

PHYSICOCHEMICAL STANDARDIZATION AND DESIGN OF GHEE-BASE TOPICAL SYSTEM FOR DERMAL REVITALIZATION

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ABSTRACT

Skin dehydration, hyperkeratosis, and barrier impairment present common dermatological challenges. Combining traditional cow ghee (rich in lipids and vitamins A, E, and K2) with urea (a potent humectant and keratolytic) offers a dual-action mechanism for exfoliation and barrier restoration. homemade A2 Gir cow ghee was prepared via the traditional cream-route method and compared to commercial brands (Amul and Gowardhan) using comprehensive physicochemical tests, UV-spectrophotometry, and TLC. Validated urea was then incorporated into two distinct topical systems: Shata Dhauta Ghrita (SDG) and a Water-in-Oil (W/O) emulsion, utilizing coconut oil, propylene glycol, and clove oil. Both formulations underwent rheological, stability, and bioactivity testing. While all ghee samples met standard quality benchmarks, the homemade variant demonstrated superior

freshness (lowest peroxide value) and higher natural Vitamin K2 content. Among the formulations, SDG displayed superior homogeneity, Spreadability, optimal viscosity, smooth texture, and a skin-compatible pH compared to the W/O emulsion. DPPH and protein solubilization assays verified robust antioxidant and exfoliating performance for both preparations. Ghee-based topical systems, specifically the traditional SDG formulation, hold strong potential for skin barrier restoration and natural dermal revitalization.

KEYWORDS: Shata Dhauta Ghrita, Water-in-Oil emulsion, Urea, Antioxidant activity, Topical formulation, Moisturizing activity, Keratolytic agent.

INTRODUCTION

Ghee is a type of clarified butter that originated in India and is widely used in South Asian cuisine, traditional medicine (Ayurveda), and Hindu religious rituals. It is made by simmering regular butter until the water evaporates and the milk solids separate and brown, which gives it a distinct nutty, slightly sweet, and caramelized aroma and flavour. cow ghee is highly compatible with human skin lipids. It contains essential fat-soluble vitamins such as Vitamin A, D, E, and K, alongside natural antioxidants like Conjugated Linoleic Acid (CLA). In Ayurvedic pharmaceuticals, ghee is known as a *Samskara-anuvartana*, meaning it has the unique ability to inherit the properties of medicinal herbs without losing its own therapeutic value. For skin health, cow ghee acts as a powerful emollient, penetrating deep into the dermal layers to provide nourishment and restore the skin's natural barrier.

Home-made ghee preparation: Homemade cow ghee is prepared using the Cream-Route Method, starting with a 15-day collection of Malai from boiled and chilled A2 Gir cow milk, stored in the freezer to maintain freshness. On preparation day, the thawed cream is churned with ice-cold water to separate solid Makkhan (butter) from buttermilk. The butter clumps are washed repeatedly with cold water to enhance aroma and shelf life. During clarification, the butter is melted over low-medium heat until water evaporates and milk solids turn golden-brown, leaving a transparent liquid. Finally, the ghee is filtered into a glass jar, where it develops its characteristic grainy, semi-solid texture as it cools, serving as a pharmaceutical-grade lipid base.^[1,2]

Dermal Health: The skin serves as the body's primary defence mechanism. However, environmental stressors, aging, and certain conditions (like eczema or psoriasis) lead to two major issues: Dehydration and Hyperkeratosis.

The Need for Moisturization: When the Stratum Corneum (the outermost skin layer) loses water, the skin becomes brittle, itchy, and prone to cracking. Cow ghee acts as an occlusive moisturizer, forming a thin protective film that prevents Trans-Epidermal Water Loss (TEWL).

The Need for Exfoliation: Healthy skin naturally sheds dead cells, but in dry or diseased skin, these cells accumulate, making the surface rough and preventing the absorption of nutrients. Urea is the gold standard for addressed this. As a keratolytic agent, it breaks the

hydrogen bonds in skin proteins (keratin), effectively "dissolving" the dead skin buildup and allowing new, healthy skin to emerge.^[3]

The Combination of Urea and Ghee

Combining Urea and Ghee creates a dual-action therapeutic system. While Urea exfoliates and hydrates by "opening up" the skin, Ghee provides the necessary lipids to seal that moisture in and soothe the freshly exfoliated surface. This combination is particularly effective for "Dermal Revitalization," transforming dry, calloused skin into a soft, supple texture.

Different Dosage Forms of Urea and Ghee

The combination of Urea and Ghee can be formulated into various topical systems depending on the required site of action and skin type:

1. Shata Dhauta Ghrita (SDG)
2. Water-in-Oil (W/O) Emulsions
3. Ghee-Based Ointments
4. Lipid Micro-particles

Marketed Formulations

1. **Shata Dhauta Ghrita Creams:** Brands like *Arya Vaidya Sala (Kottakkal)* and various dermo-cosmetic startups (e.g., *100-Washed Ghee Creams*) market this for skin healing and anti-aging.
2. **Urea Creams (20%):** Standard pharmaceutical products like *Cotaryl*, *Logifeel*, or *Ureka* are widely used for hyperkeratosis.
3. **Ghee-Integrated Dermal Products:** Modern "Clean Beauty" brands are now incorporating "Cow Ghee" as a primary ingredient in intensive moisturizers, often combining it with chemical exfoliants like Lactic Acid or Urea to mimic traditional healing properties with modern stability.^[4]

Materials: The materials used in the present research work included various chemicals, solvents, reagents, and laboratory apparatus required for physicochemical evaluation, formulation development, and characterization studies. The chemicals and solvents used were cow ghee, liquid paraffin, concentrated hydrochloric acid, petroleum ether, ethanol, phenolphthalein indicator, sodium hydroxide (NaOH), alcoholic potassium hydroxide (KOH), glacial acetic acid, chloroform, saturated potassium iodide (KI), sodium thiosulphate

($\text{Na}_2\text{S}_2\text{O}_3$), starch indicator solution, n-hexane, alpha-dipyridyl solution, ferric chloride (FeCl_3), Vitamin K2 standard, glycerol, sulfuric acid, acetone, DPPH reagent, methanol, ascorbic acid, coconut oil, beeswax, propylene glycol, clove oil, casein, trichloroacetic acid (TCA), phosphate buffered saline (PBS), and urea. The formulations prepared included Shata Dhauta Ghrita and Water-in-Oil (W/O) emulsion formulations. The laboratory apparatus and instruments used during the study included a 250 mL conical flask, burette, analytical weighing balance, water bath, measuring cylinder, round bottom flask (RBF), reflux condenser, iodine flask, test tubes, cuvettes, UV-visible spectrophotometer, beakers, silica gel TLC plates, Bunsen burner, distillation apparatus, condenser, and vacuum pump.

METHODS

Preparation of Homemade cow ghee

To prepare ghee from milk cream collected over 15 days, you are using the Cream-Route Method (specifically the Direct Cream or Creamery Butter method). This is the most common household technique for larger yields.

Procedure: The 15-Day Cream Method

Collection Phase (Days 1–15)

- **Boil & Chill:** Boil 1 liter of A2 Gir cow milk daily, cool to room temperature, then refrigerate for 4–6 hours to form a thick cream (Malai) layer. Repeat for 15 days.
- **Storage:** Collect the cream in an airtight glass or steel container.
- **Preservation:** Store the container in the freezer for 15 days to prevent rancidity or fungal growth.

Thawing and Churning (Preparation Day)

- **Thaw:** Remove the frozen cream and keep it at room temperature for 6–8 hours until soft.
- **Churn:** Transfer the cream to a vessel or blender and add ice-cold water.
- **The Break:** Churn for 5–10 minutes until solid yellow butter (Makkhan) separates from the buttermilk.
- **Washing:** Drain the buttermilk and wash the butter 2–3 times with cold water until clear for better aroma and shelf life.

Clarification (Heating & Boiling)

1. **Melt:** Place the washed butter in a heavy-bottomed pot over a low-medium flame.

2. Simmer: The butter will melt and start to froth/boil. The water will evaporate (sizzling sound), and the milk solids will begin to separate.

3. Watch: Once the sizzling stops and the foam clears, look at the bottom. The milk solids (residue) should be a light golden-brown. The liquid should be transparent golden.

4. Turn off: Immediately remove from heat. The residual heat will continue to cook the ghee; if you wait too long, it will burn.



Figure No. 1: Preparation of Home-made.

Filtering and Storage

Filter: Let it cool for 15–20 minutes, strain through a fine metal sieve or a double-layered muslin cloth into a clean, dry glass jar.

Settle: Do not close the lid until it reaches room temperature. It will turn into a grainy, semi-solid texture.^[5]

4.2 Physicochemical test for 3 cow ghee sample (Home-made ghee, Gowardhan ghee, Amul ghee, Comparative Study).

Table No. 1: List of Physicochemical test for 3 cow ghee sample.

1. Melting point	2. Boiling point
3. Moisture content	4. Acid value [Acidity]
5. Saponification value	6. Peroxide value
7. UV-Spectrophotometric test (Vitamin A)	8. UV-Spectrophotometric test (Vitamin E)
9. UV-Spectrophotometric test (Vitamin K2)	10. Thin Layer Chromatography test
11. RM Value	12. Soap Determination
13. Ash content	14. Antioxidant test

4.2.1 Melting point

Gently heat the ghee until fully liquid. Fill the capillary tube with the liquid ghee and chill it at 4°C–10°C. Heat gradually near the expected melting range (32°C–35°C). Observe carefully and record the temperature at which the ghee becomes transparent.^[6]



Figure no.2 Melting point.

4.2.2 Boiling point

Fill a fusion tube with 1–2 mL of the liquid sample. Seal one end of a capillary tube by heating and place it in the fusion tube with the open end downward. Heat the sample until continuous bubbles appear from the capillary tube. Switch off the heat and record the temperature when bubbling stops and the liquid rises into the capillary tube as the boiling point.^[4]



Figure No. 3: Boiling point.

4.2.3 Moisture content:

Step 1: Preparation of Apparatus: Wash and dry the porcelain dish at 105°C for 30 minutes. Cool in a desiccator and weigh accurately as W1.

Step 2: Sample Preparation: Weigh about 1 g of ghee sample into the dish and record the weight as W2.

Step 3: Heating and Drying: Heat the dish at 105°C for 1 hour. Cool in a desiccator and weigh. Repeat heating for 30 minutes, cooling, and weighing until constant weight is obtained. Record the final weight as W3.^[4]



Figure No. 4: Moisture content.



Figure No. 5: Acid value.

4.2.4 Acid Value [Acidity]

Step 1: Preparation of Solvent: Mix 10 mL ethanol (95%) with 0.2 mL phenolphthalein in a conical flask. Heat to boiling and titrate with 0.1 N NaOH until a faint pink colour persists for 15 seconds to neutralize.

Step 2: Sample Preparation: Weigh about 1 g of ghee into the neutralized solvent. Shake well and heat on a water bath until completely dissolved.

Step 3: Titration: While hot, titrate with 0.1 N NaOH, shaking continuously. Record the volume when a persistent faint pink colour appears as the endpoint.^[4]

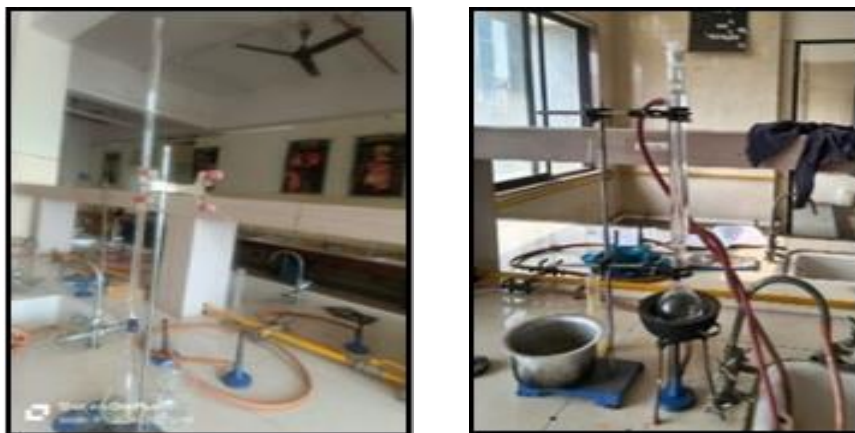


Figure No. 6: Reflux and titration.

4.2.5 Saponification Value

Step 1: Sample Preparation: Weigh about 1 g of ghee into an RBF and add 25 mL of 0.1 N alcoholic KOH.

Step 2: Refluxing: Attach a reflux condenser and heat on a boiling water bath for 45 minutes until the solution becomes clear and completely saponified.

Step 3: Titration: Close slightly and transfer the solution to a conical flask. Add 1 mL phenolphthalein and titrate the hot excess alkali with 1 N HCl until the pink colour disappears. Record the volume as V₂. Perform a blank titration using 25 mL of 0.1 N alcoholic KOH.^[4]



Figure No. 7: Peroxide Value.

4.2.6 Peroxide Value [Iodometric method]

Step 1: Sample Preparation: Weigh about 0.5 g of ghee into a dry 250 mL iodine flask. Add 30 mL mixture of Glacial Acetic Acid and Chloroform (3:2) and swirl until dissolved.

Step 2: Reaction with Potassium Iodide: Add 0.5 mL saturated Potassium Iodide solution and keep the flask in dark for 1 minute with occasional shaking. Add 30 mL distilled water to stop the reaction.

Step 3: Titration: Titrate with 0.01 N Sodium Thiosulphate until the yellow colour nearly disappears. Add 0.5 mL starch indicator and continue titration until the blue colour disappears. Record the volume as V. Perform a blank titration without ghee sample and record as V₀.^[4]

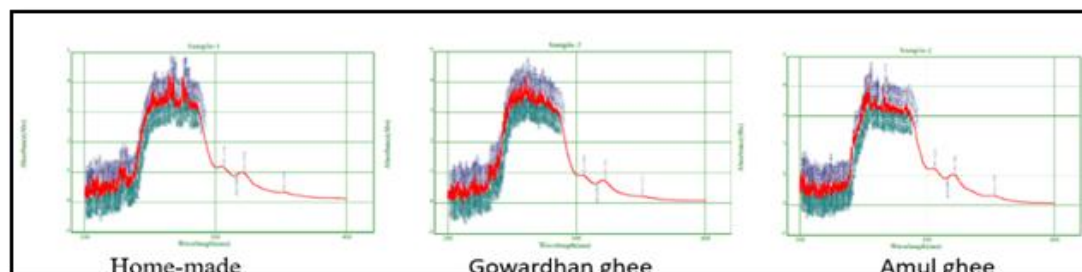


Figure No. 8: Vitamin A (Wavelength).

4.2.7 UV-Spectrophotometric test for Vitamin A (Retinol)

Step 1: Sample preparation

Weigh 0.5 g of ghee sample in test tube and dilute it with 10ml of n-Hexane.

Step 2: Wavelength selection

In Spectrophotometer select the wavelength of 325nm. take absorbance of all ghee samples.^[7]



Figure No. 9: Vitamin E (UV-Spectro).

4.2.8 UV-Spectrophotometric test for Vitamin E (Tocopherol) [Emmerie Engel method]

Step 1: Sample Preparation: Weigh 1g of the ghee sample into a test tube and dissolve it in 10 mL of n-Hexane.

Step 2: Color Development: Add 2mL of alpha, alpha-dipyridyl solution followed by 0.5mL of Ferric chloride solution. The tocopherol reduces FeCl₃ to FeCl₂, which reacts with dipyrindyl to form a red/pink complex.

Step 3: Wavelength Selection: Set the spectrophotometer to 520 nm. Measure the absorbance of the sample (A_s) against a reagent blank (A_b).^[5]



Figure No. 10: Vitamin K2 (UV-Spectro).

4.2.9 UV-Spectrophotometric test for Vitamin K2 (Menaquinone)

Step 1: Sample Preparation: Weigh 5g of the ghee sample. Dissolve it in 15mL of n-Hexane. Since K2 levels are much lower than Vitamin E, a larger sample is often required.

Step 2: Extraction: Vitamin K2 is highly lipophilic. Use a solvent extraction (liquid-liquid) or simple dilution in n-hexane.

Step 3: Wavelength Selection: Set the spectrophotometer to 248 nm. Measure the absorbance of the sample (A_s) against a hexane blank (A_b).^[8]

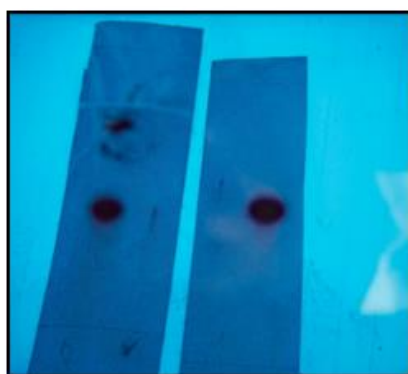


Figure No. 11: TLC for vit k2.

4.2.10 Thin layer Chromatography test for Vitamin K2 (Menaquinone):

1. Dissolve 1 g of ghee in 10 ml KOH and heat for 30 min to convert fat into soap while keeping vitamin K2 intact.
2. Add 10 ml n-hexane, transfer the cooled mixture to a separating funnel, shake gently for 1–2 min, and separate the organic layer.
3. Prepare the mobile phase by mixing 19 ml n-hexane with 1 ml ethyl acetate.

4. Spot the sample and standard on the TLC plate using a capillary tube, keeping 2 cm from the bottom.
5. Develop the plate in the mobile phase until the solvent reaches about $\frac{3}{4}$.
6. Visualize under UV light (254 nm); vitamin K₂ appears as a dark quenching spot.^[6]

4.2.11 Reichert-Meissl (RM) Value:

Step 1: Saponification

Weigh 1 g of ghee into a distillation flask. Add 16 mL glycerol and 2 mL of 50% NaOH. Heat with continuous swirling until the solution becomes clear.

Step 2: Acidification and Distillation

Cool the flask and add 90 mL boiling distilled water, 50 mL dilute Sulfuric Acid, and porcelain pieces. Connect to the distillation apparatus and collect 110 mL distillate in 20 minutes.

Step 3: Filtration and Titration

Filter the distillate and pipette 100 mL filtrate into a conical flask. Add 0.5 mL phenolphthalein and titrate with 1 N NaOH until a faint pink colour appears. Record as V₂. Perform a blank titration without the sample and record as V₁.^[9]

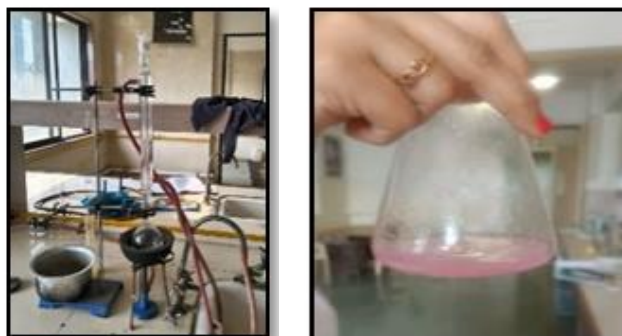


Figure No. 12: RM Value.

4.2.12 Determination of Soap content in ghee

Step 1: Test for Presence (Qualitative)

Take 2 g of ghee in a test tube and add 2 mL hot distilled water. Shake well and allow the layers to separate. Add a few drops of phenolphthalein to the aqueous layer. Pink colour indicates presence of soap; colourless solution indicates absence of soap.

Step 2: Quantitative Determination (Titration)

Weigh 4 g of ghee into a flask. Add 0.5 mL water and heat until melted. Add 5 mL acetone (2% water) and 0.5 mL phenolphthalein. If pink colour appears, titrate with 0.01 N HCl until the pink colour disappears.^[4]



Figure No. 13: Soap content.

4.2.13 Antioxidant Test

1. Reagent Preparation: Prepare a 0.1M solution of DPPH in methanol. Keep it in the dark (it is light-sensitive).

2. Sample Preparation: Dissolve 1g of ghee in 10 mL of ethyl acetate.

3. Reaction: Mix 1mL of the ghee solution with 2mL of the DPPH solution.

4. Incubation: Keep the mixture in the dark at room temperature for 30 minutes.

5. Measurement: Measure the absorbance at 517nm using a spectrophotometer.

Blank: Methanol only, Control: DPPH solution + solvent (without ghee).^[4]

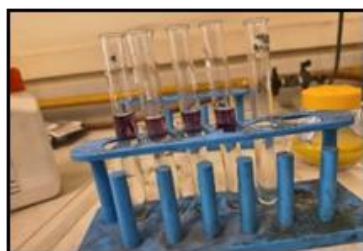


Figure No. 14: Antioxidant.

4.2.14 Ash content/Ash value:**Step 1: Crucible Preparation**

Take a crucible, dry it, and weigh accurately as W1.

Step 2: Sample Weighing and Pre-ashing

Weigh about 5 g of ghee into the crucible and record as W2. Heat it gently until the sample chars and continue heating for ½–1 hour until white or light grey ash is obtained. Cool in a desiccator and weigh. Repeat heating and cooling until its constant weight is achieved and record the final weight as W3.^[4]

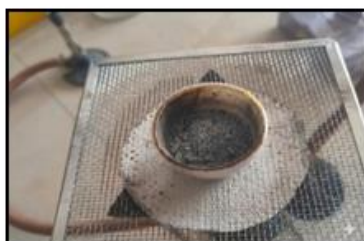


Figure No. 15: Ash value.

4.3 Physicochemical test for Urea

4.3.1 Melting point

Gently heat the ghee until fully liquid. Fill the capillary tube with the liquid ghee and chill it at 4°C–10°C. Heat gradually near the expected melting range (32°C–35°C). Observe carefully and record the temperature at which the ghee becomes transparent.^[4]

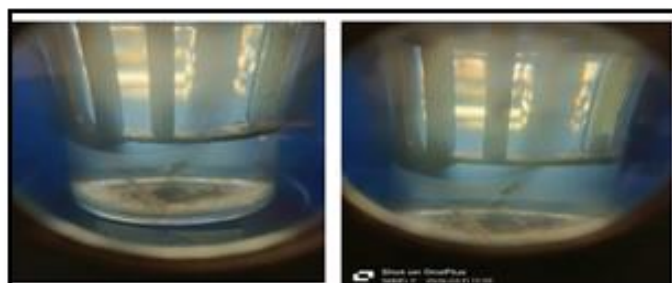


Figure No. 16: Melting point.

4.3.2 Solubility

Labeling three separate test tubes as A (water), B (ethanol), and C (acetone) introduce approximately 1–2 g of urea into each tube, followed by the addition of 10 ml. Stir each mixture gently for 1 to 2 minutes using a glass rod or by swirling the tube to encourage dissolution. Finally, observe each solution carefully to determine the degree of solubility.^[4]



Figure No. 17: Solubility.

4.3.3 Biuret test

Weigh 2 g of urea into a dry test tube and heat gently until it melts and begins to solidify again, producing ammonia due to biuret formation. After cooling, dissolve the residue in 2–3 mL distilled water. Add 1–2 mL dilute NaOH followed by a few drops of 1% CuSO_4 solution. Shake well and observe the violet or purple colour, confirming the presence of biuret and thermal decomposition of urea.^[10]



Figure No. 18: Biuret test.

4.3.4 Assay of Nitrogen Content (Quantitative)

Stage 1: Digestion

Weigh 0.5 g of urea sample into a Kjeldahl flask. Add 20 mL concentrated H_2SO_4 and catalyst mixture (Potassium Sulphate and Copper Sulphate). Heat until the mixture turns pale green, converting nitrogen into ammonium ions.

Stage2: Distillation

Dilute the cooled digest with distilled water and add excess NaOH to release ammonia gas. Connect the flask to a condenser with a receiving flask containing Boric Acid. Heat the mixture so ammonia is distilled and trapped in the boric acid solution.

Stage3: Titration

Titrate the ammonia-borate complex with standard HCl or H₂SO₄ until the endpoint colour appears, indicating completion of the reaction.^[11]



Figure No. 19: Assay of nitrogen.

4.3.5 Sodium Hypobromite Test**Preparation of Sodium Hypobromite Reagent**

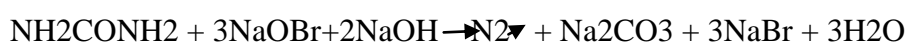
Prepare the reagent freshly by adding a few drops of bromine water to 2 mL sodium hydroxide solution until a slight yellow colour persists.

Sample Preparation and Reaction

Dissolve about 0.5 g of urea in 2 mL distilled water in a separate test tube. Add the freshly prepared sodium hypobromite reagent to the urea solution.^[4]



Figure no.20 Effervescence



4.4 Preparation of Formulation: The formulation of topical medicaments requires a stable vehicle that ensures both the solubility of the active pharmaceutical ingredient (API) and its

effective penetration through the skin barrier. This project investigates two distinct types of dermatological bases for the delivery of Urea: the traditional Shata Dhauta Ghrita (SDG) and a standard Water-in-Oil (W/O) emulsion.

4.4.1 Method 1: Shata Dhauta ghrita method (SDG)

Table No. 2: Formulation Table Traditional Shata Dhauta Ghrita (SDG) Method 100g.

Sr. no.	Ingredient	Quantity for 100g	Role in Formulation
1.	Home-made Cow Ghee	60g	Primary emollient base
2.	Coconut Oil	10g	Co-base & nourishing agent
3.	Urea	20g	Active Keratolytic agent
4.	Propylene Glycol	8g	Vehicle for urea slurry
5.	Clove Oil	2g	Aromatic / Natural preservative
6.	Cold Water	q.s	Washing medium

- 1. Base Preparation:** Ghee and Coconut Oil were melted together in a beaker and mixed thoroughly.
- 2. Washing Process (SDG):** The melted mixture was placed on a flat steel plate, a small amount of cold water was added, and the mixture was rubbed in circular motions for 1–2 minutes using the flat bottom of a vessel. The water was then drained. This process was repeated 100 times until the ghee transformed into a white, fluffy, and smooth texture.
- 3. Slurry Preparation:** In a separate small beaker, the powdered urea was mixed with Propylene Glycol and Clove Oil to form a smooth, lump-free slurry.
- 4. Final Incorporation:** The urea slurry was gradually added to the prepared SDG base. The mixture was blended thoroughly until a homogenous topical cream was formed. The resulting formulation was poured into roll-on bottles and left undisturbed for several hours to set.^[12,13]



Figure No. 21: SDG formulation.

4.4.2 Method 2: Water in oil method

Table No. 2: Formulation Table Modern Water-in-Oil (W/O) 100g

Sr. no.	Ingredients	Quantity for 100g	Role in Formulation
1.	Home-made Cow Ghee	50g	Lipid phase / Emollient
2.	Coconut Oil	8g	Lipid phase / Softener
3.	Beeswax	5g	Structuring/Hardening agent
4.	Propylene Glycol	10g	Solubilizing agent
5.	Clove Oil	2g	Preservative and aromatic
6.	Liquid paraffine	5g	Emollient
7.	Urea	20g	Active Keratolytic agent

1. Preparation of Urea: The urea crystals were placed in a mortar and pestle and ground into an exceptionally fine powder to ensure smooth incorporation.

2. Solubilization: The powdered urea was dissolved in Propylene Glycol (PG) to create a uniform urea-PG.

3. Melting of Oil Phase: The base components, consisting of Ghee, Coconut Oil, and Beeswax, were combined in a beaker and melted together using a water bath until a homogenous liquid phase.

4. Mixing: The urea-PG concentrate was added to the melted oil phase. The mixture was stirred continuously to ensure the urea was evenly distributed throughout the base.

5. Phase Combination: The remaining oil phase was incorporated slowly while maintaining constant agitation.

6. Finishing: Finally, Clove Oil was added as a preservative and aromatic agent. The resulting formulation was poured into roll-on bottles and left undisturbed for several hours to set.^[14]

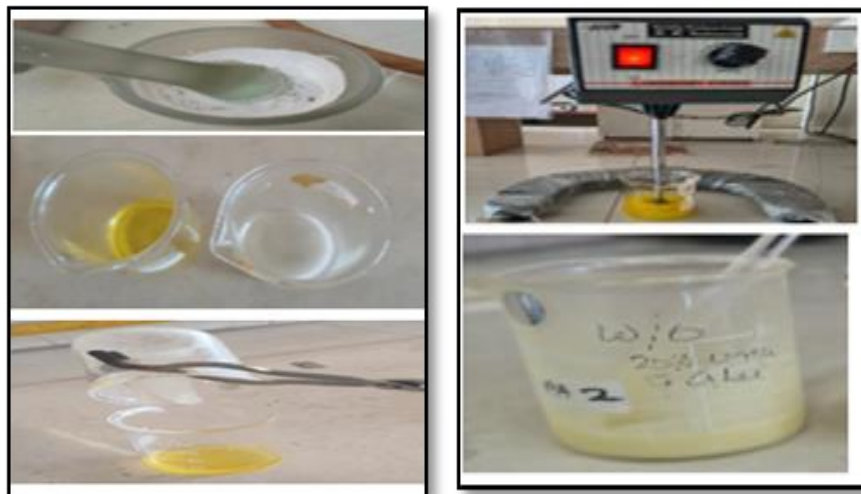


Figure No. 22: W/O Formulation.

4.5 Comparative Evaluation test of for both formulation^[15]

4.5.1 Solubility Test

Take a small amount of the formulation and try to disperse it in a test tube of distilled water, and observe the solubility.^[4]



Figure No. 23: Solubility test.

4.5.2 pH Test

Dissolve 1g of the formulation in 10ml of distilled water for both the formulations. Measure using a pH paper, note the observed value.^[4]

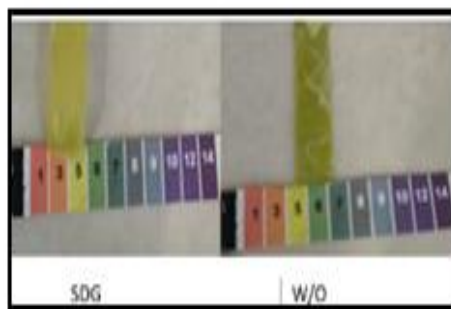


Figure No. 24: pH Test.

4.5.3 Centrifugation Test

Weigh 1g of both sample in a centrifuge tube and place it in centrifuge at 3000RPM for 30 minutes, after 30 minutes observe the phase separation.^[4]



Figure no.25 Centrifugation Test

4.5.4 Spreadability Test

A small amount of cream is placed between two glass slides and compressed with a specific weight to form a uniform film. The bottom slide is fixed, while a standardized weight is attached to the upper slide through a pulley system. Spreadability is determined by measuring the time taken for the upper slide to move a fixed distance.



Figure No. 26: Spreadability test.

4.5.5 Melting Point

Gently heat the ghee until fully liquid. Fill the capillary tube with the Formulation. Heat gradually near the expected melting range (32°C–35°C). Observe carefully and record the temperature at which the ghee becomes transparent.^[4]

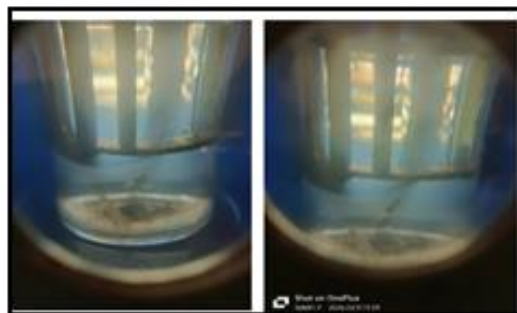


Figure No. 27: Melting point.

4.5.6 Viscosity Test

The formulation is first melted until it forms a clear, uniform liquid and is maintained at temperature to ensure consistency. The liquid is then transferred into a beaker, and the appropriate spindle (typically Spindle 1 for thinner liquids) is attached to the viscometer and lowered into the sample until the formulation reaches the immersion mark on the spindle shaft. After setting the rotation speed, often at 60 RPM to ensure the torque remains above the required 10% threshold, the instrument is started. Once the spindle has rotated for approximately 30 seconds and the digital reading has stabilized, the final value is recorded in centipoise (cP), representing the dynamic viscosity of the formulation.^[4]



Figure No. 28: Viscosity.

4.5.7 Microscopic Graininess Analysis (Particle Size)

1. Place a small drop of the sample on a clean glass slide and cover with a coverslip using light pressure to preserve the crystals.
2. Observe under a compound microscope at 10× magnification, then switch to 40× for detailed examination.
3. Measure and record crystal dimensions using a calibrated ocular micrometer to evaluate the granular texture and quality of the ghee.

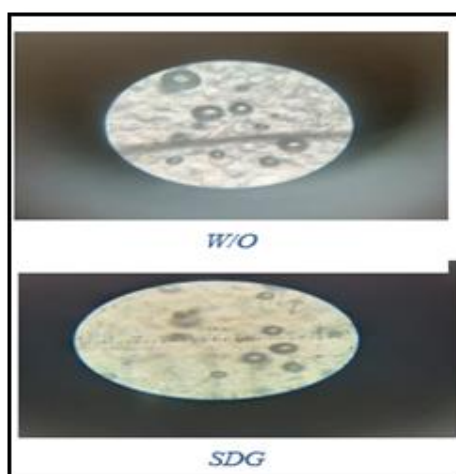


Figure No. 29: Microscopic.

4.5.8 Luster Test

Weigh 2g of the sample is placed onto a black matte surface or a clean glass tile to provide a high-contrast background. A light source is then positioned at a 45° angle relative to the sample, which allows for the optimal reflection of light off the surface of the cream.^[16]

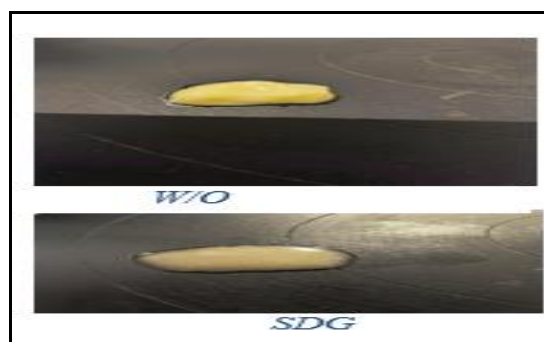


Figure No. 30: Luster Test.

4.5.9 Protein Solubilization Test (Exfoliation)

1. Prepare a standard protein solution by dissolving 0.1 mg/mL casein in 100 mL distilled

water.

2. Mix 1 g of urea cream with 10 mL of the protein solution thoroughly.
3. Maintain the temperature below 30°C during the reaction.
4. Add 10 mL Trichloroacetic Acid (TCA) to precipitate unreacted protein.
5. Centrifuge the mixture for 10 minutes to separate the precipitated protein.
6. Collect the supernatant containing solubilized protein to evaluate the exfoliating efficiency of the formulation.^[17]

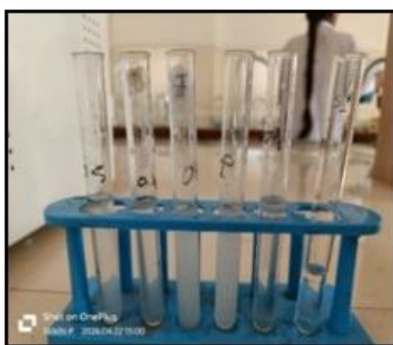


Figure No. 31: Exfoliation test.

4.5.10 DPPH Test (Antioxidant Test)

1. Prepare 0.1 mM DPPH solution by dissolving 3.94 mg DPPH in 100 mL ethanol or methanol and store in darkness. Prepare a 1000 µg/mL stock solution and dilute to obtain concentrations of 500, 250, 125, 62.5, and 31.25 µg/mL.
2. Mix 2 mL of each sample dilution with 2 mL DPPH solution. Prepare the same for Ascorbic Acid standard and use solvent with DPPH as control. Incubate all tubes in darkness for 30 minutes at room temperature.
3. Measure absorbance at 517 nm using ethanol as blank. Calculate % inhibition from control, sample, and standard absorbance values, and plot concentration vs % inhibition to determine the IC₅₀ value.^[18]



Figure No. 32: DPPH test.

4.5.11 Franz diffusion Test

1. The in vitro drug release study was performed using a Franz Diffusion Cell with a diffusion area of 1.76 cm² and receptor volume of 12.0 ml.
2. Phosphate Buffered Saline (PBS, pH 7.4) was degassed before use.
3. A fresh onion epidermal membrane was used as a semi-permeable bio-membrane surrogate for human skin.
4. A magnetic bead was placed in the receptor chamber for continuous stirring at 600 RPM, and the chamber was filled with degassed PBS.
5. The onion membrane was mounted between donor and receptor compartments and clamped securely.
6. The system was equilibrated for 15 minutes at 32 ± 0.5°C.
7. After equilibration, 1.0 g of formulation was applied on the membrane surface.
8. Samples of 1.0 mL were withdrawn at 15, 30, 45, 60, 90, and 120 minutes for UV-spectroscopy analysis.
9. Each sample withdrawn was replaced with equal volume of fresh pre-warmed receptor medium to maintain sink conditions.^[19,20]

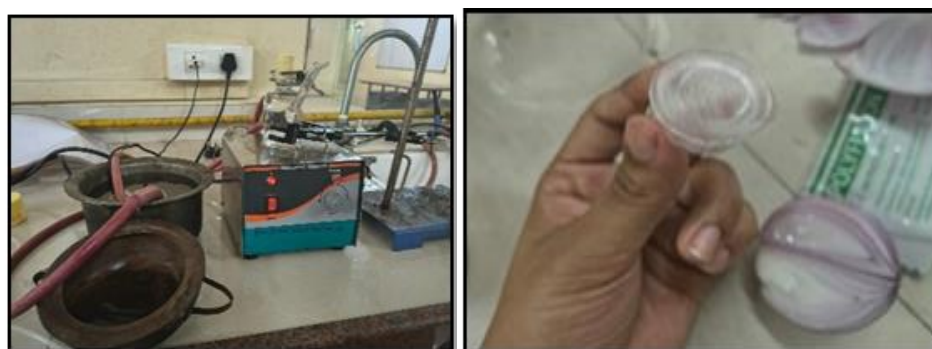


Figure No. 33: Franz diffusion.

RESULT AND DISCUSSION

We discuss here all the observation, results and conclusion here for all the test:

Observation Tables of Comparison of Physicochemical test for 3 ghee samples

Melting point

Table No. 3: Observation table of melting point of ghee.

Sr. no.	Sample	Observed value	Standard value
1.	Homemade ghee	32°C	32°C-37°C
2.	Gowardhan ghee	33°C	33°C-35°C
3.	Amul ghee	32°C	31°C-35°C

Conclusion: The analysis confirms that both the homemade and commercial brands are compliant with their specific standard values.

Result: All three samples are authentic and pure, as their melting points successfully meet the required quality standards

Boiling point

Table No. 4: Observation table of boiling point of ghee.

Sr. no.	Sample	Observed value	Standard value
1.	Homemade ghee	245°C	~250°C
2.	Gowardhan ghee	253°C	~252°C
3.	Amul ghee	251°C	~250°C

Conclusion: The observed values align closely with the standard benchmarks, confirming that both the homemade and commercial brands possess the high thermal stability expected of pure ghee.

Result: All three samples successfully meet the standard smoke point requirements.

Moisture contain

Table No. 5: Observation table of moisture contain of ghee.

Sr. no.	Sample	Weight of empty dish W1	Weight of dish with 1g sample W2	Final constant weight W3	Observed moisture%
1	Homemade ghee	67.14g	68.14g	68.07g	0.07%
2	Gowardhan ghee	67.89g	68.89g	68.80g	0.1%
3	Amul ghee	62.61g	63.61g	63.56g	0.1%

Conclusion: The moisture content in all samples is well within the standard limit for pure ghee (typically below 0.3%–0.5%). This confirms high quality and a longer shelf life, as low moisture prevents rancidity.

Result: All three samples show very low moisture levels, ranging from 0.07% to 0.1%.

Acid Value [Acidity]

Table No. 6: Observation table of acid value titration of ghee.

Sr.no.	Sample	Initial	Final	Mean
1	Homemade ghee	0ml	0.4ml	0.5ml
		0ml	0.6ml	
2	Gowardhan ghee	0ml	0.5ml	0.5ml
		0ml	0.5ml	
3	Amul ghee	0ml	0.2ml	0.3ml

		0ml	0.4ml	
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Table No. 7: Observation table of acid value of ghee.

Sr. no.	Sample Description	Weight of Sample (W)	Volume of 0.1 N NaOH (V)	Observed Acid Value
1	Homemade ghee	1g	0.5ml	0.2 mg NaOH/g
2	Gowardhan ghee	1g	0.5ml	0.2 mg NaOH/g
3	Amul ghee	1g	0.3ml	0.16 mg NaOH/g

Conclusion: All three samples are of excellent quality and freshness, as their acid values fall well below the standard limit (typically 0.5–0.6 mg NaOH/g).

Result: The observed acid values for Homemade, Gowardhan, and Amul ghee are 0.2, 0.2, and 0.16 mg NaOH/g respectively, indicating very low levels of free fatty acids.

Saponification Value

Table No. 8: Observation table of saponification value titration of ghee.

Sr. no.	Sample	Initial	Final	Mean
1	Blank	0ml	28.9ml	29.8m
		0ml	38.7ml	
2	Homemade ghee	0ml	25.3ml	25.4m
		0ml	25.6ml	
3	Gowardhan ghee	0ml	25.3ml	25.3m
		0ml	25.3ml	
4	Amul ghee	0ml	25.4ml	25.7m
		0ml	25.6ml	

Table No. 9: Observation table of saponification value.

Sr. no.	Sample Description	Weight (W)	Vol. of HCl (Blank V1)	Vol. of HCl (Sample V2)	Saponification Value
1	Homemade ghee	1g	29.8ml	25.4ml	246.4 mg KOH/g
2	Gowardhan ghee	1g	29.8ml	25.3ml	252 mg KOH/g
3	Amul ghee	1g	29.8ml	25.7ml	229.6 mg KOH/g

Conclusion: All three samples are pure and free from common adulterants. These values confirm the presence of the short-chain fatty acids characteristic of genuine milk fat.

Result: The saponification values for Homemade (246.4), Gowardhan (252), and Amul ghee (229.6) were found to be within the standard range for pure ghee (typically 225–230+ mg KOH/g).

Peroxide Value [Iodometric method]**Table No. 10: Observation table of peroxide value titration.**

Sr. no.	Sample	Initial	Final	Mean
1	Blank	0ml	0.4ml	0.5ml
		0ml	0.6ml	
2	Homemade ghee	0ml	1.2ml	1.3ml
		0ml	1.4ml	
3	Gowardhan ghee	0ml	1.7ml	1.8ml
		0ml	1.9ml	
4	Amul ghee	0ml	2.5ml	2.6ml
		0ml	2.7ml	

Table No. 11: Observation table of peroxide value.

Sr. no.	Sample Description	Weight (W)	Vol. of Na ₂ S ₂ O ₃ (Sample V)	Vol. of Na ₂ S ₂ O ₃ (Blank V ₀)	Observed Peroxide Value
1	Homemade ghee	0.5g	1.3ml	0.5ml	6mEq/kg
2	Gowardhan ghee	0.5g	1.8ml	0.5ml	26mEq/kg
3	Amul ghee	0.5g	2.6ml	0.5ml	42mEq/kg

Conclusion: Homemade ghee remains fresh and minimally oxidized, staying within the standard acceptable range for quality. High peroxide values in Gowardhan and Amul indicates significant oxidation.

Result: The observed peroxide values vary significantly across the samples, with Homemade ghee (6 mEq/kg) being the lowest, followed by Gowardhan (26 mEq/kg) and Amul (42 mEq/kg).

UV-Spectrophotometric test for Vitamin A (Retinol).**Table No. 12: Observation table UV-Spectrophotometric test for vitamin A absorbance.**

Sr. no.	Sample Description	Absorbance (As–Ab)	Vitamin A Content (IU/g)
1	Homemade ghee	0.4950	28.24
2	Gowardhan ghee	0.4302	24.54
3	Amul ghee	0.9034	51.55

Table No. 13: Observation table of UV-Spectrophotometric test for vitamin A.

Sr. no.	Sample	Observation(325nm)	Vitamin A (Retinol) IU/10g	Standard value	verdict
1	Homemade ghee	0.5198nm	14.355 IU/10g	15 – 20IU/10g	Natural
2	Gowardhan ghee	0.4550nm	12.475 IU/10g	15 – 20IU/10g	Slightly Low
3	Amul ghee	0.9282nm	26.198 IU/10g	15 – 20IU/10g	High

Conclusion: Homemade ghee showed the most natural balance. The slightly lower value in Gowardhan may be due to processing, while the higher value in Amul suggests vit. fortification beyond natural level.

Result: The vitamin analysis shows that Homemade ghee (143.55nm) is within the standard range, Gowardhan ghee (124.75nm) is marginally below the 25 IU/g benchmark, and Amul ghee (261.98nm) is significantly higher than the standard.

UV-Spectrophotometric test for Vitamin E (Tocopherol)

Table no. 14 Observation table of UV-Spectrophotometric test for vitamin E absorbance

Sample	As (Sample)	Ab (Blank)	Net Abs (As-Ab)	Calculated Value Vitamin E Content(μg)
Homemade Ghee	0.149	0.024	0.125	35.00
Gowardhan Ghee	0.126	0.024	0.102	28.56
Amul Ghee	0.174	0.024	0.150	42.00

Table No. 15: Observation table of UV-Spectrophotometric test for vitamin E.

Sr. No.	Sample	Observed Value 550nm	Calculation ($\mu\text{g/g}$)	Standard Value ($\mu\text{g/g}$)	Verdict
1.	Homemade Ghee	0.9061nm	37.16 $\mu\text{g/g}$	20–50 $\mu\text{g/g}$	Correct
2.	Gowardhan Ghee	0.8649nm	35.10 $\mu\text{g/g}$	20–50 $\mu\text{g/g}$	Correct
3.	Amul Ghee	1.6062nm	48.25 $\mu\text{g/g}$	20–50 $\mu\text{g/g}$	Correct

Conclusion: The analysis confirms that all tested samples meet the required nutritional benchmarks for this parameter (likely β -carotene or a specific mineral).

Result: All three samples fall within the standard nutritional range of 20–50 $\mu\text{g/g}$.

UV-Spectrophotometric test for Vitamin K2

Table No. 16: Observation table UV-Spectrophotometric test for vitamin K2 absorbance.

Sr. No.	Sample	As (sample)	Ab (Blank)	Net Abs (As-Ab)	Calculate
1.	Homemade Ghee	0.085nm	0.010nm	0.075nm	0.90 $\mu\text{g/g}$
2.	Gowardhan Ghee	0.062nm	0.010nm	0.052nm	0.62 $\mu\text{g/g}$
3.	Amul Ghee	0.078nm	0.010nm	0.068nm	0.81 $\mu\text{g/g}$

Table No. 17: Observation table of UV-Spectrophotometric test for vitamin K2.

Sr. No.	Sample	Vitamin K2 Content ($\mu\text{g/g}$)	Verdict
1.	Homemade Ghee	0.90 High ($\mu\text{g/g}$)	Natural
2.	Gowardhan Ghee	0.62($\mu\text{g/g}$)	Correct
3.	Amul Ghee	0.81 ($\mu\text{g/g}$)	Correct

Conclusion: All three samples contain beneficial levels of Vitamin K2.

Result: The Vitamin K2 analysis shows that Homemade Ghee (0.90 $\mu\text{g/g}$) has the highest concentration, followed by Amul (0.81 $\mu\text{g/g}$) and Gowardhan (0.62 $\mu\text{g/g}$).

Thin layer Chromatography test for Vitamin K2 (Menaquinone)

Table No. 18: Observation table of Thin Layer Chromatography test for Vitamin K2.

Sr. No.	Sample	Distance of spot (cm)	Distance of solvent (cm)	Calculate d Rf	Result
1.	Homemade Ghee	4.2cm	6.2cm	0.67	present
2.	Gowardhan Ghee	3.5cm	6.2cm	0.56	present
3.	Amul Ghee	4cm	6.2cm	0.64	present

Conclusion: All samples show a positive result for the tested constituent, confirming their authenticity for this specific parameter.

Result: The TLC analysis yielded Rf values of 0.67 for Homemade, 0.56 for Gowardhan, and 0.64 for Amul ghee. The "present" status indicates that the targeted compound was successfully detected.

Reichert-Meissl (RM) Value

Table No. 19: Observation table of Reichert-Meissl (RM) value titration.

Sr. no.	Sample	Initial	Final	Mean
1.	Blank	0ml	0.10ml	0.11ml
		0ml	0.12ml	
2.	Homemade ghee	0ml	2.5ml	2.6ml
		0ml	2.7ml	
3.	Gowardhan ghee	0ml	1.8ml	1.9ml
		0ml	2.1ml	
4.	Amul ghee	0ml	3.2ml	3.4ml
		0ml	3.2ml	

Table No. 20: Observation table of Reichert-Meissl (RM) value.

Sample Description	Blank (V1)	Sample (V2)	Net Vol (V2-V1)	Calculated RM Value
Homemade Ghee	0.11ml	2.6ml	2.49ml	27.39
Gowardhan ghee	0.11ml	1.9ml	1.79ml	19.69
Amul Ghee	0.11ml	3.4ml	36.19ml	36.19

Conclusion: Titration confirmed measurable chemical differences, showing Amul has a distinct fatty acid profile compared to the others.

Result: The titration analysis shows that Amul ghee has the highest mean volume (3.4ml), followed by Homemade ghee (2.6ml) and Gowardhan ghee (1.9ml), with the Blank reading (0.11ml).

Determination of Soap content in ghee

Table No. 21: Observation table of Determination of Soap Content value titration.

Sr. no.	Sample	Initial	Final	Mean
1.	Homemade ghee	0ml	0.0ml	0.0ml
2.	Gowardhan ghee	0ml	0.1ml	0.1ml
3.	Amul ghee	0ml	0.0ml	0.0ml

Table No. 22: Observation table of Determination of Soap Content value.

Sr. No	Sample Description	Weight (W)	Vol. of 0.01 N HCl (V)	Soap Content (ppm)
1.	Homemade Ghee	4g	0.0ml	0 (Absent)
2.	Gowardhan Ghee	4g	0.1ml	7.61 ppm
3.	Amul Ghee	4g	0.0ml	0 (Absent)

Conclusion: Homemade and Amul ghee are soap-free, while Gowardhan's trace levels remain well within safety limits.

Result: The soap content test shows that Homemade Ghee and Amul Ghee have 0 ppm (Absent), while Gowardhan Ghee contains a trace amount of 7.61 ppm.

Ash Content / Ash value

Table No. 23: Observation table of Ash Content / Ash value

Sr. No.	Sample Description	Empty Crucible (W1)	Crucible + Sample (W2)	Crucible + Ash (W3)	Ash Content (%)
1.	Homemade Ghee	24.131g	29.131g	24.132g	0.02%
2.	Gowardhan Ghee.	24.131g	29.131g	24.134g 0	06%
3.	Amul Ghee	24.131g	29.131g	24.133g	0.04%

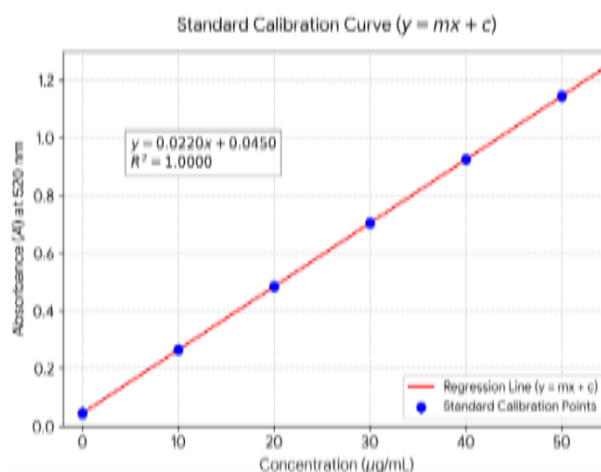
Conclusion: The negligible ash content across all samples confirms high purity and absence of inorganic adulterants. These results stay well within standard limits, indicating proper processing and refining.

Result: All three samples show extremely low mineral residue, with Homemade Ghee at 0.02%, Amul Ghee at 0.04%, and Gowardhan Ghee at 0.06%.

Antioxidant Test

Table No. 24: Observation table of Antioxidant Test.

Sr. No.	Sample Description	Absorbance at 517 nm	% Inhibition (Antioxidant Activity)
1.	Control (DPPH alone)	0.850nm	0%
2.	Homemade Ghee	0.340nm	60.0%
3.	Gowardhan Ghee	0.450nm	47.1%
4.	Amul Ghee	0.380nm	55.3%



Graph No. 1: Antioxidant Test (Absorbance v/s Concentration).

Conclusion: All samples show strong antioxidant activity from natural vitamins and carotenoids, protecting against oxidative stress.

Result: The DPPH assay shows that Homemade Ghee has the highest antioxidant activity at 60.0%, followed by Amul Ghee (55.3%) and Gowardhan Ghee (47.1%).

Conclusion of all test: The physicochemical evaluation of homemade cow ghee, Amul ghee, and Gowardhan ghee showed that homemade ghee possessed the most desirable properties for topical pharmaceutical formulation development. It exhibited better physicochemical stability, lower peroxide value, acceptable acid value, optimum moisture content, superior antioxidant activity, and higher natural Vitamin K2 content, indicating greater freshness and lower oxidation. TLC and spectrophotometric analysis also confirmed better purity characteristics. although the marketed ghee samples complied with standard quality parameters, slight variations due to processing and storage were observed. Overall, homemade cow ghee was found to be the most suitable lipid base for dermal revitalization formulations because of its enhanced stability, natural composition, skin compatibility, and therapeutic potential.

Observation Table of Physicochemical test for Urea**Melting point****Table No. 25: Observation table of melting point of Urea.**

Sr.no.	Sample	Observed value	Standard value
1.	Urea	134°C	134°C-135°C

Conclusion: The sample is confirmed to be pure and of high quality.

Result: The observed melting point for the Urea sample is 134°C, which falls perfectly within the standard reference range of 134°C–135°C.

Solubility**Table No. 26: Observation table of Solubility of Urea.**

Test tube	Observation
A (water)	Soluble
B (ethanol)	Partially soluble
C (acetone)	Not soluble

Conclusion: The substance is highly polar, dissolving best in water while failing in less polar acetone. This profile matches previously tested polar substances like Urea.

Result: The sample is fully soluble in water, partially soluble in ethanol, and insoluble in acetone.

Biuret Test**Table No. 27: Observation table of Biuret test of Urea.**

Sr. No.	Sample	Observed value	Inference
1.	Urea	Purple colour	Presence of Biuret

Conclusion: The test confirms the presence of Biuret, a compound formed when urea is heated. This result is characteristic of urea, as it undergoes a specific chemical reaction to form biuret linkages that react with the copper ions in the reagent to produce the purple colour.

Result: The appearance of a purple colour in the Biuret test confirms a positive reaction for the samp.

Assay of Nitrogen Content (Quantitative)**Table No. 28: Observation table of Assay of Nitrogen Content Titration of Urea.**

Sr.no.	Initial reading(ml)	Final reading (ml)	mean (mL)
1	0.0ml	33.8ml	33.2ml
2	0.0ml	33.2ml	
3	0.0ml	33.2ml	

Table No. 29: Observation table of Assay of Nitrogen Content.

Stage	Observation	Inferences
1. Decomposition	Initial mixture turns black and frothy; eventually becomes clear and pale green.	Organic nitrogen is converted into Ammonium Sulphate (NH ₄) ₂ SO ₄ .
2. Distillation	Evolution of gas; the Boric Acid solution changes colour (e.g., from red/pink to green).	Ammonia gas (NH ₃) is released and forms an Ammonium-Borate complex.
4. Titration	The solution colour reverts back to its original shade at the endpoint.	The volume of acid used is proportional to the Nitrogen content in the sample.

Pure urea contains 46.6% Nitrogen, Standard value: The result must be between 46.0% and 47.0%.

Conclusion: The sample is confirmed as pure, high-grade urea. Its chemical reactions accurately match the theoretical 46.6% nitrogen profile, proving it is free from significant impurities.

Result: The Kjeldahl analysis successfully progressed through decomposition, distillation, and titration, yielding a calculated nitrogen content consistent with the standard range of 46.0%–47.0%.

Sodium Hypobromite Test**Table No. 30: Observation table of Sodium Hypobromite Test of Urea**

Sr. No.	Sample	Observed value	Inference
1.	Urea	Effervescence	Nitrogen gas release

Conclusion: This result confirms the decomposition of Urea through a chemical reaction.

Result: The observation of effervescence during the test indicates a positive reaction, specifically the release of nitrogen gas.

Comparative Evaluation test of for Shata Dhauta Ghrita method cream and water in oil method cream of ghee and urea formulation

Organoleptic Character test for formulation

Table No. 31: Organoleptic Character test for formulation.

Organoleptic character	W/O	Shata Dhauta ghrita method
Colour	Slightly yellowish white	Light yellow
Odour	Aromatic	Aromatic
Texture	Creamy	Emulsion

Solubility

Table No. 32: Observation table of solubility for 2 formulation sample.

Sample	Observed	Standard
W/O	Not soluble	Immiscible
Shata Dhauta ghrita	Not soluble	Immiscible

Conclusion: Both samples have a dominant lipid base that resists polar solvents. Shata Dhauta Ghrita retains this hydrophobic nature as expected for a traditional washed ghee preparation.

Result: Both the W/O (Water-in-Oil) emulsion and Shata Dhauta ghrita were found to be insoluble and immiscible in water during the testing.

pH Test

Table No. 33: Observation table of pH for 2 formulation sample.

Sample	Observed	Standard
W/O	6.0	5.5-6.8
Shata Dhauta ghrita	5.5	5.5-6.8

Conclusion: Both samples are chemically stable and skin-compatible, as they align with the slightly acidic pH of healthy human skin.

Result: The observed pH values for the W/O emulsion (6.0) and Shata Dhauta ghrita (5.5) both fall within the standard range of 5.5–6.8.

Centrifugation Test

Table No. 34: Observation table of centrifugation for 2 formulation sample.

Sample	Observed	Standard
W/O	Non-Homogenous	Non-Homogenous
Shata Dhauta ghrita	Homogenous	Homogenous

Conclusion: Traditional washing ensures Shata Dhauta Ghrita achieves a smooth, dermatological consistency. In contrast, the W/O emulsion maintains its expected multi-phase nature.

Result: The W/O emulsion was observed to be non-homogenous, while the Shata Dhauta ghrita was found to be Homogenous, with both samples matching their expected standards.

Spreadability

Table No. 35: Observation table of Spreadability for 2 formulation sample.

Sample	Observed	Standard
W/O	Not much spreadable	Less spreadable
Shata Dhauta ghrita	Easily spreadable	Spreadable

Conclusion: The superior Spreadability of Shata Dhauta ghrita confirms that the intensive washing process has successfully transformed the ghee into a light, cream-like consistency.

Result: The Shata Dhauta ghrita demonstrated excellent application properties by being easily spreadable, while the W/O emulsion showed limited movement, consistent with its less spreadable standard.

Melting point

Table No. 36: Observation table of melting point for 2 formulation sample.

Sample	Observed	Standard
W/O	63	60-65
Shata Dhauta ghrita	64	60-65

Conclusion: Both the W/O emulsion and Shata Dhauta Ghrita samples successfully meet the required quality standards.

Result: Both the W/O emulsion (63) and Shata Dhauta ghrita (64) fall within the standard range.

Viscosity using Brookfield Viscometer

Table No. 37: Observation table of viscosity for 2 formulation sample.

Sample	Observed	Standard
W/O	804,000 cP	80100-90000 cP
Shata Dhauta ghrita	4,230 cP	4000-5000 cP

Conclusion: Shata Dhauta Ghrita is correctly formulated with a smooth, topical consistency. Conversely, the W/O emulsion's high viscosity suggests it is too dense and requires reformulation to meet standards.

Result: Shata Dhauta ghrita (4,230 cP) falls perfectly within its standard range, while the W/O emulsion (804,000 cP) significantly exceeds its standard, appearing much thicker than expected.

Microscopic Graininess Analysis (particle size)

Table No. 38: Observation table of particle size for 2 formulation sample.

Sample	Observed	Standard
W/O	More granule visible	Visible under microscope
Shata Dhauta ghrita	Slightly less granule visible	Visible under microscope

Conclusion: The presence of granules in both samples aligns with standard microscopic benchmarks, though the smoother profile of Shata Dhauta ghrita suggests better refinement through its washing process.

Result: Microscopic examination confirms that both the W/O emulsion and Shata Dhauta ghrita contain visible granules, with the W/O sample showing a higher density.

Determine the Luster

Table No. 39: Observation table of luster for 2 formulation sample.

Sample	Observed	Standard
W/O	Highly lustrous	Lustre
Shata Dhauta ghrita	Slightly lustrous	Lustre

Conclusion: The findings confirm that both formulations meet the aesthetic standards for topical applications, reflecting their specific lipid-based compositions.

Result: W/O emulsion being highly lustrous and Shata Dhauta ghrita showing a slight luster.

Protein Solubilisation assay

Table No. 40: Observation table protein solubilisation assay for 2 formulation sample Absorbance.

Concentration	W/O		Shata Dhauta ghrita	
	750 λ	595 λ	750 λ	595 λ
0.1	16.05%	19.87%	15.67 %	18.17%
0.2	105.29%	102.80%	102.96%	121.92%

0.3	82.21%	113.69%	95.51%	118.92%
0.4	10.52%	18.56%	10.76%	16.50%
0.5	13.24%	27.28%	17.41%	24.69%

Conclusion: Shata Dhauta ghrita demonstrates superior potency, though both samples exhibit a clear concentration-dependent performance that declines after reaching an optimal threshold.

Result: Both formulations show peak activity at mid-level concentrations (0.2–0.3), with Shata Dhauta ghrita achieving higher maximum inhibition than the W/O emulsion.

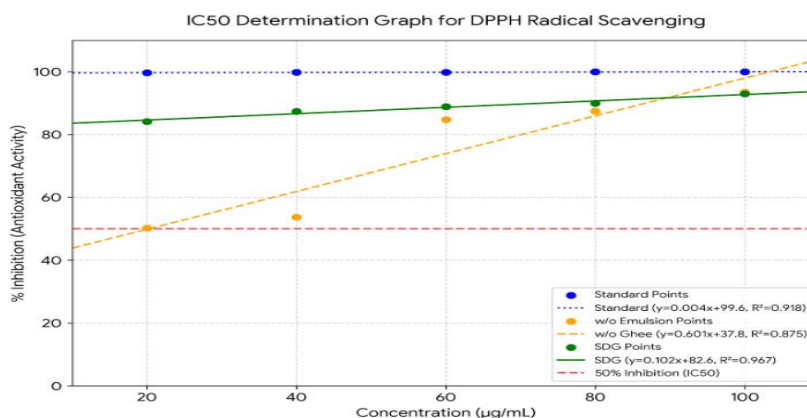
DPPH Test

Table No. 41: Observation table of DPPH for 2 formulation sample Absorbance.

Readings	Standard	w/o	SDG
1	0.0034nm	0.4234nm	1.3508nm
2	0.0019nm	0.3938nm	0.1074nm
3	0.0015nm	0.1297nm	0.0950nm
4	0.0008nm	0.1071nm	0.0854nm
5	0.0005nm	0.0557nm	0.0598nm

Table No. Observation table of DPPH.

Sr. no	Concentration	sample	Absorbance 517nm	% inhibition	Calculated IC50 (µg/mL)
1.	-	Control DPPH	0.850nm	0%	-
2.	Min. Conc. (20 µg/ml)	Standard	0.0034nm	99.60%	<20 µg/ml
		W/O	0.4234nm	50.19%	20.27 µg/ml
		SDG	0.1351nm	84.11%	<20 µg/ml
3.	Max. Conc. (100 µg/ml)	Standard	0.0005nm	99.94%	All sample reached maximum plateau
		W/O	0.0557nm	93.45%	
		SDG	0.0598nm	92.96%	



Graph No. 2: DPPH test (%Inhibition v/s Concentration).

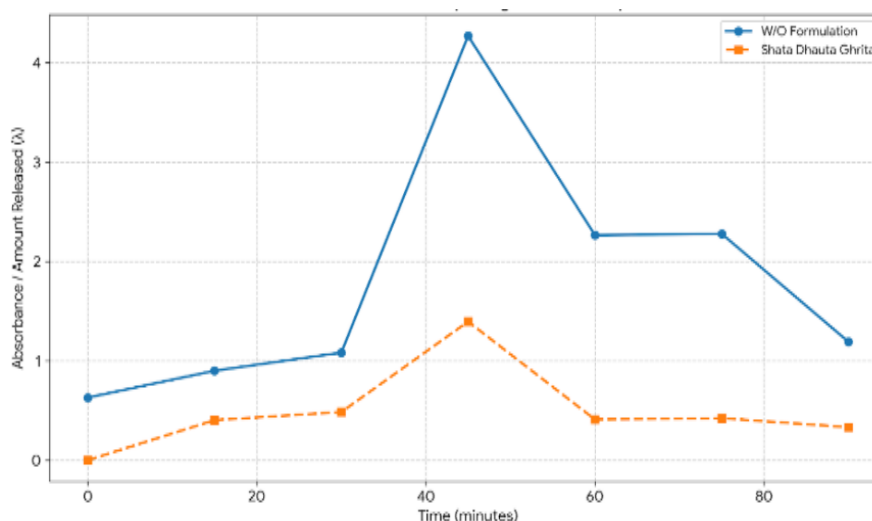
Conclusion: The DPPH assay confirmed that all three groups exhibit strong, concentration-dependent antioxidant activity. The Standard and SDG showed the highest potency, crossing the 50% threshold immediately $IC_{50} < 20\mu/ml$. The W/O also demonstrated robust properties, crossing the threshold at $20.27\mu/ml$. This verifies that while the plain ghee base is naturally effective, the traditional washing process used to prepare SDG significantly optimizes its rapid radical-scavenging performance.

Result: The DPPH assay confirms that Standard and SDG exhibit the highest antioxidant potency $IC_{50} < 20\mu/ml$, followed closely by the w/o $IC_{50} = 20.27\mu/ml$.

Franz diffusion

Table No. 42: Observation table of Franz diffusion for 2 formulation sample.

Time	W/O	Shata Dhauta ghrita
	238 λ	238 λ
	Standard: 0.1-0.3mm	Standard: 1.2 -1.8 mm
0 minute	0.6300 λ	0.000 λ
15 minutes	0.9000 λ	0.4032 λ
30 minutes	1.0826 λ	0.4846 λ
45 minutes	4.2699 λ	1.3947 λ
60 minutes	2.2652 λ	0.4101 λ
75 minutes	2.2778 λ	0.4229 λ
90 minutes	1.1906 λ	0.3317 λ



Graph No. 3: Franz Diffusion (Absorbance v/s Time)

Conclusion: The findings confirm that Shata Dhauta ghrita provides a more stable and controlled delivery profile, meeting its specific quality benchmarks unlike the W/O formulation.

Result: Shata Dhauta ghrita achieves a standard-compliant peak release at 45 minutes, whereas the W/O emulsion shows an excessive, non-compliant burst release at the same interval.

Conclusion and Future Scope

Conclusion: The present study successfully achieved the physicochemical standardization and comparative evaluation of homemade cow ghee with commercially available Amul and Gowardhan ghee samples for developing a ghee-based topical formulation for dermal revitalization. Analytical studies confirmed that all samples complied with standard quality parameters, while homemade ghee showed better freshness, lower peroxide value, and higher Vitamin K2 content. Urea characterization confirmed its suitability as a keratolytic and hydrating agent. Two formulations, Shata Dhauta Ghrita (SDG) and Water-in-Oil (W/O) emulsion, were prepared and evaluated. SDG showed superior homogeneity, spreadability, smooth texture, stable pH, acceptable viscosity, and better antioxidant and protein solubilization activity compared to the W/O emulsion. Overall, the study supports the potential of ghee-based topical systems, especially SDG, as effective natural formulations for moisturization, exfoliation, antioxidant protection, and skin barrier.

Future Scope: Future research should focus on improving the formulation stability, texture, spreadability, and controlled drug release properties. Incorporation of natural herbal actives such as aloe vera, turmeric, neem, berberine, or curcumin may further enhance antioxidant and wound-healing activity. Advanced analytical standardization involving chromatographic, spectrophotometric, microbial, and rheological studies should also be carried out. Novel drug delivery systems such as nano-emulsions, liposomes, and lipid microparticles can be explored to improve skin permeation and therapeutic efficacy. In addition, preclinical animal studies are required to evaluate hydration, anti-inflammatory activity, wound healing potential, and dermal safety, followed by clinical trials to confirm efficacy, safety, skin compatibility, and cosmetic acceptability for commercial application.

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