*EJPMR, 2026, 13(6), 754-765* **Research Article** **Impact Factor: 8.158**

**EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH**

www.ejpmr.com

**ISSN (O): 2394-3211**
**ISSN (P): 3051-2573**
**Coden USA: EJPMAG**

# A RESEARCH ON: FORMULATION, DEVELOPMENT AND EVALUATION OF BEETROOT NANOVESICLE GEL FOR ENHANCED ANTIOXIDANT ACTIVITY AND SKIN PERMEATION

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**DOI:** https://doi.org/10.5281/zenodo.20641668

**How to cite this Article:** Ajay Sunil Thorat*[1], Kalyani Jaywant Bhor[2], Payal Dnyaneshwar Nannaware[3], Soham Sandeep Lokare[4], Sharad Balu Narhe[5]. (2026). A Research On: Formulation, Development And Evaluation Of Beetroot Nanovesicle Gel For Enhanced Antioxidant Activity And Skin Permeation. European Journal of Pharmaceutical and Medical Research, 13(6), 754–765.



**Article Received on 15/05/2026** **Article Revised on 05/06/2026** **Article Published on 01/07/2026**

## 1. ABSTRACT

The present research focuses on the formulation, development, and evaluation of a beetroot (Beta vulgaris) based nanovesicle gel designed for enhanced antioxidant activity and improved skin permeation. Beetroot is a rich natural source of betalains, flavonoids, phenolic compounds, and other bioactive molecules exhibiting strong antioxidant potential. However, their poor stability and limited skin penetration restrict topical application. To overcome these limitations, nanovesicular drug delivery systems were developed to enhance the stability, bioavailability, and dermal penetration of beetroot phytoconstituents. The nanovesicles were incorporated into a gel base to improve patient compliance, spreadability, and controlled release properties. The formulated nanovesicle gel was evaluated for physicochemical properties, pH, viscosity, homogeneity, spreadability, in vitro drug release, and antioxidant activity using standard assays such as DPPH. The study demonstrates that nanovesicle-based gel significantly enhances antioxidant activity and skin permeation compared to conventional formulations, making it a promising approach for herbal dermatological applications.[1] Beetroot extract was incorporated into nanovesicles prepared using suitable phospholipids and incorporated into a gel base containing selected excipients. The developed formulation was evaluated for various physicochemical and performance parameters including appearance, colour, odour, homogeneity, pH, viscosity, spreadability, and stability. Antioxidant activity of the formulation was assessed using suitable radical scavenging methods such as DPPH and ABTS assays, while skin permeation characteristics were evaluated using appropriate diffusion studies.

**KEYWORDS:** Beetroot, Nanovesicles, Herbal Gel, Antioxidant Activity, Skin Permeation, Phytosomes, Drug Delivery System, Betalains.

## 1. INTRODUCTION

Herbal drug delivery systems have gained significant attention due to their safety, biocompatibility, and therapeutic efficiency. Natural plant extracts contain numerous bioactive compounds, but their clinical application is often limited by poor stability, low solubility, and inadequate skin permeation. Beetroot (Beta vulgaris) is a deep red-colored root vegetable rich in betalains, which include betacyanins and betaxanthins. These compounds exhibit strong antioxidant, anti-inflammatory, and wound healing properties. Despite these benefits, beetroot phytoconstituents are highly sensitive to light, temperature, and oxidation, resulting in reduced therapeutic efficacy. Nanovesicular drug delivery systems such as liposomes, niosomes, and phytosomes provide an effective strategy for encapsulating plant extracts. These vesicles enhance penetration through the stratum corneum, protect active compounds from degradation, and offer controlled release. Incorporation of nanovesicles into a gel base further improves topical delivery by increasing retention

time, enhancing patient compliance, and providing a smooth application.[2]

The global cosmeceutical and dermatological market has witnessed an unprecedented surge in demand for plant-derived bioactive ingredients that offer multi-mechanistic antioxidant, anti-inflammatory, and skin-rejuvenating properties without the adverse effects associated with synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). Among the diverse array of phytochemicals under investigation, betalains from Beta vulgaris (beetroot) have emerged as exceptionally promising candidates owing to their potent free radical scavenging capacity (ORAC value: 1,776 µmol Trolox equivalents/100 g), broad-spectrum antioxidant mechanisms, anti-inflammatory properties, and striking pigmentation that imparts anti-melanogenic benefits.

However, the topical application of beetroot extract in conventional formulations (creams, lotions, gels) is severely hampered by the inherent limitations of betalains: (i) hydrophilicity, which prevents penetration through the lipophilic stratum corneum; (ii) molecular instability, rendering them susceptible to degradation by heat, light, oxidation, and extreme pH; (iii) rapid surface washout and limited residence time on skin; and (iv) large molecular size, which impedes passive diffusion. These physico-chemical constraints demand the adoption of advanced nano-carrier strategies to deliver betalains efficiently to deeper skin layers.[3]

## 2. Rationale for Herbal Nanovesicle Gel

The rationale for developing a herbal nanovesicle gel integrating beetroot extract and aloe vera rests on the following scientifically grounded arguments:

- Overcoming Skin Barrier Limitations

The stratum corneum (SC), the outermost layer of the epidermis, is the principal rate-limiting barrier to transdermal drug delivery. Composed of corneocytes embedded in a lipid matrix (ceramides, cholesterol, free fatty acids), it effectively excludes hydrophilic macromolecules such as betalains. Nanovesicles, by virtue of their nano-scale size and flexible, deformable phospholipid bilayer, can squeeze through the intercellular spaces of the SC, creating transient pores, and deliver encapsulated actives to the viable epidermis and upper dermis — layers where antioxidant protection is most critical.[4]

- **Protection of Labile Betalains**

Betalains are notoriously unstable under processing and storage conditions. Encapsulation within phospholipid bilayers provides a physico-chemical shield against oxidation, photodegradation, and hydrolysis. The internal aqueous environment of the vesicle maintains optimum pH, while the bilayer acts as a molecular fence, preventing direct exposure to destabilising agents. Entrapment efficiency studies confirm that vesicular encapsulation can preserve up to 78.6% of betalain content, compared to rapid degradation observed in unprotected extract formulations.

- **Synergism with Aloe Vera**

Aloe barbadensis Miller (Aloe vera) gel, incorporated into the formulation as both a co-active ingredient and a gel-forming agent, contributes acemannan (a bioactive polysaccharide), aloin, anthraquinones, vitamins C and E, and amino acids that independently exhibit antioxidant, moisturising, wound-healing, anti-inflammatory, and skin-soothing activities. The synergistic combination of beetroot-derived betalains and aloe vera actives creates a multi-target antioxidant formulation with additive-to-synergistic efficacy.[5]

- **Superior Cosmeceutical Acceptability**

Gel formulations offer distinct advantages for cosmeceutical applications: non-greasy texture, ease of application, rapid absorption, high water content that provides immediate hydration, and aesthetic transparency. The incorporation of nanovesicles into the gel matrix further extends the release profile, ensuring sustained antioxidant protection over 8–12 hours of application.

### Scope of the Study

The scope of this research encompasses the following dimensions of scientific enquiry and pharmaceutical application:

- Phytochemical characterisation and standardisation of beetroot (Beta vulgaris L.) extract with quantification of total betalain content, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity.
- Systematic formulation development of nanovesicles by thin-film hydration with optimisation of lipid composition, lipid-to-drug ratio, hydration medium, probe sonication parameters, and extrusion conditions.
- Physico-chemical characterisation of nanovesicles including particle size analysis, zeta potential measurement, polydispersity index, entrapment efficiency, vesicle morphology (TEM, AFM, SEM), and in vitro drug release profiling.[6]
- Preparation and optimisation of nanovesicle gel using Carbopol 934 and HPMC gel bases combined with Aloe vera gel, with evaluation of rheological behaviour, spreadability, extrudability, and bioadhesive strength.
- In vitro antioxidant activity assessment using DPPH, ABTS, FRAP, and CUPRAC assays to quantify the radical scavenging potency of the formulated BNG compared to free extract.
- Ex vivo skin permeation studies using excised rat abdominal skin on Franz diffusion cells to determine permeation flux, permeability coefficient, enhancement ratio, and depth of penetration by confocal laser scanning microscopy (CLSM) using rhodamine B as a fluorescent marker.

- Accelerated stability studies at 40°C/75% RH and long-term stability studies at 25°C/60% RH as per ICH Q1A(R2) guidelines over 90 days.
- Safety evaluation through skin irritation studies (HET-CAM test) and dermal sensitisation assessment.

## 3. AIM OF THE STUDY

The primary aim of this study is to formulate, develop, and comprehensively evaluate a herbal nanovesicle gel incorporating standardised beetroot (Beta vulgaris L.) extract and Aloe vera (Aloe barbadensis Miller) gel, with the specific objective of enhancing the topical antioxidant activity and transdermal skin permeation of bioactive betalains, thereby establishing a scientifically validated, safe, and efficacious cosmeceutical preparation for dermal anti-ageing and photoprotective applications.[7]

## 4. OBJECTIVES

1. To prepare and standardise ethanolic and aqueous extracts of beetroot (Beta vulgaris L.) roots and quantify total betalain, phenolic, and flavonoid content along with in vitro antioxidant activity.
2. To design and optimise nanovesicles using phospholipid (soy lecithin/DPPC) and cholesterol by the thin-film hydration method, investigating the influence of lipid:drug molar ratio (5:1, 10:1, 15:1), hydration volume, and sonication time on vesicle characteristics.
3. To characterise the optimised nanovesicles for particle size, PDI, zeta potential, entrapment efficiency, morphology (TEM, AFM), and in vitro drug release kinetics.
4. To incorporate the optimised nanovesicles into gel bases (Carbopol 934, HPMC K4M, Aloe vera gel) and evaluate the resultant nanovesicle gel for physicochemical, rheological, and bioadhesive properties.
5. To conduct in vitro antioxidant studies (DPPH, ABTS, FRAP assays) on the BNG and compare efficacy with free extract and plain gel.
6. To perform ex vivo permeation studies across excised rat skin using Franz diffusion cells and determine permeation kinetics and enhancement ratio.
7. To evaluate safety via dermal irritation studies and undertake ICH-compliant accelerated stability testing. 8

## Introduction to Antioxidants and Skin Permeation

### ➢ Reactive Oxygen Species and Oxidative Stress in Skin

Free radicals and reactive oxygen species (ROS) are highly unstable molecules characterised by the presence of one or more unpaired electrons in their outer orbital shell. In biological systems, ROS are generated endogenously through mitochondrial electron transport chain leakage, xanthine oxidase activity, NADPH oxidase-mediated respiratory burst, and cytochrome P450 metabolism. Exogenously, UV radiation (UVA and UVB), ionising radiation, ozone, cigarette smoke, industrial pollutants, and chemical toxins are significant sources of skin-specific ROS.

**The major ROS implicated in skin oxidative damage include**

- Superoxide anion radical ($O_2^{\bullet -}$): Generated by one-electron reduction of molecular oxygen; initiates the ROS cascade.
- Hydrogen peroxide ($H_2O_2$): Membrane-permeable; converted to •OH by Fenton/Haber-Weiss reactions.
- Hydroxyl radical (•OH): The most reactive and destructive ROS; attacks DNA, proteins, and lipids indiscriminately.
- Singlet oxygen ($^1O_2$): Generated by UVA-mediated sensitiser reactions; attacks unsaturated fatty acids.
- Peroxynitrite ($ONOO^-$): Formed from superoxide and nitric oxide; causes protein nitration and DNA damage.[9]

### ➢ Antioxidant Mechanisms

Antioxidants neutralise ROS through multiple complementary mechanisms: (i) hydrogen atom transfer (HAT), in which a phenolic antioxidant donates a hydrogen atom to quench a radical; (ii) single electron transfer (SET), where the antioxidant reduces the radical by electron donation; (iii) metal chelation, sequestering pro-oxidant metal ions ($Fe^{2+}$, $Cu^{2+}$) to prevent Fenton chemistry; (iv) enzyme activation, upregulating endogenous antioxidant enzymes (SOD, CAT, GPx); and (v) gene expression modulation, activating the Nrf2–ARE pathway to enhance cellular antioxidant defences. Betalains in beetroot exert antioxidant activity primarily via HAT and SET mechanisms, with betacyanins demonstrating particularly high radical scavenging capacity (Trolox equivalent antioxidant capacity: 2.5–3.2 mmol/100 g dry weight).[10]

### ➢ Routes and Mechanisms of Transdermal Skin Permeation

Transdermal drug delivery exploits the skin as a portal for systemic or localised drug delivery, circumventing hepatic first-pass metabolism and gastrointestinal degradation. The primary routes of skin permeation include:

Transcellular route: Drug molecules traverse directly through corneocytes and their lipid envelopes. Although geometrically direct, this route requires the drug to partition repeatedly between lipophilic corneocyte interiors and hydrophilic lipid layers, making it energetically unfavourable for most molecules.

Intercellular route: Considered the predominant pathway for most drugs; molecules navigate the tortuous lipid matrix (ceramides, cholesterol, free fatty acids) between corneocytes. Highly lipophilic drugs preferentially use this route.

Trans-appendageal route: Drugs bypass the SC via follicular channels (hair follicles, sebaceous glands) and sweat pores. Although appendages occupy only 0.1% of total skin area, this pathway is significant for nanoparticles and ionic species.[11]

| Skin Layer | Description & Thickness |
|---|---|
| Stratum Corneum (SC) | Primary barrier — 10–20 µm |
| Viable Epidermis | Keratinocytes — 50–100 µm |
| Dermis | Collagen, blood vessels — 1–4 mm |
| Hypodermis | Fat layer — systemic absorption |

**Figure 1: Schematic Representation of Skin Permeation Pathways and Nanovesicle Penetration Mechanism.**

Nanovesicles enhance skin permeation through several mechanisms: (i) Elastic deformation, allowing flexible vesicles to squeeze through SC channels far smaller than their diameter; (ii) Osmotic driving force, vesicular water content creates a hydration gradient; (iii) Lipid exchange, vesicular phospholipids fluidise the SC lipid bilayers, reducing barrier resistance; (iv) Follicular targeting, nano-size enables follicular deposition and uptake by dendritic cells.

## 5. Plant Profie

1. Beetroot - Beta vulgaris L.

***Fig. Beetroot.***

| Taxonomic Rank | Classification |
|---|---|
| Kingdom | Plantae |
| Subkingdom | Tracheobionta |
| Division | Magnoliophyta (Angiosperms) |
| Class | Magnoliopsida (Dicotyledons) |
| Order | Caryophyllales |
| Family | Amaranthaceae (formerly Chenopodiaceae) |
| Genus | Beta |
| Species | Beta vulgaris L. |
| Common Names | Beetroot, Garden Beet, Red Beet, Table Beet, Blood Turnip |
| Parts Used | Root (tuberous tap root), leaves |
| Chromosome Number | 2n = 18 |

**Phytochemical Composition of Beetroot**

Beta vulgaris is phytochemically distinguished by its exceptionally rich content of betalains — a class of aromatic, nitrogen-containing pigments unique to plants of the order Caryophyllales. Chemically, betalains are immonium derivatives of betalamic acid (a dihydropyridine compound), which condenses with different amino or amine compounds to yield either betacyanins (red-violet) or betaxanthins (yellow-orange). The major bioactive constituents and their pharmacological significance are detailed below:

| Phytochemical Class | Major Compounds | Content (per 100g FW) | Pharmacological Activity |
|---|---|---|---|
| Betacyanins | Betanin, Isobetanin, Neobetanin | 40–210 mg | Antioxidant, anti-inflammatory, hepatoprotective |
| Betaxanthins | Vulgaxanthin I & II | 20–75 mg | Antioxidant, anti-mutagenic |
| Phenolic Acids | Caffeic acid, Ferulic acid, p-Coumaric acid | 50–350 mg GAE | Antioxidant, anti-carcinogenic |
| Flavonoids | Quercetin, Kaempferol | 12–45 mg QE | Anti-inflammatory, antioxidant |
| Inorganic Nitrates | $NO_3^-$ | 150–400 mg | Vasodilatory, skin microcirculation |
| Vitamins | Vitamin C, Folate (B9), Riboflavin | Vit C: 4.9 mg | Antioxidant, skin repair |
| Minerals | Iron, Calcium, Potassium, Magnesium | Fe: 0.80 mg | Skin enzyme cofactors |
| Dietary Fibre | Pectin, Cellulose | 2.8 g | Prebiotic, skin microbiome |
| Saponins | Triterpenoid glycosides | Trace amounts | Surfactant, penetration enhancer |

**2. Aloevera - Aloe barbadensis Miller**

***Fig. Aloevera.***

| Taxonomic Rank | Classification |
|---|---|
| Kingdom | Plantae |
| Order | Asparagales |
| Family | Asphodelaceae (Xanthorrhoeaceae) |
| Genus | Aloe |
| Species | Aloe barbadensis Miller |
| Common Names | Aloe vera, Ghrit kumari, Burn Plant, Miracle Plant |
| Parts Used | Leaf gel (inner parenchyma), latex (exudate) |
| Active Constituents | Acemannan, Aloin, Barbaloin, Anthraquinones, Vitamins A, C, E, B12 |

**7. Development and Formulation Consideration of Beetroot Nanovesicle Gel**

The development and formulation of beetroot nanovesicle gel involves careful selection of herbal extract, vesicle-forming agents, gel base, and evaluation parameters to ensure stability, enhanced antioxidant activity, and improved skin permeation.[12]

**1. Selection of Active Ingredient**

Beetroot (Beta vulgaris) extract is selected as the active ingredient because of its high antioxidant content and dermatological benefits.

**Major Active Constituents**

- Betalains (Betacyanin and Betaxanthin)
- Phenolic compounds
- Flavonoids
- Vitamins and minerals

**Role in Formulation**

- Antioxidant activity
- Skin protective effect
- Anti-inflammatory action
- Natural coloring and healing property.

**2. Selection of Nanovesicle System**

Nanovesicles are microscopic lipid or surfactant vesicles used to encapsulate active phytoconstituents and improve their delivery through the skin.

**Purpose of Nanovesicle Incorporation**

- Protection of beetroot bioactive compounds from oxidation
- Improved skin penetration
- Enhanced drug stability
- Sustained and controlled release
- Increased therapeutic efficacy

**Common Nanovesicle Types**

- Liposomes
- Niosomes
- Phytosomes
- Transferosomes

For beetroot gel, phospholipid-based nanovesicles using soya lecithin are commonly preferred due to good biocompatibility and skin affinity.

**3. Selection of Excipients**

Proper excipient selection is essential for stable gel formation and efficient delivery.

**I. Aloe vera Gel**

**Function**

- Natural gel base
- Moisturizing agent
- Cooling and soothing effect
- Improves skin hydration

**II. Soya Lecithin**
**Function**
- Vesicle-forming phospholipid
- Enhances encapsulation
- Improves skin permeation

**III. Glycerine**
**Function**
- Humectant
- Maintains moisture
- Improves smooth texture

**IV. Preservatives**
**Examples**
1. Methyl paraben
2. Propyl paraben
3. Sodium benzoate

**Function**
- Prevent microbial contamination
- Improve shelf life

**4. Formulation Considerations**
**I. Particle Size**
Nanovesicle size plays an important role in skin penetration.
- Ideal size range: 50–300 nm
- Smaller vesicles provide better permeation and uniform distribution.

**II. Encapsulation Efficiency**
Encapsulation efficiency indicates the amount of beetroot extract entrapped inside vesicles.
- Higher encapsulation:
- Improves stability
- Enhances drug delivery
- Provides prolonged release

**III. pH Adjustment**
The gel pH should be compatible with skin.

**-Ideal pH Range: 5.5–6.5**
**Improper pH may cause**
- Skin irritation
- Instability of phytoconstituents
- Reduced therapeutic effect

**IV. Viscosity Consideration**
Viscosity affects application and drug release.

**Ideal gel viscosity should**
- Allow easy spreading
- Prevent leakage
- Maintain uniformity

Typical range: 3000–50000 cP depending on gel composition.

**V. Homogeneity**
A good formulation should be:
- Smooth
- Lump-free
- Uniform
- Free from phase separation.[13]

**5. Preparation Method Consideration**
The preparation process affects vesicle formation and gel quality.

**Step 1: Beetroot Extract Preparation**
- Wash and peel beetroot
- Crush or blend
- Filter extract
- Concentrate if required

**Step 2: Nanovesicle Formation**
- Dissolve soya lecithin in suitable solvent
- Add beetroot extract
- Stir and homogenize
- Sonicate for nanosize vesicles

**Step 3: Gel Preparation**
- Prepare Aloe vera or polymer gel base
- Add glycerine and preservatives
- Mix thoroughly

**Step 4: Incorporation of Nanovesicles**
- Slowly add nanovesicle suspension into gel
- Stir gently
- Adjust pH and viscosity

**6. Stability Considerations**
Stability is essential for maintaining therapeutic activity.

**Factors affecting stability**
- Temperature
- Light exposure
- Oxidation
- Microbial contamination

**Preventive Measures**
- Use airtight containers
- Store at cool temperature
- Add antioxidants/preservatives
- Protect from direct sunlight.[14]

**7. Quality Control and Evaluation Considerations**
Final formulation should be evaluated for:
- Appearance
- Color and odor
- pH
- Viscosity
- Spreadability
- Homogeneity
- Drug content
- Antioxidant activity
- In vitro diffusion study
- Stability study.[15]

## 8. MATERIALS AND METHODS

### Materials

| Sr. No | Ingredient | Grade / Supplier | Role |
|---|---|---|---|
| 1. | Beetroot Extract | Laboratory prepared / Analytical grade | Antioxidant, active ingredient |
| 2. | Aloe vera Gel | Pharmaceutical grade / Herbal supplier | Moisturizer, soothing agent |
| 3. | Soya Lecithin | Pharmaceutical grade / HiMedia or equivalent | Vesicle former, permeation enhancer |
| 4. | Cholesterol | Analytical grade / Loba Chemie or equivalent | Stabilizer |
| 5. | Glycerin | Analytical grade / Merck or equivalent | Humectant |
| 6. | Carbopol 934 | Pharmaceutical grade / Lubrizol or equivalent | Gelling agent |
| 7. | Vitamin E Oil | Pharmaceutical grade / Herbal or pharma supplier | Antioxidant stabilizer |
| 8. | Triethanolamine | Analytical grade / Loba Chemie or equivalent | pH adjuster |
| 9. | Distilled Water | Laboratory grade | Vehicle/ Solvent |

- **Extraction of Beetroot Phytoconstituents**

Fresh beetroot roots were washed thoroughly with tap water followed by distilled water, peeled, sliced (2 mm thickness), and dried in a hot air oven at 40°C ± 2°C for 48 hours to obtain consistent moisture content (<8%). The dried slices were ground to a fine powder (mesh #40) using a laboratory mill. Extraction was carried out by the cold maceration method: 100 g powder was macerated with 500 mL of 70% ethanol in a sealed amber-coloured vessel at 25°C for 72 hours with periodic stirring every 8 hours. The macerate was filtered through Whatman No. 1 filter paper followed by coarse cotton cloth. The filtrate was concentrated under reduced pressure at 45°C using a rotary evaporator (Buchi R-300) to yield the concentrated ethanolic extract. The extract was standardised for: total betalain content (pH-differential method at 538 nm and 600 nm), total phenolic content (Folin-Ciocalteu method, expressed as mg GAE/g), total flavonoid content (aluminium chloride colorimetric method, expressed as mg QE/g), and antioxidant activity (DPPH and ABTS assays).[16]

***Fig. Beetroot Extraction.***

- **Phytochemical Screening**

The beetroot extract was subjected to standard qualitative phytochemical screening tests to identify the presence of major secondary metabolite classes, including alkaloids (Dragendorff's test), saponins (froth test), tannins (ferric chloride test), phenolics ($FeCl_3$ test), flavonoids (Shinoda test), cardiac glycosides (Keller-Killiani test), steroids (Liebermann-Burchard test), and betalains (visual observation and spectrophotometric confirmation at 538 nm). The results confirmed predominant presence of betalains, phenolics, flavonoids, and saponins.[17]


***Fig. Phytochemical Screening.***

**Method of Preparation**

**Step 1: Preparation of Beetroot Extract**

1. Take fresh beetroot and wash thoroughly with distilled water.
2. Peel and cut into small pieces.
3. Grind using a mixer to obtain juice/paste.
4. Filter through muslin cloth or filter paper.
5. Concentrate the filtrate using water bath at low temperature.
6. Store the extract in refrigerator until use.

**Step 2: Preparation of Nanovesicles**

1. Accurately weigh soya lecithin and cholesterol.
2. Dissolve them in a small quantity of ethanol/chloroform mixture.
3. Evaporate solvent using rotary evaporator or gentle heating to form thin lipid film.
4. Hydrate the film using beetroot extract with continuous stirring.
5. Sonicate the dispersion for few minutes to reduce vesicle size and obtain nanovesicles.


**Step 3: Preparation of Gel Base**

1. Disperse Carbopol 934 slowly in distilled water with continuous stirring.
2. Allow it to soak and swell for 30–60 minutes.
3. Add glycerin and aloe vera gel slowly with stirring.
4. Add Vitamin E oil and mix uniformly.


**Step 4: Incorporation of Nanovesicles**

1. Add prepared beetroot nanovesicle dispersion slowly into gel base.
2. Stir continuously to obtain uniform dispersion.

**Step 5: Adjustment of pH**

1. Add triethanolamine dropwise.
2. Stir continuously until transparent gel is formed.
3. Adjust pH near skin pH (about 6–7).

**Step 6: Final Preparation and Storage**

1. Make up the final volume with distilled water.
2. Mix thoroughly to obtain smooth homogeneous gel.
3. Transfer gel into airtight containers.
4. Store in cool and dry place.[18]

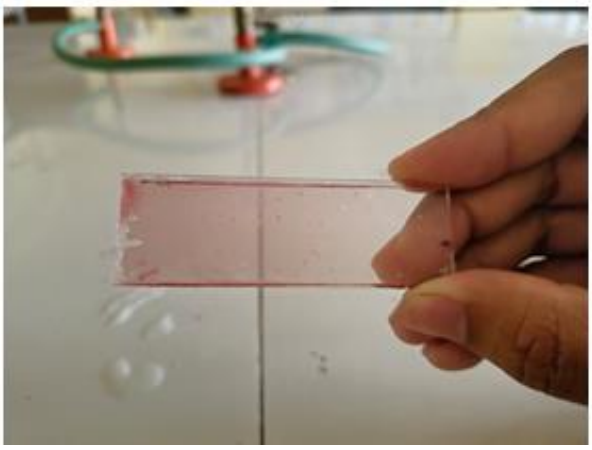
**9. Evaluation parameter**

**1. Physical Evaluation**

1. Color (reddish, uniform)
2. Odor (characteristic herbal smell)
3. Appearance (smooth, homogeneous gel)
4. Texture (non-gritty, non-lumpy)

**2. Homogeneity Test**

Homogeneity is evaluated by visual inspection and touch. A small quantity of gel is pressed between thumb and index finger to detect the presence of lumps or coarse particles. A homogeneous gel should be smooth, uniform, and free from grittiness.[19]



**3. pH Determination**

The pH of the gel is determined using a calibrated digital pH meter. About 1 g of gel is dispersed in 10 mL of distilled water and allowed to stand for complete dissolution. The pH electrode is immersed into the sample and the reading is recorded. The ideal pH range for topical gel is 5.5–6.5 to ensure skin compatibility and avoid irritation.


**4. Viscosity Determination**

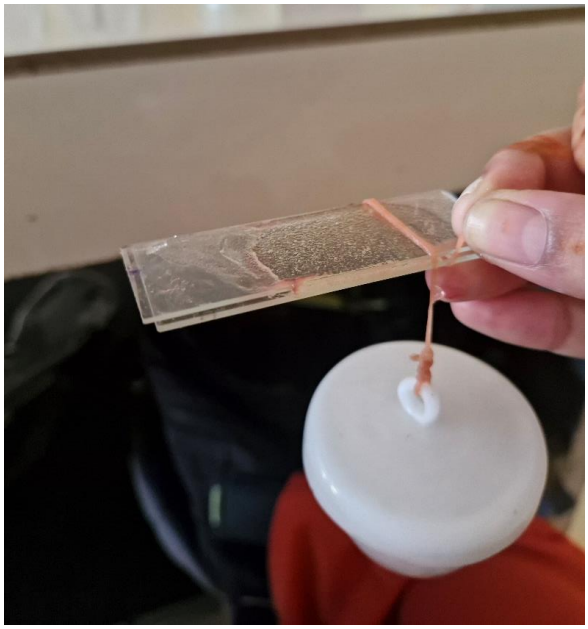
Viscosity is measured using a Brookfield viscometer at room temperature. Approximately 50 g of gel is placed in a beaker and spindle rotation is adjusted at suitable rpm. The viscosity value is recorded in centipoise (cP). Proper viscosity ensures easy application and retention on the skin.[20]


**5. Drug Content Determination**

Drug content analysis determines the amount of active phytoconstituent present in the formulation. A weighed quantity of gel is dissolved in suitable solvent and filtered. The solution is analyzed using UV-visible spectrophotometry at the appropriate wavelength. Uniform drug content indicates proper mixing and formulation consistency.[21]

**6. Spreadability Test**

Spreadability determines the ease with which the gel spreads over the skin surface. A known quantity of gel is placed between two glass slides. A standard weight is applied on the upper slide and the diameter or time required for spreading is measured. Good spreadability indicates uniform application and patient acceptability.



**7. Skin Irritation Test**

The irritation study is performed by applying a small quantity of gel on shaved animal skin or human volunteer patch area under ethical approval. The treated site is observed for redness, swelling, or itching over 24–48 hours. Absence of irritation indicates safety for topical use.

**8. Stability Study**

Stability studies are conducted to evaluate the effect of storage conditions on formulation quality. The gel is stored at different temperatures such as room temperature and accelerated conditions for a specified period. Parameters including appearance, pH, viscosity, and drug content are evaluated periodically to determine formulation stability.skin permeation.

**9. Skin Permeation Study**

Skin permeation study evaluates the penetration of active constituents through biological membrane. Excised animal skin or synthetic membrane is mounted on a Franz diffusion cell. The gel is applied to the donor side and receptor fluid is analyzed periodically. The amount of drug permeated is calculated to assess permeation efficiency.[22]

**10. Solubility Test**

The solubility test is performed to determine the ability of the formulation or extract to dissolve in different solvents. Solubility influences drug release, stability, and skin permeation of the formulation.[23]


## 11. RESULTS AND DISCUSSION

The formulated beetroot nanovesicle gel exhibited smooth texture, uniform consistency, and good homogeneity without any phase separation. The pH was found within the range of 5.5–6.5, making it suitable for topical skin application. The gel showed satisfactory viscosity and spreadability, ensuring easy application and good skin retention. Particle size and entrapment efficiency confirmed successful nanovesicle formation. In-vitro release studies demonstrated sustained release of active phytoconstituents, while skin permeation studies showed enhanced penetration through the skin barrier. The DPPH assay indicated significant antioxidant activity due to the presence of betalains and polyphenolic compounds in beetroot extract. Stability studies revealed no significant changes in physical appearance, pH, or consistency, confirming good formulation stability and supporting its potential effectiveness as an antioxidant and skin permeation enhancing topical gel.[24]

## 12. CONCLUSION

The beetroot nanovesicle gel represents an advanced herbal topical delivery system with enhanced antioxidant activity and improved skin permeation. The use of nanotechnology significantly improves the stability and bioavailability of beetroot phytoconstituents. This formulation has strong potential in dermatological applications such as anti-aging, skin repair, and oxidative stress reduction. Further clinical studies may establish its effectiveness for commercial and therapeutic use.[25]

## 13. ACKNOWLEDGMENT

We sincerely acknowledge the continuous guidance, motivation, and constructive feedback provided by our mentor, Ass. Prof. Kalyani J. Bhor Mam. Their expertise and patience have been instrumental in shaping this research paper.

We also extend our gratitude to Samarth Institute of Pharmacy, Belhe for providing the necessary academic environment and resources that supported this work.

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