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Garbage to Beauty: Development and In-Vitro Evaluation of Antioxidant Rich Microsponge Sunscreen Lotion from Fish Scale and Mango Kernels

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ABSTRACT

This study aims to develop and assess a herbal sunscreen formulation for skin protection against sun damage, utilizing ingredients from plant extracts such as Mangifera indica (mango seed kernels) and animal extract from Sardinella longiceps (pearl essence extracted from fish scales). Mango seed kernels, known for their high stability and antioxidant activity, offer a valuable commodity by repurposing waste. The pearl essence, derived from fish scales, contains guanine, an iridescent substance found in the epidermal layer and scales.

The herbal sunscreen formulation incorporates fixed oils, medicinal plants, and animal materials. Mango seed kernels are extracted using a soxhlet apparatus, while pearl essence is obtained through a solvent diffusion method. Regular use of sunscreen has been shown to reduce the development of actinic keratosis, squamous cell carcinoma, and melanoma by absorbing or reflecting the sun's ultraviolet radiation, protecting the skin. The escalating incidence of skin cancers and the detrimental effects of ultraviolet radiation have amplified the use of sunscreening agents, demonstrating their efficacy in mitigating symptoms.

The prepared sunscreen, enriched with mango kernel extract and pearl essence, as well as curcumin microsponge, offers a multifaceted sunblocking action due to high antioxidant activity and imparts a shimmering effect on the skin. The inclusion of curcumin microsponge aids in the penetration of the lotion into the skin, ensuring a comprehensive effect. Evaluation of the sunscreen lotion encompassed parameters such as pH, viscosity, spreadability, and stability, confirming the safety of the herbal sunscreen for skin use.

Key words: *Herbal sunscreen, Mango kernal, Pearl essence, Microsponge drug delivery system, Antioxidant activity.*

INTRODUCTION

Sunscreen has become an essential element of skincare routines, providing protection against ultraviolet (UV) radiation. The initial experiments on UV radiation's harmful effects on the skin led to the invention of the first sunscreen in 1928. Over the last two decades, the sunscreen market has expanded significantly, incorporating various ingredients and technologies to offer products claiming to

be broad-spectrum, water-resistant, non-comedogenic, paraben-free, fragrance-free, and mineral-enriched, with diverse sun protection factor (SPF) ranges and UVA star ratings.

From traditional ingredients like jasmine, rice, and olive oil to modern formulations with a mix of chemical and mineral components, sunscreen has evolved with increased awareness about sun protection to prevent sunburns, skin cancers, and premature aging.

Present-day parents exhibit improved attitudes toward photoprotection for their children, employing hats, sunglasses, protective clothing, and high-protection sunscreen systematically. The FDA recommends avoiding sunscreen use in infants under 6 months and keeping them away from direct sunlight. For older children, the FDA suggests using mineral-based sunscreens to avoid irritation from chemical sunscreens.

In recent discoveries, dermatologists must recommend suitable sunscreens for their patients, emphasizing additional sun protection measures, such as seeking shade during peak sunlight hours, wearing protective clothing, and using sunglasses, as no sunscreen provides 100% protection.

Sun protection factor (SPF) on sunscreen labels measures protection against ultraviolet B (UVB) rays, the main cause of sunburns. However, SPF does not indicate a sunscreen's ability to block ultraviolet A (UVA) rays, which contribute to skin cancer. Higher SPF numbers offer greater protection from UVB rays, but no sunscreen can block all UVB rays. For instance, SPF 30 sunscreen blocks about 97% of UVB rays, while SPF 50 blocks about 98%.

Sunscreen, also known as sunblock or sun cream, protects the skin from harmful rays by reflecting, absorbing, and scattering both UVA and UVB radiation. It comes in various forms such as lotions, sprays, gels, foams, powders, and other topical products, often complementing other photoprotective measures like sunglasses and sunhats.

Benefits of sunscreen include reducing the risk of skin cancer, protecting against sunburn, preventing inflammation and redness, avoiding early onset of wrinkles and fine lines, preventing blotchy skin and hyperpigmentation, and stopping DNA damage induced by sunlight.

Advantages of wearing sunscreen include reducing the risk of skin cancer, protecting against sunburn and premature aging. However, disadvantages may include potential side effects such as acne, burning, itching, or stinging of the skin.

Application of sunscreen is crucial for protecting the skin from UV radiation, with regular use helping prevent sunburn, skin cancer, and premature aging. Microsponges, a patented polymeric delivery system, offer controlled release of active ingredients in sunscreens, reducing oiliness and shine from the skin.

Microsponges, tiny spheres with porous structures, entrap various active ingredients like sunscreens, emollients, and anti-inflammatory agents. Their benefits include controlled release, stability over a wide pH range, resistance to high temperatures, and a relatively high payload. However, disadvantages include product entrapment of unreacted monomers, non-uniform structure, and the need for a two-step method for thermosensitive drugs.

Applications of microsponges in sunscreens provide long-lasting efficacy with improved protection against sunburn and related injuries. They also benefit anti-acne formulations by maintaining efficacy with decreased skin irritation, anti-inflammatory formulations with long-lasting activity and reduced skin allergies, anti-fungal formulations with sustained release of activities, and anti-pruritic formulations with extended and improved activity.

Mango kernel oil, extracted from the stone of mango fruit, is known to produce edible oil and is rich in bioactive compounds and antioxidants. It protects the skin from UV damage, making it a potential ingredient in sunscreens.

METHODOLOGY

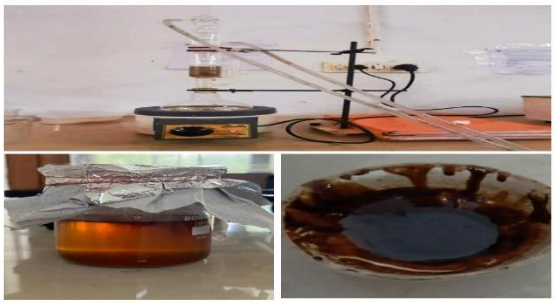
The mango seed were initially washed using a running tap water to remove the dirt or dust and are dried under shade for 20 to 25 days at 30°C during summer and the size reduced by sevieing in 120 mesh size. The Powder sample was weighed and stored in an airtight container and extraction commenced.

The seeds were dried under shade for 4 weeks to make it suitable for grinding and also to avoid the degeneration of active constituents. Grind the seeds into powder and put in an airtight container and perform extraction using soxhlet apparatus.

Extraction of Mangifera indica kernel oil by Soxhlet apparatus

The extraction of mango seed oil utilized a Soxhlet extractor with n-hexane as the solvent. A total of 300 ml of hexane was introduced into the round-bottom flask of the Soxhlet apparatus. Following this, 20g of crushed mango seed was placed in the thimble, which was then inserted into the Soxhlet extractor. The apparatus was assembled, and the solvent was heated, causing vapor that was subsequently condensed by circulating water in the condenser.

This cycle of heating and cooling persisted until a sufficient quantity of mango seed kernels was obtained. Upon completion of the extraction, the thimble was removed, and any remaining solvent was recharged into the round-bottom flask. Finally, the setup was reassembled and heated again to recover the extract.



PEARL ESSENCE

Pearl essence is extracted from fish scales. Guanine is a substance that found in the epidermal layer and scales. The suspension of guanine in a solvent is called " essence of pearls". It can be used in skin make up for hiding blemishes. It provides a shimmering effect on cosmetics

Methodology

Guanine deposit on the fish scales is more removable as compared to that on epidermis. Freshly removed scales are collected and washed to removed adhering foreign matter. Scales can be preserved in 10-15% brine. The brine is later drained off and the scales are squeezed in muslin cloth bags and compressed. The compressed mass can be stored at 0˚C.

Pearl essence can be prepared as an aqueous or non-aqueous suspension. Washed scales are agitated with minimum quantity of water containing little ammonia in an agitator. The mixture is then passed through a strainer to remove the scales. The pearly substance present as a suspension in the liquor is purified by settling in a cool atmosphere.

PHYSICOCHEMICAL EVALUATION

Determination Of Extractive Values

This method determines the number of active constituents in each amount of plant material when extracted with the solvent. The extractive value used as a means of evaluating crude drug which are not readily estimated by other means.

% Extractive value =weight of extract obtained / weights of sample × 100

Determination Of Alcohol Soluble Extractive Value

Weigh about 5-gram powdered drug with 100 ml of alcohol in a stoppered flask for 24 hours and shaking for 6 hours. Filter rapidly through filter paper. Taking precaution against excessive loss of alcohol. Evaporate 25 ml of alcoholic extracted to dryness in a tared flat-bottomed dish (32). Dry at 800C and weigh. Keep it in a desiccator. Calculate the percentage w/w of alcohol soluble extractive with the reference to the air-dried drug.

Determination of Water-Soluble Extractive Value

Determination of water-soluble extractive value follows the procedure as above using chloroform. Water is used instead of alcohol.

Determination of Ash Value

Ash value is helpful in determining the quality and purity of a crude drug. On incineration crude drug normally leaves an ash usually consisting of carbonate, phosphate and silicate

of sodium, potassium, calcium and magnesium.

PROCEDURE

Weigh about 3 gram of the powdered drug in a tared silica crucible and incinerate the powdered drug by gradually increasing the temperature until free from carbon and cool it. Keep it in a desiccator (37). Weigh the ash and calculate the percentage of total ash with reference to the added dried sample.

Total ash = weight of ash /initial weight ×100

Determination Of Moisture Content

Their principle of determining the thermogravimetric method of moisture content is defined as the weight loss of mass that occurs when the material is heated. The sample weight is taken prior to heating and again after reaching a steady-state mass subsequent to drying (15). Presence of moisture content in a crude drug can lead to its deterioration due to the either activation of certain enzyme or growth of microbes. Moisture content can be determined by heating the drug at 60 0C in an oven to a constant weight and calculating the loss of weight.

Procedure

Place 10 gram of drug in a tared evaporating dish. For unpowered part the sample was prepared by cutting. Placing the drug in a tared evaporating dish dry at 105°C for 5 hour(7). Then weigh it and continue the drying and weighing at one hour interval until the difference between two successive weight corresponding to not more than 0.25%. constant weight is reached when two consecutive weight after drying for 30 minutes and cooling for 30 minutes in a desiccator.

CALCULATION

Weight of weighing bottle= W1
Weight of bottle + drug= W2
Weight of drug =W2-W1
Final weight of the drug =W4
Moisture content =W3 -W4/W3 ×100

Phytochemical screening

Phytoconstituents	Test	Observations
Alkaloids	Hager's Test : 2ml extract + few drops of hager's reagent	Yellow precipitate
Flavonoids	Ammonia test : Filter paper dip in alcoholic solution of drug was exposed to ammonia vapour	Formulation of yellow spots on filter paper
Carbohydrates	Molisch's Test : 2ml extract + 10ml water + 2ml drops ethanolic alpha naphthol (20%) + 2ml concentrared. sulphuric acid	Reddish violet ring at the junction
Glycosides	Liebermann's test : add 1 ml extract + 2ml chloroform acetic acid	Violet to blue to green color
Tannins	Braymers Test: 2ml extract+ 2ml water + 2 drops of ferric chloride (5%)	Green precipitate
Steroids	Salkowski Test: 2ml extract + 2ml chloroform + 2ml concentrated sulphuric acid	Reddish brown ring at the junction
Proteins	Ninhydrin Test : 1ml extract + 2ml ninhydrin reagent	Violet precipitate
Saponins	Foam Test : 5ml extract + 5ml water + heat	Froth appear
Phenols	Ferric chloride Test: extract was treated with 3-4 drops of ferric chloride.	Formation of bluish black colour

FORMULATION

Phase A: Oil in water (o/w) emulsion-based cream (semisolid formulation) was formulated. The emulsifier (stearic acid) and other oil soluble components (cetyl alcohol, almond oil) were dissolved in the oil phase (Phase A) and heated to 75 c.

Phase B: The preservatives and other water soluble components (methyl paraben, propyl paraben, triethanolamin, propylene glycol, all extract) were dissolved in the aqueous phase (Phase B) and heated to 75 c.

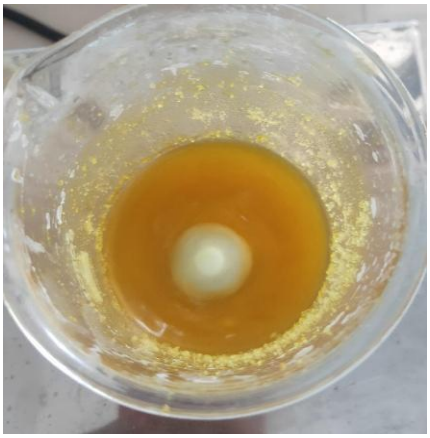
After heating, the aqueous phase was added in portions to the oil phase with continuous stirring until cooling of emulsifier took place.

Composition of sunscreen lotion formulation	F1 w/w%	F2 w/w%	F3 w/w%	F4 w/w%	F5 w/w%	F6 w/w%
Kernel Oil	0.5	1	1.5	2	2.5	3
Pearl Essence	0.5	1	1.5	2	2.5	3
Stearic Acid	8.5	8.5	8.5	8.5	8.5	8.5
Cetyl Alcohol	3.5	3.5	3.5	3.5	3.5	3.5
Starch	1.5	1.5	1.5	1.5	1.5	1.5
Sodium Lauryl Sulfate	1	1	1	1	1	1
Almond Oil	15	15	15	15	15	15
Glycerol	3	3	3	3	3	3
Methyl Paraben	0.2	0.2	0.2	0.2	0.2	0.2
Rose Oil	15	15	15	15	15	15
Triethanolamine	0.5	1	1.5	2	2.5	3

Formulation of curcumin microsponge

Curcumin microsponges were prepared using the quasi-emulsion solvent diffusion method. The internal phase was composed of ethyl cellulose (2% w/v) dissolved in dichloromethane. The drug (100–500 mg) was incrementally introduced into the ethyl cellulose solution with continuous stirring at 600 rpm. Subsequently, the internal phase was added dropwise into the aqueous external phase containing polyvinyl alcohol (0.5% w/v). After 2 hours of stirring, the microsponges were formed through the evaporation of dichloromethane from the system. The resulting microsponges were filtered, dried in a hot air oven at 40 °C until a constant weight was achieved, and then stored in an airtight container.

Formulation	Curcumin	Ethyl cellulose	Dichloro methane	Poly vinyl alcohol
M1	500	100	5	0.5
M2	500	100	5	0.5
M3	500	100	5	0.5
M4	500	100	5	0.5
M5	500	100	5	0.5



EVALUATION OF SUNSCREEN LOTION

pH of the Cream:

The pH meter was calibrated using standard buffer solution. About 0.5g of the cream was weighed and dissolved in 50.0 ml of distilled water and its pH was measured.

Viscosity:

Viscosity of the formulation was determined by Brookfield Viscometer at 100 rpm, using spindle no 7.

Dye test:

The scarlet red dye is mixed with the cream. Place a drop of the cream on a microscopic slide covers it with a cover slip, and examines it under a microscope. If the disperse globules

appear red the ground colourless. The cream is o/w type. The reverse condition occurs in w/o type cream i.e. the disperse globules appear colourless in the red ground.

Homogeneity:

The formulations were tested for the homogeneity by visual appearance and by touch.

Appearance:

The appearance of the cream was judged by its color, pearl scence and roughness and graded.

After feel:

Emolliency, slipperiness and amount of residue left after the application of fixed amount of cream was checked.

Type of smear:

After application of cream, the type of film or smear formed on the skin were checked.

Removal:

The ease of removal of the cream applied was examined by washing the applied part with tap water.

Acid value:

Take 10 gm of substance dissolved in accurately weighed, in 50 ml mixture of equal volume of alcohol and solvent ether, the flask was connected to reflux condenser and slowly heated, until sample was dissolved completely, to this 1 ml of phenolphthalein added and titrated with 0.1N NaOH, until faintly pink color appears after shaking for 30 seconds.

Acid value = n X 5.61/w

n = the number of ml of NaOH required, w = the weight of substance.

Saponification value:

Introduce about 2 gm of substance refluxed with 25 ml of 0.5 N alcoholic KOH for 30 minutes, to this 1 ml of phenolphthalein added and titrated immediately, with 0.5 N HCL.

Saponification value = (b-a) X 28.05/w

The volume in ml of titrant = a

The volume in ml of titrant =b

The weigh of substance in gm = w

Irritancy test:

Mark an area (1sq.cm) on the left hand dorsal surface. The cream was applied to the specified area and time was noted. Irritancy, erythema, edema, was checked if any for regular intervals up to 24 hrs and reported.

Antioxidant study:

A diverse array of antioxidants can be incorporated into sunscreens to fulfill various functions. These antioxidants play a crucial role in mitigating oxidative stress within the skin, thereby diminishing the signs of skin aging. Additionally, they prove beneficial in the treatment of certain UV-sensitive dermatoses, such as polymorphic light eruption, prurigo aestivalis, solar urticaria, and porphyria. In the context of photodermatoses, both oral and topical administration of antioxidants serves to neutralize free radical species, preventing and combating their assault on cellular structures. This underscores the significant role of antioxidants in UV-induced skin dermatoses.

The skin inherently possesses multiple antioxidant defense systems, encompassing both enzymatic (e.g., the glutathione peroxidase-reductase enzyme system and superoxide dismutase) and non-enzymatic components (e.g., vitamin C, vitamin E, glutathione, and coenzyme Q10). Topical application of antioxidants has the potential to enhance the intrinsic defense mechanisms of the skin. Furthermore, the delivery of antioxidants topically can augment the photoprotective function of UV filters. Numerous studies have demonstrated that certain topical antioxidants utilized in sunscreen formulations exhibit photoprotective properties, leading to a reduction in erythema, sunburn cell development, and immunosuppression.

Moreover, several organic UV filters employed in sunscreens have exhibited instability upon exposure to solar radiation, resulting in the generation of oxidized by-products. This instability heightens the phototoxicity of these compounds, subsequently diminishing their photoprotective efficacy. Cosmetic ingredients

endowed with antioxidant activity can contribute to the stability of UV filters, thereby reducing damage induced by free radicals. The quest for new antioxidant compounds to incorporate into cosmetic formulations remains ongoing, with nature being a preferred source. Botanical compounds, owing to their polyphenolic structures, have long been recognized as potent antioxidants. With continuous innovation in the cosmetic industry, multifunctional compounds are imperative for the development of new cosmetic products.

More recently, the marine environment has been extensively explored as a source of natural products with diverse biological activities, including anti-aging and antioxidant properties. This exploration has led to the integration of certain marine-derived products into cosmetic formulations. This work aims to provide readers with an up-to-date overview of the most commonly utilized antioxidants in commercial sunscreens, offering a comprehensive understanding of their mechanisms of action and their impact on photoprotective effectiveness.

EVALUATION RESULT

pH of the cream

The Ph of the whole herbal sunscreen lotion was found to be 6.95. The ideal pH range is in between 5-7.5, which was sufficient for the skin, suggesting that the herbal sunscreen lotion was suitable for the skin.

F1	F2	F3	F4	F5	F6
6.54	7.11	6.95	7.10	7.02	7.30

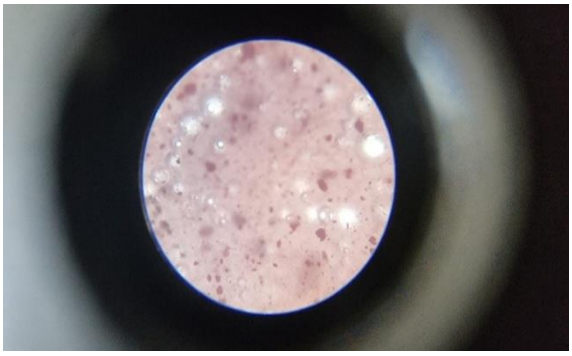
Viscosity

The viscosity measurement was performed and recorded using Brooke field viscometer. The viscosity was found to be 16640cps.

F1	F2	F3	F4	F5	F6
16032	15780	17202	16640	17856	15326

Dye test

The dye test confirms that the formulated cream was o/w type emulsion cream.



Homogeneity

By visual examination of the appearance and presence of any lumps, flocculates or aggregates, the produced herbal sunscreen lotion was checked for homogeneity. The homogeneity of prepared sunscreen lotion has been shown to be fine.

Appearance

The physical appearance, colour and feel of the prepared herbal sunscreen lotion are visually tested lotion formulation was light yellowish preparation with a smooth texture and with good consistency.

After feel

It is well absorbed so no slipperiness and no residue are left after the application of fixed amount of formulation.

Type of smear

After application of the cream, type of film formed is noted.

Removal

Removal of cream was done by washing the applied part with tap water.

Spreadability

The spreadability of semisolid preparations is commonly determined using the parallel plate method. In this study, a modified laboratory apparatus was employed for the assessment of spreadability. The experimental setup comprised two glass slides positioned on a tripod stand, with an excess of cream (3g) applied between them. The upper slide was movable, while the lower slide was securely fixed to the stand. Applying a 100 g weight for 5 minutes compressed the cream to a uniform thickness, and any excess cream at the edges was carefully removed. Subsequently, a 50 g weight was added to one side of the slide, and the slide was pulled to cover a distance of 10 cm. The time in seconds required for the separation of the two glass slides over this 10 cm distance served as the measure of spreadability. A shorter duration indicated better spreadability. Spreadability was calculated using the specified formula.

S=m.l/t

Where, S=Spreadability, m=Weight tied to upper glass slide, l=Length of glass slide, t=Time taken to separate them.

F1	F2	F3	F4	F5	F6
17.09	17.85	17.04	18.52	18.45	17.95

Acid Value

SL1	SL2	SL3	SL4	SL5	SL6
29.73	29.85	29.67	29.73	30.04	29.83

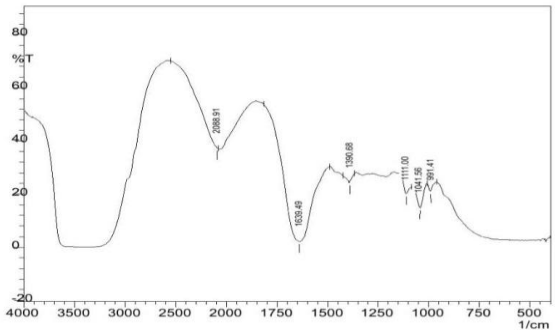
Saponification Value

SL1	SL2	SL3	SL4	SL5	SL6
37.86	37.75	37.69	38.04	38.12	37.79

Irritancy Test

The formulated cream exhibits no redness, edema, inflammation and irritation during irritancy studies.

FTIR



SPF STUDY

The effectiveness of sunscreen formulation was illustrated in terms of SPF. The SPF of different formulations was determined using UV spectrophotometric analysis. The SPF values of different formulations range between 9.388 to 19.878.The maximum SPF was observed in F1.

Wavelength (nm)	EE Employed	F1	F2	F3	F4
290	0.0150	0.018	0.080	0.061	0.073
295	0.0817	0.020	0.090	0.95	0.105
300	0.2874	0.310	0.160	0.120	0.136
305	0.3278	0.560	0.280	0.535	0.587
310	0.1864	1.680	0.395	0.624	0.739
315	0.837	1.730	0.535	0.734	0.843
320	0.0180	1.810	0.743	0.896	0.893

Absorbance of sunscreen formulation

F1	F2	F3	F4
19.878	9.388	10.041	10.654

SPF values of different formulations

ANTI OXIDANT STUDY

The DPPH free radical method is easy, fast and widely used for evaluation of the antioxidant potential of substances. It can be measured spectrophotometrically.

Calculated by using formula:

%Scavenging capacity of DPPH=A0-A1/A0*100

A0=Absorbance of blank , A1=Absorbance of sample

F1	F2	F3	F4	F5	F6
78.52	65.23	59.5	69.78	75.86	72.1

CONCLUSION

In conclusion, this study underscores the potential utilization of fish scale and mango kernel waste as eco-friendly ingredients in crafting antioxidant-rich microsponge sunscreen lotion. The in vitro findings reveal promising results, showcasing high SPF and UVAPF values, coupled with commendable antioxidant activity. While further investigations are necessary to assess the lotion's in vivo efficacy and safety, the study suggests its potential as a valuable addition to the sunscreen market.

The study constitutes a noteworthy contribution to the realm of sustainable cosmetics by demonstrating the practicality of repurposing waste materials for the creation of high-quality products with beneficial attributes. These findings not only have the potential to curtail the environmental impact of the cosmetics industry but also advocate for the development of more sustainable and eco-friendly alternatives.

Beyond environmental advantages, incorporating fish scale and mango kernel waste into sunscreen lotion holds economic promise. Currently underutilized, these materials could open up new markets and opportunities for businesses operating in the cosmetics industry. The formulation of this lotion marks a positive stride towards fostering a more sustainable and eco-friendlier cosmetics sector. With continued research, it may emerge as a valuable asset in the sunscreen market, contributing to a reduction in the environmental footprint of the cosmetics industry.

The future of sustainable cosmetics appears promising, as evidenced by this study. As

consumer awareness grows regarding the environmental repercussions of their choices, there is likely to be an increasing demand for sustainable cosmetics. This study serves as a guide for developing novel and innovative sustainable cosmetics products that cater to consumer needs while safeguarding the environment.

Declaration by Authors

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Conflict of Interest: The authors declare no conflict of interest.

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