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Review Article

# Formulation and Evaluation of Betacyanin- Rich Herbal Cream

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## ABSTRACT

The increasing preference for natural and skin-friendly cosmetic products has intensified the exploration of plant-derived pigments with therapeutic potential, particularly betacyanins obtained from beetroot (Beta vulgaris). These pigments possess strong antioxidant, antimicrobial, anti-inflammatory, and skin- brightening properties, making them highly suitable for topical use. This report provides a comprehensive overview of the phytochemical nature of betacyanins, their biological relevance, and modern extraction approaches that enhance purity and yield. Special focus is given to formulation techniques for developing stable betacyanin-based creams, highlighting factors such as pH control, excipient selection, encapsulation methods, and antioxidant support to prevent degradation. Real-world case studies demonstrate the successful incorporation of betacyanin extracts into gels, emulsions, powders, and lip products. Additionally, essential evaluation parameters—including physical, chemical, microbial, and performance assessments—are examined to ensure product quality and safety. Regulatory requirements governing herbal topical products are also discussed to support compliant cosmetic development. Overall, betacyanin-rich formulations represent a promising category of natural cosmeceuticals with multifunctional benefits, and future research integrating nanotechnology, sustainable extraction techniques, and clinical validation may further advance their commercial potential.

## INTRODUCTION

In recent years, the cosmetics and dermatology industries have witnessed a significant shift toward natural and plant-based products. Consumers are becoming increasingly conscious of the potential side effects associated with synthetic chemicals and are instead seeking safer, sustainable, and biocompatible alternatives[1]. This rising demand has fueled the development of herbal formulations, often referred to as *cosmeceuticals*, which combine cosmetic appeal with therapeutic benefits[2].

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Among the wide variety of phytochemicals explored for topical applications, natural pigments stand out due to their dual functionality: they impart attractive coloration while also offering biological activity [3]. A particularly promising group of natural pigments is the betacyanins, which belong to the betalain family. These water-soluble, nitrogen-containing compounds are well recognized for their antioxidant, anti-inflammatory, antimicrobial, and skin-brightening properties[4].

Beetroot (*Beta vulgaris* L.) is one of the richest sources of betacyanins, especially the pigment betanin. Traditionally used as a food colorant and in natural dyes, beetroot has more recently attracted attention in the pharmaceutical and cosmetic sectors[5]. Its intense red-purple coloration, coupled with strong antioxidant activity, makes it a unique ingredient for topical products[6]. Beyond its visual appeal, beetroot extract has demonstrated the ability to neutralize free radicals, protect skin from oxidative stress, and promote an overall healthier complexion[7]. These features highlight beetroot as a valuable raw material for the formulation of herbal creams, gels, and color cosmetics[8].

### 1.1 Betacyanin-Rich Plants for Topical Use.

Betacyanins are unique in that they replace anthocyanins in plants of the order *Caryophyllales*. They provide shades ranging from red to purple and have strong biological activity, making them attractive not only as colorants but also as active ingredients in skincare[9]. Unlike synthetic dyes or certain chemical actives, betacyanins are **biodegradable, non-toxic, and safe for human use**, aligning perfectly with the current demand for clean-label and eco-friendly cosmetics[10].

In topical applications, betacyanins serve two main purposes. From a **biofunctional perspective**, they act as antioxidants, protect skin cells from oxidative damage, regulate melanogenesis to reduce hyperpigmentation, and promote wound healing[1]. At the same time, they play an **aesthetic role**, imparting natural coloration to creams, gels, blushes, and lipsticks, thereby enhancing consumer appeal[5].

Despite these advantages, the use of betacyanins in formulations is not without challenges. These pigments are highly sensitive to environmental factors such as **pH, light, temperature, and oxygen**, which can lead to rapid degradation and color loss[1]. Consequently, developing stable formulations requires careful selection of excipients and the use of techniques such as encapsulation, pH buffering, and antioxidant support[10].

### 1.2 Scope and Objectives of the Review

The purpose of this review is to provide a **comprehensive analysis** of the use of betacyaninrich plant extracts—particularly beetroot—in the development of topical herbal creams and related formulations. This work brings together insights from existing literature to present a consolidated framework for researchers, formulators, and students working in the field of herbal pharmaceutics[10].

## 2. Phytochemistry of Betacyanin-Rich Plants

### 2.1 Introduction to Betalains

Betalains are a class of water-soluble, nitrogen-containing pigments found almost exclusively in plants belonging to the order *Caryophyllales[11]*. They are subdivided into two main groups: **betacyanins**, which impart red to purple coloration, and **betaxanthins**, which provide yellow to orange hues[12]. Unlike anthocyanins, which are widely distributed in nature, betalains are restricted to a smaller group of plants and therefore hold special interest in both botanical and pharmaceutical research[13].

Among these pigments, betacyanins are particularly significant due to their **vibrant coloration, strong antioxidant properties, and therapeutic potential[14]**. Their unique chemical structure not only allows them to serve as natural colorants but also provides biological functions beneficial for human health, including anti-inflammatory and antimicrobial effects[15]. Because of these dual roles, betacyanins are now considered valuable ingredients for both food and cosmeceutical applications[16].

## 2.2 Structure and Chemical Properties of Betacyanins

Chemically, betacyanins are glycosides derived from **betalamic acid**. When betalamic acid condenses with cyclo-DOPA (a derivative of L-DOPA), it produces the characteristic redpurple chromophore that defines betacyanins[17]. The most abundant and well-studied betacyanin is **betanin**, the major pigment in beetroot (*Beta vulgaris*)[18].

**Key structural and chemical features include:**

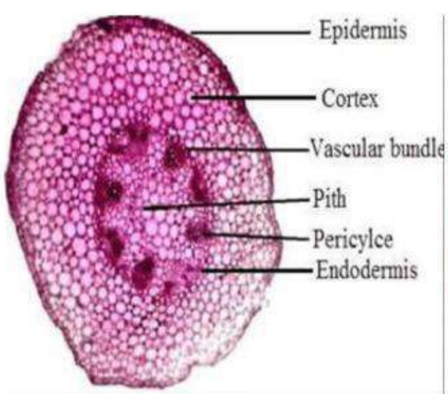
**Water solubility**: Betacyanins dissolve easily in aqueous systems, making them suitable for topical formulations[18].

**pH sensitivity**: They remain most stable in slightly acidic conditions (pH 4–5) but degrade rapidly in alkaline environments[19].

**Environmental sensitivity**: These pigments are prone to degradation when exposed to light, oxygen, or elevated temperatures[19].

**Molecular formula of betanin**: $C_{24}H_{26}N_2O_{13}$ with a molecular weight of approximately 550.5 g/mol[20].

Such sensitivity to external factors makes betacyanins both fascinating and challenging for formulation scientists. Without stabilization, their functional and aesthetic properties may be compromised during storage or use [21].



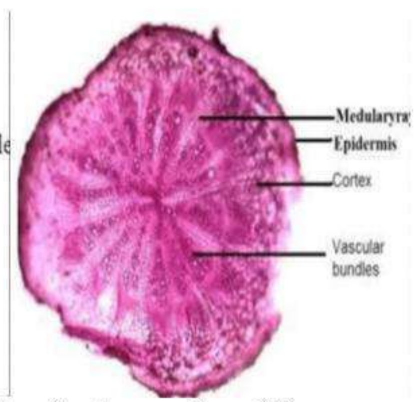




**Figure.2.1** **Transverse section of root** **Figure.2.2**

## 2.1 Sources of Betacyanin-Rich Extracts

While beetroot is the most commercially viable source of betacyanins, several other plants also contain these pigments in varying amounts[16].

**Table 2.1 Notable sources include:**

| Plant Source | Scientific Name | Part Used | Betacyanin Content |
|---|---|---|---|
| Beetroot | *Beta vulgaris* | Root | 300–600 mg/100 g |
| Red Amaranth | *Amaranthus cruentus* | Leaves, flowers | 150–300 mg/100 g |
| Cactus Pear | *Opuntia ficus-indica* | Fruit | 120–200 mg/100 g |
| Bougainvillea | *Bougainvillea glabra* | Bracts | Variable |
| Celosia | *Celosia argentea* | Flowers | ~100 mg/100 g |

Among these, **beetroot is favored** because it yields high concentrations of betanin, is inexpensive, widely cultivated, and recognized as **Generally Recognized as Safe (GRAS)** by regulatory agencies such as the FDA[20]. This status makes beetroot extracts especially attractive for incorporation into skincare products.

## 2.2 Extraction Techniques

Efficient extraction of betacyanins is critical for maximizing yield and preserving pigment integrity[21]. A variety of methods are available:

### A. Conventional Solvent Extraction

Uses ethanol, methanol, or water, often with acidifiers like citric acid or hydrochloric acid to maintain low pH. Simple and cost-effective but time-consuming, with a risk of pigment instability.

### B. Ultrasound-Assisted Extraction (UAE)

Employs ultrasonic waves to disrupt plant cell walls, enhancing pigment release. Faster and more efficient than conventional methods.

### C. Microwave-Assisted Extraction (MAE)

Uses microwave energy to heat the solvent and plant material rapidly. Energy-efficient, though excessive heating can degrade betacyanins.

### D. Supercritical Fluid Extraction (SFE)

Utilizes carbon dioxide under high pressure as a solvent. Environmentally friendly and highly selective, though complex and expensive. Each technique has its own trade-offs between efficiency, cost, and stability. For herbal cream formulations, UAE and MAE are often considered optimal due to their balance between yield and pigment preservation[22].

## 2.3 Stability Considerations

The biggest limitation in using betacyanins is their **instability** under environmental stress[23]. Table 2.2 Factors influencing their degradation include:

| Factor | Effect on Betacyanins |
|---|---|
| pH | Degrades rapidly above pH 6 |
| Light | Promotes oxidative degradation |
| Temperature | High heat disrupts pigment bonds |
| Oxygen | Causes oxidative color fading |
| Metal ions | $Cu^{2+}$ and $Fe^{3+}$ catalyze degradation |

**Stabilization strategies include:**

Encapsulation within polymers such as chitosan or alginate microspheres. Addition of antioxidants like ascorbic acid or tocopherol. Maintaining acidic pH conditions and storing in light-protective packaging. Such approaches are essential for ensuring that creams and gels retain both their functional activity and visual appeal over time[24].

### 1.1 Analytical Techniques for Betacyanin Identification

Accurate characterization of betacyanins is necessary to confirm their presence, concentration, and stability within formulations.

Common analytical tools include:

**UV-Visible Spectrophotometry**: Betanin shows maximum absorbance at ~538 nm.

**High-Performance Liquid Chromatography (HPLC)**: Separates and quantifies individual betalain compounds[25].

**Fourier-Transform Infrared Spectroscopy (FTIR)**: Identifies functional groups and bonding.

**LC-MS/MS (Liquid Chromatography–Tandem Mass Spectrometry)**: Provides detailed structural and compositional analysis.

These methods help ensure standardization of extracts and consistency in formulation development.

**Figure.2.3 Beetroot**

## 3. Biological and Dermatological Activities of Betacyanin-Rich Extracts

Herbal extracts have become increasingly popular in dermatology and cosmetology due to their natural origin, safety profile, and multiple therapeutic benefits. Betacyanin-rich plants, particularly beetroot (*Beta vulgaris*), are an excellent example of such multifunctional ingredients. These pigments not only provide attractive coloration but also deliver a wide range of **biological effects** that are highly relevant for topical applications. Their antioxidant, antiinflammatory, antimicrobial, wound healing, and skin-brightening properties make them ideal candidates for incorporation into creams, gels, and other cosmetic formulations[26].

### 3.1 Antioxidant Activity

One of the most widely studied properties of betacyanins is their **antioxidant capacity** Betacyanins act as potent free radical scavengers, neutralizing reactive oxygen species (ROS) such as superoxide anion ($O_2^-$), hydroxyl radicals ($OH^-$), and hydrogen peroxide ($H_2O_2$) .These ROS are known to contribute to **skin aging, inflammation, and hyperpigmentation** by damaging cellular structures and breaking down collagen[27].

Experimental studies have shown that **betanin**, the primary pigment in beetroot, possesses a higher antioxidant potential compared to common antioxidants like ascorbic acid and tocopherol. Assays such as DPPH, ABTS, and FRAP consistently demonstrate strong radical scavenging activity at relatively low concentrations of beetroot extrac.

**In topical use, this antioxidant activity translates to:**

- Protection against oxidative stress and photoaging.
- Preservation of collagen fibers and skin elasticity.
- Slowing down the appearance of fine lines and wrinkles.

This makes betacyanin-rich formulations promising candidates for **anti-aging creams and protective skincare products[28]**.

### 3.2 Anti-Inflammatory Effects

Inflammation is a common underlying factor in many skin disorders, including acne, eczema, and sunburn. Betacyanins help alleviate inflammation by reducing the expression of key proinflammatory mediators such as:

- **TNF-α (Tumor Necrosis Factor-alpha)**
- **IL-6 (Interleukin-6)**
- **COX-2 (Cyclooxygenase-2)[28].** This occurs primarily through the inhibition of the **NF- κB signaling pathway**, a major regulator of inflammatory response.By modulating these pathways, betacyanins help reduce redness, irritation, and inflammatory skin reactions caused by environmental stressors.

As a result, betacyanin-based formulations are suitable for **sensitive skin products, anti-acne creams, and post-sunburn treatments[29]**

### 3.3 Antimicrobial Activity

Beetroot extract also displays **broad-spectrum antimicrobial activity**, making it valuable for both dermatological and preservative purpose. It has been shown to be effective against:

- Gram-positive bacteria: *Staphylococcus aureus*
- Gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*
- Fungi: *Candida albicans*

The mechanism involves **disruption of microbial cell membranes** and interference with protein and DNA synthesis, which reduces microbial growth.

**Applications include:**

- **Acne treatment formulations**, where microbial overgrowth contributes to skin lesions.
- **Wound care products**, where antimicrobial activity supports healing.
- **General hygiene products**, where microbial safety is important.

Additionally, the antimicrobial effect contributes to **formulation stability**, helping reduce microbial contamination in water-based creams and gels[30].

### 3.4 Wound Healing Properties

Betacyanins also play a role in **accelerating wound repair**. They stimulate fibroblast proliferation, enhance collagen synthesis, and promote angiogenesis (formation of new blood vessels) . Together, these processes support faster tissue regeneration and reduced scarring[31]. In vitro scratch assays and animal studies have confirmed that betanin-enriched formulations result in **faster wound closure** and improved skin recovery compared to controls. This makes

### 3.5 Skin-Brightening and Anti-Hyperpigmentation Effects

Another attractive property of betacyanins is their ability to inhibit **tyrosinase**, the key enzyme involved in melanin synthesis. By reducing melanin production, they help lighten dark spots and create a more even skin tone.

Studies using beetroot extracts at concentrations of 1–5% have demonstrated moderate tyrosinase inhibition in enzyme assays. Small-scale clinical observations also suggest that topical beetroot-based gels improve skin brightness and texture over short application periods[33].

This positions betacyanins as a **natural alternative to conventional skin-lightening agents**

such as hydroquinone or kojic acid, which often carry safety concerns.

### 3.6 Anti-Aging and Anti-Wrinkle Potential

Through their combined **antioxidant and anti-inflammatory effects**, betacyanins help protect the skin against collagen degradation and UV-induced damage. They reduce the formation of fine lines, wrinkles, and pigmentation associated with photoaging.

Their effectiveness may be enhanced by combining them with other well-known anti-aging actives such as:

- **Vitamin C**, which boosts collagen synthesis.
- **Hyaluronic acid**, which improves hydration.
- **Green tea extract**, another potent natural antioxidant[34].

This makes betacyanin-based products ideal for **anti-aging serums and day creams.**

### 3.7 Photoprotective Potential

Although not a replacement for sunscreen, betacyanins exhibit mild **UV-absorbing properties**. They can reduce UV-induced oxidative stress and skin damage when used in combination with standard UV filters. This suggests potential for use in **sun care products**, where they can serve as supportive ingredients that enhance photoprotection[35].

**Table 3.1 Summary of Bioactivities**

| Biological Activity | Mechanism/Effect | Topical Application |
|---|---|---|
| Antioxidant | ROS scavenging, lipid peroxidation inhibition | Anti-aging creams, protective products |
| Anti-inflammatory | NF-κB pathway inhibition, reduced cytokines (TNF-α, IL-6) | Acne creams, sensitive skin, eczema care |
| Antimicrobial | Disruption of microbial membranes | Acne gels, wound care, hygiene creams |
| Wound Healing | Fibroblast activation, collagen synthesis | Healing creams, burn gels, post-procedural care |
| Skin-Brightening | Tyrosinase inhibition, melanin reduction | Brightening creams, spot correctors |
| Anti-Aging | Collagen preservation, UV protection | Wrinkle-reducing serums, anti-aging day creams |

## 4. Formulation Approaches for Topical Delivery of Betacyanin Extracts

The formulation of topical creams and related products involves far more than simply combining active ingredients with a base. For sensitive compounds like betacyanins, stability and bioavailability are central challenges. Betacyanins are prone to degradation when exposed to light, heat, oxygen, and alkaline pH, which can lead to loss of both color and biological activity[36]. Therefore, successful formulation strategies must address these limitations while ensuring **cosmetic elegance, consumer acceptability, and therapeutic efficacy**.

This chapter explores the different dosage forms, excipient choices, stabilization strategies, and case studies that inform the design of herbal creams and related products containing betacyanin- rich extracts.

### 4.1 Dosage Forms for Topical Betacyanin Delivery

Betacyanins can be incorporated into several topical dosage forms, each offering distinct advantages depending on the intended use[37]:

- **Creams**: Emulsion systems (oil-in-water or water-in-oil) that provide hydration, smooth texture, and ease of application. They are widely used in cosmetics due to their versatility.
- **Gels**: Hydrophilic, non-greasy formulations ideal for oily or acne-prone skin. Often prepared with polymers like hydroxypropyl methylcellulose (HPMC), Carbopol, or chitosan.
- **Lotions**: Lower-viscosity emulsions suitable for covering larger skin areas quickly and comfortably.
- **Ointments**: Greasy, occlusive preparations that provide intense hydration and protection but are less cosmetically appealing for daily use.
- **Makeup Products**: Betacyanins also function as natural pigments in blushes, lipsticks, and tinted creams, adding both color and bioactivity.

Among these, **creams and gels** are the most widely researched for betacyanin delivery due to their balance of functionality and consumer acceptability.

### 4.2 Selection of Cream Base

The cream base plays a critical role in maintaining stability, ensuring good skin feel, and controlling the release of active ingredients [38].

**Table 3.2 A typical herbal cream base includes:**

| Component | Function |
|---|---|
| Oil phase | Provides emollience and helps dissolve lipophilic actives |
| Water phase | Provides hydration and dissolves hydrophilic actives like betacyanins |
| Emulsifiers | Stabilize the oil–water interface (e.g., Tween 80, Span 60) |
| Thickeners | Control viscosity (e.g., HPMC, Carbopol) |
| Preservatives | Prevent microbial contamination (e.g., parabens, phenoxyethanol) |
| Antioxidants | Protect sensitive actives from oxidation (e.g., Vitamin E, ascorbic acid) |
| Humectants | Improve skin hydration (e.g., glycerin, propylene glycol) |

Since betacyanin extracts are **water-soluble**, they are best incorporated into the **aqueous phase** of emulsions to preserve their functionality[39].

### 4.3 pH Adjustment for Stability

Maintaining the correct **pH range (4–5)** is essential for preventing betacyanin degradation. At higher pH values, pigments lose their stability and rapidly change color. To control pH, formulators often use:

- **Citric acid** to adjust acidity.
- **Sodium citrate** or other buffers to maintain stability throughout storage.

### 4.4 Encapsulation and Controlled Release Strategies

Encapsulation has emerged as one of the most effective methods to stabilize betacyanins and enhance their delivery.Several approaches are used:

- **Chitosan Microparticles**: Biodegradable and film-forming carriers that encapsulate betacyanins, prolong release, and improve stability. For instance, a gel containing 0.5% HPMC and chitosan microparticles demonstrated excellent pigment retention and noticeable skin-brightening effects[40].
- **Ethyl Cellulose Microcapsules**: Provide a barrier between betacyanins and other formulation components, reducing interactions that might lead to degradation.
- **Liposomes and Niosomes**: Vesicular systems made from phospholipids or surfactants that protect betacyanins while enhancing skin penetration[41]. These systems are more expensive but highly effective.
- **Nanoemulsions**: Stable colloidal systems that allow better solubility, controlled release, and improved skin absorption, often enhancing both efficacy and sensory properties[42].

Encapsulation not only prevents oxidative degradation but also ensures **sustained delivery**, enhancing product performance.

### 4.5 Role of Natural Stabilizers and Antioxidants

The inclusion of natural stabilizers is crucial for protecting betacyanins during storage and use Examples include:

**Ascorbic acid (Vitamin C)** and **tocopherol (Vitamin E)**, which neutralize oxidative stress.

- **Chelating agents** such as EDTA, which bind metal ions (e.g., $Fe^{3+}$, $Cu^{2+}$) that catalyze pigment degradation.
- **Opaque packaging**, which minimizes light exposure and slows pigment breakdown[43].
- **Rosemary extract** – Contains rosmarinic acid, which prevents oxidation and enhances color retention.
- **Green tea polyphenols** – Provide photoprotective and anti- inflammatory benefits.
- **Grape seed extract (proanthocyanidins)** – Strengthens antioxidant profile of topical formulations.

These combined measures significantly extend the shelf life of betacyanin-rich formulations.

**Table 4.1 Example of a Betacyanin Cream Formulation**

| Ingredient | % w/w | Function |
|---|---|---|
| Beetroot extract | 2.0 | Active ingredient |
| Stearic acid | 3.0 | Emulsifier |
| Cetyl alcohol | 2.0 | Thickener, emollient |
| Glycerin | 5.0 | Humectant |
| Liquid paraffin | 3.0 | Emollient |
| Preservative | 0.2–0.3 | Microbial stability |
| Triethanolamine (pH 5) | q.s. | pH adjustment |
| Distilled water | Up to 100 | Solvent/base |

**Procedure**:

**Procedure for Preparing Beetroot1 Cosmetic Cream:**

- Preparation: Prepare oil and water phases separately.
- Heating and Mixing: Heat both phases to ~70°C, then mix with high-shear stirring.
- Cooling and Incorporation: Cool gradually to 40°C while incorporating beetroot extract and pH adjusters.

**Table 4.2 Factors Influencing Cream Performance**

| Factor | Description / Influence on Performance |
|---|---|
| pH of the Formulation | Betacyanins are stable at acidic pH (4–5). Higher pH accelerates pigment degradation, causing discoloration and reduced antioxidant activity. |
| Temperature / Storage Conditions | Elevated temperature enhances oxidation and breakdown of betacyanin structure, reducing shelf-life. Cool and dry storage improves long- term stability. |
| Light Exposure | UV and visible light degrade pigments, affecting color intensity and therapeutic potency. Opaque/UV-resistant packaging is recommended. |
| Type of Emulsion (O/W or W/O) | O/W creams provide better spreadability and user acceptance, while W/O emulsions offer stronger occlusive and moisture-retaining properties. Emulsion choice impacts release rate of betacyanins. |
| Viscosity and Texture | Optimal viscosity enhances spreadability, absorption, and patient compliance. Too high viscosity reduces ease of application; too low may affect retention on skin. |
| Antioxidants / Stabilizers Used | Natural stabilizers (Vitamin C, Vitamin E, rosemary extract) slow oxidation, preserve color, and improve overall product shelf-life and efficacy. |
| Encapsulation Techniques | Microcapsules, liposomes, and nanoemulsions protect betacyanins from chemical degradation, enhance delivery, and prolong release on skin. |
| Choice of Oils and Emollients | Oils influence occlusive effect, hydration, and solubility of active components. Suitable emollients improve softness and reduce transepidermal water loss (TEWL). |

## 5. Case Studies and Benchmarks from Literature

The practical application of betacyanin-rich extracts in topical formulations has gained momentum in recent years, with multiple studies highlighting their potential as both functional actives and natural colorants. Case studies not only provide experimental evidence for the stability and efficacy of these formulations but also act as **benchmarks for future product development[44]**By reviewing examples from literature, we can identify successful strategies, common challenges, and areas where further research is required.

### Case Study 1: Chitosan Microparticle Gel for Skin Brightening

One of the most innovative approaches reported is the use of **chitosan microparticles** to encapsulate beetroot extract. Abubakar et al. (2024) developed a gel formulation in which beetroot extract was encapsulated using ionic gelation, then incorporated into a **0.5%**

**hydroxypropyl methylcellulose (HPMC) gel base[45]**.

- **Evaluation**: The gel maintained an ideal pH of 5.0–5.2, stable viscosity, and consistent color for up to 30 days at room temperature. Spreadability tests showed smooth application, and preliminary user observations indicated noticeable **skin-brightening effects within two weeks**.

- **Key Finding**: Encapsulation with chitosan significantly enhanced pigment stability and prolonged activity, making this system promising for skin-lightening products.

**Case Study 2: Polyherbal Cream with Beetroot, Neem, and Carrot**

A study published in the *International Journal of Pharmaceutical Research* (2025) formulated a **polyherbal cream** combining beetroot, carrot, and neem extracts[74]. The formulation was prepared in an oil-in-water emulsion base with beetroot (2%), carrot (2%), and neem (1.5%).

- **Evaluation**: The cream exhibited a stable pH of 5.5, no phase separation after 30 days, and strong **antibacterial activity against *Staphylococcus aureus* and *Escherichia coli***. Irritation testing on rabbit skin revealed no erythema or redness, confirming safety.

- **Key Finding**: The synergistic action of the three extracts resulted in a multifunctional cream with antibacterial, antioxidant, and brightening properties, demonstrating the benefits of **polyherbal formulations**.

**Case Study 3: Herbal Blush and Compact Powder Using Beetroot Extract**

Researchers in the *Indonesian Journal of Chemistry* (2021) evaluated beetroot extract as a natural pigment in color cosmetics, specifically blush and compact powder formulations [46]

- **Formulation**: A 6% beetroot extract was incorporated into both compact powder and cream blush.

- **Evaluation**: The compact powder retained stable color and pH (4–5) over 28 days at room temperature, while the cream blush showed reduced stability at 30°C. User preference studies indicated higher satisfaction with the powder formulation compared to the cream.

- **Key Finding**: Beetroot extract is highly suitable as a natural pigment in **powder-based cosmetics**, but cream-based systems require stabilization measures to prevent heatinduced degradation.

## 6. Stability and Evaluation Parameters of Topical Betacyanin Formulations

The stability of a topical formulation is one of the most critical factors determining its **safety, efficacy, and consumer acceptability**. For herbal creams and gels containing betacyanins, this becomes especially important, as these pigments are highly sensitive to **pH, temperature, light, oxygen, and metal ions**. Even slight degradation can lead to undesirable changes in color, reduced antioxidant activity, or compromised therapeutic potential[47].

This chapter discusses the evaluation protocols used to assess the **physical, chemical, microbial, and functional stability** of betacyanin-based formulations, along with standard methods to ensure their long-term performance.

### Types of Stability Testing

To predict the shelf life and ensure consistency, stability testing is performed under controlled conditions.

- **Accelerated Stability Studies**: Conducted according to ICH guidelines, typically at **40**

**± 2°C / 75% RH, 30 ± 2°C / 65% RH, and 25 ± 2°C / 60% RH** for 1–6 months. These studies simulate long-term changes in a shorter period.

- **Real-Time Stability Testing**: Carried out under ambient storage for 6–12 months to replicate actual shelf-life conditions.
- **Freeze–Thaw Cycles**: Repeated exposure to temperatures between 4°C and 45°C helps identify physical instabilities such as **phase separation, cracking, or pigment precipitation[48]**.

Together, these tests provide a comprehensive picture of formulation robustness.

### 6.1 Physical Evaluation Parameters

**Table.6.1 Physical properties directly influence consumer perception and product quality [49].**

| Parameter | Purpose | Method/Observation |
|---|---|---|
| Color | Indicates pigment degradation | Visual inspection, colorimetry |
| Odor | Detects microbial growth or rancidity | Sensory evaluation |
| pH | Ensures pigment stability and skin compatibility | pH meter (1% dilution in water) |
| Viscosity | Controls spreadability and texture | Brookfield viscometer |
| Spreadability | Assesses ease of application | Slip weight or glass plate method |
| Homogeneity | Ensures uniform pigment distribution | Visual/microscopic examination |
| Phase Separation | Indicates emulsion stability | Storage observation over time |

### 6.2 Chemical Stability Parameters

**Table.6.2 Chemical testing ensures that active compounds like betanin remain intact[50].**

| Parameter | Purpose | Method |
|---|---|---|
| Betanin content | Confirms pigment retention | UV-Vis spectrophotometry at ~538 nm |
| Antioxidant activity | Confirms biofunctional efficacy | DPPH, ABTS, or FRAP assays |
| Oxidation markers | Detects oxidative breakdown | HPLC, peroxide value tests |
| pH drift | Indicates changes chemical/microbial | Monitored over time |

### 6.3 Microbial Stability Testing

Because herbal creams contain water, they are prone to microbial contamination. Microbial testing ensures safety and shelf life.

- **Total Bacterial Count**: Should be below $10^4$ CFU/g.
- **Fungal Count**: Should not exceed $10^2$ CFU/g.
- **Pathogen Detection**: Screening for *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.
- **Preservative Efficacy Testing**: Confirms that added preservatives can control microbial growth during storage[51].

### 6.4 Functional Evaluation Parameters

Stability must be complemented by performance testing to ensure the formulation delivers the intended benefits.

- **In-vitro Antioxidant Activity**: DPPH or FRAP assays confirm that antioxidant potential is preserved after formulation.
- **Skin Irritation Test**: Conducted on animals or human volunteers (patch test) to evaluate erythema, itching, or swelling. Betacyanin-rich products with pH 4.5–6.5 generally show good tolerance.
- **Skin Brightening Tests**: Ex-vivo or small-scale clinical studies are performed for 2–4 weeks to assess reductions in melanin content or improvements in skin tone.
- **Spreadability Test**: Evaluates how easily the cream spreads under pressure, influencing consumer perception[52].

### 6.5 Organoleptic Evaluation

**Table 6.3 Organoleptic properties are important for consumer satisfaction and acceptance [53]:**

| Feature | Acceptable Criteria |
|---|---|
| Appearance | Smooth, uniform, glossy or matte |
| Color | Stable reddish-pink to purple |
| Odor | Mild herbal fragrance, no rancid smell |
| Texture | Non-gritty, smooth consistency |
| After-feel | Non-sticky, easily absorbed |

### 6.6 Packaging and Storage Considerations

Packaging plays a vital role in maintaining stability. Betacyanin formulations require:

- **Opaque containers** to prevent light-induced degradation.
- **Air-tight or pump-based packaging** to minimize oxygen exposure.
- **Storage at 15–25°C**, away from direct sunlight and humidity. Such measures greatly improve product longevity[54].

**Table 6.4 Summary of Key Evaluation Parameters**

| Category | Tests |
|---|---|
| Physical Parameters | Color, odor, viscosity, spreadability, phase stability |
| Chemical Stability | Betanin content, antioxidant assays, pH drift |
| Microbial Stability | Bacterial/fungal count, preservative efficacy |
| Functional Evaluation | Antioxidant activity, skin brightening, irritation test |
| Safety & Packaging | Patch test, packaging compatibility studies |

## 7. Future Prospects and Research Opportunities

The global demand for **natural, safe, and effective skincare products** continues to expand rapidly. Consumers are increasingly favoring plant-based actives over synthetic alternatives due to concerns regarding safety, sustainability, and long-term health. Within this context, **betacyanin-rich plant extracts**, especially from beetroot (*Beta vulgaris*), represent a promising class of bioactive compounds. Although considerable progress has been made in understanding their properties and incorporating them into formulations, many opportunities remain to enhance their utility through **advanced technologies, novel dosage forms, and indepth clinical validation[55]**.

This chapter highlights the unexplored areas, innovative approaches, and emerging directions that could shape the future of betacyanin-based topical products.

### 7.1 Unexplored Research Areas

Despite significant interest, several important questions remain unanswered regarding betacyanin applications in skincare:

- **Mechanism of Action**: While their antioxidant and tyrosinase-inhibiting effects are established, the detailed molecular pathways through which betacyanins reduce hyperpigmentation or inflammation need further exploration[56].

- **Optimal Concentration**: Standardized studies are required to identify the ideal concentration of betanin for achieving maximum efficacy while ensuring safety in longterm topical use[57].

- Skin Penetration Studies: More research is needed to clarify whether betacyanins act primarily at the skin surface or penetrate into deeper dermal layers[58].
- Synergistic Combinations: The interactions of betacyanins with other herbal actives such as curcumin, aloe vera, or niacinamide should be systematically studied to evaluate potential synergy[59].
- Long-Term Safety: Comprehensive clinical trials are necessary to assess any side effects from daily, prolonged application of betacyanin-based formulations[60].

### 7.2 Advancements in Formulation Technologies

Modern formulation science offers several innovative platforms to enhance the stability and efficacy of betacyanin extracts.

- Nanotechnology: Nanoemulsions, solid lipid nanoparticles (SLNs), and liposomes can improve skin penetration, controlled release, and pigment stability[127]. Future research should compare the performance of traditional emulsions with these advanced nanoformulations.
- Hydrogel and Film-Forming Systems: Transdermal films, peel-off masks, and hydrogel patches represent convenient delivery systems for wound healing, skinbrightening, or anti-inflammatory applications[61]. These systems can enhance retention time and local efficacy.
- Encapsulation Innovations: Biopolymer-based systems such as chitosan, alginate, or novel polysaccharides could be further optimized for protecting betanin from degradation.

### 7.3 Biotechnology and Green Extraction Methods

As sustainability becomes a global priority, greener extraction methods must replace conventional solvent-based processes.

- Eco-friendly solvents like water, ethanol, or deep eutectic solvents offer safer alternatives.
- Ultrasound-assisted and microwave-assisted extractions provide higher yield and shorter extraction times.
- Standardization protocols using advanced tools such as HPLC and LC-MS are necessary to ensure reproducibility and quality across batches.
- Developing standardized beetroot extracts with defined betanin concentrations would facilitate

regulatory approval and large-scale commercialization[62].

### 7.4 Personalized Skincare and AI Integration

The future of skincare is moving toward personalization. Emerging technologies such as AIdriven formulation design and skin microbiome analysis can be integrated with betacyaninbased products[63].

**Potential applications include:**

- Customizing cream formulations based on an individual's skin type, pH, or microbiome profile.
- Using AI algorithms to predict stability outcomes and optimize excipient selection.
- Developing diagnostic tools that recommend betacyanin-rich formulations tailored to specific skin concerns.

### 7.5 Clinical Trials and Regulatory Recognition

Most current research on betacyanin formulations is limited to in-vitro assays, animal studies, or short-term human patch tests. To establish credibility, there is an urgent need for:

- Randomized controlled clinical trials (RCTs) on human volunteers[64].
- Long-term safety and efficacy assessments.
- Comparative studies against established actives like hydroquinone or vitamin C.

Well-structured clinical evidence will not only support product claims but also pave the way for regulatory recognition of betacyanins as dermatological actives[65].

### 7.6 Exploring Alternative Betacyanin Sources

**Table.7.1 Although beetroot is the primary source, other betacyanin-rich plants remain underutilized [66]:**

| Plant Source | Potential Benefits |
|---|---|
| *Amaranthus spp.* | Strong antioxidant, anti-inflammatory |
| *Opuntia ficus-indica* (Prickly Pear) | Hydration and polysaccharide-rich extracts |
| *Chenopodium quinoa* | Vitamins, flavonoids, and anti-aging effects |
| *Gomphrena globosa* | Natural colorant with potential anti-aging properties |

Comparative studies on these sources may lead to **new and unique herbal formulations**.

### 7.2 Patentable Innovations

The future also holds potential for innovation and patenting in:

- **Novel delivery systems** such as sprayable gels, melt-in creams, or microneedle patches[66].
- **Stabilization technologies** using natural biopolymers and encapsulation methods[67].
- **Synergistic formulations** combining betacyanins with vitamins, peptides, or other herbal actives[68].

Such innovations not only enhance efficacy but also create intellectual property opportunities for researchers and companies.

### 7.3 Global Market and Commercial Potential

The herbal cosmetics market is projected to exceed **USD 25 billion by 2030**, with significant growth expected in:

- **Natural skin brighteners** and fairness products.
- **Anti-aging serums and day creams**.
- **Color cosmetics** using plant-derived pigments.

Betacyanin-based products are well positioned to meet this demand due to their **clean-label appeal, multifunctional benefits, and consumer trust in herbal actives**.

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## 8. Review of Literature

| No | Author's Name | Study | Published in Journal | Findings |
|---|---|---|---|---|
| 1 | Cai, Y., Sun, M., & Corke, H. (2005) | Characterization of betalains from Amaranthus species | Journal of Agricultural and Food Chemistry | Amaranth identified as a stable source of betacyanin suitable for pharmaceutical cream coloring and antioxidant benefits. |

| | | | | |
|---|---|---|---|---|
| 2 | Herbach, K. M., et al. (2006) | Thermal and pH degradation kinetics of betacyanin pigments | Journal of Agricultural and Food Chemistry | Betacyanin degrades quickly under alkaline pH and high temperature; recommended controlled processing and cold storage. |
| 3 | Stintzing, F. C., & Carle, R. (2007) | Betalains in food: properties and applications | Trends in Food Science & Technology | Concluded that betalains provide strong pigmentation, antioxidant action, and are biocompatible and safe for topical use. |
| 4 | Azeredo, H. M. C. (2009) | Betalains: Properties, sources, applications, and stability | International Journal of Food Science & Technology | Betacyanins are heat, light, and alkaline sensitive but stable in acidic pH (4–6). Antioxidants and light-protective packaging improve stability. |
| 5 | Jain, P., & Bari, S. (2010) | Betalain-rich extracts as topical antioxidants | Journal of HerbMed Pharmacology | Betalain topical applications improved skin hydration, antioxidant activity, and nourishment. |
| 6 | Nithyanandam, R., et al. (2012) | Extraction and characterization of red dragon fruit betacyanin | Journal of Pharmacy Research | Red dragon fruit shows strong antioxidant capacity; pigment stability enhanced with ascorbic acid and citrates. |
| 7 | Gandía-Herrero, F., & García- Carmona, F. (2013) | Biosynthesis and potential applications of betalains | Planta | Betalains show strong antioxidant activity and protect cells from oxidative stress, suitable for dermatological and cosmetic use. |
| 8 | Sreekumar, S., et al. (2014) | Stability and antioxidant potential of red beet betalains | Journal of Food Science & Technology | Betalains show improved stability under refrigeration and light protection, suggesting acidic formulation conditions. |

## CONCLUSION

Betacyanin-rich herbal cream offers a promising natural approach for improving skin health due to its strong antioxidant, anti-inflammatory, and skin-brightening properties. Although betacyanins are sensitive to light, heat, and pH, an optimized cream formulation with suitable stabilizers, pH control, and protective excipients can enhance their stability and effectiveness. Overall, this formulation represents a safe and eco-friendly alternative to synthetic skincare ingredients, with potential use in cosmetic and dermatological applications. Further studies on standardization, stability, and clinical evaluation will support its development as a reliable herbal skincare product.

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