Research Article

Formulation and Evaluation of Sunscreen Containing Extract of *Pithecellobium dulce*

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ABSTRACT

Introduction: In the realm of skincare, our research delves into the artistry of sun protection, unveiling a revolutionary chapter: the formulation and evolution of sunscreen enriched with the essence of Pithecellobium Dulce. This research narrative introduces a paradigm shift in sun care, sparking curiosity and innovation within the scientific community. We embark on a journey deep into the heart of Pithecellobium Dulce, extracting its essence with precision to create a sunscreen that transcends its traditional role.

Materials and Methods: Our sunscreen formulation stands as a testament to meticulous craftsmanship, weaving together cutting-edge science and ancient botanical wisdom. This paper meticulously outlines the formulation process, highlighting the incorporation of Pithecellobium Dulce extract. Through rigorous experimentation and analysis, we unravel the sunscreen's evolution- its texture, efficacy, and its ability to shield against harmful UV rays.

Results: Among six formulated batches, Batch Number 4 emerged as the optimal formulation, exhibiting superior stability, texture, and spread ability. With an SPF value of 24.51, Batch 4 demonstrated enhanced performance, making it the most effective and user-friendly sunscreen formulation.

Conclusion: This study successfully formulated and evaluated a Pithecellobium dulce-based sunscreen, with Batch 4 showing promising results (SPF 24.51). The extract's potential in developing effective, natural sunscreens was demonstrated. Further research is essential to optimize this approach, encouraging exploration in sunscreen technology for safe, effective, and natural products.

Keywords: Pithecellobium dulce, sunscreen, ultraviolet, UV.

INTRODUCTION

The radiant sun, while the source of life and warmth, also harbors a hidden peril - the relentless barrage of ultraviolet (UV) rays that can leave an indelible mark on our skin. In our quest to harness the power of nature to shield ourselves from this celestial adversary, we turn to the enchanting world of botanicals. Among these botanical treasures, Pithecellobium Dulce, an unassuming tropical plant with a rich heritage, beckons us with its promises of both protection and rejuvenation[1].

Addressing the Need for Sun Protection:

The sun, with its golden embrace, brings life to our world. Yet, its rays hold a paradoxical duality, nurturing life while also inflicting harm. Sunburn, premature aging, and the menacing Specter of skin cancers are the tolls extracted by excessive sun exposure. As we stand at the crossroads of nature and science, the incorporation of nature's defenses into our skincare arsenal gains profound relevance. Pithecellobium Dulce, with its botanical secrets, offers us a gateway to effective sun protection and potential skincare enchantments[2].

Pharmacognosy of Pithecellobium Dulce: Pithecellobium dulce, commonly known as Manila tamarind, is a tropical tree of the Fabaceae family with promising UV protection properties. Its pharmacognostic study reveals that various parts such as leaves, bark, seeds, and fruit contain chemical constituents beneficial for skin protection.

Figure 1: Image of Pithecellobium dulce and its leaf

The leaves, containing parenchyma cells, stomata, and trichomes, are particularly rich in flavonoids known for their antioxidant effects, which help protect the skin from UV-induced damage. The bark, with secondary xylem and phloem, fibres, and sclereids, also contributes to this protective activity. Notably, the plant's rich content of flavonoids, tannins, and terpenoids provides a potent combination of UV absorption and antioxidant properties, reducing oxidative stress caused by UV exposure[3]. Traditionally, extracts from Pithecellobium dulce have been used in poultices for skin conditions, reflecting its role in skin protection and healing. Current research focuses on isolating these bioactive compounds to develop effective natural sunscreens and skin care products, leveraging the plant's ability to protect against harmful UV radiation and maintain skin health[4,5].

Crafting Sunscreen: The Art and Science: As tradition meets innovation, this paper embarks on an exploration of the synergy between Pithecellobium Dulce and the art of sunscreen formulation. Our primary quest is twofold: to

offer effective sun protection and to explore the potential skincare wonders these formulations, enriched with quercetin, may unveil. This voyage commences with a comprehensive unveiling of the sunscreen production process, blending the meticulous science of photoprotection with the artistry of botanical extraction using ethanol as a solvent. Subsequent chapters delve into the sun-defying qualities of these formulations, their potential skincare enchantments, and the considerations for their widespread embrace[1].

In this research, we celebrate not only the botanical heritage of Pithecellobium Dulce but also endeavor to propel the dialogue on the harmonious integration of natural remedies into the modern canvas of skincare choices. Through these sunscreens, we endeavor to deliver not just protection but also the poetry of nature's embrace, inviting all who cherish the fusion of science and tradition to partake in this transformative journey[6].

Advantages of Sunscreen Formulation with Pithecellobium Dulce Extract

The utilization of sunscreen enriched with Pithecellobium dulce extract offers several potential advantages:

- **Natural UV Protection:** Pithecellobium dulce extract contains bioactive compounds with natural UV-protective properties, making it a promising ingredient for sunscreen formulations.
- **Broad-Spectrum Protection:** The extract may offer broad-spectrum protection against both UVA and UVB radiation, enhancing its effectiveness in shielding the skin from harmful UV rays[7].
- **Antioxidant Benefits:** Pithecellobium dulce extract may provide additional antioxidant benefits, helping to combat the oxidative stress induced by UV radiation.

METHODOLOGY

Collection and Authentication: Leaves of Pithecellobium Dulce were collected from the local area of Sidhari, Azamgarh, Uttar Pradesh, washed with sterile water and dried in shades. Then the samples were powered in mechanical grinder. The plants were examined by Prof. Nawal Kishore Dubey (FNASc, FNAAS, Centre of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi-221005.

Extraction:

- Grind the dried Pithecellobium dulce leaves into a fine powder using a mortar and pestle or a grinder.
- Place the powdered leaves in a glass container with a lid.
- Add enough ethanol to completely immerse the plant material. Ensure that the solvent covers the plant material adequately[8].
- Seal the container with the lid and gently shake it to ensure thorough mixing of the solvent and plant material[9].
- Store the sealed container in a cool, dark place for an extended period, usually several days to allow for efficient extraction. Periodically shake the container to facilitate the extraction process.
- After the extraction period, filter the mixture to separate the liquid extract from the solid plant material. Use filter paper or a fine mesh strainer for this step.
- Collect the liquid extract in a glass beaker.
- To remove the ethanol, you can employ a water bath or gentle heat source for evaporation. Avoid excessive heat, as high temperatures may degrade quercetin [10].
- Optionally, a vacuum pump or rotary evaporator can be used for more precise and efficient solvent removal.
    - As the solvent evaporates, a concentrated extract containing quercetin will be left behind.
    - Allow the extract to air dry in a controlled environment to remove any residual moisture[11,12].

Preliminary Phytochemical Analysis of Pithecellobium Dulce:

- **Phytochemical testing for mucilage**: Mucilage was tested for presence of various phytochemicals like alkaloids, flavonoids, glycosides, tannins, carbohydrates and proteins.
- **Fluorescence Test (UV Lamp):** The fluorescence analysis in this test, plant material (like leaves) is dried, powdered, and then treated with various solvents or reagents such as ethanol, chloroform, hydrochloric acid (HCl), sulfuric acid (H2SO4), and water. The treated samples are observed under ultraviolet (UV) light to detect fluorescence, which can help in the identification and characterization of chemical constituents present in the plant material[13].
- **Powder Microscopy (Compound Microscope):** The powder microscopy, involves examining powdered sample under a microscope to analyse their physical and chemical properties. This technique is commonly used in field such as pharmacognosy, forensic science and materials science. It helps to analyse particle size and shape, Crystallinity and crystal structure, Purity and contaminants, homogeneity, chemical composition, surface morphology and texture, and thermal properties[14].

Physio-Chemical Analysis:

- **Ash Value Determination:** To determine the total ash value of plant leaf dried powder, start by accurately weighing 2-3 grams of the sample. Place the sample in a pre-weighed crucible and gradually incinerate it by increasing the heat to 500-600°C in a muffle furnace until the sample turns white, indicating the complete removal of carbon. After incineration, allow the crucible to cool in a desiccator to prevent moisture absorption.

Once cooled, weigh the crucible containing the ash. The total ash value is then calculated using the formula:

%ASH = [(ashed wt.) – (crucible wt.)] × 100 \ [(crucible and sample wt.) – (crucible wt.)] **Eq. 6**

- **Swelling index determination:** Using the BP technique, 1gm of mucilage powder was placed in a 100 ml graduated cylinder. 25 ml of water was then added, and the mixture was shaken continuously every 10 minutes for an hour. The mixture was then left to settle for a full day. The swelling index was computed by taking the mean of the three measurements and dividing it by the volume covered mucilage.

Swelling index = V2 – V1\ W1 **Eq. 1**

Were, V2: Final volume of the mixture

V1: Initial volume of the water

W1: Weight of the powder

- **Angle of Repose:** A measurement of the powder's flow characteristics is its angle of repose. It is the greatest angle formed between the powder heap's surface and the horizontal plane. The formula was used to calculate the angle of repose. The finely ground mucilage was transferred via a funnel onto graph paper, with the funnel's height kept constant. The height and base of the powder heap that had developed were measured, and equation was used to determine the angle of repose in accordance with the USP[15].

$$\tan\theta = h/r$$

$$\theta = \tan^{-1}(h/r) \quad \text{Eq. 1}$$

Where, θ represents the angle of repose,

H is height in cm

R is radius/base in cm.

- **Bulk density (BD):** This is the proportion of the powder's bulk volume to its overall mass. Weigh out 50 gm of powdered mucilage precisely, and then pour it into a 100 ml container graduated barrel. The mixture's initial apparent volume (Vo) of mucilage was meticulously levelled. The formula for calculating loose bulk density can be used, and the result is represented in g/ml[16].

$$\rho b = M/Vb \quad \text{Eq. 2}$$

Where ρb=bulk dernsity, M=bulk weight of blend, Vb= bulk volume of the blend.

- **Tap density (TD**): This is the proportion of the powder's total mass to its tapped volume. Weigh precisely 40 gm of the powder combination, which was placed in a 100 ml container cylinder for measuring. After three manual taps (1250, 750, and 500) on the sample-containing cylinder, the final tapped volume (Vf) was determined. The formula for calculating tapered bulk density can be used, and the result is stated in g/ml**.**

$$\rho t = M/Vt \quad \text{Eq. 3}$$

Where, ρt=Tapped density, M=weight of blend, Vb= tapped volume of the blend.

- **Compressibility Index (Carr's Index):** The ratio of the bulk density to the tapped density and the difference between the two is known as the compressibility index. It is expressed in and quantifies the flowability of powder proportion.

Carr's Index (%) = (Dt-Db/Dt) *100 **Eq. 4**

Where Dt = Tapped density of the powder, Db=bulk density of the powder.

- **Hausner ratio:** The Hausner ratio is a number that is correlated to the flowability of a powder or granular material. It is an indirect index to measure the ease of powder flow. A measure of a powder or granular material's flowability is called the Hausner ratio. It is a metric that measures the powder flow's easiness indirectly [17].

Hausner ratio= $\rho b/\rho t$ **Eq. 5**

Where, ρb=Tapped density of the powder, ρt =Bulk density of the powder.

FORMULATION OF SUNSCREEN:

Table 1: Ingredient used to make Sunscreen

S.No	Ingredients	For 100 gm	For 10 gm
1	Beeswax	4 gm	0.4 gm
2	Stearic Acid	1.5 gm	0.14 gm
3	Cetyl Alcohol	2 gm	0.2 gm
4	Olive Oil	12 gm	1.2 gm
5	Coconut Oil	16 gm	1.6 gm
6	Span 80	1.5 gm	0.15 gm
7	Aloe Veera Gel	40 gm	4 gm
8	Tween 80	1.4 gm	0.14 gm

Preparation of the Oily Phase:

- Measure out the following ingredients:
  a. Beeswax- 0.4 grams
  b. Stearic Acid- 0.14 grams
  c. Cetyl Alcohol- 0.2 grams
  d. Olive Oil- 1.2 gram
  e. Coconut Oil- 1.6 grams
  f. Span 80- 0.15 grams
- Combine all the oily phase ingredients in a heat-resistant container.
- Heat the mixture in a water bath at approximately 70-75°C until all the ingredients have melted and combined thoroughly.

- Stir the mixture continuously to ensure uniformity[18,19].

Preparation of the Water Phase:

- Measure out the following ingredients:
    a. Aloe Vera Gel- 4 grams
    b. Tween 80- 0.14 grams
- Combine the Aloe Vera Gel and Tween 80 in a separate heat-resistant container.
- Heat the mixture in a water bath at approximately 70-75°C until the Tween 80 is fully dissolved in the Aloe Vera Gel.
- Stir the mixture continuously to ensure uniformity.

Emulsification:

- Slowly add the heated water phase to the heated oily phase while continuously stirring.
- Use a high-shear mixer or a homogenizer to blend the mixture thoroughly for a few minutes until a uniform emulsion is formed.
- Continue to stir the mixture while allowing it to cool down to room temperature. This helps to stabilize the emulsion and ensure a smooth texture.

Final Adjustment and Packaging:

- Once the mixture has cooled to room temperature, check the consistency and texture of the sunscreen. If necessary, adjust the consistency by adding small amounts of either phase and mixing thoroughly.
- Transfer the prepared sunscreen into suitable containers.
- Label the containers with the product name, date of preparation, and any other relevant informatio[20].

Storage:

- Store the sunscreen in a cool, dry place away from direct sunlight and heat to maintain its stability and efficacy[21,22]

Figure 2: Different batches of Sunscreen

Table 2: Different Batches of Sunscreen

Ingredients of Sunscreen	Batches					
	B1	B2	B3	B4	B5	BLANK
Beeswax	0.4 gm	0.4 gm	0.4 gm	0.4 gm	0.4 gm	0.4 gm
Stearic Acid	0.14 gm	0.14 gm	0.14 gm	0.14 gm	0.14 gm	0.14 gm
Cetyl Alcohol	0.2 gm	0.2 gm	0.2 gm	0.2 gm	0.2 gm	0.2 gm
Olive Oil	1.2 gm	1.2 gm	1.2 gm	1.2 gm	1.2 gm	1.2 gm
Coconut Oil	1.6 gm	1.6 gm	1.6 gm	1.6 gm	1.6 gm	1.6 gm
Span 80	0.15 gm	0.15 gm	0.15 gm	0.15 gm	0.15 gm	0.15 gm
Aloe Veera Gel	4 gm	5 gm	6 gm	7 gm	8 gm	9 gm
Tween 80	0.14 gm	0.14 gm	0.14 gm	0.14 gm	0.14 gm	0.14 gm
Herbal Extract	0.2 gm	0.4 gm	0.6 gm	0.8 gm	1 gm	---

Evaluation of Sunscreen:

- **In Vitro SPF assay:** The in vitro screening method is performed by making 10% solution of herbal cream with 95% ethanol. The absorbance was tested at 290-320nm for each sample at a definite interval of 5 nm, using UV-Visible spectrophotometer.
- The following formula was used to get the measured absorbance values at 5-nm intervals (290-320 nm):

$$SPF_{spectrophotometer} = CF \times \sum_{290}^{320} EE(\lambda) \times 1(\lambda) \times (\lambda) \times Abs(\lambda)$$

- Where EE (λ) is the erythrogenic effect of light at wavelength λ, Abs (λ) is the spectrophotometric absorbance values at wavelength λ, and CF is the correction factor. The constant values may be found in EE (λ)xI (λ).
- pH **of the cream (Digital pH Meter):** Standard buffer solution was used for pH metre calibration. After weighing and dissolving around 0.5g of the cream in 50.0 ml of distilled water, the pH of the mixture was determined.
- **Viscosity (Viscometer):** Using spindle number seven, the Brookfield Viscometer was used to measure the formulation's viscosity at 100 rpm[23,24].
- **Dye test:** The cream is combined with the scarlet crimson colour. After applying a cover slip and placing a drop of the cream on a tiny slide, it is examined under a microscope. If the scattered globules seem crimson, the ground will be colourless. It is an o/w variety of cream. In w/o type cream, the situation is the opposite; that is, the scattered globules look colourless in the red ground.
- **Homogeneity:** The uniformity of the formulations was assessed by touch and appearance.
- **Appearance:** The cream's colour, pearlescence, and roughness were used to assess its appearance.
- **After feel:** Checks were made for emollience, slipperiness, and quantity of residue remaining after a certain amount of cream was applied.
- **Removal:** By using tap water to wash the area where the cream was applied, the cream's ease of removal was assessed.
- **Acid Value:** Dissolve 10 gm of substance in 50 ml of alcohol and solvent ether, connect the flask to a reflux condenser and slowly heat until completely dissolved. Add 1 ml of phenolphthalein and titrate with 0.1N NaOH until a faint pink colour appears after 30 seconds of shaking.

  Were,

  Acid value = n X 5.61/w

  n = the no. of ml of NaOH required

  w = the weight of substance

- **Saponification Value:** Reflux 2 gm of material with 25 ml of 0.5 N alcoholic KOH for 30 minutes. Add 1 ml of phenolphthalein and titrate with 0.5 N HCL[25,26].

  Saponification value = 56.1 × N × (V_b – V_s)/W

  Were,

  56.1 is the molecular weight of KOH

  N is the normality of KOH

  V_b is the vol. of KOH used for the blank

  V_s is the vol. of KOH used for the sample

  W is the weight of the sunscreen

- **Irritation Test:** Mark a 1cm square spot on the left dorsal surface. The cream was administered to a specific region and time was recorded. Irritation, erythema, and oedema were assessed at regular intervals for up to 24 hours and reported[21,22].

RESULT AND DISCUSSIONS

1. Preliminary Phytochemical Analysis and physio chemical analysis of Pithecellobium Dulce:

Table 3: Result of Phytochemical Analysis and physio chemical analysis of Pithecellobium Dulce

Phytochemicals Tested	Extraction by Methanol
Alkaloids	++
Cardiac Glycosides	--
Flavonoids	++
Proteins	++
Tannins	++
Terpenoids	++
Saponins	--
Sterols	++
Sugars	++

Table 4: Result of Phytochemical Analysis

S. No.	Physicochemical Analysis	Result
1	Swelling Index	3.5 ± .5 ml/g
2	Angle of Repose	22° ± 5°
3	Bulk Density	0.3 to 0.4 g/ml
4	Tap Density	0.2 to 0.6 g/ml
5	Carr's Index	15 ± 5 %
6	Hausner Ratio	1.2 to 1.5
7	Ash Valve	9 ± 2 %
8	Acid Soluble Ash	1.32 ± 0.25 %

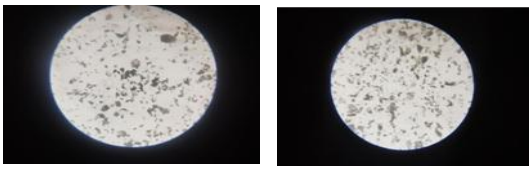

Figure 3: Microscopy of Pithecellobium

Table 5: Test of Fluorescence

S. No.	Solutions	Visible light	UV Light (254nm)	UV Light (365nm)
1	Distilled Water	Green	Dark Green	Black
2	1N HCL	Brown	Dark Brown	Black
3	1N NaOH	Brownish	Green	Black
4	Ammonia Solution	Green	Yellowish	Black
5	Ethanol	Brown	Green	Green
6	Methanol	Green	Green	Dark Green
7	Chloroform	Light green	Green	Black
8	Acetone	Geen	Dark Green	Yellow green
9	Petroleum Ether	Dark green	Dark Green	Black
10	Ethyl Acetate	Light Brown	Dark Green	Dark Brown
11	Benzene	Black	Dark Green	Brown
12	Glacial Acetic Acid	Brown	Dark green	Orange

2. Results and discussion of evaluation of sunscreen:

Table 6: Result of in Vitro SPF assay

SNO.	Wavelength (nm)	EE (λ)X I(λ) Employed	Absorbance	EE(λ)X I (λ)X absorbance(A)
1	290	0.0151	3.107±0.03	0.0469157
2	295	0.0815	2.872±0.01	0.234068
3	300	0.2875	2.654±0.01	0.763025
4	305	0.328	2.417±0.02	0.792776
5	310	0.1863	2.236±0.01	0.4165668
6	315	0.0839	1.982±0.01	0.1662898
7	320	0.0182	1.725±0.01	0.031395
8	**TOTAL**	2.4510363		
9	**SPF**	24.51		

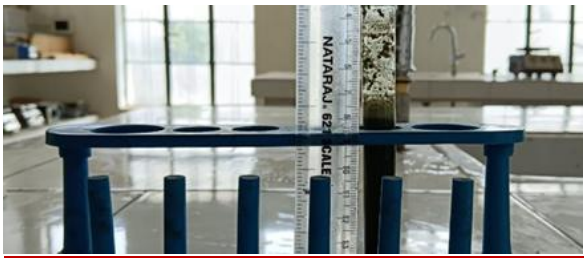

Figure 4: Test of Foaming index

Table 7: Result Evaluation of the Cream

S.NO	Evaluation Parameter	Batch(B4)
1	Colour	Dark Green
2	Texture	Smooth and non-greasy
3	Ph	6.73±0.0321
4	Spreadability (g*cm/sec)	0.61±0.0305
5	Saponification Value	82±5 mg KOH/g
6	Viscosity(cps)	11228

CONCLUSION

This study successfully formulated and evaluated a sunscreen containing the extract of Pithecellobium dulce and batch 4 was found best among other 6 batches. The formulation process was complemented by a comprehensive preliminary chemical analysis of the plant. The extraction method was meticulously performed to obtain the desired plant extract for the sunscreen formulation. The evaluation of the sunscreen involved testing the in vitro SPF assay, revealing its potential efficacy in providing sun protection. Furthermore, the sunscreen's acid value and saponification value were determined, alongside an irritation test, to ensure its safety and effectiveness. The findings highlight the promising potential of Pithecellobium dulce extract in developing effective sunscreen formulations. However, further research and development are essential to fully optimize this innovative approach. The scientific community is encouraged to explore this field further, as it holds significant potential for advancements in sunscreen technology. Continued research and exploration in this area are crucial for the development

of safe, effective, and natural sunscreen products that can provide superior protection against harmful UV radiation.

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