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Characterization of caffeinated facial creams with *Diploknema butyracea* butter as an emollient

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Abstract. *Subedi S, Koirala S, Pokharel P, Bhandari DR. 2025. Characterization of caffeinated facial creams with* Diploknema butyracea *butter as an emollient. Asian J Nat Prod Biochem 23: 11-18.* Caffeine, a versatile ingredient in cosmetics, is known for its vasoconstrictive, anti-inflammatory, UV protective, and anti-aging properties. The plant *Diploknema butyracea*, a medium-sized tree belonging to the Sapotaceae family, native to Nepal, India, and Bhutan, is known as *chyuri*, and its seeds contain about 55% fat, known as *chyuri* butter. *Chyuri* butter is rich in polyunsaturated fatty acids and vitamins (A and E) to hydrate, nourish, soften skin and serve as an effective emollient. In this study, we formulated caffeinated facial creams with *D. butyracea* butter and meticulously evaluated their physical and chemical properties. Caffeine was extracted via liquid-liquid extraction. Four formulations (F1 to F4) with varying concentrations of *chyuri* butter and caffeine were assessed for pH, stability, acid and saponification values, spreadability, in-vitro occlusivity, centrifugation, after-feel, smear type, removal, and dilution. The pH of the creams ranged from 6.7 to 7.6. All formulations, except F1, remained stable and homogeneous at different temperatures. Acid values (9.5-11.2) and saponification values (23.5-26.8) were satisfactory. The creams demonstrated good spreadability (4.3-4.5 cm) and improved water retention due to *chyuri* butter. No phase separation occurred during centrifugation. The creams formed a non-greasy film, were easily removable with tap water, and were confirmed as O/W (oil in water) type. Our findings not only validate the efficacy of caffeine and chyuri butter in cosmetic cream formulations but also open up new possibilities for their use in skincare products.

Keywords: Caffeine, chyuri, cosmetics, *Diploknema butyracea*, facial cream

INTRODUCTION

Throughout history, different natural and synthetic ingredients have been used for beauty. Due to the presence of secondary metabolites, various compounds of herbal plants possess antimicrobial, anticancer, anti-inflammatory, and other pharmacological properties (Larbie et al. 2019; Katyal 2022). Natural ingredients and their potent compounds are gaining popularity in cosmetic products. Cosmeceutical products containing natural ingredients provide the skin with essential nutrients such as vitamins A, C, and E, phenolic compounds, flavonoids, and terpenoids. These components act as antioxidants and anti-inflammatory agents and have anti-aging and skin-brightening effects, ultimately promoting and improving skin health (Lohani et al. 2019). Cosmetic products are extensively used to safeguard the skin from harmful external and internal elements to improve the skin's appearance and beauty (Kaur and Saraf 2010; Liu 2022). Cosmeceuticals enhance external attractiveness and promote longevity by mitigating skin disorders (Datta and Paramesh 2010; Kumar et al. 2024).

Caffeine (1, 3, 7-Trimethylpurine-2,6-dione), an alkaloid found in coffee and tea, is a central nervous system stimulant. Caffeine is widespread in the cosmetics industry due to its potent biological activity and ability to penetrate the skin barrier (Blanco-Llamero et al. 2024). It exhibits significant antioxidant and anti-aging activities, shielding cells from UV radiation and decelerating the skin's photoaging process (Herman and Herman 2012). Caffeine also possesses anticellulite properties by inhibiting adrenergic receptors and hindering excessive fat buildup through accelerated lipolysis. The impact of a caffeine-containing emulsion on adipose cells was investigated by topically applying it to Wistar female mice for 21 days, resulting in a 17% decrease in adipose cell diameter compared to the control group (Velasco et al. 2008). Furthermore, caffeine has been proven to increase vasoconstriction, tighten the skin, and reduce swelling (Lee et al. 2019), providing a solid foundation for its use in cosmetic products and instilling confidence in its effectiveness.

According to the FDA, animal testing is not specifically required for marketing a cosmetic product; however, any testing relied upon should be scientifically sound. FDA regulations state that cosmetic products and ingredients, except for color additives, do not require FDA approval before being introduced. However, they must not be "adulterated" or "misbranded." When applied near the eyes, lotions containing caffeine and theobromine are limited to maximum concentrations of 1.5 and 0.0025%, respectively (Cherian 2018).

Emollient bases improve skin moisture and flexibility by forming a protective layer on the skin's surface. This layer helps prevent water loss from the underlying skin layers (occlusive effect) and traps moisture from the surrounding environment (moisturizing effect) (Stojiljković

et al. 2013). Despite the growing popularity of cosmetic creams, several challenges have emerged, including the instability of the chemical base, the need for multiple excipients to ensure stability, the high production costs of synthetic bases, and the potential for the base to cause skin hypersensitivity reactions. These challenges have driven the investigation into more effective emollients from natural sources (Pandey et al. 2021).

Diploknema butyracea plant, a medium-sized tree belonging to the family Sapotaceae, is native to Nepal, India, and Bhutan and found at altitudes ranging from 300 to 1,500 meters above sea level. It grows mainly in the sub-Himalayan tracts on steep slopes and cliffs. Known as "*chyuri*" in Nepal and "Indian-butter nut" in English, its primary product is "*ghee*" or butter extracted from the seeds, named "*chiuri ghee.*" The *chyuri* seed contains approximately 55% fat, known as *chyuri* butter, serving as a vegetable oil and massage oil for conditions such as rheumatism, headache, acne, and boils. *Chyuri* fat is a valuable natural emollient, particularly during winter, for moisturizing and nourishing cracked feet and hands (Anand et al. 2024). Furthermore, numerous commercial enterprises have experimented with developing various products such as chyuri syrup, chyuri jam, and chyuri cream (Pandey et al. 2021).

Among various topical preparations in cosmeceuticals, creams are semisolid uniform preparations intended for use on the skin's surface or external mucous membranes. In this study, caffeinated facial cream, including *D. butyracea* butter as an emollient, was formulated and evaluated for physical and chemical parameters. The study aimed to develop cosmetic creams using locally available herbal ingredients with a novel approach. Instead of using whole plant extracts, the formulation utilized a single potent compound of the plant as an active ingredient.

MATERIALS AND METHODS

Chemical and ingredients

Table 1 contains a list of the chemicals and ingredients used in the experiments, along with their corresponding brand names and suppliers.

Extraction of caffeine

We extracted caffeine from coffee using the method by Mumin et al. (2006) with slight modifications. Coffee beans were locally purchased from Pokhara Metropolitan, Nepal, and were grounded into fine powder particles using a mixer grinder. A 10% w/v solution of powdered coffee was prepared using acidic water (pH adjusted to 2 using hydrochloric acid). The mixture was boiled for 20 min at 100°C while stirring and then filtered. Sodium carbonate was added to the filtrate to make the solution basic (pH 10). Next, a 500 mL separatory funnel was placed on an iron ring attached to a ring stand. The basic filtrate solution was poured into the separatory funnel, adding approximately 10 mL of dichloromethane. The solution was thoroughly shaken, and the stopcock was opened to release any vapors. After separating the layers, the lower layer (dichloromethane) was carefully collected into a 100 mL beaker. This separation process was repeated three times, using 10 mL of dichloromethane each time, and all the collected dichloromethane layers were combined in the beaker. Dichloromethane was evaporated using a rotary evaporator. The temperature was kept at a controlled low level, ranging from 30 to 35°C. As a result of the dichloromethane evaporation, crude caffeine solidified at the bottom of the beaker. Finally, to obtain pure caffeine crystals, 10 mL ethanol was used for recrystallization.

Measurement of the extraction yield

The weight of pure caffeine crystals obtained was measured, and the yield percentage was calculated using the following formula:

Extraction yield (%) = [Weight of caffeine crystals obtained/Weight of coffee powder taken] ×100

Table 1. Chemicals and ingredients used in this study with their brand and supplier

Chemicals and ingredients	Brand	Supplier
Coffee beans	Whole bean/Arabica Coffee	Machhapuchhre Organic Coffee
Hydrochloric acid (Chlorane)	Paskem	Paskem Fine Chem Pvt. Ltd.
Sodium carbonate	Himedia	Himedia Laboratories Pvt. Ltd.
Dichloromethane	CDH	Central Drug House Pvt. Ltd.
Ethanol	-	Changshu Hongsheng Fine Chemical Pvt. Ltd.
Stearic acid (octadecanoic acid)	Nike	Nike Chemical India
Cetostearyl alcohol	Loba Chemie	Loba Chemie Pvt. Ltd.
Triethanolamine (2,2′,2″-Nitrilotri(ethan-1-ol))	Alpha Chemika	Alpha Chemika
Glycerol propan-1,2,3-triol	Qualigens	Thermo Fisher Scientific India Pvt. Ltd.
Propylparaben (Propyl 4-hydroxybenzoate)	Loba	Loba Chemie Pvt. Ltd.
Chyuri butter	Himalayan bio	Daraz, Nepal
Ether (Ethoxyethane)	CDH	Central Drug House Pvt. Ltd.
Phenolphthalein 3,3-bis(4-hydroxyphenyl)-2-benzofuran-1(3□H)-one ()	Qualigens	Thermo Fisher Scientific India Pvt. Ltd.
Potassium hydroxide	Qualigens	Thermo Fisher Scientific India Pvt. Ltd.

Table 2. Concentration (% w/w) of ingredients used for four cream formulations and their uses

Ingredients	F1	F2	F3	F4	Uses
Stearic acid	5	5	5	5	Emollient, emulsifier, and stabilizer
Cetostearyl alcohol	3.75	3.75	3.75	3.75	Emulsifier
Triethanolamine	0.62	0.62	0.62	0.62	pH adjuster and emulsifier
Glycerol	2.5	2.5	2.5	2.5	Humectant
Propyl paraben	0.04	0.04	0.04	0.04	Preservative
Chyuri butter	0	20	20	20	Emollient
Caffeine	0	0	0.5	1	Active ingredient
Distilled water	88.09	68.09	67.59	67.09	Solvent

Cream formulation

The cream was formulated using the method by Farboud et al. (2011) with slight modifications. The necessary quantity of the aqueous and lipid phases was individually heated to 70±2°C. The aqueous phase was then carefully added to the lipid phase while continuously stirring with a hand blender until it solidified. Four different formulations were developed, each containing 0% (F2), 0.5% (F3), and 1% (F4) caffeine, with varying amounts of chyuri butter serving as the emollient and control with 0% caffeine and 0% chyuri butter (F1). These formulations, with their specific caffeine and chyuri butter concentrations, were subjected to various evaluations. The components employed in the cream formulation are provided in Table 2. According to the FDA, ingredients used in cosmeceuticals should be safe per the safety data provided by Cosmetic Ingredient Review (CIR). All the ingredients listed are safe per the safety guidelines available on the CIR website (CIR 2016).

Cream formulation

Formulated creams were stored at three distinct temperature settings: 8±2°C, 25±2°C, and 40±2°C. A stability study was conducted on the 0^{th}, 7^{th}, 14^{th}, and 21^{st} days (Smaoui et al. 2017). The cream (0.5 g) was carefully weighed and dissolved in 50 mL of distilled water. Subsequently, the pH of the solution was determined by employing a pH meter. The color and homogeneity of formulations were judged through visual observation and touch (Saheb et al. 2018).

Acid value

The acid values of creams were assessed using the method outlined by Saraf et al. (2010). Next, 5 g of cream was dissolved in a 50 mL equimolar mixture of ethanol (95%) and ether. The flask was attached to a reflux condenser and heated slowly until the cream dissolved. In the mixture, 1 mL of phenolphthalein was introduced and titrated with 0.1 N NaOH until a faintly pink color was observed. The acid value was then calculated using the following formula:

$$\text{Acid value} = [N \times 5.61]/W$$

Where:
N: the number of mL of NaOH required
W: the weight of the substance.

Saponification value

The saponification values of creams were assessed using the method outlined by Aswal et al. (2013). Briefly, 2 g of the formulated cream was mixed with 25 mL of 0.5 N ethanolic KOH and refluxed for 30 minutes. After adding 1 mL of phenolphthalein indicator, the solution was titrated with 0.5 N HCl.

The saponification value (SV) was calculated by applying the following equation:

$$SV = [(b-a) \times 28.05]/W$$

Where:
a: the volume in ml of titrant
b: the volume in ml of the titrate
SV: the saponification value
W: the weight of the substance in g

Spreadability

To measure the spreadability of the test formulation, 0.5 g of the sample was placed within a 1 cm diameter circle marked on a glass plate. Then, it was covered with another glass plate, and a weight of 500 g was placed on the top glass plate for 5 minutes. Spreadability refers to the area covered by a fixed amount of the cream sample after evenly spreading it on the glass slide. The diameter increase caused by the test formulation's spreading was recorded (Maru and Lahoti 2018).

In vitro occlusivity test

The occlusivity of the formulations were determined using the method of Maru and Lahoti (2018). Beakers with a diameter of 3.2 cm and height of 4.6 cm were used for the occlusivity test. In each beaker, 10 g of distilled water was placed by covering the top with Whatman filter paper (with pores of size 0.45 µm), and 200 mg of the sample was evenly spread on the filter paper. After that, these beakers were kept at a constant temperature of 37±2°C and relative humidity of 60±5% for 48 hours. All the formulations and negative control (where the filter paper was left uncovered) were examined to measure the amount of water vapor that passed through the samples and determine the occlusivity of the formulations.

The occlusion factor F was calculated as follows:

$$F = (A-B)/A \times 100$$

Where:

A: Water flux through an uncovered filter (percent water loss)

B: Water flux through the filter when covered by test preparation (percent water loss)

Centrifugation tests

Centrifugal tests were conducted on freshly prepared emulsions. The tests were performed at 5,000 rpm and 25°C for 10 minutes, with 10 g of each sample placed in separate centrifugal tubes (Smaoui et al. 2017).

After feel and type of smear

Emollience, type of smear and the quantity of residue after applying a fixed amount of cream were assessed using the method by Dhase et al. (2014). An area was marked on the dorsal side of the left hand with a marker. 0.5 g of the formulations were uniformly applied over the marked area spreading it gently to form an even layer. The treated area was examined for changes in texture, smoothness and softness which indicate the emollience. The type of smear was categorized as greasy and non-greasy reflecting the absorbability of the formulations. The quantity of residue was measured qualitatively by lightly wiping the area with a clean towel and observing the transfer.

Removal

The ease of removal of the cream applied to the treated area was evaluated by rinsing it with tap water after half an hour of application (Mishra et al. 2014). The treated area was subjected to a gentle stream of tap water at ambient temperature. After rinsing, the treated area was patted dry with a clean towel, and the skin was examined for any lingering residue, greasiness, or film.

Dilution test

The dilution test was conducted to identify the emulsion type of the cream, either oil-in-water (O/W) or water-in-oil (W/O). 1 g of the cream formulation was placed into each of two beakers. In the first beaker, 10 mL of distilled water was gradually added while stirring. In the second beaker, 10 mL of oil was added under the same conditions. The mixtures were examined for any phase separation, or clumping. If the cream formulation mixed uniformly with water without separation, it was classified as an O/W emulsion. If the cream mixes uniformly with oil but not with water, it was classified as W/O emulsion (Dhase et al. 2014).

RESULTS AND DISCUSSION

Caffeine extraction and cream preparation

The extraction yield of caffeine crystals (shown in Figure 1.A) was 0.13%. Four formulations labelled F1, F2, F3 and F4 (shown in Figure 1.B) with varying concentrations of caffeine and chyuri butter (shown in Figure 1.C) were prepared. The concentrations of each ingredient are listed in Table 2. These formulations were evaluated for various parameters to assess their properties and performance.

Stability of creams

The ideal pH range for cosmetic creams should lie between 5 and 8 (Saraf et al. 2010). The formulations' pH was within the specified range of 6.7-7.6 (Figure 2). The pH of the formulations remained relatively constant during the study. Formulations containing chyuri butter (F2, F3, and F4) were white, while formulations not containing chyuri butter were transparent white (Figure 3). The appearance study of all the formulations showed homogeneity. Their appearance did not change at 8±2°C, 25±2°C, and 40±2°C except for formulation F1, which showed non-homogeneity with grittiness at 8±2°C (Figure 3). The centrifugation test relies on centrifugal force to separate substances of different densities. This test is used to evaluate and forecast the stability of emulsions (Khan et al. 2010). In this study, there was no phase separation of all the formulations on centrifugation (Figure 3).

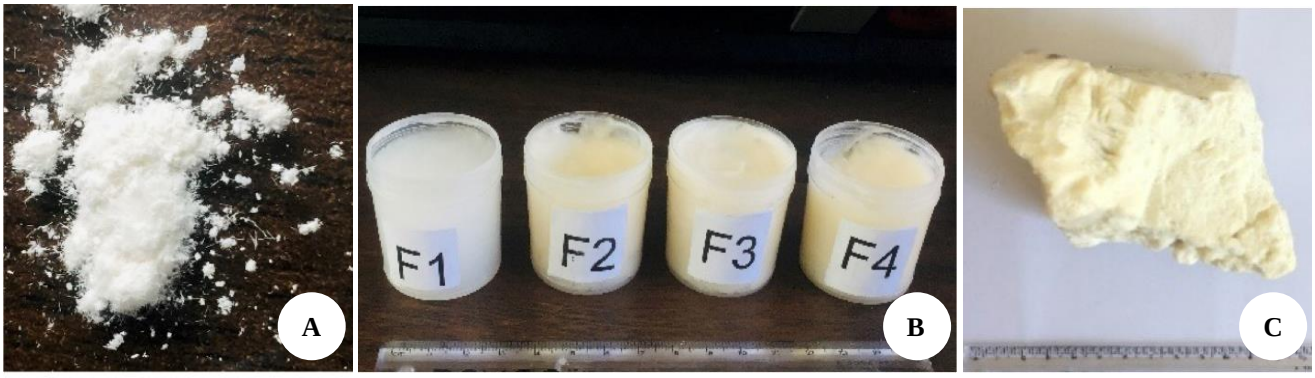

Figure 1. A. Extracted caffeine crystals; and B. Prepared four formulations with concentrations of 0, 0.5, and 1% caffeine; C. Incorporating *chyuri* butter

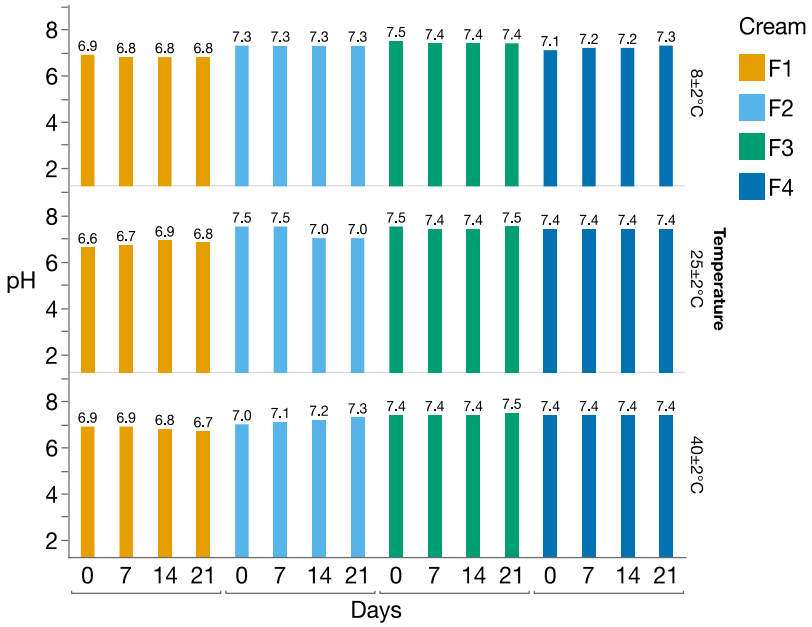

Figure 2. The pH range of the four formulations at three different temperatures (8±2°C, 25±2°C, and 40±2°C) in 0th, 7th, 14th, and 21st days

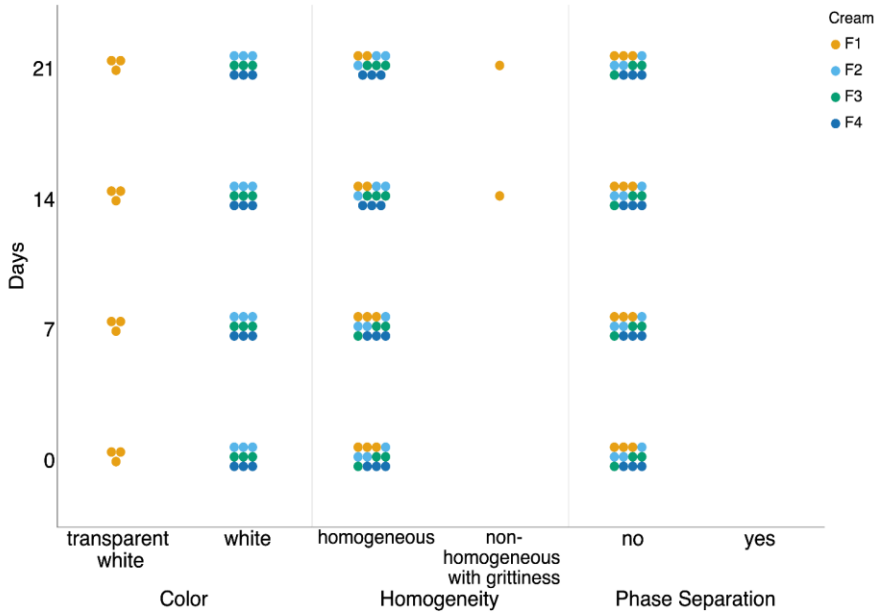

Figure 3. Color (left), homogeneity (middle) and phase separation (right) of the four formulations. Each point shows whether the formulations are transparent white or white (left), homogeneous or non-homogeneous with grittiness (middle) and phase separated (right)

Physical parameters

The acid and saponification values of all cream formulations were under the acceptable range (between 9.5-11.2 and 23.4-26.7, respectively) (Figure 4). This value indicates that the cream's stability would be higher, reducing the likelihood of degradation or rancidity (Panda and Mishra 2018). The spreadability of formulated creams were between 4.3-4.5 cm, which indicates good spreadability (Figure 4) (Maru and Lahoti 2018). The higher the occlusivity value, the more water loss is prevented in a higher proportion from the system and vice versa. The rate of water loss from the skin (water flux) is influenced by the occlusive properties of the formulations applied to the skin. The cream's water-retaining properties were improved by adding *chyuri* butter, which was evident from the increased percentage of in-vitro occlusivity (Figure 4).

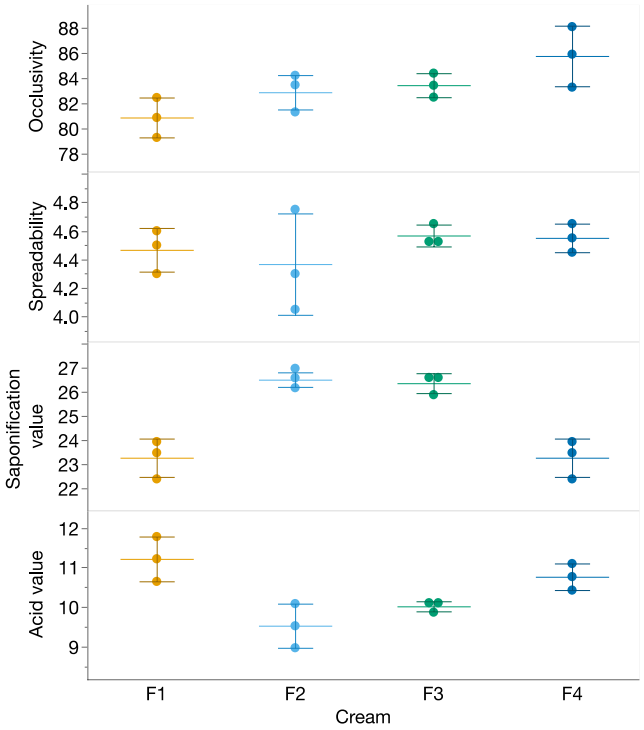

Figure 4. Acid value, saponification value, spreadability, and occlusivity of the four formulated creams. Each horizontal bar represents the mean (±SD) of the physical parameters

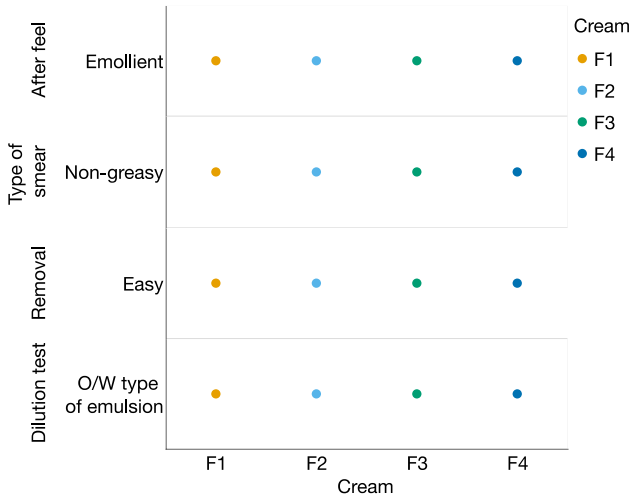

Figure 5. Each point represents parameters such as emulsion type, easy removal, greasiness, and emollience

Other parameters

The investigation revealed that the creams created a non-greasy layer on the skin's surface with emollient characteristics upon application. Moreover, no residue was left on the skin surface after applying, and removal was found easy under tap water. All of the formulated creams were found to be O/W (oil in water) type by dilution test (Figure 5).

Discussion

Nepal's involvement in commercial coffee cultivation is renowned in global markets for its unique flavor and aroma. Coffee is produced at elevated altitudes, distant from the primary coffee-growing Capricorn and Cancer Belt (beyond 230 latitude). Coffee beans from Nepal are primarily exported to countries like Japan, the United States, and various European nations with the trend now expanding to other regions. Caffeine, a significant component in coffee, offers a plethora of health benefits. Caffeine, which is a significant component of coffee, diminishes puffiness around the eyes, hastens the drainage of the lymph system from fatty tissue, enhances blood microcirculation in capillary vessels, has anti-cellulite properties, triggers lipolysis, and releases excess fat from adipocyte cells by decreasing their size (Herman and Herman 2012).

Chyuri butter is rich in polyunsaturated fatty acids and vitamins (A and E) that help hydrate, nourish, and soften our skin. Polyunsaturated fatty acids present in chyuri butter are palmitic acid (50-60%), oleic acid (30-40%), linoleic acid (2-10%), and stearic acid (0-5%) (Dahal et al. 2021). Due to these constituents, *chyuri* fat has a rich historical significance in economic and ethnomedicinal importance among various ethnic groups in Nepal. It has been widely employed as a key ingredient in numerous commercial formulations by cosmetic, pharmaceutical, and soap companies (Pandey et al. 2021).

The economic potential of *D. butyracea* (*chyuri*) butter in Nepal is significant due to its diverse uses, ranging from culinary and medicinal applications to cosmetics and industrial products. With a growing demand for natural products globally, *chyuri* butter could be a high-demanded product for international consumers. For example, between 2011 and 2016, approximately 20 metric tons of *chyuri* butter were exported annually from Nepal to the European market. Cosmetic items incorporating *chyuri* butter have already been introduced into the European market. Brands like Officina Naturae and Cime have already unveiled skincare products, utilizing *chyuri* butter as a fundamental ingredient for creams, ointments, and lotions. Cosmetic items incorporating *chyuri* butter have already been introduced into the European market. Brands such as Officina Naturae and Cime have already launched skincare products, utilizing *chyuri* butter as a base for creams, ointments, and lotions (Devkota et al. 2019). The production and commercialization of chyuri butter can create income opportunities for local communities, foster small-scale industries, promote value chain development, and enhance both rural and national economies, all while encouraging environmentally responsible practices and preserving traditional knowledge.

The properties mentioned earlier make caffeine and chyuri butter important biological compounds that can be used in different cosmetic products. The ingredients used in our formulation constitute both acidic and basic components. Stearic acid and propylparaben contribute to acidic pH, while cetostearyl alcohol, triethanolamine, glycerol, chyuri butter, and caffeine contribute to basic pH. Assessing the pH is essential for evaluating the stability of

pharmaceutical products and cosmeceuticals. Any changes in pH represent potential interactions and degradation, impacting the product's quality (Smaoui et al. 2017). Most cosmetic and pharmaceutical creams are formulated as either "Oil in Water" (O/W) or "Water in Oil" (W/O) emulsions. Oil in water emulsion is non-greasy and is easily removable from the skin surface.

In contrast, water in oil emulsion is greasy and not water-washable. Water-soluble medications are released more rapidly from oil-in-water emulsions (Barkat et al. 2011). Caffeine is water soluble; thus, the creams in our study are formulated as O/W emulsions. The instability of emulsion can be due to various factors such as phase inversion, creaming (or sedimentation), coalescence, or breaking (Masmoudi et al. 2005). This research subjected the formulations to different storage conditions, including 8, 25, and 40°C. The formulations were examined for changes in pH, color, and phase separation. The pH of samples was found to be acidic to slightly basic. The pH of the formulations remained relatively constant during the study. The prepared creams containing chyuri butter were white. Their appearance did not change at 8±2°C, 25±2°C, and 40±2°C except for formulation F1, which showed non-homogeneity with grittiness at 8±2°C. The change in appearance in F1 appeared from the 14th day and persisted up to the 21st day of the analysis period. The alteration in the appearance of F1 throughout the observation period could likely be attributed to the separation of the oily phase, which was encouraged by low temperatures. Moreover, formulation F1 did not contain chyuri butter as an emollient, which suggests that *chyuri* butter increases the stability of the formulation.

This study found creams' acid and saponification values between 9.53-11.21 and 23.47-26.77, respectively (Figure 4). Cream with 1% caffeine exhibited an acid value of 10.77±0.46 and a saponification value of 26.76±0.46 (Figure 4). In a previous study, a caffeinated fairness cream with 1% caffeine was formulated, demonstrating an acid value of 5.9 and a saponification value of 25.7 (Panda and Mishra 2018). The disparity in acid value and saponification value could be attributed to variations in the ingredients utilized in the formulations. The saponification value of the formulation increases with a higher concentration of fatty acids, and the formulation has more chance of microbial degradation (Saraf et al. 2010). In our investigation, we discovered that the saponification value of the cream (Figure 4) increased with the addition of chyuri butter; therefore, creams containing chyuri butter were more prone to microbial growth than creams not containing *chyuri* butter.

Our study found that the in vitro occlusivity percentage of creams increased with adding *chyuri* butter. The improved occlusivity observed in creams containing chyuri butter can be due to the enhanced lipid content of the developed formulations, leading to more effective prevention of water evaporation (Maru and Lahoti 2018). Thus, *chyuri* butter can moisturize and soften the skin, which helps to provide a protective barrier on the skin's surface, helping to retain moisture and prevent dryness, making the skin feel smoother. Thus, it can be used as an emollient on skin care products.

There was no phase separation of all the formulations on centrifugation, indicating that the emulsion forming the cream was stable (Figure 3). This could result from the appropriate homogenization speed during the emulsion formulation, which could have prevented the formulations from breaking on centrifugation (Nour and Yunus 2006). Hence, the findings revealed potential possibilities for utilizing caffeine and chyuri butter in cosmetics creams. The discovery of novel biological properties of caffeine is expected to expand caffeine utilization in the fields of cosmetology and dermatology in the future (Herman and Herman 2012). The study suggests that an O/W cream formulated with stearic acid, cetostearyl alcohol, triethanolamine, glycerol, propylparaben, distilled water, 20% chyuri butter, and 0.5-1% caffeine yields stable creams with good physical characteristics.

Future studies should focus on validating the chemical and physical stability of these formulations containing caffeine and chiuri butter at different climactic and storage conditions using rheological and quantitative chemical analysis techniques such as HPLC (Guaratini et al. 2006; Temova Rakuša et al. 2021). While the negative impacts of ingested caffeine are well documented, the side effects of topical applications require more extensive investigation. Its efficacy and potential toxicity on various skin types as indicated by Baumann Skin Type Indicator (BSTI) and interactions of caffeinated facial creams with other skin care products or medication need to be further investigated (Baumann 2008; Visconti et al. 2020; Gajbhiye and Pal 2024). Ethical issues must be strongly considered following the principles of “four Rs” (Reduction, Refinement, Replacement and Responsibility) for studies that require animal experimentation (Kiani et al. 2022).

Diploknema butyracea (chiuri) plant is primarily found in the sub-Himalayan regions and is native to Nepal, India, and Bhutan. Beside its ethnomedicinal uses, many commercial ventures have explored creating a range of products, including *chyuri* syrup, *chyuri* jam, and *chyuri* cream. Medicinal plants are disappearing rapidly, necessitating effective conservation and sustainable use strategies. Ecological and environmental implications of harvesting chiuri plant need to considered for sustainable practices and minimize environmental impacts.

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