coefficients between various assays provide insights into the relationships among different properties of the cream (Table 4).

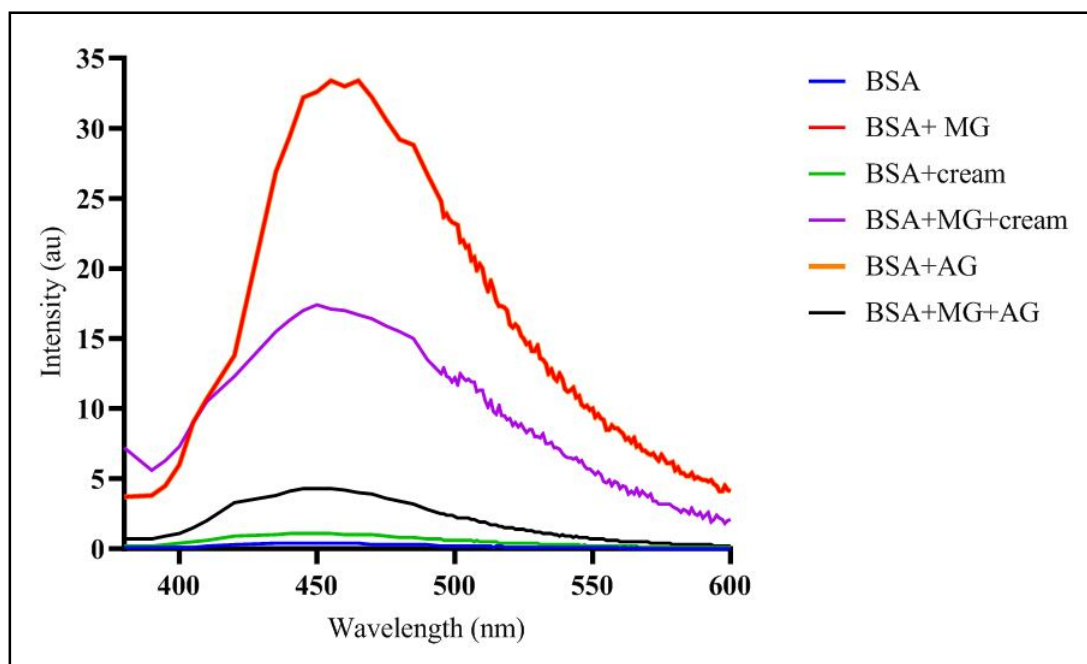
**Table 4: Correlation study of various activities of the cream**

Assays	ABTS	DPPH	NO	Antityrosinase
ABTS	1			
DPPH	0.991	1		
NO	0.927	0.877	1	
Antityrosinase	0.994	0.978	0.942	1

The ABTS decolorization assay shows strong positive correlations with the DPPH assay (correlation coefficient = 0.995) and the NO radical scavenging assay (correlation coefficient = 0.944), indicating consistent antioxidant results across these methods. The DPPH and NO assays also exhibit a strong positive correlation (correlation coefficient = 0.877).

### 3.11 Preventive effect of cream on MG-mediated on AGE formation

The *S. platensis* extract-infused cream was evaluated for its ability to inhibit the formation of AGEs, which contribute to skin issues like wrinkles and loss of elasticity. The reduction in AGE formation was assessed using the fluorescence intensity of the BSA-MG system.

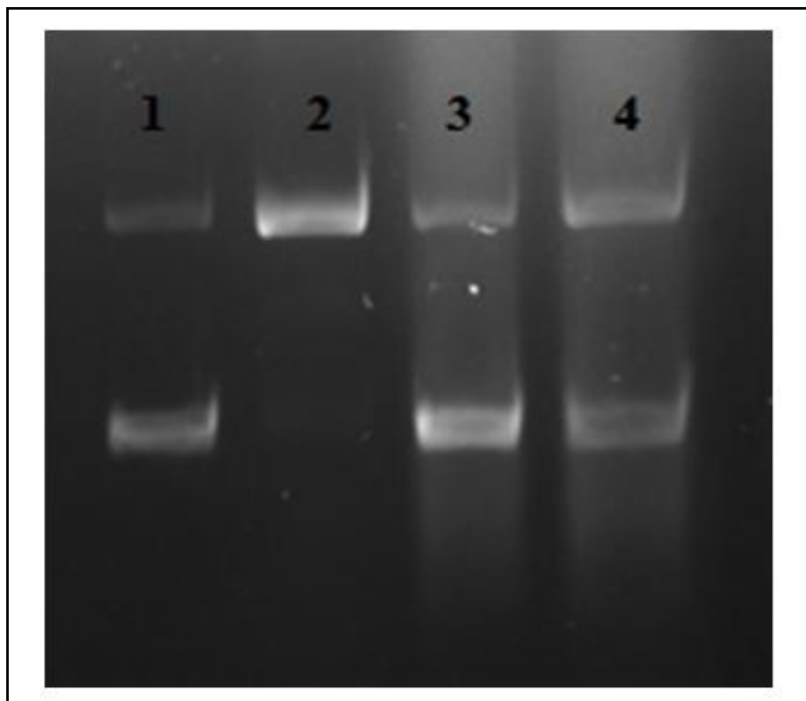
**Figure 7: Inhibition of total AGEs by skin cream.**

[Results are expressed in intensity (au)]

After 28 days of incubation, the cream demonstrated an inhibition rate of approximately 58.98% (Figure 7), compared to an 81.73% inhibition by the positive control, AG. This is slightly lower than the 65% glycation prevention observed in hemoglobin in our previous study (Paramanya *et al.*, 2023a).

### 3.12 Effect of cream on DNA damage

Plasmid DNA pBR322 exhibited two distinct bands when analyzed alone (lane 1) – band 1, representing a linear conformation, and the lower band, identified as band 2, representing a supercoiled conformation (Figure 8).



**Figure 8: Glycooxidative DNA damage and its subsequent prevention by cream.**  
[Lanes: 1 – DNA alone; 2 – DNA + Glycation system (GS); 3 – DNA + cream; 4 – DNA + GS + cream; Incubation time: 180 min; Stained with EtBr]

Glycation induced strand breaks in DNA, converting the supercoiled form to a linear form, as seen in lane 2 (pBR322 + GS). Lane 3, with DNA and the cream, closely resembled lane 1 (DNA alone), indicating no significant impact on DNA structure. Lane 4 (DNA + GS + cream)

showed a similar pattern to lane 1 but with a reduced intensity of the linear band and increased intensity of the supercoiled form. This suggests that the cream helps reverse or prevent glycation-induced strand breaks.

**Table 5: Comparison of IntDen between DNA Lanes in Band 1 (Linear Form) and Band 2 (Supercoiled Form)**

Band 1			Band 2		
Lane	IntDen	Per cent increase/decrease	Lane	IntDen	Per cent increase/decrease
1	1378.134	0	1	5869.296	0
2	9785.388	+610.04	2	188.021	-96.79
3	2014.69	+46.18	3	8565.418	+45.93
4	3178.418	+130.63	4	4079.933	-30.48

[Lanes: 1 – DNA alone; 2 – DNA + GS; 3 – DNA + cream; 4 – DNA + GS + cream. The increase and decrease have been indicated with + and – signs before the value, respectively.]

Methylglyoxal (MG) induced a substantial 96.79% reduction in the supercoiled form of DNA, converting it to the linear form (Table 5). However, the application of cream appeared to significantly mitigate this damage, reducing it to only 30.48%. This indicates the cream's potential in reversing or preventing the adverse effects of MG-induced DNA damage.

## 4. Discussion

The pale green color of the cream suggests the presence of bioactive compounds from *S. platensis* extract, contributing to both its aesthetic appeal and functional benefits. The smooth texture enhances user experience and facilitates even application, which is crucial for consumer satisfaction (Biswas *et al.*, 2016). Its odorless nature



benefits users with sensitive olfactory systems, and the near-neutral pH helps maintain the skin's acid mantle, preventing irritation and supporting natural barrier function (Sabale *et al.*, 2011). The cream's demonstrated thermal stability, with no changes in color, texture, or phase separation, underscores its robustness, ensuring efficacy and longevity under various conditions (Abdel-Moneim *et al.*, 2022).

The minimal moisture absorption observed indicates a low propensity for environmental moisture uptake, which is beneficial for maintaining the cream's consistency and effectiveness over time. This stability enhances its suitability as a cosmetic product, ensuring it remains effective throughout its shelf life (Abdel-Moneim *et al.*, 2022; Singh and Nanda, 2014). The cream's strong antioxidant potential is highlighted by its ability to neutralize various radicals, as evidenced by the concentration-dependent responses in the ABTS, DPPH, and NO assays (Gunes *et al.*, 2017). This antioxidant capacity is promising for combating oxidative stress and skin ageing. Additionally, the cream's tyrosinase inhibitory effect improves with concentration, aligning with findings by Mapoung *et al.* (2020) and Augustina *et al.* (2021). This suggests the cream's potential in addressing pigmentation concerns and promoting skin luminosity through effective modulation of the tyrosinase enzyme. The mixed inhibition pattern observed, where the cream affects both  $K_m$  and  $V_{max}$ , indicates complex interactions between the cream's bioactive compounds and the tyrosinase enzyme. This type of inhibition is consistent with substances like Kojic acid (Wang *et al.*, 2022) and natural inhibitors such as flavonolignins and glycolic acid (Kim *et al.*, 2023), highlighting the intricate modulation by compounds like phycocyanin from *S. platensis*.

High correlation coefficients between the antityrosinase activity and antioxidant assays (ABTS: 0.991, DPPH: 0.994, NO: 0.978) suggest a close relationship between the cream's tyrosinase inhibition and its antioxidant properties. The ability of the cream to reduce AGE formation suggests it can mitigate glycation-related skin damage, helping preserve skin elasticity and reduce wrinkles. Although, its effectiveness is slightly lower than the positive control, the cream shows promise in preventing age spots and overall skin ageing. The restoration of supercoiled DNA structure, as indicated by reduced linear DNA and increased supercoiled DNA, further suggests that the cream can counteract glycation effects, maintaining DNA integrity and aligning with findings from Balyan and Ali (2022) and Paramanya *et al.* (2023a).

## 5. Conclusion

This study highlights the significant potential of a cream formulated with *S. platensis* extract for cosmetic applications. The cream demonstrates strong anti-inflammatory, antiglycating, antityrosinase, and antioxidant activities, while adhering to industry standards for stability and usability. Its notable glycation-reducing capability is particularly relevant for addressing skin ageing and inflammation. The anti-inflammatory effects contribute to skin protection and soothing, while the antiglycating properties help prevent glycation-induced skin damage, enhancing its anti-ageing efficacy. Additionally, the antityrosinase and antioxidant activities are essential for pigmentation control and reducing oxidative stress, crucial for skin health. This study provides a comprehensive assessment of the cream's multifaceted benefits, supporting its potential as a versatile

and effective component in advanced skincare formulations and serving as a reference for further research into its mechanisms of action and applications in the cosmetics industry.

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## Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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