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Development and Evaluation of a Transfersomal Loaded Herbal Dual Action Serum Containing Hibiscus and Green Tea Extract for Anti-Aging and Skin Brightening Therapy

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ABSTRACT

The growing demand for multifunctional, plant-based cosmeceuticals has driven the exploration of advanced drug delivery systems that enhance efficacy and safety. This review focuses on a dual-functional approach employing herbal transfersomal-loaded serum for simultaneous anti-aging and skin brightening therapy using *Hibiscus rosa-sinensis* and *Camellia sinensis* (green tea) extracts as bioactive agents.

Despite their therapeutic potential, the clinical application of these phytoconstituents is limited by poor skin penetration and stability. Transfersomes, ultra-deformable vesicular carriers, offer a promising solution by enhancing transdermal delivery, improving bioavailability, and enabling targeted release of active compounds into deeper skin layers. This review systematically discusses the formulation strategies, physicochemical characterization, and evaluation parameters of transfersomal systems incorporated into serum-based topical formulations. Additionally, it highlights the synergistic benefits of combining anti-aging and skin brightening activities in a single nano herbal transfersomal serum formulation.

Keywords: Transfersomal serum, ethanol injection method, *Hibiscus rosa-sinensis*, *Camellia sinensis*, anti-aging, skin brightening serum.

INTRODUCTION

TOPICAL DRUG DELIVERY SYSTEM

Topical drug delivery systems have gained significant importance in the management of skin-related conditions due to their ability to deliver active agents directly to the site of action. This approach minimizes systemic side effects, enhances local drug concentration, and improves patient compliance. In recent years, cosmetic serums have emerged as advanced topical formulations designed to overcome such limitations. Serums are lightweight, fast-absorbing preparations containing high concentrations of active ingredients, allowing deeper skin penetration and enhanced efficacy compared to conventional creams and lotions.

The skin is the largest organ of the human body and serves as the primary protective barrier against environmental, physical, chemical, and microbial insults. Healthy skin is not only for physiological functions such as thermoregulation, sensation and immune defence but also for maintaining an individual's appearance and confidence and overall quality of life.

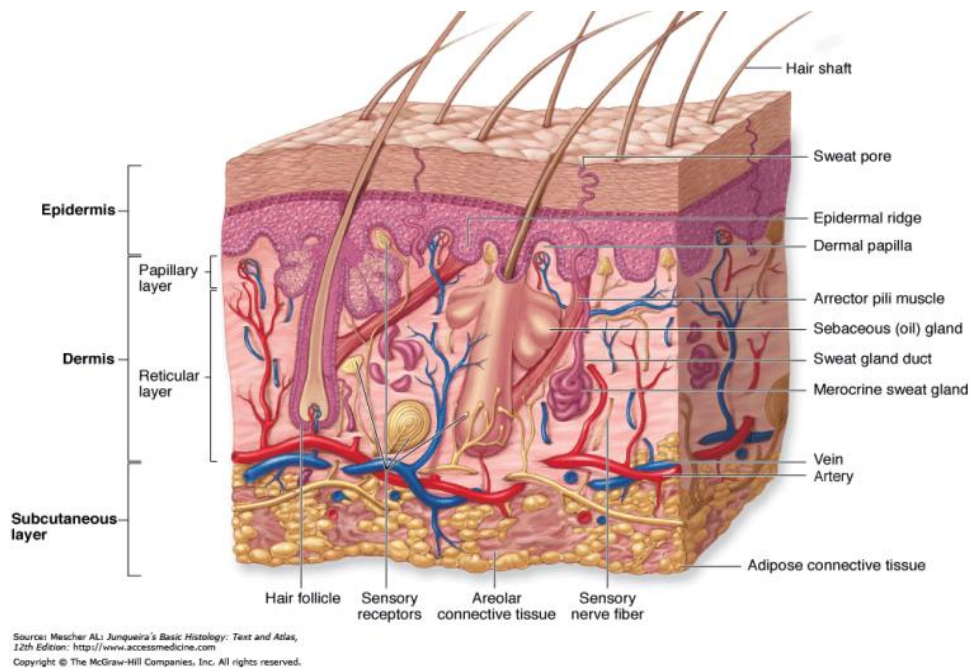


FIG-1 Structure of skin

FUNCTIONS OF SKIN:

Skin is the largest organ of the human body and performs the following vital functions:

1. Protection:

The skin serves as a primary anatomical barrier that prevents entry of pathogens and protects against mechanical, chemical, and physical injury. Langerhans cells present in the skin contribute to adaptive immune defence.

2. Sensation:

The skin is richly innervated with sensory receptors and nerve endings that detect thermal stimuli (heat and cold), touch, pressure, vibration, and pain.

3. Thermoregulation (Heat Regulation):

The skin helps in maintaining body temperature. Vasodilation (dilation of blood vessels) enhances cutaneous blood flow and facilitates heat loss, whereas vasoconstriction (constriction of vessels) reduces blood flow to conserve body heat.

4. Control of Evaporation / Barrier to Fluid Loss:

The skin forms a relatively dry and semi-impermeable membrane that restricts transepidermal water loss. Loss of this function, as seen in burns, results in massive fluid loss.

5. Aesthetics and Communication:

Skin appearance reflects emotional state, health status, mood, and physical attractiveness. It plays a vital role in social interaction and communication.

6. Storage and Synthesis:

The skin acts as a reservoir for lipids and water. It is also involved in synthesis of Vitamin D upon exposure to ultraviolet (UV) radiation.

7. Water Resistance:

The skin provides a water-resistant barrier that prevents leaching and washing out of essential nutrients and electrolytes from the body.



FIG-2 Difference between healthy and damaged skin

A healthy skin diagram shows a tightly packed cellular structure (stratum corneum) that retains moisture alongside thick, organised network of collagen and elastin. In contrast, a damaged skin diagram reveals a weakened moisture, barrier with gaps, allowing water loss, bacteria and UV penetration, accompanied by fragmented collagen fibres.

The visible differences in Anatomy: - a medical illustration comparing healthy and damaged skin will typically highlight these cellular and structural changes:

The Epidermis (The outer layer)

- Healthy skin: Features smooth, uniform outer surface rich in ceramides and lipids. Cells are densely packed to form a sturdy protective seal known as the acid mantle.
- Damaged skin: Depleted of natural lipids, the barrier flakes and cracks. These microscopic fissures allow moisture to escape and environmental irritants, uv rays, and bacteria to enter.

The Dermis (The Middle layer)

- Healthy skin: contains robust, intertwined strands of collagen and elastin, which provide plumpness, firmness and flexibility.
- Damaged skin: shows degradation from uv exposure (photodamage) or sugar binding (glycation). Collagen and elastin fibers are fragmented, unorganized, or destroyed, leading to sagging and deep wrinkles.

SKIN AGING:

Cutaneous aging is a multifactorial, progressive physiological process involving alterations in structure, function, and appearance of skin. It arises due to interplay of intrinsic chronological factors and extrinsic environmental influences, resulting in both clinically visible and histological changes in skin architecture.



FIG 3. Skin aging

Types of Skin Aging

Skin aging is broadly classified into two main types:

a) Intrinsic Aging (Chronological Aging):

Intrinsic aging is a natural, genetically determined process that occurs over time independent of external factors. It is inevitable and influenced by genetic makeup, hormonal changes, and metabolic processes. Characteristics include fine wrinkles, thinning of epidermis and dermis, reduced collagen production, decreased elasticity, and dryness due to reduced sebum production.

b) Extrinsic Aging (Photoaging):

Extrinsic aging is caused by external environmental factors and lifestyle habits. Ultraviolet (UV) radiation, pollution, smoking, stress, and poor nutrition are the most significant contributors. Characteristics include deep wrinkles, coarse texture, hyperpigmentation (age spots, melasma), loss of firmness, and increased risk of skin cancers.

Causes of skin aging:

Genetic Factors

Genetics play a key role in determining the rate and pattern of aging. Genes regulate collagen synthesis, antioxidant defense systems, and cellular repair mechanisms. Variations in these genes influence susceptibility to wrinkles, pigmentation, and elasticity loss.

Hormonal Changes

- Hormones, particularly UV radiation
- Pollution
- Smoking

Ultraviolet (UV) Radiation

UV radiation is the most critical extrinsic factor in skin aging.

- **UVA rays** penetrate deep into the dermis and cause collagen degradation.
- **UVB rays** affect the epidermis and lead to sunburn and DNA damage.

UV exposure activates enzymes such as matrix metalloproteinases (MMPs), which degrade collagen and elastin fibers, resulting in wrinkle formation and loss of skin elasticity.

Environmental Pollution

Pollutants such as particulate matter (PM), ozone, and toxic chemicals generate free radicals, leading to oxidative damage, inflammation, and pigmentation disorders.

Lifestyle Factors

- **Smoking:** Reduces blood flow and oxygen supply, leading to dull and wrinkled skin.
- **Poor diet:** Lack of vitamins (A, C, E) and antioxidants affects skin repair.
- **Stress:** Increases cortisol levels, which accelerates collagen breakdown.
- **Sleep deprivation:** Impairs skin regeneration.

HYPER PIGMENTATION

Hyperpigmentation is a common dermatological condition characterized by the darkening of certain areas of the skin due to excessive production or uneven distribution of melanin, the natural pigment responsible for skin colour. It is one of the most visible and prominent signs associated with skin aging and environmental damage.

Uneven skin tone refers to irregularities in skin coloration, where patches of skin appear darker or lighter compared to the surrounding areas. This condition results from localized variations in melanin synthesis, vascular changes, and skin damage. Both hyperpigmentation and uneven skin tone significantly affect aesthetic appearance and are key targets in cosmeceutical and dermatological treatments.

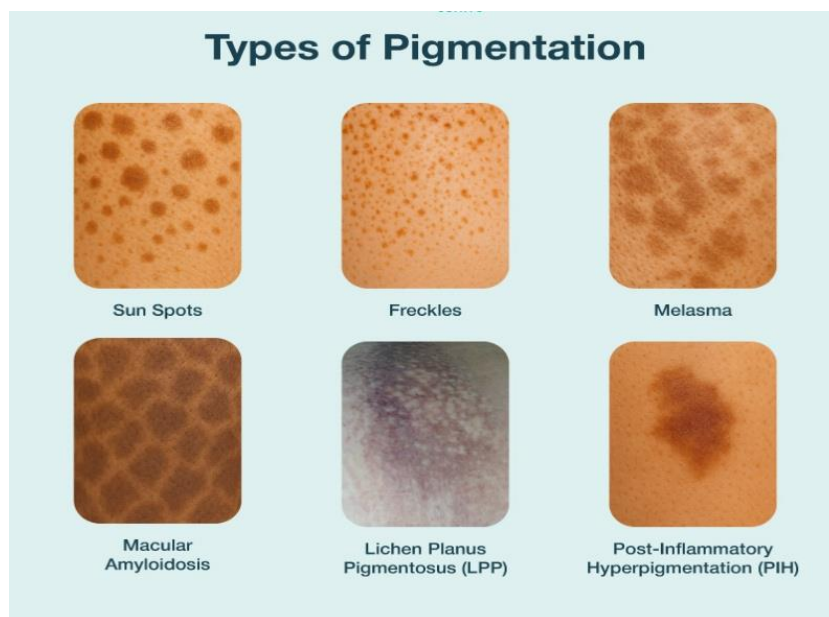


FIG 4. Types of pigmentation

Factors causing pigmentation:

Hyperpigmentation and uneven skin tone are influenced by a combination of internal and external factors that disrupt normal melanin production in the skin. One of the primary factors is excessive exposure to ultraviolet (UV) radiation, which stimulates melanocytes to produce more melanin, leading to dark patches. Environmental pollution and oxidative stress also play a significant role by generating reactive oxygen species that damage skin cells and alter pigmentation patterns. Hormonal changes, such as those occurring during pregnancy or due to hormonal therapies, can trigger conditions like melasma, resulting in irregular skin tone. Inflammatory skin conditions, including acne, eczema, or minor injuries, may lead to post-inflammatory hyperpigmentation as the skin heals. Additionally, aging contributes to uneven pigmentation by slowing down cell turnover, causing accumulation of melanin in certain areas. Genetic predisposition and improper skincare practices, such as harsh chemical use or lack of sun protection, further aggravate the condition, collectively leading to hyperpigmentation and uneven skin appearance.

SERUM

A serum is a lightweight, fast-absorbing topical formulation designed to deliver a high concentration of active ingredients directly into deeper layers of skin. Unlike conventional creams or lotions, which primarily act on surface and contain higher amounts of occlusive agents, serums are typically water-based or hydroalcoholic systems with smaller molecular structures that allow enhanced penetration through stratum corneum. This characteristic makes serums particularly effective in targeting specific concerns such as hyperpigmentation, dehydration, and signs of aging including fine lines and wrinkles.

Types of serum

Type of Serum	Key Ingredients	Primary Function	Target Skin Concern
Hydrating Serum	Hyaluronic acid, Glycerin	moisture and improves skin hydration	Dry, dehydrated skin
Anti-aging Serum	Retinol, Peptides, Vitamin C	Stimulates collagen synthesis, reduces wrinkles	Fine lines, wrinkles, aging
Brightening Serum	Vitamin C, Niacinamide, Liquorice extract	Inhibits melanin production, enhances skin radiance	Hyperpigmentation, dull skin
Anti-acne Serum	Salicylic acid, Tea tree oil, Zinc	Reduces sebum, antibacterial, anti-inflammatory	Acne, oily skin
Antioxidant Serum	Vitamin E, Green tea extract, Ferulic acid	Neutralizes free radicals, protects from oxidative damage	Environmental damage, premature aging
Exfoliating Serum	AHAs (Glycolic acid), BHAs (Salicylic acid)	Removes dead cells, promotes cell turnover	Rough texture, clogged pores

Type of Serum	Key Ingredients	Primary Function	Target Skin Concern
Soothing/Repair Serum	Aloe vera, Centella asiatica, Panthenol	Reduces irritation, promotes skin healing	Sensitive, irritated skin
Nano/Advanced Serum	Liposomes, Niosomes, Transferosomes	Enhances penetration and bioavailability of actives	Targeted drug delivery (cosmeceuticals)

TABLE 1: Types of Serums and Their Functions

Mechanism of action of serum

Application of Serum on Skin Surface → Rapid Absorption due to Low Molecular Weight & Light Base → Penetration through Stratum Corneum (Intercellular / Transcellular / Follicular Pathways) → Delivery of Active Ingredients to Epidermis/Dermis → Interaction with Target Cells/Enzymes → Biological Effects:

- Antioxidants → Neutralize free radicals
- Hydrators → Bind water & improve moisture
- Anti-aging agents → Stimulate collagen synthesis
- Depigmenting agents → Inhibit tyrosinase enzyme
- Anti-acne agents → Reduce sebum & bacteria

Advantages:

High concentration of active ingredients, Penetrates deeply, Fast-absorbing and lightweight, Non-greasy suitable for oily skin, Targets specific concerns, Quicker visible results.

Disadvantages:

May cause irritation, Not sufficient for moisturizing alone, Needs to be followed by moisturizer, Can be expensive, Overuse may damage skin barrier.

NOVEL DRUG DELIVERY SYSTEMS.

Novel drug delivery systems (NDDS) have gained considerable attention in recent years due to their ability to reduce dosing frequency, improve bioavailability, achieve site-specific delivery, and minimize adverse effects.

TRANSFEROSOMES

Transferosomes, also known as ultra-deformable vesicles, are specialized topical carriers composed of a phospholipid bilayer along with an edge activator and an aqueous core. Depending on its lipophilicity, the active moiety can be encapsulated within the aqueous core or intercalated within the lipid bilayer. Unlike conventional liposomes, transferosomes exhibit superior flexibility and can penetrate into deeper skin layers after topical application.

Structure of Transferosomes

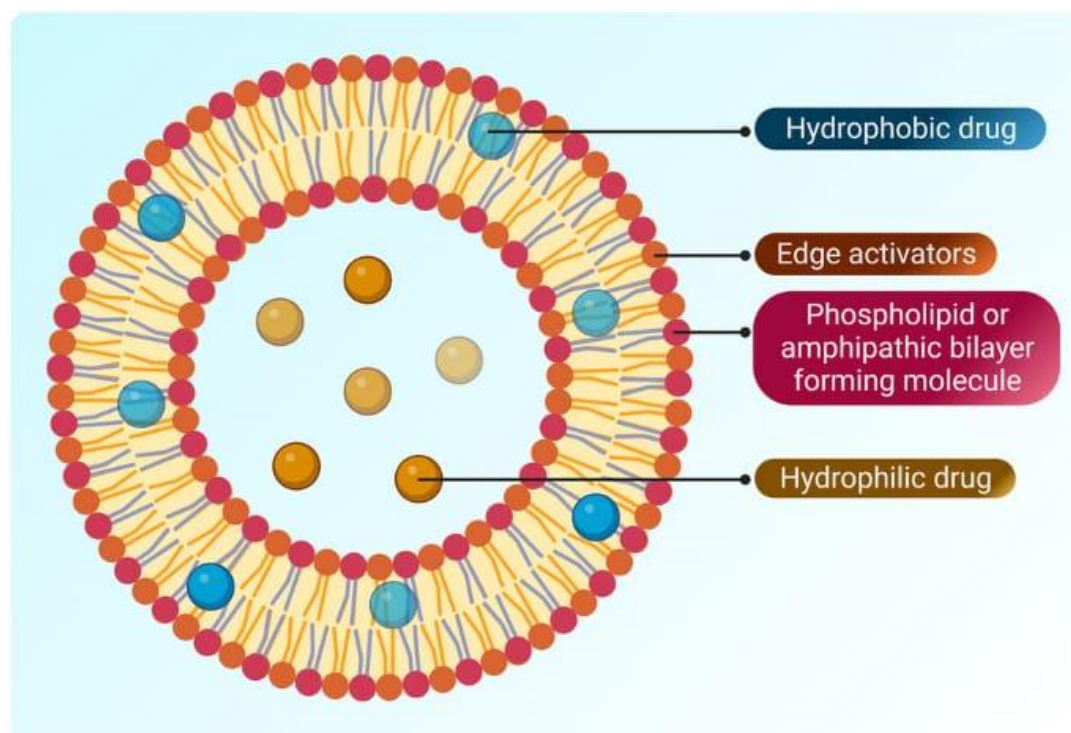


FIG 5 – Structure of transferosomes

SL. No	Example	Class	Use
1	Egg phosphatidyl choline soya phosphatidyl choline, dipalmitoylphosphatidyl choline	Phospholipids	Vesicles forming component
2	Ethanol, Methanol, isopropyl alcohol, chloroform	Solvents	As a solvent
3	Sodium cholate, sodium deoxycholate, Tween-80, Span-80, Tween-20	surfactants	Vesicles forming compound (Edge activators)
4	Saline phosphate buffer (PH 6.4), Phosphate buffer PH 7.4	Buffering agent	As a hydrating medium
5	Rhodamine-123 Rhodamine-DHPE Fluorescein-DHPE Nile-red	Dye	For CSLM Study

TABLE 2. Classification of materials used in transferosomal preparation

Mechanism of Action of Transferosomes

Recent investigations indicate that transferosomes are capable of traversing intact skin. Their penetration is driven by trans-epidermal hydration gradient: interaction of lipids with water leads to hydration and movement of vesicles towards deeper water-rich layers. The variation in water content between stratum corneum and viable epidermis creates an osmotic gradient that facilitates permeation. Owing to their self-optimizing deformability, transferosomes can reversibly alter membrane flexibility to squeeze through narrow intercellular pores. Application under non-occlusive conditions is essential to maintain this gradient; evaporation of water from the formulation generates xerophobic (hydrotactic) behavior that drives vesicles toward deeper hydrated layers.

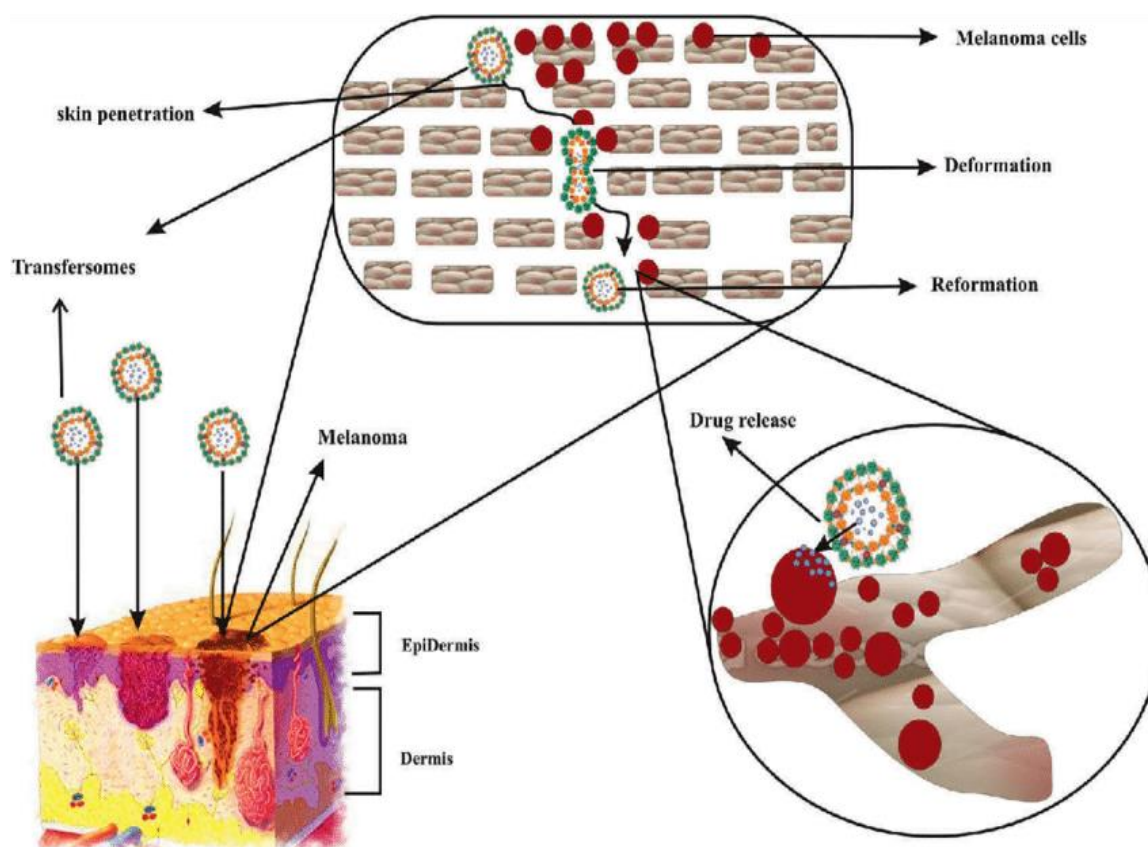
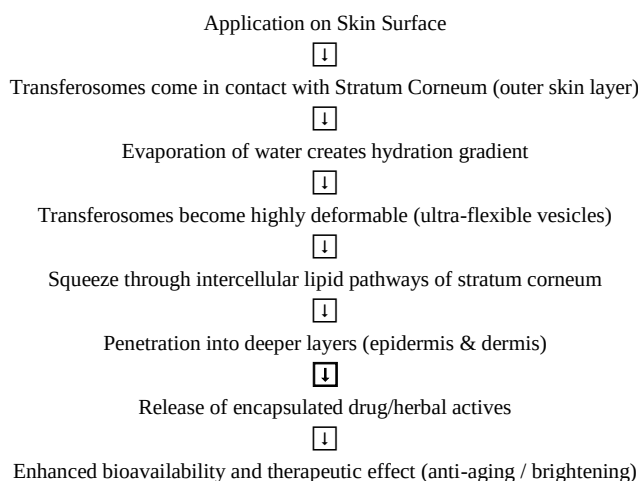


FIG 6: Mechanism of ultra-deformable transfersomes penetrating through stratum corneum via deformation and reformation for targeted drug delivery to melanoma cells



Methods of preparation of Transfersomes

1. Rotary Film evaporation method

This technique is also known as the modified hand-shaking method and is one of the most widely used methods for transfersome preparation. In this process, the active pharmaceutical ingredient (API), lecithin (phospholipid), and edge activator are dissolved in a 1:1 mixture of chloroform and ethanol with manual agitation at a temperature above the lipid phase transition temperature. The resulting clear solution is transferred to a round-bottom flask and subjected to evaporation under reduced pressure using a rotary evaporator to remove the organic solvent. A thin lipid film is formed on the walls of the flask. The film is kept overnight in a desiccator to ensure complete removal of organic solvent. The film is subsequently hydrated by rotating it at 60 RPM for 1 hour at room temperature with phosphate buffer of pH 6.5. The resulting vesicles are allowed to swell for 2 hours at room temperature. Finally, the dispersion is sonicated at room temperature using a bath sonicator to obtain small, uniform-sized vesicles.

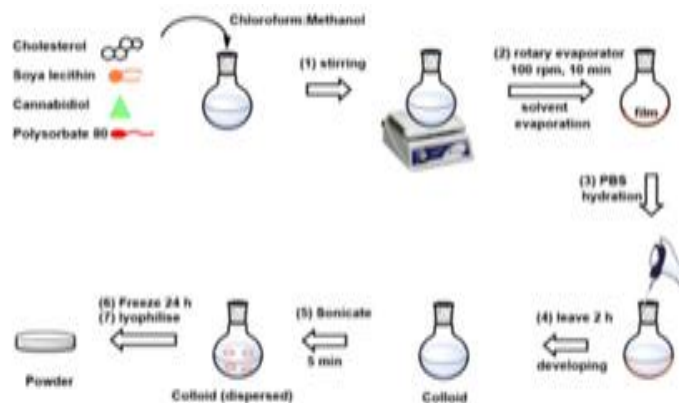


FIG 7 Rotatory funnel operator

2. Reverse phase evaporation method

In this method, lipids and organic solvents are taken in a round-bottomed flask under nitrogen purging along with aqueous phase containing edge activators. Depending on the solubility of the drug, it is incorporated into either lipophilic or hydrophilic phase. The mixture is sonicated to form a homogeneous dispersion and allowed to stand for 30 minutes. The organic phase is then eliminated under reduced pressure. The system transforms into a viscous gel which spontaneously forms vesicles upon further agitation. This method is particularly suitable for hydrophilic drugs and provides high entrapment efficiency.

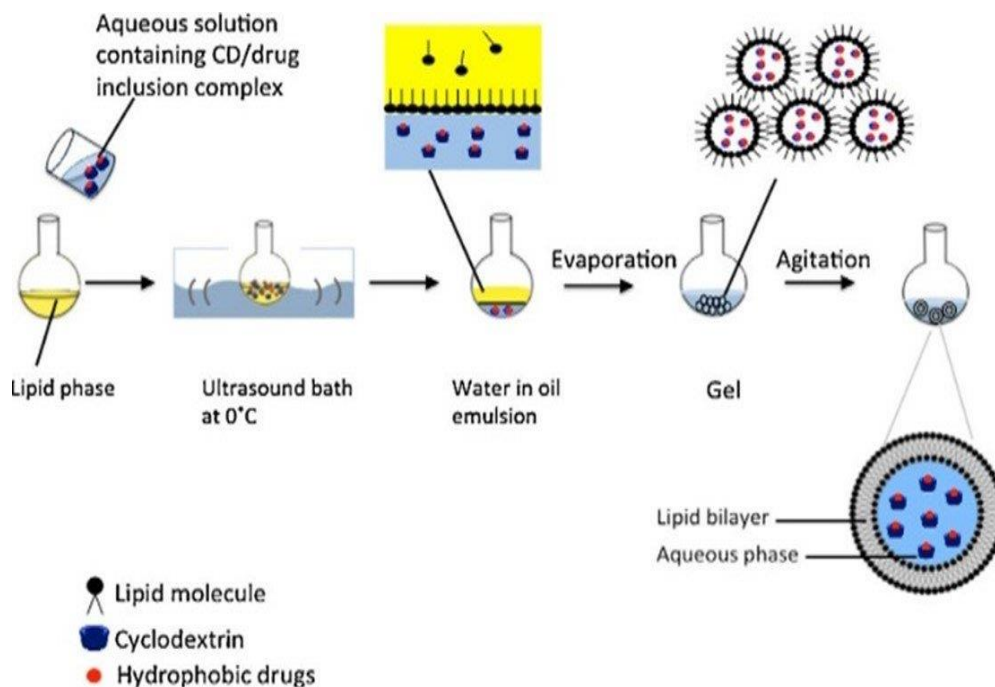


FIG 8 . Reverse phase evaporation method

3. Vortex or sonication method

In this procedure, edge activators and phospholipids are dispersed in phosphate buffer by continuous vortexing or swirling. A milky, turbid suspension is formed, which is then sonicated in a bath sonicator for 10-20 minutes to reduce vesicle size. The resulting dispersion is subsequently extruded through polycarbonate membranes of defined pore size (100-200 nm) to obtain uniform, small unilamellar vesicles. This method is simple and does not require organic solvents.

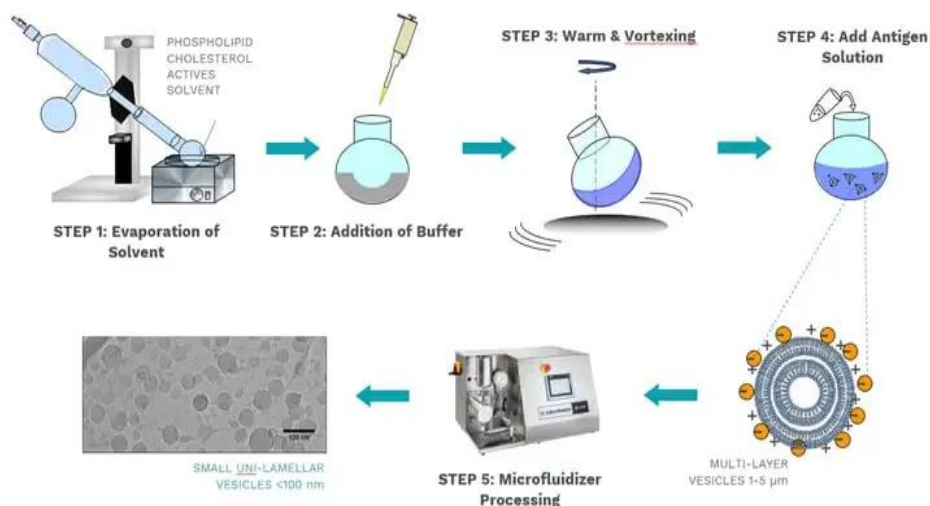


FIG 9 Vortex or sonication method

4. Freeze thaw method

This technique involves subjecting the multilamellar vesicle (MLV) suspension to repeated freeze-thaw cycles to improve lamellarity and entrapment efficiency. The prepared MLV suspension is transferred to a centrifuge tube and frozen by immersing it in a liquid nitrogen bath at -30°C for 30 seconds. After complete freezing, the suspension is thawed by placing it in a water bath at higher temperature. This cycle is repeated 8-9 times. The repeated freezing and thawing leads to formation of unilamellar vesicles with improved encapsulation of active ingredients.

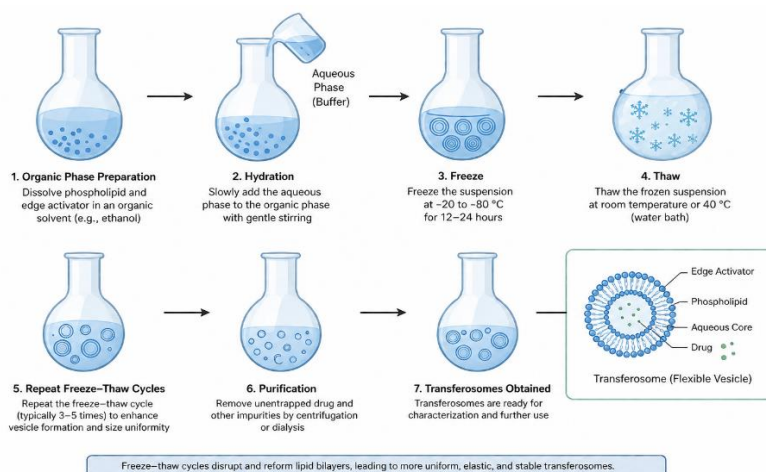


Fig 10 Freeze thaw method

5. Ethanol Injection Method

The ethanol injection method offers significant advantages in terms of simplicity, reproducibility, and scalability, and is the method used in your Hibiscus and Green Tea formulation. In this approach, the organic phase consisting of vesicle-forming components like phospholipid (soya lecithin) and edge activator (Tween 80) is dissolved in absolute ethanol at 35 – 40°C with continuous stirring to obtain a clear solution. The aqueous phase containing water-soluble herbal extracts dissolved in phosphate buffer of pH 6.4–7.4 is heated separately to 40 – 45°C . The warm ethanolic lipid solution is then injected dropwise into the warm aqueous solution using a syringe with continuous high-speed stirring at 45 – 50°C . Lipids self-assemble into bilayered vesicles entrapping the extract upon dilution of ethanol in water. Stirring is continued for 30–60 minutes to ensure complete evaporation of ethanol. Finally, size reduction is carried out using a bath or probe sonicator for 10–20 minutes at room temperature to obtain uniform nano-sized transferosomal vesicles. The final dispersion is cooled and stored in an airtight amber container at 2 – 8°C .

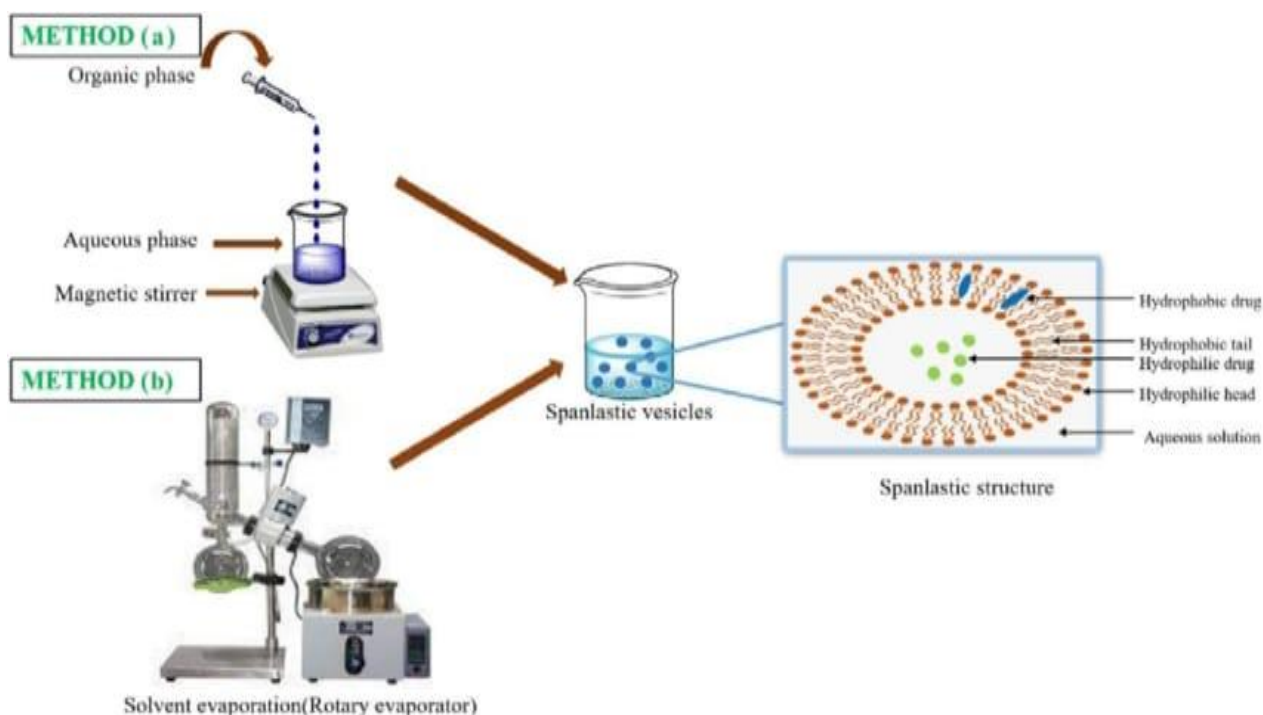


Fig 11 Ethanol Injection Method

Since both Acne and Skin aging involve inflammation and oxidative stress, there is growing interest in developing multifunctional cosmetic formulations capable of addressing both conditions simultaneously. Herbal bioactive compounds rich in anti-oxidants and anti-inflammatory phytochemicals have gained considerable attention because of their efficacy, safety, and reduced risks of adverse effects compared with many synthetic agents.

The contemporary cosmeceutical industry is rapidly evolving toward the development of multifunctional skincare systems that not only enhance aesthetic appeal but also deliver therapeutic benefits. In order to bring out the novelty in cosmetic science, Advanced delivery systems such as transfersomes further enhance the penetration and bioavailability of these natural actives, improving their therapeutic and cosmetic performance. Therefore, the development of transfersome-loaded herbal serum represents a promising approach for the effective management of acne while simultaneously preventing premature skin aging, offering improved skin health and enhanced cosmetic benefits.



FIG 12 Design and Mechanism of Herbal Transfersomal Serum

Methods Extraction of Materials:

1. **Hibiscus:** For hibiscus petal extraction, two weeks shadowed dried flowers (petal) where taken and soaked in 80% ethanol for 48 hours. Water bath was heated up to 40 degree for 10 minutes with the soaked petals. Extract was obtained.



FIG 13: Shadowed dried hibiscus petal soaked in 80% ethanol for 48 hours

2. **Green Tea:** In this method, dried green tea leaves where soaked in 96% ethanol and sealed in glass jar tightly, store in cool and dark place for one week. After one week the extract was filtered out and stored in amber colour bottle.



FIG 14: Dried green tea leaves soaked in 96% ethanol for one week

Pre formulation studies:**A. Hibiscus:****1. Organoleptic Properties:**

- color

- odour
- texture

2. pH Determination:

Use a calibrated digital pH meter to ensure the extract falls between 4.5 and 6.0 to prevent skin damage.

3. Phenols Test and Tannins test:

The extracts were mixed with three to four drops of the ferric chloride test. The presence of phenols and tannins is indicated if a blue-black color develops.^[14]

4. Flavonoids Test:

Some drops of lead acetate solution were added to the extracts. The presence of flavonoids would be indicated by the production of yellow precipitate.^[14]

5. Alkaloids Test:

Adding dragendroff's reagent to the extract yields an orange-coloured precipitate.^[14]

6. Viscosity:

Assessed using a Brookfield or falling-sphere viscometer to ensure the extract has a lightweight consistency that facilitates deep skin penetration.

7. Determination of Partition Coefficient:

10 ml hibiscus liquid extract was mixed with 10 ml ethyl acetate in separating funnel. Shaken for 2 hours and allowed to separate. Both phases evaporated and weighed.

$D = W_e/W_w$ calculated.

B. Green Tea:

1) Organoleptic properties:

- Color
- Odour
- Texture

2) pH Determination:

Essential to match skin physiology. Use a calibrated digital pH meter to ensure the extract falls between 4.5 and 6.0 to prevent barrier damage.

3) Viscosity:

Assessed using a Brookfield or falling-sphere viscometer to ensure the extract has a lightweight consistency that facilitates deep skin penetration.

4) Heavy Metal Limit Test:

The test is performed in Nessler cylinders by comparing the colour of sample with standard lead solution. 1g of green tea extract is dissolved in water, filtered, and 25ml of filtrate is taken in a 50ml Nessler cylinder. pH is adjusted to 3.0–4.0 using dilute acetic acid or dilute ammonia and volume made up to 40ml with water. To this, 2ml of sulphide reagent is added and mixed. 20 ppm standard lead solution is treated similarly as reference. The brownish-black colour of the sample should not be more intense than the standard, confirming heavy metals are within permissible limit.

5) TLC Method for Catechins:

Thin Layer Chromatography was performed for the identification of catechins using Silica gel 60 F254 pre-coated plates as the stationary phase. The standard and sample solutions were spotted on the plate and developed in a saturated chamber using Chloroform: Ethyl acetate: Glacial acetic acid (4:4:2 v/v) as the mobile phase. For specific detection, the plate was sprayed with 5% Ferric chloride (FeCl_3) solution. Catechins appeared as green to bluish-black spots due to the formation of complexes with phenolic hydroxyl groups. The R_f values were calculated and compared with the standard catechin for confirmation of identity and purity.

Stationary phase: Silica gel 60 F254

Mobile phase: Chloroform: Ethyl acetate: Glacial acetic acid (4:4:2)

Detection: 5% FeCl_3 spray → Green-Black spots

R_f range: Typically 0.4 - 0.7 for catechins in this system

6) Determination of Partition Coefficient:

10 ml green tea liquid extract was mixed with 10 ml ethyl acetate in separating funnel. Shaken for 2 hours and allowed to separate. Both phases evaporated and weighed.

$D = W_e/W_w$ calculated.

Formation of transfersomes using Ethanol Injection Method

Organic Phase: Weigh phospholipid (100-200 mg) and edge activator (10-30 mg). Dissolve in absolute ethanol, stir till clear.

Aqueous Phase: Dissolve herbal aqueous extract in phosphate buffer pH 6.4. Filter if needed.

Heating: Heat both phases separately at 45-50°C.

Ethanol Injection: Inject organic phase dropwise into aqueous phase with high-speed stirring.

Evaporation: Stir at 45-50°C for 30-60 min to remove ethanol.

Size Reduction: Sonicate 10-20 min.

Storage: Cool, centrifuge if needed, store at 2-8°C.

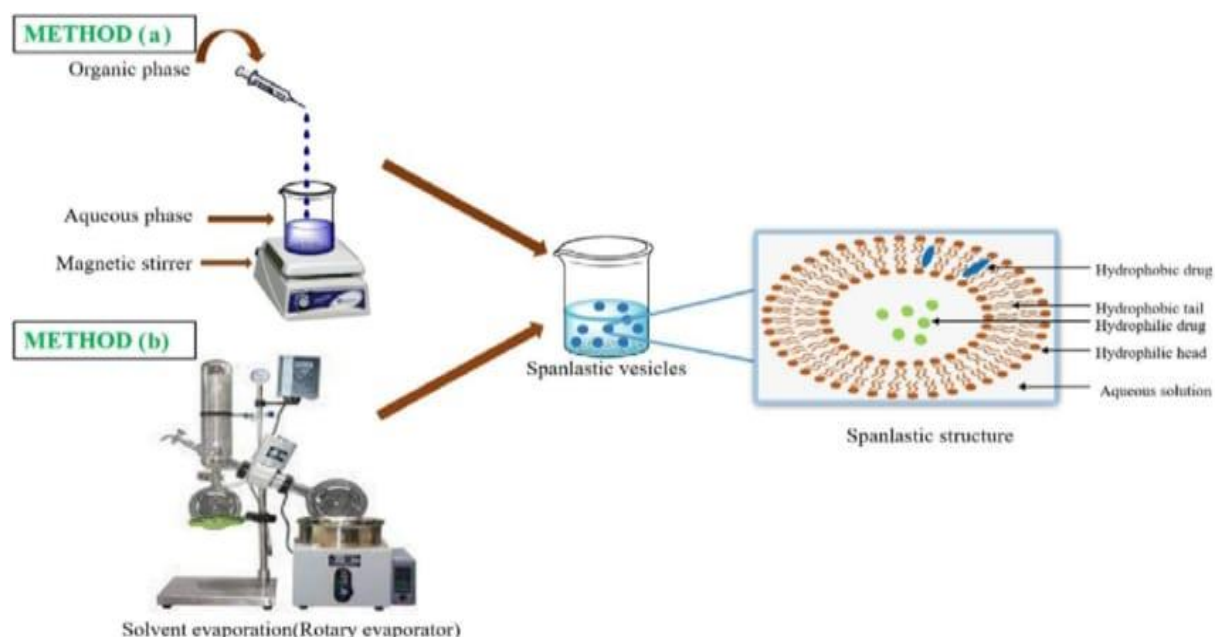


FIG 15 Ethanol injection method

Evaluation parameter of transfersomes:

1) **Zeta potential and particle size determination:** The particle size and zeta potential of the formulation are measured at 25°C using a Malvern Zeta Sizer based on dynamic light scattering. The sample is diluted with filtered water before analysis. Results give average vesicle size, PDI and surface charge which indicates physical stability. ^[19]

2) **Vesicle morphology:** Transmission Electron Microscopy and Phase Contrast Microscopy are used to visualize transfersome vesicles. The size, shape and lamellarity of vesicles are observed to determine structural integrity and stability over time. ^[19]

3) **Entrapment efficiency:** Untrapped drug is separated by micro-column centrifugation. Vesicles are disrupted using 0.1% Triton X-100 or 50% n-propanol.

$$\% EE = (\text{Amount entrapped} / \text{Total amount}) \times 100 \quad [19]$$

Preparation of Transfersomes:

Transfersomes are prepared by the solvent evaporation method. The organic phase consists of vesicle forming components like phospholipid/cholesterol and an edge activator such as Tween 80, dissolved in ethanol at 35–40°C with stirring to obtain a clear solution. The aqueous phase contains water-soluble API dissolved in phosphate buffer of pH 5.4–8 and heated to 40–45°C. The organic phase is then added drop wise to the aqueous phase using a syringe at a controlled flow rate to form lipid bilayers. The mixture is stirred for 10–15 min and heated to 40°C to ensure complete evaporation of ethanol. Finally, size reduction is carried out using a homogenizer to obtain uniform transfersomal vesicles.

Procedure for Preparation of Nano Transfersomal herbal serum:

Organic Phase: Accurately weigh phospholipid (100-200 mg) and edge activator (10-30 mg). Dissolve both in absolute ethanol and stir until a clear solution is obtained.

Aqueous Phase: Dissolve herbal aqueous extract in phosphate buffer of pH 6.4 or 7.4. Filter if needed to remove particulate matter.

Heating: Heat both organic and aqueous phases separately in a water bath at 45°C-50°C.

Ethanol Injection: Inject the warm ethanolic lipid solution dropwise into the warm aqueous solution using a syringe with continuous high-speed stirring. Lipids self-assemble into bilayered vesicles entrapping the extract.

Solvent Evaporation: Continue stirring at 45°C-50°C for 30-60 min to evaporate ethanol. A rotary evaporator can also be used.

Size Reduction: Sonicate the dispersion using a bath or probe sonicator for 10-20 min at room temperature to obtain uniform nano-sized vesicles.

Storage: Cool to room temperature. Centrifuge if required to remove untrapped material. Store the final dispersion in an airtight amber bottle at 2°C-8°C.

Evaluation parameters of face serum: [4, 7, 10]**1) Physical evaluation:**

The formulation is evaluated based on visual appearance and touch.

- Color
- Texture
- odour

2) pH: Using a standard buffer solution, a pH meter was calibrated. The pH of the combination was determined by accurately measuring and blending almost 1 mL of face serum with 10 mL of clean water. The skin has an acidic pH, and skin serums should have a pH between 4.1 to 6.7.

3) Spread ability: The serum spreads over the filter paper, indicating the area to which it was delivered. For each of the filter paper sizes selected, the total area (A1) and weight (W1) are measured. The correct entry for this measurement is A2.

% Spread by Area = $(A2/A1)100$.

4) Absorbance time: After applying the serum to the skin and timing how long it takes it to absorb, note the time.

5) Viscosity: Using a spindle type model S64, the viscosity of the formulation was measured at 100 rpm using a Brookfield viscometer. After dipping the spindle in 5 ml of the serum in a beaker for approximately 5 minutes, readings were obtained.

6) Stability Testing: Monitored for 30 days at room temperature at 40°C; observed for changes in colour, pH, viscosity and odour- criteria for shelf life

7) Wash ability test: Few drops of prepared serum is exposed to the skin surface and then wash ability is determined manually.

8) Irritancy Test: Patch testing confirmed non-irritant nature, validating safety for daily use.

9) Assay of polyphenol in Hibiscus and Green tea extract by KMnO₄ Titration:**Principle:**

Polyphenols present in green tea and hibiscus have reducing properties. In acidic medium, potassium permanganate oxidizes these phenolic compounds. The excess KMnO₄ is titrated and the amount consumed corresponds to the polyphenol content. The reaction is based on redox titration.

Reagents Required: 0.1N KMnO₄ - Standardized titrant, pink color 2N H₂SO₄ - Acidic medium Oxalic acid 0.1N - For standardization of KMnO₄ Distilled water

Procedure:**Preparation of Sample:**

Weigh accurately about 1g of dried hibiscus / green tea extract. Dissolve in 100ml distilled water and filter. Pipette out 10ml of filtrate into a conical flask.

Titration:

Add 10ml of 2N H₂SO₄ to the flask. Titrate with 0.1N KMnO₄ solution till a permanent pale pink color appears that persists for 30 seconds. Note the burette reading. This is the sample titration - V1 ml.

Blank Titration:

Repeat the same procedure without the sample using 10ml distilled water + 10ml 2N H₂SO₄. Note the reading - V₂ ml.

Calculation:

$$\% \text{ Polyphenols} = [(V_2 - V_1) \times N \times 0.02904 \times 100] / W$$

Where, V₂ = Volume of KMnO₄ for blank in ml
V₁ = Volume of KMnO₄ for sample in ml
N = Normality of KMnO₄
W = Weight of sample taken in grams
0.02904 = Equivalent weight of catechin /10

Result:

The % content of total polyphenols expressed as catechin in hibiscus/green tea extract = ____ % w/w

Note: Green tea is mainly rich in EGCG, ECG, EGC which are strong reducing agents → High KMnO₄ consumption. Hibiscus contains anthocyanins + flavonoids which also reduce KMnO₄ → Gives total antioxidant value. Titrate in acidic medium only, otherwise MnO₂ precipitate forms.

Endpoint: Disappearance of pink color of KMnO₄ → Pale pink persistent

Formulation Chart:

Si no.	Ingredients	Action
01	Soy lecithin	A phospholipids, amphiphilic structure, emulsifier, emollient, penetration enhancer, vesicles forming component .
02	Tween 80	An edge activator, non-ionic surfactant, flexible vesicles for easy penetration into tiny pores, amphiphilic nature, penetration enhancer.
03	Ethanol	A solvent, improve drug solubility, usage of ethanol injection method.
04	Hibiscus extract	Bio active ingredient
05	Green tea extract	Bio active ingredient
06	Phosphate buffer	Maintains stability, skin friendly pH(5.5-6.5), stability of vesicles
07	Glycerine	Humectant, enhances anti-aging effect, provides elasticity
08	Propylene glycol	Humectant, penetration enhancer, improves drug delivery and skin hydration.
09	Benzyl alcohol	Preservative, prevent microbial growth
10	Rose water	Perfume

TABLE 3 Formulation Chart

Formulation Code for transferosome loaded serum base:

Ingredients	F1	F2	F3	F4
Soy lecithin	1.5mg	2.0mg	2.5mg	3.0mg
Tween 80	0.25mg	0.50mg	0.75mg	1.0mg
Ethanol	q.s	q.s	q.s	q.s
Hibiscus extract	2.0ml	2.0ml	2.0ml	2.0ml
Green tea extract	2.0ml	2.0ml	2.0ml	2.0ml
Phosphate buffer	q.s	q.s	q.s	q.s
Benzyl alcohol	1.0ml	1.0ml	1.0ml	1.0ml
Glycerin	15ml	15ml	15ml	15ml
Rose water	q.s	q.s	q.s	q.s

TABLE 4 Formulation Code for transferosome loaded serum base

Preformulation test for Hibiscus:

(Hibiscus flower extract)

Result:**1. Appearance:**

No.	Test	Observation
01	Color	Wine Red color
02	Odor	Ethanolic-floral odor
03	Texture	Smooth sticky texture

2. pH:

No.	Preparation	pH meter value
01	For 2ml of extract 10ml of Distilled water is added and mixed thoroughly. Then pH is measured by pH meter.	6.58

3. Solubility:

No	Solvent	Solubility
01	Distilled water	Sparingly soluble
02	Ethanol	Soluble

4. Chemical test:

No.	Test	Experiment	Observation	Inference
01	Phenols	The extracts were mixed with three to four drops of the ferric chloride test.	Dark green or blue black color is observed.	Phenols are confirmed
02	Alkaloids	Adding dragendroff's reagent to the extract	White or yellow ppt.	Alkaloids are confirmed
03	Flavonoids	Some drops of lead acetate solution were added to the extracts	Bright yellow color that becomes colorless upon adding dil. acid	Flavonoids are confirmed

5. Viscosity:

No	Viscometer used	Value
01	Brookfield Viscometer	102.4mpa.s

6. Determination of Partition Coefficient:

Extract	Residue in Water phase (Ww) mg	Residue in Ethyl acetate phase (We) mg	Distribution Coefficient $D = W_e / W_w$	Log D
Hibiscus extract	2.4 mg	27.6 mg	0.38	-0.42

Preformulation test for Green tea:

(Green tea leaves extract)

Result:**1. Appearance:**

No.	Test	Observation
01	Color	Greenish brown color
02	Odor	Ethanolic green tea odor
03	Texture	Smooth sticky texture

2. pH:

No.	Preparation	pH meter value
01	For 2ml of extract 10ml of Distilled water is added and mixed thoroughly. Then pH is measured by pH meter.	6.58

3. Solubility:

No	Solvent	Solubility
01	Distilled water	Sparingly or partially soluble
02	Ethanol	Freely Soluble

4. Viscosity:

No	Viscometer used	Value
01	Brookfield Viscometer	103.9mpa-s

5. Limit test:

No.	Experiment	Preparation	Observation	Inference
01	Standard	2ml of standard lead solution is added to the cylinder, pH is checked and adjusted to 3-4 using acetic acid or dilute ammonia, addition of 10 ml H ₂ S solution, and dilute with water to the 50ml mark.	Turbid solution is observed	Standard color produced corresponds to the limit of lead.
02	Sample	2ml of Green tea extract is added to the cylinder and made up to 25ml with distilled water. pH is checked and adjusted to 3-4 using acetic acid or dilute ammonia, addition of 10 ml H ₂ S solution, and dilute with water to the 50ml mark.	Light green colored solution	The heavy metal content is below the prescribed limit. The extract passes the limit test.

6. TLC Catechins:

No.	Experiment	Preparation	Rf value
01	Standard	Dissolve standard Catechins of 2gm in diluent (ethanol: water 4:1v/v) and centrifuge it.	0.74
02	Sample	Dissolve plant extract of 2ml in diluent (ethanol: water 4:1v/v) and centrifuge it.	0.67

7. Partition coefficient :

Extract	Residue in Water phase (Ww) mg	Residue in Ethyl acetate phase (We) mg	Distribution Coefficient $D = W_e / W_w$	Log D
Green Tea extract	54.8 mg	45.2 mg	0.82	-0.08

Evaluation parameter of transfersomes:

1) Zeta potential and particle size determination:

Formulation code	Mean particle size nm $\pm$ SD	Polydispersity index(PDI)	Zeta potential (mV) $\pm$ SD	Inference
F1	210.5 $\pm$ 4.2	0.385 $\pm$ 0.02	-18.4 $\pm$ 1.1	High PDI, moderate stability
F2	185.2 $\pm$ 3.6	0.292 $\pm$ 0.01	-25.6 $\pm$ 1.5	Good homogeneity, and charge
F3 (Optimized)	152.5 $\pm$ 2.8	0.215 $\pm$ 0.01	-32.0 $\pm$ 1.8	Ideal size, high stability
F4	268.0 $\pm$ 5.1	0.410 $\pm$ 0.03	-14.2 $\pm$ 0.9	Particle growth, lower stability

2) Vesicle morphology:

Formulation code	Surfactant used (Edge Activator)	Morphological appearance(SEM observation)	Structural Integrity and Lamellarity
F1	Tween 80	Well-defined, rigid spherical vesicles. Clear boundaries, no aggregation.	Unilamellar to oligolamellar. Slightly rigid due to insufficient edge activator.
F2	Tween 80	Spherical, smaller vesicles but with slightly irregular or blurred edges.	Perdominantly unilamellar. High surfactant concentration initiates minor membrane destabilization.
F3 (Optimized)	Tween 80	Perfectly spherical, uniform Nano vesicles. Fully dispersed with zero signs of fusion or leakage.	Highly flexible unilamellar vesicles. Optimum structural integrity and maximum ultra-deformability.
F4	Tween 80	Disrupted highly irregular vesicular structures. Co-existence of mixed micelles and free fragments.	Broken bilayers.Excess edge activator solubilizes the lipid matrix, destroying vesicular morphology.

3) Entrapment efficiency:

Formulation code	Lipid: Surfactant ratio (Soya Lecithin: Edge Activator)	Mean Particle size (nm)	Entrapment efficiency(%EE $\pm$ SD,n=3)
F1	6:1	210.5 $\pm$ 4.2	79.15 $\pm$ 1.8%
F2	4:1	185.2 $\pm$ 3.6	82.5 $\pm$ 2.1
F3 (Optimized)	10:3	152.5 $\pm$ 2.8	88.2 $\pm$ 1.5%
F4	3:1	268.0 $\pm$ 5.1	65.3 $\pm$ 2.4%

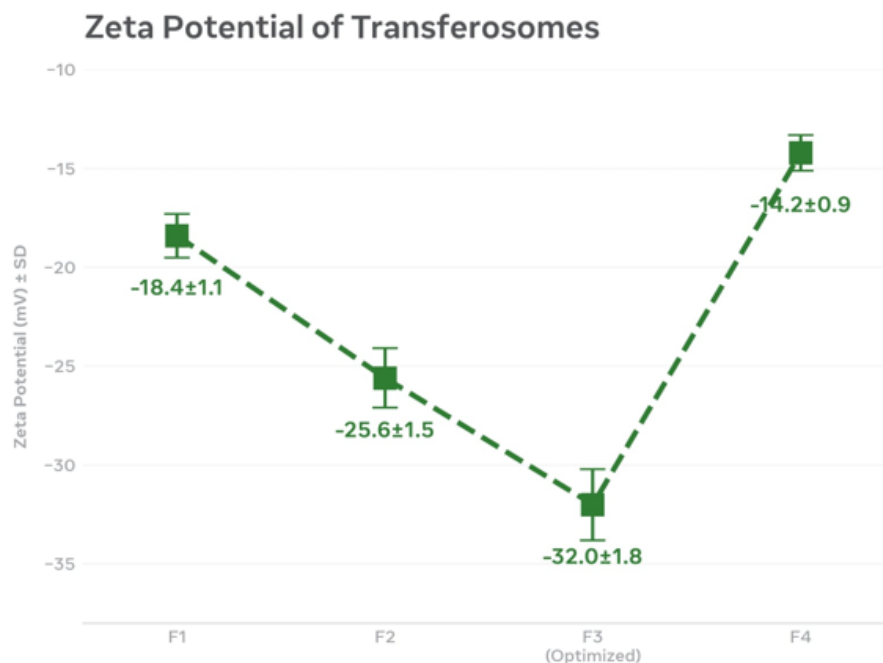


FIG 16: Line graph showing Zeta Potential (mV) of different transfersome formulations

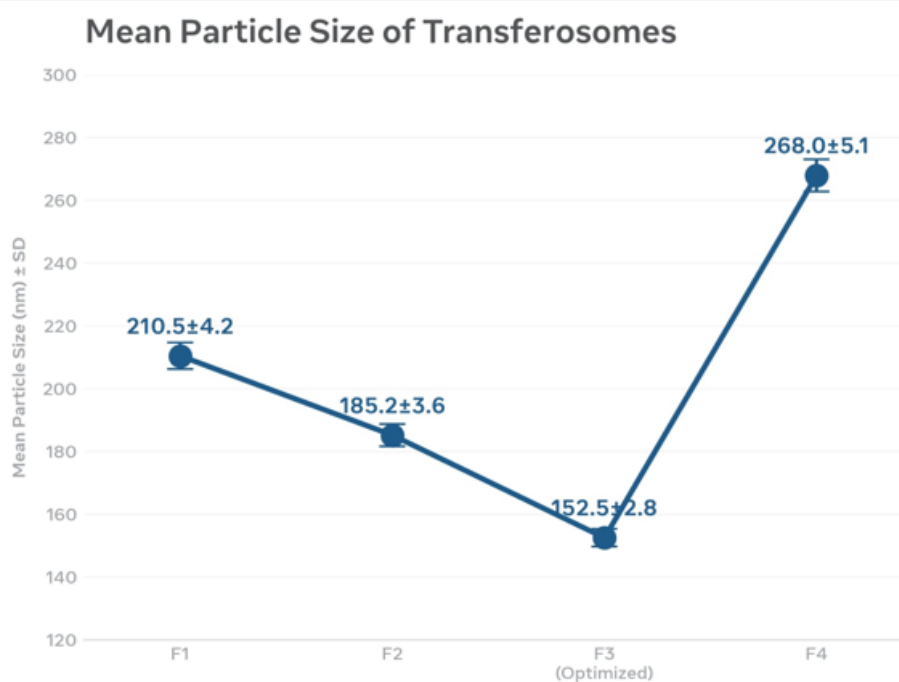


FIG 17: Line graph showing Mean Particle Size (nm) of different transfersome formulations (F1-F4)

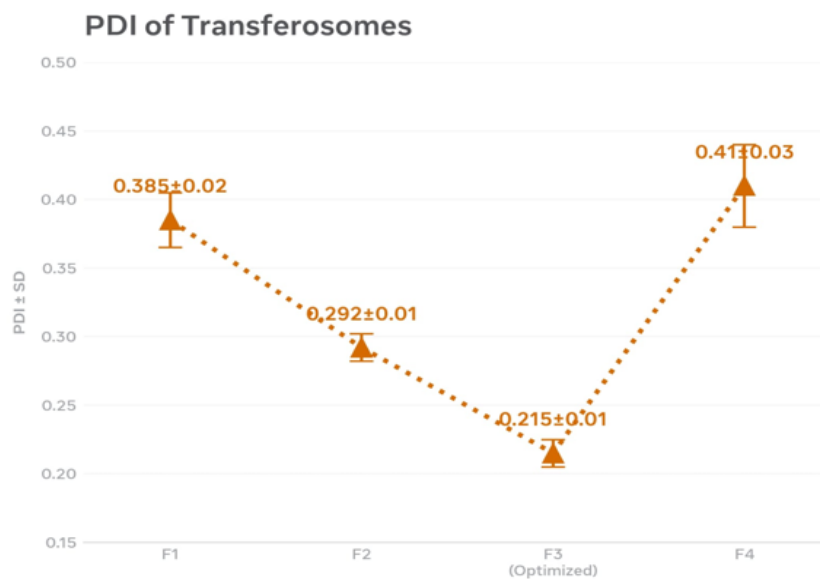


FIG 18 : Line graph showing PDI of different transferosome formulations

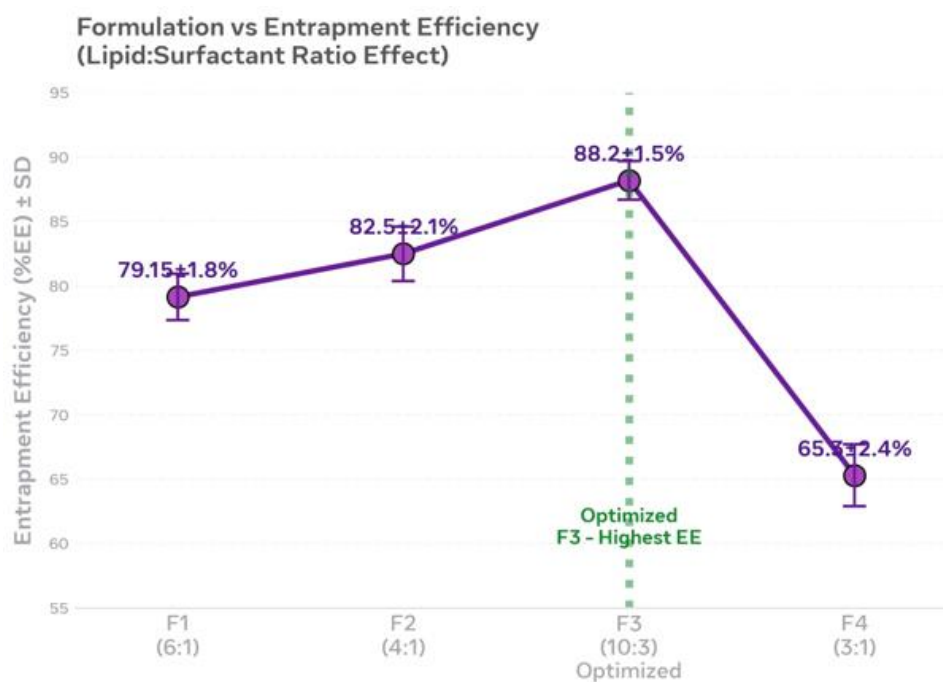


FIG 19: Line graph showing Entrapment Efficiency (%EE) vs Formulation code (Effect of Lipid:Surfactant ratio)

EVALUATION OF HERBAL SERUM:

1. Physical evaluation:

Characteristics	F1	F2	F3	F4
Color	Transparent golden yellow	Transparent golden yellow	Transparent yellow	Pale milky yellow
Odour	Ethanol odour	Ethanol odour	Ethanol odour	Ethanol odour
Homogeneity	Fairly homogenous	Fairly homogenous	Fairly homogenous	Poorly homogenous
Texture	smooth	smooth	smooth	Slightly smooth
Consistency	Good	Good	Good	Poor

2. pH:

Test	F1	F2	F3	F4
pH	6.58	6.59	6.63	6.64

3. Spread ability:

Mass	Time	F1	F2	F3	F4
50g	60sec	6.25gcm/s	5.83gcm/s	6.66gcm/s	5.0gcm/s
100g	60sec	14.1gcm/s	15.6gcm/s	16gcm/s	10.5gcm/s
150g	60sec	25gcm/s	25.5gcm/s	26.5gcm/s	16gcm/s

4. Absorbance time:

Test	F1	F2	F3	F4
Absorbance time	2.20min	2.48min	1.40min	6.40min

5. Viscosity:

Test	F1	F2	F3	F4
Viscosity	315.1 mpa.s	512.7mpa.s	478.6mpa.s	513.5mpa.s

6. Specific gravity:

Test	F1	F2	F3	F4
Specific gravity	1.04g/ml	1.07g/ml	1.10g/ml	1.25g/ml

8. Wash ability test:

Test	F1	F2	F3	F4
Wash ability test	Easily washable	Easily washable	Easily washable	Easily washable

9. Irritancy Test:

Test	F1	F2	F3	F4
Irritancy Test	Non-irritant	Non-irritant	Non-irritant	Non-irritant

9. Assay:

No.	Formulation	% Purity
01	F1	99.3%v/v
02	F2	102.2%v/v
03	F3	101.3%v/v
04	F4	89%v/v

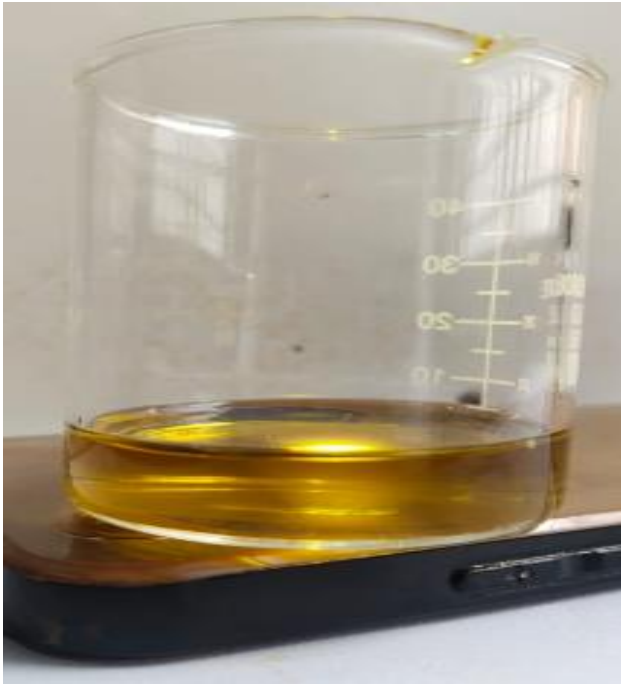


FIG 20: Formulation 3

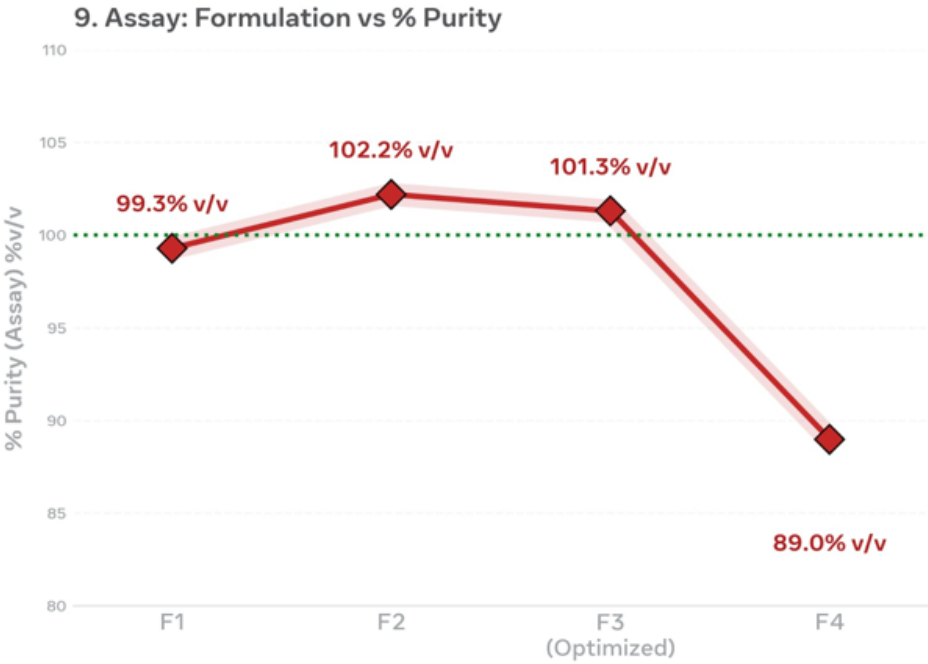


FIG 21: Line graph showing Assay (% Purity) of different transferrin formulations (F1-F4)

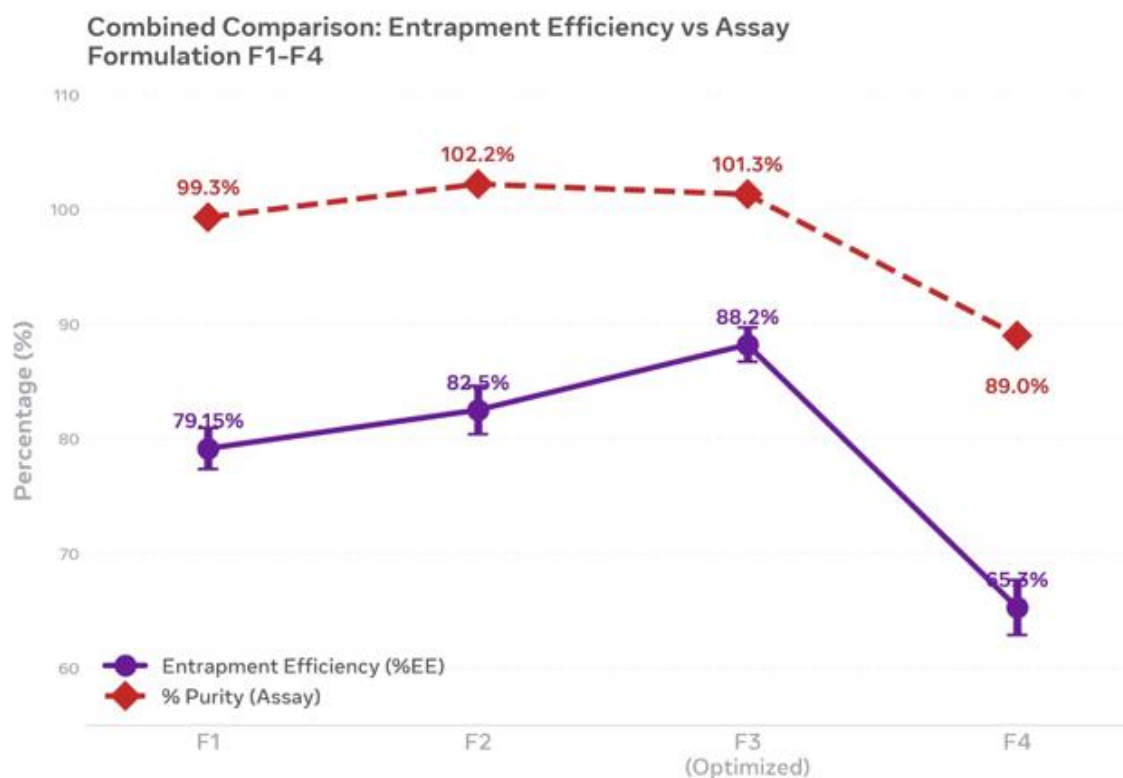


FIG 22: Combined line graph showing correlation between Entrapment Efficiency (%EE) and Assay (% Purity) for formulations F1-F4

Conclusion

The brightening and anti-aging serum using hibiscus, green tea extract by using transferosomes. This preparation gives moisturizing and glowing activity on skin, reduce the signs of aging, and improve overall skin health. The use of transferosomes as a drug delivery system further enhances the effectiveness of these herbal ingredients by improving their penetration into the deeper layers of the skin and increasing their stability. Herbal face serums address many different skincare needs, from nourishing and hydrating to relaxing and revitalizing.

They improve the stability, controlled release, and bioavailability of active compounds, allowing better delivery of herbal ingredients to the target site. This makes transferosomes a promising carrier for developing advanced herbal skincare products.

Overall, the combination of hibiscus and green tea extracts with transferosomal technology represents an innovative and effective approach in cosmeceutical research. It combines the therapeutic benefits of natural plant extracts with an advanced drug delivery system to improve skin health and reduce the signs of aging.

Reference:

- [1] Kadu P, More H, Shaikh T, Kakad S, & Mahajan J. Formulation and Evaluation of Herbal Cosmetics Serum. *Int J Pharm Sci Rev Res.* 2015; 31(2):17.
- [2] Ghosh A, & Ghosh T. Phytochemical composition and pharmacological activities of *Hibiscus rosa-sinesis*. *J Nat Prod.* 2018; 50(1): 50-6.
- [3] Chatterjee P, Pradhan D, & Gupta S. *Hibiscus rosa-sinensis*: A Review of Phytochemistry and Pharmacological Aspects. *Int J Pharm Sci.* 2018; 10(3): 45-52.
- [4] Jadhav N, & Patil S. Preparation and Evaluation of Herbal Serum for Face Brightening. *J Drug Deliv Ther.* 2020; 10(3): 125-30.
- [5] Ashish Tawar, Thormote Dipak, Bhojane Ankita, *et al.* From Tradition to Innovation Hibiscus in Modern Anti-Aging Skincare. *Int J Adv Res Sci Commun Technol.* 2025; 5(7): 662.
- [6] Singh, R., & Kaur, N. Hibiscus as a Natural Source of Antioxidants. *J Med Plants Stud.* 2014; 2(3): 55-9.
- [7] Komal Gadekar, Vaishali Kailas Gaikwad, *et al.* Formulation and Evaluation of Herbal Anti-Acne Serum using *Hibiscus rosa-sinesis*. *Int Res J.* 2026; 13(4): 5-11.
- [8] Nahid Anjum Hafizuddin Chishti, ZohebZaheer Shaikh, Patil Nikita Dnyaneshwar, & Saundarkar Aditi Gajanan. Formulation Development and Evaluation of Anti-Aging Vitamin E Serum. *Int J Pharm Appl.* 2025; 10(1): 3-4.
- [9] Lim J.T, Tham, S.N, & Lui K. F. Glycolic acid and Kojic acid combination in the treatment of melasma. *Dermatol Surg.* 1999; 25(4): 282-84.

- [10] Sharma V, & Tripathi A. Formulation and Evaluation of Herbal Face Serum. *Int J Drug Dev Res*. 2020; 9(2): 46-54.
- [11] Desai K, & Mehta D. Stability and Safety Assessment of Herbal Cosmetic Products. *Int J Pharm Sci Res*. 2018; 9(7): 2800–05.
- [12] Shalini K. Development and evaluation of herbal face serum using Hibiscus rosa-sinensis extract. *Int J Mod Pharm Res*. 2024; 8(2): 120–26.
- [13] Vincenta Khristi, & V. H. Patel. Therapeutic potential of Hibiscus rosa-sinensis: a review. *Int J Nutr Diet*. 2016; 4(2): 105-23.
- [14] Singh M, & Kaur N. Evaluation of flavonoids and phenolic compounds in Hibiscus rosa-sinensis flowers. *J Pharmacogn Phytochem*. 2017; 6(5): 120–12.
- [15] Tushali Khanna, & Swati Joshi. Formulation and Evaluation of Anti Acne Face Serum. *J Med Plants Stud*. 2024; 12(3): 2-4.
- [16] Bhowmik D, Chiranjib K.P, & Sampath K.P. Pharmacological properties of Hibiscus rosa-sinensis and its role in skin disorders. *Int J Pharm Sci Rev Res*. 2012; 3(2): 534–38.
- [17] Thorat PS, Bhadane HB, Wagh SS, Gaikwad MS, Chhajed SS. General review on face serum. *World J Pharm Res*. 2023; 12(6): 445.
- [18] Khabiya, K, & Somani S. A Review on Skin, Skin Cosmetic and Relative Herbal Ingredients. *Int J Innov Sci Res Technol*. 2023; 12(8): 867-73.
- [19] P. Kranthi Kumar, & R. Santosh Kumar. Review on Transfersomal and Transfersomal Gels. *J Pharm Res Int*. 2021; 33(43B): 115-20.
- [20] Jain S, Jain V, & Mahajan SC. Lipid Based Vesicular Drug Delivery Systems. *Adv Pharm. Hindawi*. 2014; 1-12
- [21] Bhaskar A, Nithya V, & Vidhya V. G. Phytochemical screening and in vitro antioxidant activities of the ethanolic extract of Hibiscus rosa-sinensis L. *Ann Biol Res*. 2011;2(5): 653-61.
- [22] Sivaraman CM, & Sanju F. Medicinal value of Hibiscus rosa-sinensis: a review. *Int J Pharmacogn Chem*. 2021; 2(1): 1-11.
- [23] Jena J, Maurya N, Rai J. K, *et al*. Evaluation on Pharmacognostical and Phytochemical Parameters of Hibiscus rosa-sinensis. *YMER*. 2023; 22(07): 228.
- [24] Bijauliya RK, Alok S, Kumar M, Chanchal DK, Yadav S. a Comprehensive Review on Herbal Cosmetics. *Int J Pharm Sci Res*. 2017; 8(12): 4930-49.
- [25] Khristi V, & Patel V. H. Therapeutic Potential OF Hibiscus rosa-sinensis: A Review. *Int J Nutr Diet*. 2017; 4(2): 105–23.
- [26] Sharma P, Patel P, & Mehta T. Formulation and Evaluation of Herbal Face Serum for Treatment of Hyperpigmentation. *Res J Top Cosmet Sci*. 2024; 15(2): 113-20.
- [27] Missoum A. An Update Review on Hibiscus rosa-sinensis Phytochemistry and Medicinal Uses. *J Ayurvedic Herb Med*. 2018; 4(3): 135-46.
- [28] Mishra D, & Sharma N. Innovative Herbal Face Serum: An Ayurvedic and Modern Fusion Approach. *Int J Adv Res*. 2025; 12(3): 305-15.
- [29] SAĞLAM A, AŞAN ÖZÜSAĞLAM M, & ÇELİK İ. Determination of Antimicrobial Activity of Cream Formulation Developed with Hibiscus rosa-sinensis Extract and Probiotic. *Manas J Agric Vet Life Sci*. 2023; 13(2): 126–32.
- [30] Ashawat M.S, & Saraf S. Formulation and Development of Botanicals-Based Herbal Serum. *ISFCP PharmAspire*. 2009; 11(1): 27-36.
- [31] Wasnik V, Bhude P, Ajane P, Dharmale P, & Gaddekar P. Formulation and Evaluation of Herbal Face Serum for Treatment of Hyperpigmentation. *Res J Top Cosmet Sci*. 2024; 15(1).
- [32] Cabrera C, Artacho R, & Giménez R. Beneficial Effects of Green Tea - A Review. *J Am Coll Nutr*. 2006; 25(2): 79-99.
- [33] Reddy K. Evaluation of Stability and Safety of Herbal Cosmetic Formulations. *Int J Pharm Res*. 2021; 13(3): 300–05.
- [34] Mehta P, & Jain R. Development and Optimization of Polyherbal Cosmetic Formulations. *J Cosmet Dermatol*. 2019; 18(3): 850–56.
- [35] Sharma V. Safety and Dermatological Evaluation of Herbal Topical Preparations. *Clin Cosmet Investig Dermatol*. 2022; 15: 120–26.
- [36] Sharma P. Standardization of Extraction Process for Hibiscus rosa-sinensis. *J Pharmacogn Phytochem*. 2018; 7(3): 1012–16.
- [37] Verma S, & Singh R. Preparation and Evaluation of Herbal Extracts from Hibiscus rosa-sinensis. *Asian J Pharm Clin Res*. 2020; 13(4): 85–90.
- [38] Pavithra T, Akash Gowda, Monika Chavan, Nagaveni A. P, Nagushree K. C, & Neha P. Herbal Face Serum in Modern Dermatology: A Natural Alternative for Skin Health. *Int J Pharm Sci*. 2025; 3(09): 2-9.
- [39] Devi N, Kumar A, Garg A, Hussain A, & Khathuriya R. A Review on Herbal Cosmetics. *World J Pharm Res*. 2018; 7(8): 298-10.
- [40] Arsha Mathew Puthanmadathil, Mohammed Bilal R, Sivadethu M G, & Anuroop U P. Formulation And Evaluation of Herbal Face Serum Using Clitoria Ternatea. *IJNRD ORG*. 2024; 9(8): 104-18.
- [41] Kaveri Sonawane. Innovative Herbal Face Serum: Synergistic Approach to Skincare. *Int J Adv Res (IJAR)*. 2025; 13(10): 1711-15.

- [42] Vaishali Wasnik, Pratiksha Bhude, Priya Ajane, Punam Dharmale, & Purvaja Gadekar. Formulation and Evaluation of Herbal Face Serum for Treatment of Hyperpigmentation. *Res J Topic Cosmet Sci.* 2024; 17(1): 4-10.
- [43] Sakshi Dhotre, T.K Kedar, & Sanjay K. Standardization of Green Tea Extract: An Overview of HPLC-Based Approaches. *Int J Pharm Sci.* 2025; 3(12): 1335-54.
- [44] Chen Z.M, & Lin Z. Tea and Human Health: Biomedical Functions of Tea Active Components and Current Issues. *J Zhejiang Univ Sci B.* 2015; 16(2): 87 - 102.
- [45] Rekha Bharat Maske, & Kalyani Patil. Formulation and Evaluation of Herbal Face Cream with Green Tea Extract. *Int J Sci Dev Res.* 2025; 10(6): 100-05.
- [46] Sreya T.C, Reshmi K.S, & P Sudharsan. Review Article on: Herbal Anti-Aging Face Serum. *World J Pharm Pharm Sci.* 2024; 12(6): 57-62.
- [47] Dr. Gyati Shilakari Asthana, G. Viswas Chowdary, & R. Sai Yasvitha. Natural Synergy in Skincare: Polyherbal Face Serum Composition and its Application. *World J Pharm Res.* 2025; 14(10): 398-04.
- [48] Gite AV, Udapurkar P, & Sanap AS. Formulation and Development of Face Serum. *Int J Creat Res Thoughts.* 2023; 11(6).
- [49] Sahil S shaikh, Sayali A Deshmukh, Rohit B Satpute, Vijay V Parwar, & Hemant H Gangurde. Formulation and Evaluation of Green Tea-Based Herbal Anti-Aging Cream for Effective Skincare. *J Pharm Sci Comput Chem.* 2025; 1(2): 73-8.
- [50] Singh K, Chauhan V, & Deshmukh S. Accelerated Stability Testing of Herbal Cosmetic Products: A Case Study on Face Serum. *Int J Cosmet Sci.* 2021; 43(5): 402–8.