

Original Research Article

Prodigiosin development and its entrapped topical microstructured sunscreen for laser and UV radiation protection and antioxidant activity

Thaddeus Harrison Gugu¹, Anthony Amaechi Attama², Emmanuel Chinedum Ibezim², Ganesh Dinkarrao Basarkar³, Somchai Sawatdee⁴, Teerapol Srichana⁵

¹Department of Pharmaceutical Microbiology and Biotechnology, University of Nigeria, Nsukka, ²Department of Pharmaceutics, University of Nigeria, Nsukka, Nigeria, ³Department of Pharmaceutics, SNJB's Shriman Sureshdada Jain College of Pharmacy, Chandwad, Savitribai Phule Pune University, Pune, Maharashtra State, India, ⁴Drug and Cosmetics Excellence Center and School of Pharmacy, Walailak University, Nakhon Si Thammarat 80161, Thailand, ⁵Drug Delivery System Excellence Center, Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

*For correspondence: **Email:** thaddeus.gugu@unn.edu.ng; **Tel:** +234 8034152833

Sent for review: 15 February 2025

Revised accepted: 21 July 2025

Abstract

Purpose: To develop prodigiosin (PG) cream for laser and ultraviolet radiation (UV) protection.

Methods: The cream containing PG (PG-C) was prepared and characterized. In vivo skin irritation was evaluated on Wistar rats by shaving the animals and applying the calculated amount on the animal skin daily for 3 days, while occlusivity was carried out in vitro. Trans-epithelial water loss and melanin contents were evaluated in rats and antioxidant studies of PG were done using DPPH (0.1 mM) and ABTS (7 mM) protocols. In vitro drug release was conducted using dissolution tester and paddles. Furthermore, laser obscuration and sun protective factor (SPF) were screened.

Results: The PG-C appeared pinkish with pH of 6.63 ± 0.21 , viscosity of 2957 ± 2 Pa·s and spreadability of $36.85 \text{ g} \cdot \text{cm}^2/\text{sec}$. There was no skin irritation during the 14-day treatment. Transmission electron microscope (TEM) and scanning electron microscopy (SEM) revealed the sizes of PG and PG-C to be 50 nm. The IC_{50} of PG was $28.74 \pm 0.89 \mu\text{g/mL}$ (DPPH) and $11.9 \pm 0.75 \mu\text{g/mL}$ (ABTS), while the average IC_{50} for PG-C was $21.33 \pm 1.91 \mu\text{g/mL}$ (DPPH) and $24.0 \pm 0.82 \mu\text{g/mL}$ (ABTS) with ascorbic acid at $43.89 \pm 1.41 \mu\text{g/mL}$. The PG-cream was able to block the laser and UV light. The PG release at 4 h from cream was 2 – 10 %. The loss of water content from human skin was minimal, while melanin content was not affected.

Conclusion: This study has shown that PG-C offered protection against laser and UV light and demonstrates the presence of remarkable antioxidants.

Keywords: Prodigiosin, Sunscreen cream, Laser obscuration, Sun-protection factor, Antioxidant

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Scopus, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Prodigiosin (PG) is a natural secondary metabolite and red tripyrrole pigment that belongs to the prodiginine group of tripyrrole acid.

It is mainly produced by different species of microorganisms such as *Streptomyces variegatus*, *Pseudomonas magnesorubra*, *Colwellia psychrerythraea*, and *Hahella chejuensis* with some biological activities such as

anti-proliferation, immunosuppression, antioxidant and antimicrobial [1,2]. It has a molecular formula of $C_{20}H_{25}N_3O$ and molecular weight of 232.44 Da. The physicochemical properties of PG include a melting point of 151 – 152 °C, boiling point of 554.3 ± 50 °C, soluble in non-polar solvents and partially soluble in alcohol and ether [3].

Prodigiosin has been studied for its use in cosmetics applications, such as soap coloring [4] and potent antioxidant activities when compared with 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzthiazoline-6- sulphonic acid; ABTS), which are commonly used antioxidant scavengers against ultraviolet (UV) rays, as a sun protection factor (SPF) and thus protector of skin [5]. It has been reported that PG confers significant protection against UV stress in *Vibrio spp* (DSM 14379) as the absence of pigment expression induces susceptibility to UV stress in the non-pigmented mutant [6]. This pigment has demonstrated effective sunscreen protective effect in combination with other extracts of photo-protective medicinal plants and therefore functions as additive in some marketed products [7]. Although some literature has reported the effective activity of PG on sun protection, there exists a lack of formulation development of PG as a sunscreen product. In addition, the effective dose of PG in cosmeceuticals is unknown, which is an important issue that needs to be addressed. Previous report shows that PG exhibits remarkable UV-absorbing properties, particularly in the UVA range, and has also been reported as an active agent against cancer cells [8]. By incorporating PG into a sunscreen formulation, it can effectively shield the skin from UVA radiation, which is known to cause long-term damage, premature aging, and also potential high risk of skin cancer. This unique pigment offers the potential for broad-spectrum protection when combined with established sunscreen agents, providing comprehensive defense against both laser radiation, UVA, and UVB rays [9], and laser beam as a function of its skin sun protection factor (SPF). Therefore, this study is aimed at using this bio-based natural product from bacteria through topical delivery to protect skin from UV radiation and sunburn effects.

EXPERIMENTAL

Preparation of cream containing prodigiosin (PG-C)

Prodigiosin was extracted and purified from gram-negative bacteria, *S. Marcescens*, by

adopting a previous protocol [10]. The cream was prepared by a combination of aqueous and oil cream phases. Briefly, the oil phase components were weighed into a glass flask and heated until the contents melted completely at 80 °C. Thereafter, the required amounts of the PG were weighed out and incorporated into the oil part to form a homogenous mixture.

Similarly, the aqueous part containing hydrophilic substances was prepared in 100 mL by weighing the calculated amount of glycerin (MySkinRecipes®), potassium hydroxide (SIGMA®), methyl and propyl paraben (FLUKA®) into a glass flask and heated to the equilibrium temperature with the oil phase. The oil phase was added into the aqueous phase slowly with constant stirring using an IKA® RW 20 digital homogenizer mixer at 3000 rpm until the cream was formed. Four (4) sample formulations were prepared using the formula in Table 1 for both PG and blank creams, respectively. Finally, PG was incorporated in an oil phase at about 40 °C in order to ensure heat stability of the PG compound.

Physicochemical evaluations

The resulting cream formulations (PG-C1, PG-C2, PG-C3, PG, and Ascorbic acid) were evaluated for their physicochemical properties such as pH, spreadability, colour, viscosity, and physical texture using scanning and transmission electron microscopy.

pH measurement

The pH was determined on each cream formulation using a LABMAN digital pH meter (CL180 Chemiline, India) by immersing the sensor electrode into 20 g of the creams, allowing for 2 – 5 min meter value stabilization and then recording the value. The process was repeated in triplicate for accurate results.

Viscosity

The viscosity of the formulated creams at 35 °C was determined by the Brookfield paddle method [11]. About 10 g each of the cream was weighed into a 25 mL beaker and then fixed onto the digital Brookfield DV-III Ultra rheometer (Brookfield, USA) fitted with an LV spindle No. 6 at 10 rpm. The viscosity was determined and recorded while the rheometer was controlled by the Brookfield Rheocalc operating software (version 3.1-1). The same process was repeated in all the formulations and blank cream base and the experiment was done in triplicate.

Table 1: Composition of the prodigiosin sunscreen cream

Batch code	Lipid phase				Aqueous phase			Prodigiosin (mg; %)	Distilled water to (mL)
	Stearic acid (g)	Stearyl alcohol (g)	Cetyl alcohol (g)	Methyl paraben (g)	Propyl paraben (g)	Glycerin (g)	Potassium hydroxide (g)		
PG-C1	13.0	1.0	1.0	0.1	0.05	10.0	0.9	100	100
PG-C2	13.0	1.0	1.0	0.1	0.05	10.0	0.9	200	100
PG-C3	13.0	1.0	1.0	0.1	0.05	10.0	0.9	300	100
Blank-C	13.0	1.0	1.0	0.1	0.05	10.0	0.9	-	100

*PG-C1, PG-C2 and PG-C3 are creams formulated with 0.1, 0.2 and 0.3 % PG, respectively, and Blank-C represents cream base without PG

Spreadability studies

The spreadability studies of the formulations were determined *in vitro* following the standard method [12]. Coefficient of spreading was determined by the principle of 'Slip' and 'Drugs' characteristics of the cream formulations. Two glass slides of 18.7 cm in length were used for this experiment, and the first was ground glass fixed on a wooden flat surface with the aid of strong gum. A known quantity (about 1 g) of the cream was placed on the ground slide and covered with the second slide, previously attached with a tread and weight carrier at one end. A standard weight of 200 g was placed on the cream-sandwiched slides for 3 – 5 min to remove air bubbles and also to provide a uniform film of the cream between the two slides.

Thereafter, measured standard weights were added to the attached pan until the second slide slipped off the ground, thereby spreading the cream towards the pulley and weight carrier. The following parameters were determined using a standard formula adopted previously, which includes the quantity of standard weight that was pulled off the second slide, the time taken to slip off the second slide from the cream in seconds and distance of cream spread. The spreadability was calculated by using the formula in Eq 1 with some modifications [13].

$$S = MxL/T \dots\dots\dots (1)$$

Where M = Quantity of standard weight that slipped off the second slide (g), L = Length of slide covered by the cream spread (cm), T = Time it takes to separate the two slides.

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM)

Following a previous protocol, the structural images and sizes of the PG and cream were studied using a transmission electron microscope (JEOL Model JM 2100, Japan) at an accelerating voltage of 100 kV [14]. Similarly, the surface morphology and images of the formulated creams were examined and captured by scanning electron microscopy (HITACHI SU3900, Japan) at 20 kV.

Antioxidant studies

Antioxidant studies were carried out to establish the ability of the PG and its cream formulations to scavenge free radicals. This was performed using both the DPPH and ABTS free radical scavenging assays.

DPPH radical scavenging assay

The DPPH assay for the free radical scavenging activity was studied on the formulations following the protocols of Hussen [15]. The proton-donating ability of the PG extractives in the cream was examined by the colour changes of DPPH methanolic solution. A 0.1 mM DPPH solution was prepared using methanol (BDH) and thereafter, 100 μ L of DPPH solution containing 170 μ g/mL was mixed with 100 μ L of PG-cream (30 μ g/mL) in de-ionized water. Also, different concentrations (12.5 – 200 μ g/mL) were prepared. The reaction mixture was mixed properly and allowed to stand for 30 min in the dark conditions. Absorbance of resultant mixtures was read using a Varioskan lux microplate reader (Thermo Scientific) at 515 nm. Ascorbic acid (50 μ M) was used as a reference.

ABTS radical scavenging assay

This assay was performed using a modified procedure as described [16]. The ABTS stock solution (7 mM) was prepared by the reaction of 7 mM ABTS and potassium persulfate (2.45 mM) as the reference antioxidant agent (1:1 v/v). The ABTS^{•+} working solution was obtained by diluting the stock solution in ethanol (AR) to obtain an absorption value of 0.70 ± 0.02 at 734 nm using microplate reader. Thereafter, 30 μ L of PG (30 μ g/mL) and PG-C (30 μ g/mL) samples were added separately to 195 μ L of ABTS^{•+} solution and kept at room temperature for 30 min. Subsequently, the absorbances of both PG and PG-cream samples in the ABTS mixture were determined at 734 nm. Percentage inhibition of ABTS^{•+} (PI) for both samples was calculated with reference to the standard by Eq 2.

$$PI = (A_c - A_s/A_c)100 \dots\dots\dots (2)$$

Where: A_c = absorbance of control (no sample), A_s = absorbance of the samples.

The sample IC₅₀ was calculated from the graph of % inhibition versus concentration.

In vitro PG release studies

Release profile of PG was determined *in vitro* using a single medium dissolution condition in phosphate buffer (pH 5.5) with a modified paddle method [17]. The spectrophotometric method was adopted to determine the PG amount released from 100 mg PG-creams (PG-C1, PG-C2, and PG-C3) containing 100, 200 and 300 μ g PG as compared with their corresponding physical mixtures. The release studies were

performed in 100 mL of phosphate buffer at 37 °C using a digital dissolution apparatus (Advanced Technology TMAT Dissolution Test Station, Thai Mahal, Thailand) with eight (8) paddles at 100 rpm. A 5 mL sample aliquot was taken manually from the dissolution mixture at 30 min time intervals for 4 h and replaced with fresh buffer. The amount of PG contained in the aliquot sample was determined using UV-VIS spectrophotometer (ABL&E-Jasco, Hungary) at 552 nm. The percentage cumulative release of PG was calculated and recorded using a standard curve. Each experiment was performed in triplicate.

Skin irritation test in Wistar rats

A total of twenty-five (25) Wistar rats (100 to 190 g) were used for the *in vitro* skin irritation studies with five (5) animals each of five (5) different groups, which were treated as follows: Group A (PG), Group B (PG-C1), Group C (PG-C2), Group D (PG-C3) and Group E (Blank-C). Animals were allowed to acclimatize in the Animal House for 72 hours, with free access to feed and water. The study protocols were approved by the India Animal Ethical Committee (SSDJ/IAEC/20-21/01). Safety of the formulation was conducted according to an established protocol [18] at the Animal Facility, SNJB'S College of Pharmacy, Chandwad, Nashik, India. A portion of the animal hairs was removed from the flanks and back (4 to 6 cm dorso-caudal area) of the animals with razor blades and the areas were covered with moist cotton wool. The shaved parts were cleaned with 70 % alcohol and after that, the animals were kept and allowed access to feed and water for 24 h to ensure stabilization of striated skin tissues before the experiment proper. Subsequently, 0.5 g of PG cream samples were applied on the shaved site as follows: on the side, both proximal (abraded site) 0.50 g each of both the formulated Blank-C (cream base) and sunscreen cream formulations containing PG of 0.5 mg (PG-C1), 1.0 mg (PG-C2) and 1.5 mg (PG-C3) and 0.5 mg (PG only) was applied and the left side distal (non-abraded site) was kept as a control for the control group. After treatment, the four areas were covered with paraffin and cotton gauze with adhesive tape. The animals were immobilized in a restraining device for 4 h. After 4 h, paraffin and adhesive tapes were removed; the shaved area was cleaned using cotton swabs moist with 70 % ethanol and allowed to dry for 3 – 5 min. All study areas were examined for eschar (sloughing), erythema (redness), and edema, and this was done daily at all the sites for the next 14 days. The skin irritation score of the abraded and intact sites was observed and recorded accordingly.

Occlusivity test

The occlusivity of the formulations was determined *in vitro* following the standard method [19]. Occlusivity of PG creams was studied by determining the percent water loss. A 50 g distilled water was placed in different beakers of 100 mL capacity and covered with a Whatman filter paper (9.0 cm). The cream formulations were used to cover the surface of the filter paper in order to prevent and estimate the water loss. The beakers containing sample preparations were kept at $30 \pm 2^\circ\text{C}/60 \pm 5\%$ relative humidity (RH) for 48 h. All the formulations were evaluated in triplicate, keeping all the conditions constant. The same study was also done using blank cream. Percent water loss was calculated with reference to the control using Eq 3.

$$F = ((A-B)/B) 100 \dots\dots\dots (3)$$

Where A = Water flux through uncovered filter (percentage water loss), B = Water flux through filter when covered by test preparation (water loss).

In vitro laser obscuration evaluations

The percentage of cream obscurations was determined *in vitro* using a Laser Spraytech NSS (Malvern instrument, UK) following the standard protocol with a little modification [20]. The flow cell embedded centrally with glass was first mounted on the stage between the laser aperture (light source) and laser detector, and the system was calibrated to zero. Using a flexible applicator stick, 1.0 mg of the PG-C1 was applied on the flow cell glass surface and the percentage obscuration was determined and recorded. Similar procedure was done on other samples, including pure PG and blank cream. The analysis was repeated three times for the entire sample and their mean values as well as standard deviations were determined. The mechanism is illustrated in Figure 1.

Melanin content (MC), stratum corneum (SC) and transepidermal water loss (TWEL)

For the determination of MC, SC and TWEL effects of PG creams, oral consent was obtained from ten (10) participants between the ages of 25 – 45 years. One gram (1 g) of the formulation was used for each procedure [21]. Thereafter, measurements were carried out before and 30 min after application.

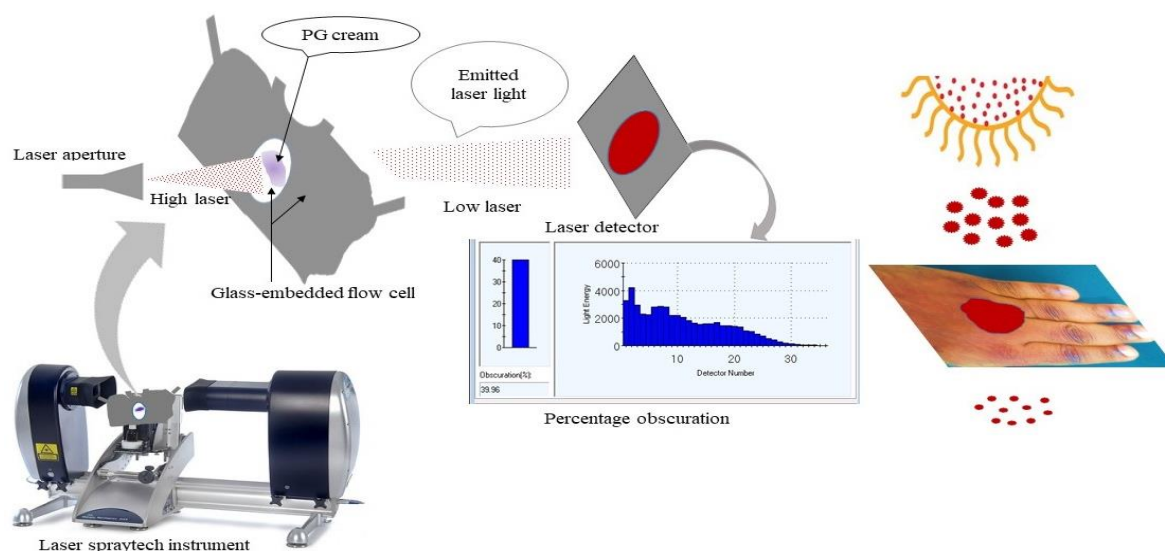


Figure 1: Mechanism of laser obscuration as a function of PG cream skin protection

The *stratum corneum* water content and accumulated water by the epidermis were determined using Corneometer® CM 852 (Courage and Khazaka Electronic Co., Ltd., Köln, Germany). Similarly, the amount of water lost from the skin (TWEL) was determined using Tewameter® TM 300 (Courage and Khazaka Electronic Co., Ltd., Germany). The extrinsic skin elasticity was measured using Cutometer® MAP 580 (Courage and Khazaka Electronic Co., Ltd., Germany) while melanin index, indicating the skin redness and pigment amount, was determined using Mexameter® MX 18 (Courage and Khazaka Electronic Co., Ltd., Germany).

Spectrophotometric evaluation of sunscreen protective factor (SPF)

The sun protection factor of the creams was evaluated *in vitro* using the Spectrophotometric method (Optometrics Corporation, USA) according to an earlier procedure [22]. A 1.0 g each of the creams was weighed into a 100 mL volumetric flask containing 50 mL aqueous ethanol (70 %). The dispersion was subjected to ultrasonication for 5 min and then filtered through Whatman filter paper (number 1). Subsequently, 5.0 mL of sample aliquot was transferred into a 50 mL conical flask and diluted with ethanol. The sample was further diluted by taking 5.0 mL aliquot into a 25 mL volumetric flask and then made to the mark with ethanol. Different spectra absorptions of each sample were obtained in the range of 290 to 550 nm using ethanol as a blank solvent. Data were obtained at every 10 nm, in triplicate. The SPF was calculated using the Mansur formula in Eq 4.

$$\text{SPF}_{\text{spectrophotometric}} = CF * \sum_{290}^{320} EE(\lambda) * I(\lambda) * Abs(\lambda) \quad \dots\dots\dots (4)$$

Where: EE = (I) – erythemal effect spectrum; I = (I) – solar intensity spectrum; Abs = (I)- absorbance of sunscreen product; CF = correction factor (= 10).

Data analysis

All *in vitro* data were analyzed using SPSS software (version 23) by one-way analysis of variance (ANOVA) and all Figures were plotted using GraphPad Prism software (version 8.0) and OriginPro (version 8.5). Values of $p < 0.05$ were statistically significant.

RESULTS

Physicochemical properties of creams containing PG

Table 2 shows the physicochemical properties and bioloards of the formulated PG cream at different concentrations of PG. The formulations were pinkish according to the concentration of PG incorporated, while the blank was whitish. Also, the topical preparation was creamy in texture with an average pH of 6.6. Similarly, the results in Table 2 show the spreadability properties as a resultant effect of cream viscosity, where the time and distance are critical factors in the spread of the product. The PG cream is a shear-thinning system. The batch formulated with 200 mg% (PG-C2) had a spreadability of 31.73 g.cm/sec, while the blank cream was 34.37 g.cm/sec without drug. Similarly, the batch formulated with 300 mg%

(PG-C3) had the highest spreadability of 97.36 g.cm/sec, which is significantly higher than the blank ($p > 0.5$). Furthermore, occlusivity tests were conducted to ascertain the skin safety and measurement of epidermal water loss and to avoid occlusion, which ensures skin protection. The results show an average occlusivity profile of 12.47 % and 15.6 % at 6 h and 24 h, respectively.

Scanning electron microscopy and transmission electron microscopy of PG creams

The SEM and TEM micrographs of both PG-cream, placebo-cream and PG are presented in

Figure 2. The SEM revealed the internal structure of the drug-excipient interaction, which formed a homogenous mixture of the product without cracks. Also, PG-C1, PG-C2 and PG-C3 show the spherical-shaped structure of lipid mixed with PG, while PG had a sticky structure and Blank-C had a burst irregular shape. Similarly, TEM showed a clear morphology of PG, PG formulation (PG-C) and Blank-C at 5 and 0.5 μm , respectively. Also, PG in Figure 2, when compared with the size and morphology of PG-cream, showed compatibility and miscibility of the compound and cream base. Furthermore, PG-C appeared light pinkish with a semi-solid and creamy texture as shown in Figure 2.

Table 2: Physicochemical properties of prodigiosin (PG) cream and blank cream

Sample codes	Physicochemical			Spreadability	Occlusivity (%)	
	Appearance		pH	(g.cm/sec)		
	Colour	Consistency			6 h	24 h
PG-C1	Light pink	Creamy	6.63	41.45	12.1	14.2
PG-C2	Light pink	Creamy	6.63	37.36	12.5	16.7
PG-C3	Light pink	Creamy	6.63	31.73	12.8	15.9
Blank-C	White	Creamy	6.84	34.37	10.0	13.2

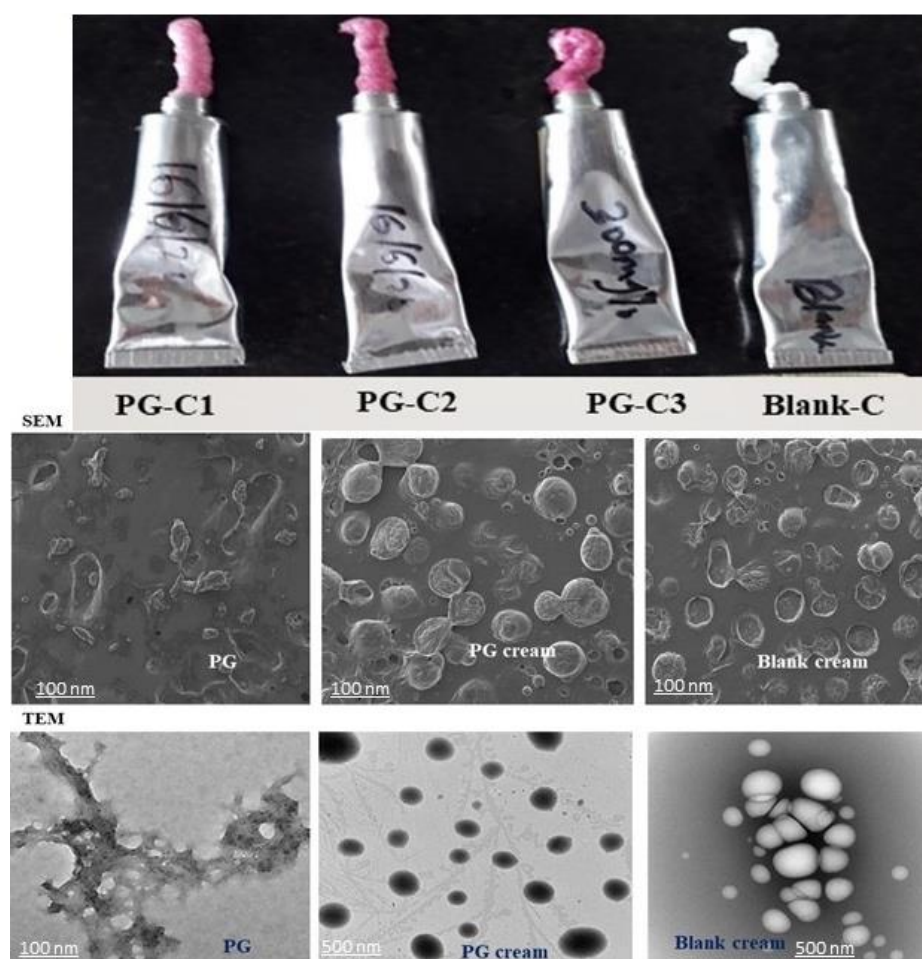


Figure 2: The physicochemical properties, SEM and TEM of PG-Sun protection cream formulations

Viscosity

Viscosities of the formulated PG-cream (sunscreen) and blank cream base were evaluated to ascertain consistency of the product, which is one of the critical factors in topical formulations. The result of viscosity tests in Figure 3 shows that, while the rate of revolution per minute increases, the viscosity decreases, which was observed at 5, 10, 20 and 50 rpm for both PG formulations and base-control. The sample batch, formulated with 100 and 200 mg% of PG concentration maintained consistent viscosity ranging from 1962.33 ± 36.69 to 4912 ± 60.39 Pa.s, with a significantly better performance when compared with some marketed cream (positive control), which showed an average viscosity of 4296 ± 49.96 Pa.s. However, the formulation with higher concentration of 300 mg% (PG-C3) showed 4347.33 ± 51.08 Pa.s at 5 and 10 rpm, suggesting evidence of good viscosity property.

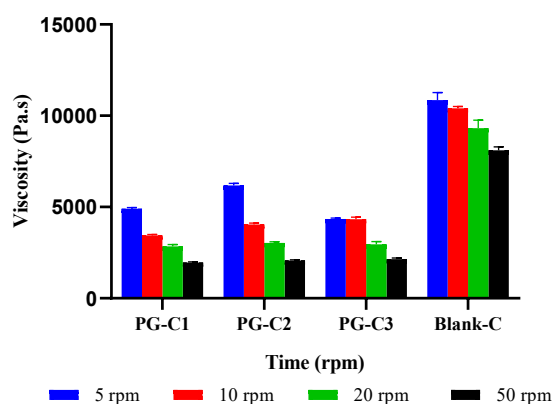


Figure 3: Viscosity of PG-C1, PG-C2, PG-C3 and Blank-C within 5 – 50 rpm

Antioxidant activities

Percentage DPPH scavenging antioxidant activities showed a better performance such that PG had up to 67.8 ± 3.19 % at $80 \mu\text{g/mL}$ as the maximum concentration used, while the formulations (PG-C1, C2, C3) were 71.4 ± 0.06 , 75.4 ± 0.96 , 75.7 ± 1.10 , respectively, and ascorbic acid as control was 64.4 ± 0.79 at $70 \mu\text{g/mL}$ concentration. The IC_{50} results, when evaluated, showed PG to be $28.74 \pm 0.89 \mu\text{g/mL}$, while PG-C1, PG-C2 and PG-C3 showed 12.22 ± 0.79 , 24.17 ± 0.49 and $27.60 \pm 1.91 \mu\text{g/mL}$, respectively. Similarly, the result of ABTS scavenging activity showed that PG at $80 \mu\text{g/mL}$ expressed 72.5 ± 1.24 % free radical ABTS scavenging for antioxidant activity, while the cream formulations (PG-C1, PG-C2 and PG-C3) were 43.4 ± 1.59 , 52.3 ± 2.13 and 36.8 ± 3.28 %, respectively.

The IC_{50} in Figure 4 shows the results of PG and formulations with PG ($11.9 \pm 0.75 \mu\text{g/mL}$), PG-C1 ($25.2 \pm 1.01 \mu\text{g/mL}$), PG-C2 ($25.2 \pm 0.94 \mu\text{g/mL}$) and PG-C3 ($21.6 \pm 0.84 \mu\text{g/mL}$), which were significantly ($p > 0.05$) lower than the IC_{50} ($43.89 \pm 1.41 \mu\text{M}$) of ascorbic acid.

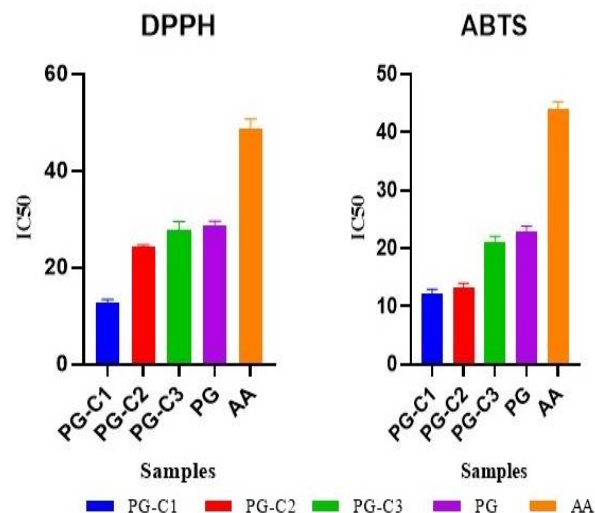


Figure 4: IC_{50} of PG and PG cream formulations by DPPH and ABTS methods. AA: Ascorbic acid

Prodigiosin release from cream base

The result of release studies in Figure 5 showed the percentage cumulative *in vitro* release of the PG from cream at time intervals in phosphate buffer (pH 5.5). Considering the release profile, the formulated PG-cream followed zero order to release maximally within 3 – 4 h. However, the cream loaded with 0.3 % (PG-C3) of the PG showed the highest percentage release of 10.22 % at 4 h while that of 0.1 % (PG-C1) and 0.2 % (PG-C2) showed the lower percentage release of 2.16 % and 8.10 %, respectively.

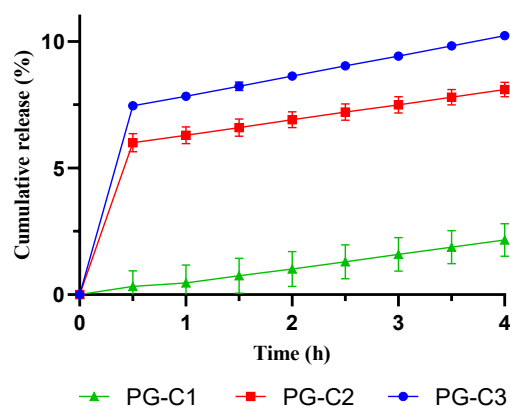


Figure 5: The PG released from the PG formulations (PG-C1, PG-C2, PG-C3; mean $\pm$ sd, $n = 3$)

Skin irritation test

The *in vitro* skin irritation test was conducted to ascertain skin safety. The results in Figure 6 show that after 14 days of the study, the formulation was safe without any irritation on the skin of the animals.

In vitro laser obscuration evaluations

The percentage obscuration and transmittance by the cream formulations and PG are shown in Figure 7 and Figure 8, respectively. While PG-C3 showed a significant increase in percentage obscuration ($43.04 \pm 3.36 \%$), PG-C1 ($26.67 \pm$

1.45%) and PG-C2 ($30.70 \pm 1.25 \%$) showed the lower percentage obscuration, respectively. Also, the pure PG exhibited a remarkable percentage of obscuration of $53.97 \pm 1.64 \%$.

Transepidermal water loss, melanin contents and sun protection factor

The results of melanin content, stratum corneum water content, percentage trans-epidermal water loss (TEWL) and SPF are shown in Figure 9. During exposure, melanin levels was 245.33 (77.8 %) in PG-C3 and average melanin content of 322.67 for the formulations (PG-C1 and PG-C2) and control blank of 319.83 at 0.5 h.



Figure 6: Skin irritation studies for 24 h – 14 days on Wistar rats (A) group treated with 0.1 % (PG-C1), (B) group treated with 0.3 % (PG-C3), and (C) group treated with cream base (Blank-C), respectively. There was no skin reaction except some minor wounds caused by shaving off hairs from the skin

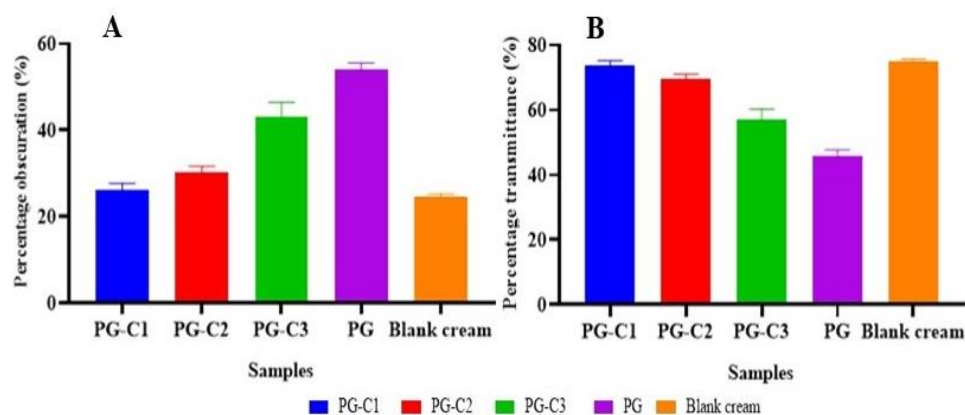


Figure 7: The percentage of laser obscuration (A) and transmittance (B)

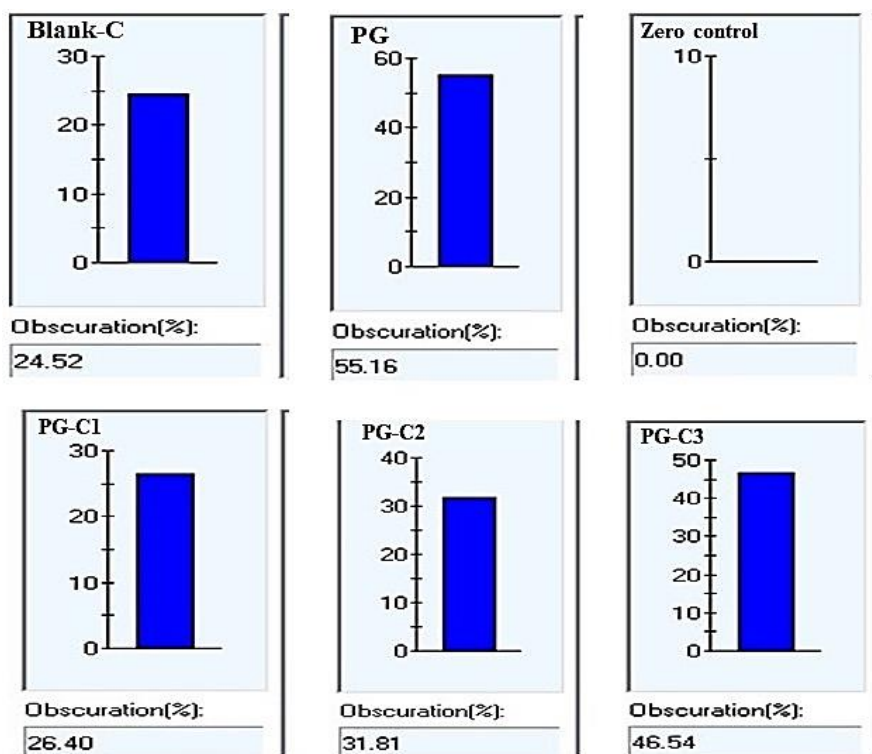


Figure 8: Percentage (%) laser obscuration histograms

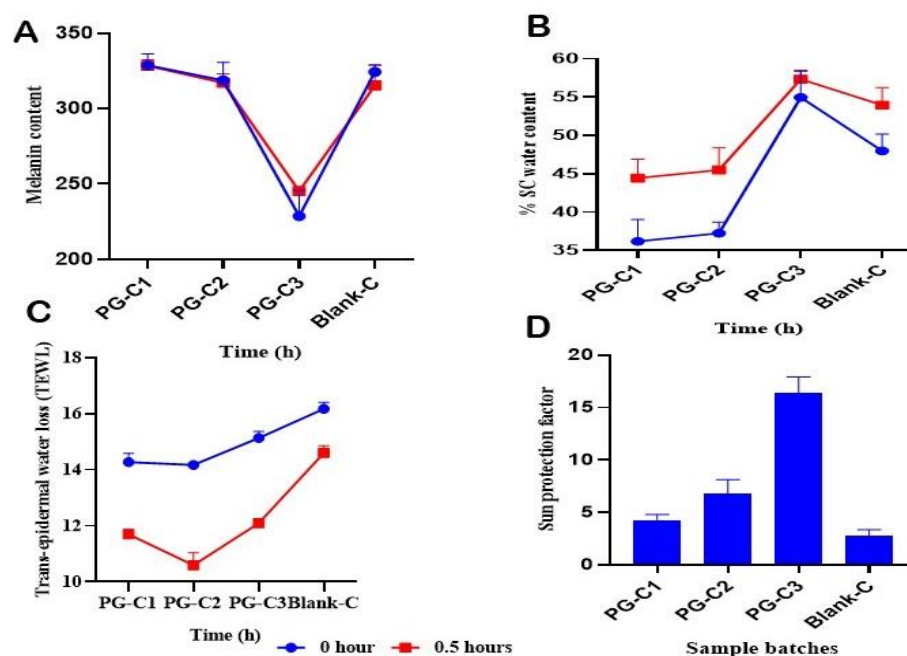


Figure 9: The melanin content (A), stratum corneum water content (B), percentage trans-epidermal water loss (TEWL; C) and sun protection factor (D) of PG, PG creams and ascorbic acid (AA)

Similarly, SC water content increased as the percentage increased up to 57.34 % (PG-C3) after 0.5 h and 54.96 % at 0 h, while TEWL were 15.13 and 12.1 % for PG-C3 at 0 and 0.5 h, respectively. Also, PG-C1 and PG-C2 had average TEWL of 14.22 and 11.15 % at 0 and 0.5 h. The result of SPF showed 16.4 ± 1.53

(PG-C3) and 6.80 ± 1.30 (PG-C2), while SPF of blank cream base (Blank-C) was not considered due to the spectrophotometric method adopted in the study, which only showed the absorbance of PG loaded in the cream at 552 nm used in calculating the SPF of the PG formulations.

DISCUSSION

The sample formulations with different PG concentrations are consistent in their aesthetic appeals, such as texture and colour (light pink) with a significant moisturizing value, which are characteristics of a good cream for topical use [23]. The pH results when compared with the normal skin pH of 5 – 5.9, were within an acceptable range that could not cause skin irritation or skin brunt upon application, as observed by some topical formulations. There is not much variation in the viscosity of creams formulated with different concentrations of PG. Prodigiosin cream with a lower concentration had better spreadability when compared with the blank cream (Blank-C). This pH and spreadability behaviour are caused by the intermolecular forces between the lipid components and pyrrole carboxylic acid contained in the PG at a lower concentration [24].

The cream formulated with PG shows evidence of a homogenous mixture of the cream base and PG drug when compared with the structural formation of the placebo cream. Similarly, TEM micrograph revealed morphological differences and sizes between PG, PG-cream (PG-Cs) and cream base (Blank-C). However, TEM showed that when PG was loaded in the cream, the droplet sizes were larger with a denser image, which shows that PG was properly incorporated in the cream base.

In addition, the scavenging effect increased with increasing concentrations of PG in creams for both DPPH and ABTS. Prodigiosin has been reported to exhibit good antioxidant activity as well as strong scavenger of DPPH [3]. Free radical scavenging activity of the PG compounds is concentration-dependent, as the concentration of the test compounds increased, the radical scavenging activity increased and lower IC₅₀ value reflects better protective action against oxidative stress. The results showed that PG and its cream formulations were able to reduce the stable free radical DPPH to the pale-yellow colored diphenylpicrylhydrazine, indicating free radical scavenging activity when compared with ascorbic acid (standard), a stronger antioxidant.

The cumulative release followed the zero-order kinetics for PG-C1, which slowly and increasingly released maximally at 3.5 h with a very low percent within the study period, while PG-C2 and PG-C3 had burst release effects. All three formulations slowly released PG from the cream base. This could be indicative of PG retention in the cream base without releasing its full content on the skin surface, which is an expected benefit

of sunscreen products. Therefore, this result suggests that PG from the PG cream remains in the cream base without much deposition on the skin, subsequently exhibiting a sun protective effect on the skin without toxicity. Also, the low percentage release of PG from the cream could be considered a good topical product intended for sunscreen. This property has been reported mostly for skin protection from environmental or climatic changes against skin cells [25]. In addition, the skin irritation of the PG and its formulated creams exhibited a safety profile on the skin during and after application on the experimental animals used. Therefore, it is an indication of the chemical and biocompatibility of both the formulation materials and PG in combination as a topical agent for possible protection of the skin, without any form of irritation. Such safety and qualitative attributes are characteristics of most topical formulations designed for skin protection.

The PG formulations showed minimal water loss, which suggests better occlusivity. This characteristic further enhances the hydration status of the skin, which can aid the retention of the formulations on the skin. The water loss from the various formulations was compared with the control whose value was considerable. The PG cream showed a significant decrease in the percent epidermal water loss after 24 h when compared with the blank cream. Subsequently, the epidermal water loss results in improved hydration status of the skin, which enhances drug penetration into deeper skin tissues.

The melanin content was not affected when exposed to PG-C1 and PG-C2 applications compared with the blank control. This effect is in line with the epidemiological data which supports the photoprotective role of melanin, considering an existing inverse correlation between skin pigmentation and the incidence of sun-induced skin cancers [26]. However, the same report suggests that melanin plays a serious role in photoprotection of skin and not merely as a sunscreen. The SPF shows how well sunscreen can protect skin against ultraviolet B (UVB) rays. The SPF results showed that PG creams could protect the skin significantly when the PG concentration increased. Research has shown that UVB rays are usually the cause of skin sunburns and also contribute to or cause skin cancer through ultraviolet A (UVA) rays [13]. Similarly, the laser radiation was diffracted and obscured by the activity of PG and PG cream formulations. The percentage obscurations of the PG and PG-C formulations were extrapolated through the light transmittance across the samples. This percentage obscuration effect is

an indication of the cream's potential for skin protection against both laser and UV radiation, which has been reported as part of the factors that cause skin aging [27].

CONCLUSION

Prodigiosin has been formulated into a topical sunscreen product that offers protection against laser and UV light for skin protection. The formulated cream's performance depends on concentration, pH, spreadability, and viscosity. These findings support the use of PG-creams as an option for sun protection for the skin.

DECLARATIONS

Acknowledgement/Funding

The authors acknowledge the SNJB's College of Pharmacy, Chandwad, India and DDSEC, Prince of Songkla University, Thailand, for providing us with the enabling environment and facilities to carry out the study.

Conflict of interest

No conflict of interest is associated with this work

Authors' contribution

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. GTH secured the fund, carried out the research and drafted the manuscript. AAA, EIC and GDB conceptualized and supervised the work; SS and TS provided reagents and analyzed the data.

REFERENCES

1. Sajjad W, Ahmad S, Aziz I, Azam SS, Hasan F, Shah AA. Antiproliferative, antioxidant and binding mechanism analysis of prodigiosin from newly isolated radio-resistant *Streptomyces* sp. strain WMA-LM31. *Mol Biol Rep* 2018; 45(6): 1787-1798.
2. D'Alessio R, Bargiotti A, Carlini O, Colotta F, Ferrari M, Gnocchi P, Isetta A, Mongelli N, Motta P, Rossi A, et al. Synthesis and immunosuppressive activity of novel prodigiosin derivatives. *J Med Chem* 2000; 43(13): 2557-2565.
3. Nguyen TH, Wang SL, Nguyen VB. Recent advances in eco-friendly and scaling-up bioproduction of prodigiosin and its potential applications in agriculture. *Agronomy* 2022; 12: DOI: 10.3390/agronomy12123099.
4. Silva LARd, Santos RAd, Mendonça RdS, Sá AVP, Andrade RFdS, Rodríguez DM, Campos-Takaki GM. Cost-effective production of stable prodigiosin by *Serratia marcescens* UCP 1549 and application in soap coloring. *Res Soc Dev* 2022; 11(4): e9711427078.
5. Lin SR, Chen YH, Tseng FJ, Weng CF. The production and bioactivity of prodigiosin: quo vadis? *Drug Discov Today* 2020; 25(5): 828-836.
6. Borić M, Danevčič T, Stopar D. Prodigiosin from *Vibrio* sp. DSM 14379; a new UV-protective pigment. *Microb Ecol* 2011; 62(3): 528-36.
7. Darshan N, Manonmani HK. Prodigiosin and its potential applications. *J Food Sci Technol* 2015; 52(9): 5393-5407.
8. Nguyen SLT, Nguyen TC, Do TT, Vu TL, Nguyen TT, Do TT, Nguyen THT, Le TH, Trinh DK, Nguyen TAT. Study on the anticancer activity of prodigiosin from variants of *Serratia marcescens* QBN VTCC 910026. *Biomed Res Int* 2022; 2022: 4053074.
9. Solano F. Photoprotection and Skin Pigmentation: Melanin-Related Molecules and Some Other New Agents Obtained from Natural Sources. *Molecules* 2020; 25: DOI: 10.3390/molecules25071537.
10. Bhagwat A, Padalia U. Optimization of prodigiosin biosynthesis by *Serratia marcescens* using unconventional bioresources. *J Genet Eng Biotechnol* 2020; 18(1): 26.
11. Kurkin EI, Chertykovtseva VO, Zakhvatkin YV. Processing a Brookfield Rotational Viscometer Measurement Results in MATLAB. *Key Eng Mat*; 2020; 834: 82-89.
12. Aiyalu R, Govindarajan A, Ramasamy A. Formulation and evaluation of topical herbal gel for the treatment of arthritis in animal model. *Braz J Pharm Sci* 2016; 52(3): 493-507.
13. Jain A, Gautam SP, Gupta Y, Khambete H, Jain S. Development and characterization of etoconazole emulgel for topical drug delivery. *Der Pharmacia Sinica* 2010; 1(3): 221-231.
14. Arora D, Khurana B, Nanda S. DoE directed optimization, development and evaluation of resveratrol loaded ultradeformable vesicular cream for topical antioxidant benefits. *Drug Dev Ind Pharm* 2020; 46(2): 227-235.
15. Hussien EM, Endalew SA. In vitro antioxidant and free-radical scavenging activities of polar leaf extracts of *Vernonia amygdalina*. *BMC Compl Med Ther* 2023; 23(1): 146.
16. Aadesariya MK, Ram VR, Dave PN. Evaluation of antioxidant activities by use of various extracts from *Abutilon pannosum* and *Grewia tenax* leaves in the Kachchh region. *MOJFPT* 2017; 17(1): 359.
17. Alagusundaram M, Jain NK, Begum MY, Parameswari SA, Nelson VK, Bayan MF, Chandrasekaran B. Development and characterization of gel-based buccoadhesive bilayer formulation of nifedipine. *Gels* 2023; 9(9): 688.
18. Merdana IM, Arjana AAG, Widyastuti SK, Tetrania T, Budiasa K, Sudimartini LM, Sudira IW. Assessment of the dermal acute irritation potential of natural veterinary

- medicine minyak rajas in albino rabbits. *J Pharm Res Int* 2020; 32(10): 17-24.
19. Kenechukwu FC, Attama AA, Ibezim EC, Nnamani PO, Umeyor CE, Uronnachi EM, Gugu TH, Momoh MA, Ofokansi KC, Akpa PA. Surface-modified mucoadhesive microgels as a controlled release system for miconazole nitrate to improve localized treatment of vulvovaginal candidiasis. *Eur J Pharm Sci* 2018; 111: 358-375.
 20. Dayal P, Shaik MS, Singh M. Evaluation of different parameters that affect droplet-size distribution from nasal sprays using the Malvern Spraytec. *J Pharm Sci* 2004; 93(7): 1725-1742.
 21. Chirikhina E, Chirikhin A, Xiao P, Dewsbury-Ennis S, Bianconi F. In vivo assessment of water content, trans-epidermal water loss and thickness in human facial skin. *Appl Sci* 2020; 10(17): 6139.
 22. Dutra EA, Oliveira DAGdCe, Kedor-Hackmann ÉRM, Santoro MIJRBDCCDS, Determination of sun protection factor (SPF) of sunscreens by ultraviolet spectrophotometry. *Braz J Pharm Sci* 2004; 40 (3):
 23. Chauhan L, Gupta S. Creams: A review on classification, preparation methods, evaluation and their applications. *J Drug Deliv Ther* 2020; 10: 281-289.
 24. Grumetto L, Carpentiero C, Di Vaio P, Frecentese F, Barbato F. Lipophilic and polar interaction forces between acidic drugs and membrane phospholipids encoded in IAM-HPLC indexes: their role in membrane partition and relationships with BBB permeation data. *J Pharm Biomed Anal* 2013; 75: 165-172.
 25. Raina N, Rani R, Thakur VK, Gupta M. New insights in topical drug delivery for skin disorders: from a nanotechnological perspective. *ACS Omega* 2023; 8(22): 19145-19167.
 26. Brenner M, Hearing VJ. The protective role of melanin against UV damage in human skin. *Photochem Photobiol* 2008; 84(3): 539-549.
 27. Amaro-Ortiz A, Yan B, D'Orazio JA. Ultraviolet radiation, aging and the skin: prevention of damage by topical cAMP manipulation. *Molecules* 2014; 19(5): 6202-6219.