RESEARCH ARTICLE

Enhancing Topical Anti-Inflammatory Activity with *Psidium guajava* L. and *Moringa oleifera*

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ABSTRACT

The main goal of this research was to formulate and assess herbal topical cream incorporating *Psidium guajava* and *Moringa oleifera* extracts to explore their antimicrobial, anti-inflammatory, moisturizing, and skin-protective properties. Three cream formulations (F1, F2, and F3) were prepared by varying the concentrations of the plant extracts while keeping other ingredients constant. The formulations were created through emulsification, where the oil phase (stearic acid, emulsifying wax, propylene glycol, and propyl paraben) and aqueous phase (plant extracts, glycerin, cetyl alcohol, and sodium stearate) were combined at 70°C before cooling and adding perfume.

The prepared formulations were assessed for the parameters such as pH, physical stability, spreadability, viscosity, stability testing, antimicrobial activity, anti-inflammatory efficacy and skin irritation potential. All formulations demonstrated physical stability, with F2 providing an optimal balance of spreadability, viscosity. Antimicrobial activity increased with higher extract concentrations, with F3 exhibiting the largest inhibition zones against bacterial strains. The anti-inflammatory activity, assessed by protein denaturation inhibition and heat-induced hemolysis assays, showed a dose-dependent trend. F3 exhibited the highest inhibition rates (74.5% for protein denaturation and 68.9% for heat-induced hemolysis), followed by F2 and F1.

The study confirmed that incorporating *Psidium guajava* and *Moringa oleifera* extracts in cream formulations enhances their antimicrobial and anti-inflammatory properties, providing both cosmetic and therapeutic benefits. These findings support further optimization and clinical exploration for skincare applications.

Keywords

Cream Formulation, anti-inflammatory Activity, Synergistic action and phytochemicals

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INTRODUCTION

The skin, as the largest organ in the human body, serves a vital function in shielding contrary to environmental factors such as microbial infections, pollutants, and harmful chemicals. Maintaining healthy skin requires effective skincare products that offer moisturizing, antimicrobial, anti-inflammatory, and protective benefits. In recent years, herbal creams have garnered significant attention due to their natural ingredients, lower risk of side effects, and therapeutic potential. The inclusion of plant extracts in cosmetic formulations represents a growing trend, offering both functional and aesthetic benefits, and positioning herbal creams as a preferred alternative to synthetic products.[1]

Psidium guajava (guava) and *Moringa oleifera* (moringa) are well-known medicinal plants with rich phytochemical profiles and numerous health benefits. *Psidium guajava* is renowned for its antioxidant, antimicrobial and anti-inflammatory effects, which are due to its rich content of polyphenols, flavonoids, and essential oils. Likewise, *Moringa oleifera* is a highly valued plant in traditional medicine, recognized for antioxidant, antibacterial, moisturizing, and anti-inflammatory potentials due to bioactive compounds such as vitamins, phenolics, and alkaloidsThe integration of these extracts into a single cream formulation has the potential to enhance antimicrobial efficacy, improve skin hydration, reduce inflammation, and promote skin healing.[2-9] Inflammation is a key factor in various skin conditions, and the ability to mitigate it is crucial for effective skincare formulations. Anti-inflammatory activity can be evaluated through assays like protein denaturation inhibition and heat-induced haemolysis, which measure the ability of compounds to stabilize proteins and protect cell membranes under stress. By incorporating *Psidium guajava* and *Moringa oleifera* extracts, the study aims to leverage their synergistic anti-inflammatory properties to develop a cream with enhanced therapeutic potential.[10]

This research focuses on formulating and evaluating herbal creams containing *Psidium guajava* and *Moringa oleifera*

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Table 1a: Materials used for formulation

Ingredient	Function	Source
Psidium guajava extract	Active ingredient	Prepared in the lab
Moringa oleifera extract	Active ingredient	Prepared in the lab
Stearic acid	Thickening agent	Commercial supplier
Emulsifying wax	Emulsifier	Commercial supplier
Propylene glycol	Humectant	Commercial supplier
Propyl paraben	Preservative	Commercial supplier
Glycerin	Moisturizer	Commercial supplier
Cetyl alcohol	Emulsifier	Commercial supplier
Sodium stearate	Emulsifier/stabilizer	Commercial supplier
Perfume	Fragrance	Commercial supplier
Distilled water	Solvent	Lab-grade

Table 1b: Formulation Table for Cream Batches (F1, F2, F3)

Ingredient	F1 (%)	F2 (%)	F3 (%)
Stearic acid	5.0	5.0	5.0
Emulsifying wax	8.0	8.0	8.0
Propylene glycol	4.0	4.0	4.0
Glycerin	2.5	2.5	2.5
Cetyl alcohol	1.0	1.0	1.0
Sodium stearate	3.0	3.0	3.0
Psidium guajava extract	2.0	3.0	4.0
Moringa oleifera extract	2.0	3.0	4.0
Propyl paraben	0.2	0.2	0.2
Perfume	0.5	0.5	0.5
Distilled water	q.s. upto 100	q.s. upto 100	q.s. upto 100

extracts as active ingredients. The formulations were prepared by emulsifying oil and aqueous phases containing these extracts. The resulting creams were then subjected to a series of evaluations, including physical properties, stability, anti-inflammatory activity, antimicrobial efficacy, and consumer acceptability.[11-13]Three formulations (F1, F2, and F3) with varying concentrations of the extracts were compared to determine the optimal balance of efficacy, stability. This research offers significant insights into the formulation of plant-based skincare products that offer both cosmetic and therapeutic benefits.

MATERIALS AND METHODS

Materials

The herbal cream formulations were prepared using the materials compiled in table 1a and 1b

Methods

Preparation of Plant Extracts

Table 2: pH Measurements of three batches (F1, F2, F3)

Formulation	Initial pH	After 30 Days
F1	5.9	5.7
F2	6.0	5.8
F3	6.1	5.9

Table 3: Spreadability of three batches (F1, F2, F3)

Formulation	Spreadability (g·cm/sec)
F1	5.5
F2	5.2
F3	4.8

Table 4: Viscosity Measurements of three batches (F1, F2, F3)

Formulation	Viscosity (cP)
F1	2800
F2	3100
F3	3500

Extract of Psidium guajava

Fresh leaves of *Psidium guajava* were collected, washed, dried, and powdered. The powdered leaves were subjected to extraction using ethanol in a Soxhlet apparatus. The extract was concentrated by using a rotary evaporator to obtain a thick, viscous extract.[14-16]

Extract of Moringa oleifera

Fresh leaves of *Moringa oleifera* were processed similarly, with ethanol used as the solvent for extraction. The extract was concentrated and kept in an airtight container for storage until required.[14-16]

Herbal Cream formulation

The cream was prepared using the oil and aqueous phase emulsification method as follows.[17-19]

Step 1: Preparation of the Oil Phase

The required quantities of stearic acid, emulsifying wax, propylene glycol, and propyl paraben were weighed.

These ingredients were placed in a beaker and heated to a temperature of 70°C with constant stirring until all components dissolved.

Step 2: Preparation of the Aqueous Phase

In a separate beaker, *Psidium guajava* extract and *Moringa oleifera* extract were mixed with glycerine, cetyl alcohol, and sodium stearate.

The mixture was stirred while being heated to 70°C to ensure uniform mixing.

Step 3: Emulsification

The aqueous phase was slowly incorporated in the oil phase at 70°C through constant stirring.

Stirring continued till a smooth, homogeneous cream was achieved.

The cream was then cooled to room temperature, perfume was added and mixed thoroughly.

Step 4: Packaging

The cream was transferred to clean, sterilized containers.

The containers were labelled as F1, F2, and F3 based on the different extract concentrations.

Evaluation Methods

Physical Appearance and Texture

Table 5: Stability testing of three batches (F1, F2, F3)

Test	F1	F2	F3
Centrifuge Test	No any change	No any change	No any change
Freeze-Thaw Test	No any change	No any change	No any change
Accelerated Storage	No signs of phase separation	No signs of phase separation	No signs of phase separation

Table 6: Antimicrobial Efficacy of three batches (F1, F2, F3)

Formulation	Zone of Inhibition (mm)
F1	11
F2	15
F3	18

Table 7: Skin irritation test of three batches (F1, F2, F3)

Formulation	Irritation Score (0-5)
F1	0
F2	0
F3	1

Table 8: Protein Denaturation Test of three batches (F1, F2, F3)

Formulation	Test Sample % Inhibition	Average % Inhibition
F1	68.4	68.9
	69.2	
	69.1	
F2	71.5	71.7
	71.8	
	71.9	
F3	74.3	74.5
	74.6	
	74.5	
Diclofenac Standard	76.2	76.06
	75.9	
	76.1	

F1: Exhibited moderate inhibition of protein denaturation, with an average of 68.9%, showing lower efficacy compared to the standard and other batches.
F2: Demonstrated improved inhibition with an average of 71.7%, closely aligning with the test sample results.
F3: Showed the highest inhibition among the formulations, achieving an average of 74.5%, which is comparable to the standard Diclofenac's inhibition of 76.06%.

The physical properties of the cream, such as color, texture, homogeneity, and odor, were evaluated visually and by touch.[17-19]

pH Measurement

To measure the pH of each formulation, 1 g cream was taken and it was dissolved in 10 mL distilled water, and the pH was then recorded by using a calibrated pH meter.

Spreadability Test

The cream's spreadability was evaluated using the glass slide technique. A sample was positioned sandwiched in to two glass slides, which were then pressed simultaneously with a 30-gram weight for five minutes to form a uniform film. After adding an additional 30 grams to the pan, a line connected to a hook was used to pull the upper slide. The time required for the top slide to move 5 cm over the lower slide was measured and recorded. The spreadability (S) was estimated using the following equation.

Spreadability (S) = (Weight attached to the top glass slide) M× distance covered by the upper glass slide L/ time required for the movement T

Viscosity Measurement

The viscosity of the cream was determined by using a Brookfield viscometer set at a speed of 20 rpm.

Stability Testing

*Centrifuge Test***:** The cream was centrifuged at 3000 rpm for 30 minutes to evaluate potential phase separation.

Freeze-Thaw Cycle: The cream was subjected to freeze-thaw cycles between -5°C and 40°C to assess stability.

Accelerated Storage: The cream was stored at 45°C for 30 days, and changes in color, odor, texture, and pH were recorded.

Antimicrobial Efficacy (Zone of Inhibition Test)

The antimicrobial effectiveness of the cream was tested by using the agar well diffusion method. Nutrient agar plates were inoculated with bacterial strains (*E. coli* and *Staphylococcus aureus*). Wells were filled with 1 mL of cream, and incubation of plates was carried out at 37°C for the time of 24 hours. The inhibition zone was recorded in millimeters.[16-17]

Test for Skin Irritation

The test for skin irritation was conducted on a group of 10 human volunteers. A patch test was performed by spreading over a small quantity of cream to the forearm for 24 hours. The area was then observed for any signs of redness, itching, or swelling.

Method for Anti-Inflammatory Activity

Protein Denaturation Inhibition Assay

Preparation of Reaction Mixtures

A reaction mixture was assembled with a total 10 ml volume comprising, 0.4 ml -fresh hen's egg albumin, 5.6 ml - phosphate-buffer solution (PBS, pH 6.4), 4 ml - experimental samples at a final concentration of 100 g/ml.. In control group, distilled water replaced the test extracts. Diclofenac sodium served as the reference standard.[21-27]

Incubation and Heating

Incubation of the reaction mixtures were carried out at 37°C ± 2°C for time of 15–20 minutes in a water bath. Subsequently, the mixtures were heated to temperature of 70°C for 5 minutes and mixtures were subjected to cool to room temperature for the time of 15 minutes.

Measurement of Absorbance

The absorbance of the mixtures was recorded at 680 nm by using a UV spectrophotometer, and each experiment was conducted in triplicate.

Calculation of Inhibition

The percentage of protein denaturation inhibition was determined by using the formula:

% inhibition = At- Ac/ Ac x100

Where,
At = Absorbance of the test solution
Ac = Absorbance of the control

Heat-Induced Hemolysis Assay

Preparation of Reaction Mixtures:

A 2 ml reaction mixture was prepared, including;10% RBC suspension (1 ml), 1 ml of the experimental sample. In the control group, saline replaced the test extracts. Diclofenac sodium served as the reference standard.

Incubation and Centrifugation

The incubation of above prepared reaction mixtures was carried out at a temperature of 56°C for 30 minutes in a water bath, followed by cooling under running water.

The mixtures were then centrifuged at 2500 rpm for the time of 5 minutes.

Measurement of Absorbance

Absorbance of the supernatant liquid was recorded at 560 nm. The assay was conducted in triplicate.

Calculation of Inhibition:

The following formula was used for calculation of Percentage inhibition of hemolysis:

$$\%\,inhibition = \frac{Abs\ Control - Abs\ Test}{Abs\ Control} \times 100$$

RESULTS AND DISCUSSION

The herbal cream formulations namely F1, F2, and F3 containing *Psidium guajava* and *Moringa oleifera* extracts were evaluated for various physical, chemical, microbial, and consumer-related parameters to assess their stability, efficacy, and overall acceptability. The results of each evaluation are discussed below.

Physical Appearance and Texture

All three formulations appeared smooth, creamy, and homogenous without any signs of phase separation. The color of the creams ranged from light beige to greenish, depending on the concentration of the plant extracts.

F1: Light beige, smooth texture
F2: Slightly darker with a smooth and thick texture
F3: Darker greenish tint, thick and slightly greasy

The texture was consistent across all formulations, but F3 showed a slightly greasy feel due to the higher concentration of extracts. The homogeneity and smooth texture indicate proper emulsification and stability of the formulations. The slight greasiness in F3 may affect consumer preference, but it may also provide enhanced moisturizing properties.

pH Measurement

The values of pH of the formulations fell within the acceptable range for safe topical use. (5.0 to 6.5). The slight decrease in pH over 30 days indicates good stability of the formulations. Slightly acidic pH is important for skin health, as it helps to maintain the skin's natural barrier and also avoids microbial growth.

Spreadability

The spreadability was evaluated by measurement of the diameter of the spread under different weights. F1 showed the best spreadability, indicating ease of application. F2 and F3 had slightly lower spreadability due to the higher concentration of extracts, which increased the viscosity.

Table 9: Heat-Induced Hemolysis Test of three batches (F1, F2, F3)

Formulation	Test Sample % Inhibition	Average % Inhibition
F1	55.2	55.7
	56.1	
	55.8	
F2	63.4	63.5
	63.6	
	63.5	
F3	68.7	68.9
	69.1	
	68.9	
Diclofenac Standard	71	71.3
	71.3	
	71.6	

F1: Exhibited a moderate inhibition of heat-induced hemolysis with an average of 55.7%, indicating the least efficacy among the formulations.

F2: Demonstrated significant inhibition with an average of 63.5%, closely matching the test sample results and showing better membrane stabilization compared to F1.

F3: Showed the highest inhibition among the formulations with an average of 68.9%, approaching the efficacy of the Diclofenac standard at 71.3%.

Viscosity

The viscosity measurement of the formulation was done by using a Brookfield viscometer. The viscosity increased with the concentration of plant extracts. F3, with the highest extract concentration, had the highest viscosity. Higher viscosity can improve the moisturizing effect but may affect the ease of application.

Stability Testing

The stability of the cream formulations was evaluated using centrifuge, freeze-thaw cycles, and accelerated storage tests. All formulations were found to be stable under various conditions, indicating that the emulsification process was successful and that the creams can withstand temperature fluctuations without phase separation.

Antimicrobial Efficac

The antimicrobial activity was tested by measurement of the zone of inhibition against *E. coli* and *Staphylococcus aureus*.

The antimicrobial efficacy improved with increasing concentration of plant extracts. F3 showed the highest zone of inhibition, indicating strong antibacterial activity against both bacterial strains. The bioactive compounds present in *Psidium guajava* and *Moringa oleifera* contributed to the antimicrobial properties.

Skin Irritation Test

The skin irritation test showed no significant adverse reactions in any of the volunteers. All formulations were found to be safe for use on human skin. F3 showed a very mild irritation in one volunteer, possibly due to the higher concentration of plant extracts. However, the irritation was negligible and did not cause any discomfort.

Protein Denaturation Inhibition

The experimental samples demonstrated a dose-dependent protein denaturation inhibition, with higher concentrations of *Psidium guajava* and *Moringa oleifera* extracts showing enhanced activity. The inhibition percentage of the test samples was comparable to the standard drug, Diclofenac sodium. These results confirm the dose-dependent activity of the formulations, with F3 being the most effective due to its higher concentration of plant extracts.

Heat-Induced Hemolysis

The test samples exhibited significant protection against heat-induced hemolysis of red blood cells. This protective effect was also dose-dependent, with formulations containing higher extract concentrations (F3) showing superior inhibition. These results confirm that the anti-inflammatory activity of the formulations follows a dose-dependent trend, with F3 being the most effective due to its higher concentration of ***Psidium guajava*** and ***Moringa oleifera*** extracts.

These results confirm that the developed formulations, especially F3, possess significant anti-inflammatory potential, warranting further exploration in clinical settings.

CONCLUSION

The study successfully demonstrated the development and evaluation of herbal creams containing ***Psidium guajava*** and ***Moringa oleifera*** extracts. These formulations exhibited promising stability, antimicrobial properties, anti-inflammatory efficacy. In conclusion, the developed herbal creams, particularly **F3**, possess remarkable anti-inflammatory and antimicrobial properties, making them promising candidates for further exploration in clinical and dermatological applications. However, formulation F2 offers the best balance of functional and sensory attributes, making it ideal for consumer use. These findings advocate the potential of herbal creams as effective and natural skincare solutions.

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