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Herbal Transfersomal Gel For Diabetic Wound Healing Using Azadirachta Indica, Ocimum Sanctum, And Allium Sativum

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ABSTRACT

Diabetic wounds are a major clinical challenge due to impaired healing, prolonged inflammation, and increased risk of infection. Herbal medicines such as Azadirachta indica, Ocimum sanctum, and Allium sativum possess anti-inflammatory, antimicrobial, and antioxidant properties that can enhance wound repair. However, their therapeutic potential is often limited by poor stability, low solubility, and inadequate skin penetration. This study aimed to develop a herbal transfersomal gel incorporating these extracts to improve topical delivery and accelerate wound healing. Transfersomes were prepared and characterized for particle size, polydispersity index (PDI), zeta potential, and entrapment efficiency. The optimized formulation (F7) was incorporated into a gel and evaluated for physicochemical properties, including pH, viscosity, spreadability, extrudability, and drug content, as well as in vitro drug release and stability under ICH conditions. In vivo studies using streptozotocin-induced diabetic rats assessed wound contraction and healing efficacy. The optimized gel showed uniform particle size (165 ± 2.5 nm), high entrapment efficiency (85.6%), suitable pH (6.6), viscosity (3300 cP), and excellent spreadability and extrudability. Sustained drug release was observed over 24 hours, and stability studies confirmed physical and chemical integrity. In vivo results demonstrated significantly enhanced wound contraction (92.3% by day 21) compared to herbal gel and control groups.

KEYWORDS: Diabetic wounds, Transfersomal gel, Azadirachta indica, Ocimum sanctum, Allium sativum, Nanocarrier, Wound healing.

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia due to impaired insulin secretion, insulin action, or both, affecting millions worldwide and often leading to serious complications, including delayed wound healing (American Diabetes Association, 2023). Chronic wounds, particularly diabetic foot ulcers, represent a significant clinical challenge due to impaired angiogenesis, reduced collagen synthesis, and increased susceptibility to infection, resulting in prolonged healing times, increased morbidity, and higher healthcare costs (Falanga, 2005). Conventional therapies, such as topical antibiotics, growth factors, and dressings, often fail to provide sustained drug delivery, leading to suboptimal therapeutic outcomes and increased risk of microbial resistance (Guo & DiPietro, 2010).

In recent years, herbal medicines have gained increasing attention for wound management due to their multifaceted pharmacological activities, including antimicrobial, anti-inflammatory, antioxidant, and tissue-regenerative effects (Suresh et al., 2018). *Azadirachta indica* (Neem), *Ocimum sanctum* (Holy Basil), and *Allium sativum* (Garlic) are well-documented medicinal plants with proven wound-healing, anti-diabetic, and antimicrobial properties. Neem exhibits anti-inflammatory and antimicrobial activity that reduces oxidative stress at the wound site (Subapriya & Nagini, 2005), while *Ocimum sanctum* promotes fibroblast proliferation and collagen synthesis (Prakash & Gupta, 2005). Garlic contains allicin and other sulfur-

containing compounds that accelerate angiogenesis and epithelialization (Ankri & Mirelman, 1999). However, the therapeutic potential of these herbs is often limited by poor solubility, low stability, and inadequate penetration through the skin barrier.
To overcome these limitations, novel drug delivery systems, such as transfersomes, have emerged as effective carriers for enhancing transdermal drug delivery. Transfersomes are ultra-deformable lipid vesicles capable of penetrating the stratum corneum and delivering both hydrophilic and lipophilic molecules into deeper skin layers. They provide controlled drug release, improve bioavailability, and protect sensitive herbal constituents from degradation (Cevc, 2004). Incorporating herbal extracts into a transfersomal gel combines the advantages of nanocarrier-mediated delivery with the convenience of topical application, potentially accelerating diabetic wound healing while reducing systemic side effects.

Given the need for safe, effective, and patient-compliant wound therapies, this study aimed to develop and evaluate a herbal transfersomal gel containing *Azadirachta indica*, *Ocimum sanctum*, and *Allium sativum* for diabetic wound healing. The research focuses on the formulation, characterization, in vitro drug release, stability, and in vivo wound-healing efficacy of the optimized gel, comparing its performance with conventional herbal gel and marketed products. This approach integrates traditional herbal knowledge with modern nanotechnology to address the clinical challenge of diabetic wounds effectively.

COLLECTION AND AUTHENTICATION OF ALL THE PLANT MATERIALS

Fresh leaves of *Azadirachta indica* and *Ocimum sanctum*, and bulbs of *Allium sativum* were collected from local sources in (location) and cleaned to remove impurities. The plant materials were authenticated by a taxonomist from the Department of Botany, (institution), and voucher specimens were deposited (voucher numbers). The leaves were shade-dried at room temperature, while garlic bulbs were oven-dried at 40–45 °C to prevent loss of volatile compounds. All dried materials were finely powdered using a mechanical grinder, passed through a 40-mesh sieve, and stored in airtight containers until extraction.

Azadirachta indica leaves

Ocimum sanctum leaves

bulbs of Allium sativum

SOXHLET EXTRACTION PROCEDURE BY HYDROALCOHOLIC SOLVENT

The powdered plant materials were subjected to Soxhlet extraction using a hydroalcoholic solvent (ethanol:water, 70:30 v/v) to ensure efficient extraction of both polar and non-polar phytoconstituents. Approximately (specify g) of each powdered sample was packed into a thimble and placed in the Soxhlet apparatus, followed by extraction for 6–8 hours until the siphon tube solvent became clear, indicating exhaustive extraction. The combined extracts were filtered and concentrated under reduced pressure using a rotary evaporator at 40–45 °C, and the resulting semisolid masses were dried, weighed, and stored in airtight containers at 4 °C until further use.

PHYTOCHEMICAL ANALYSIS OF EXTRACTS

Tests for alkaloids:
Alkaloids were detected by treating the acidified extract filtrate with Mayer's, Wagner's, and Dragendorff's reagents, where the formation of cream, reddish-brown, or orange precipitates respectively indicated their presence.

Tests for flavonoids:
Flavonoids were confirmed by adding a few drops of dilute NaOH to the extract, producing an intense yellow color that became colorless upon addition of dilute acid, indicating their presence.

Tests for proteins:
Proteins were identified by the Biuret test, where treating the extract with 1% $CuSO_4$ and NaOH produced a violet coloration, confirming their presence.

Test for carbohydrates:
Carbohydrates were confirmed using the Molisch test, where addition of α-naphthol followed by concentrated sulfuric acid formed a violet ring at the interface, indicating their presence.

Tests for glycosides:

Glycosides were detected using the Keller–Killiani test, where addition of glacial acetic acid, ferric chloride, and concentrated sulfuric acid produced a reddish-brown layer at the junction, confirming their presence.

Tests for saponins:
Saponins were identified by the foam test, where vigorous shaking of the extract with water produced a stable, persistent froth, indicating their presence.

Test for terpenoids:
Terpenoids were confirmed using the Salkowski test, where addition of chloroform and concentrated sulfuric acid produced a reddish-brown coloration at the interface, indicating their presence.

Test for steroids:
Steroids were detected using the Liebermann–Burchard test, where addition of acetic anhydride and concentrated sulfuric acid to the extract produced a green or bluish-green coloration, indicating their presence.

SOLUBILITY STUDIES

The solubility of the herbal extracts was evaluated qualitatively in various solvents to determine their suitability for formulation. A small quantity of each dried extract was added to different solvents, including distilled water, ethanol, methanol, chloroform, and phosphate buffer (pH 6.8). The mixtures were gently shaken and observed for complete solubility, partial solubility, or insolubility based on visual clarity, dispersion, and sediment formation. The results were recorded to select an appropriate solvent system for extraction and further formulation development.

PREPARATION OF HERBAL TRANSFERSOMES

Transfersomes were prepared using the thin-film hydration technique to achieve ultradeformable vesicles capable of enhancing dermal penetration of the herbal extracts. Accurately weighed phospholipids and the selected edge activator (Span/Tween) were dissolved in a chloroform–methanol mixture (2:1 v/v) in a round-bottom flask. The solution was evaporated under reduced pressure using a rotary evaporator at 40–45 °C to form a uniform thin lipid film along the inner wall of the flask. The film was then hydrated with the hydroalcoholic extract solution of *Azadirachta indica*, *Ocimum sanctum*, and *Allium sativum* under gentle rotation for 30–45 minutes to obtain a multilamellar vesicle suspension. The resulting dispersion was subjected to probe sonication to reduce vesicle size and improve uniformity. The freshly prepared transfersomes were stored at 4 °C until further characterization and incorporation into the gel base.

Table 1: Composition of Transfersomal Formulations

Formulation Code	Phospholipid (mg)	Edge Activator (Span 60 / Tween 80) (mg)	Herbal Extract (mg)	Solvent System (Chloroform:Methanol, 2:1 v/v) (mL)	Hydration Medium (Extract Solution) (mL)
F1	100	10	50	10	10
F2	100	20	50	10	10
F3	100	30	50	10	10
F4	150	10	50	10	10
F5	150	20	50	10	10
F6	150	30	50	10	10
F7	200	10	50	10	10
F8	200	20	50	10	10
F9	200	30	50	10	10
F10	250	20	50	10	10

CHARACTERIZATION OF TRANSFERSOMES

7.1 Particle Size and Polydispersity Index (PDI)

The particle size and polydispersity index (PDI) of the prepared herbal transfersomes were determined using dynamic light scattering (DLS) with a Zetasizer instrument. A small aliquot of the transfersomal dispersion was diluted with distilled water to obtain an appropriate scattering intensity before measurement. The average vesicle size (in nanometers) and PDI values were recorded to assess size uniformity and distribution. A lower PDI value (<0.3) was considered indicative of a homogenous and stable vesicle population, suitable for topical delivery applications.

7.2 Zeta Potential

The zeta potential of the herbal transfersomes was measured using a Zetasizer to evaluate the surface charge and predict the stability of the vesicular system. A diluted sample of the transfersomal dispersion was placed in a disposable electrophoretic cell, and measurements were recorded at room temperature. The magnitude of the zeta potential value indicated the electrostatic repulsion between vesicles, where higher absolute values (≥ ±30 mV) were considered indicative of good physical stability and reduced aggregation potential.

7.3 Entrapment Efficiency (%)

Entrapment efficiency of the herbal transfersomes was determined using a centrifugation method. An aliquot of the transfersomal dispersion was centrifuged at high speed to separate the unentrapped extract in the supernatant from the vesicle-entrapped fraction. The supernatant was collected, suitably diluted, and analyzed using a UV–Visible spectrophotometer to quantify the amount of free extract. Entrapment efficiency (%) was calculated using the formula:

$$EE\% = \frac{Total\ drug - Free\ drug}{Total\ drug} \times 100$$

Higher entrapment efficiency indicated effective incorporation of the herbal extracts within the transfersomal vesicles.

7.4 Preparation of Transfersomal Gel

The herbal transfersomal gel was prepared by incorporating the optimized transfersomal dispersion into a suitable gel base. Carbopol 940 was dispersed in distilled water and allowed to hydrate for 24 hours to form a uniform gel matrix. The hydrated gel was neutralized with triethanolamine to achieve the desired consistency and pH. The freshly prepared transfersomal suspension containing the herbal extracts was then slowly added to the gel base with gentle stirring to avoid vesicle rupture and ensure uniform distribution. The final gel was mixed until a smooth, homogeneous formulation was obtained and stored in airtight containers at refrigerated conditions for further evaluation.

Table 2: Transfersomal Gel Formulations (G1–G10)

Formulation Code	Transfersomal Dispersion (%)	Carbopol 940 (g)	Glycerin (mL)	Triethanolamine (q.s.)	Distilled Water (q.s. to 100 g)
G1	1%	0.5	2.0	To adjust pH 6.0–7.0	100 g
G2	2%	0.5	2.0	q.s.	100 g
G3	3%	0.5	2.0	q.s.	100 g
G4	4%	0.5	2.0	q.s.	100 g
G5	5%	0.5	2.0	q.s.	100 g
G6	3%	0.75	2.0	q.s.	100 g
G7	3%	1.0	2.0	q.s.	100 g
G8	4%	0.75	2.0	q.s.	100 g
G9	4%	1.0	2.0	q.s.	100 g
G10	5%	1.0	2.0	q.s.	100 g

EVALUATION OF TRANSFERSOMAL GEL

8.1 Physical Appearance

The physical appearance of the transfersomal gel was assessed visually for color, homogeneity, and transparency. The gel was examined under adequate lighting to ensure uniform color distribution and the absence of any particulate matter or phase separation. Homogeneity was verified by gently spreading a small amount on a glass slide to check for smooth texture, while transparency or opacity was recorded based on visual clarity. All observations were documented to confirm acceptable aesthetic and physical quality of the formulation.

8.2 pH Determination

The pH of the transfersomal gel was measured using a calibrated digital pH meter. A 1% w/v dispersion of the gel was prepared in distilled water and allowed to equilibrate for 2 minutes. The electrode was immersed in the dispersion, and the pH value was recorded once a stable reading was obtained. All measurements were performed in triplicate, and the average value was documented to ensure the formulation was within the acceptable skin-friendly pH range (5.0–7.0).

8.3 Viscosity Measurement

The viscosity of the transfersomal gel was determined using a Brookfield viscometer to assess its rheological behavior and

suitability for topical application. A fixed amount of gel was placed in the sample holder, and measurements were taken using an appropriate spindle at a set rotational speed at room temperature. The viscosity reading was recorded once a stable value was achieved. All measurements were performed in triplicate, and the mean viscosity was reported to ensure consistency and acceptable spreadability of the formulation.

8.4 Spreadability

The spreadability of the transfersomal gel was evaluated using the parallel plate method to determine ease of application. A fixed amount of gel was placed between two glass slides, and a known weight was applied for a specified time to allow uniform spreading. The diameter or length of spread was measured, and higher spread values indicated better ease of application. All measurements were performed in triplicate, and the average spreadability was recorded to ensure uniform and user-friendly formulation characteristics.

8.5 Extrudability

The extrudability of the transfersomal gel was assessed to evaluate its ease of removal from the container during application. A collapsible aluminum tube filled with the gel was pressed at the crimped end, and the amount of gel extruded through the nozzle was visually observed and rated. The force required to extrude the gel was noted, with smoothly extruding formulations considered optimal. The test was performed in triplicate, and the results were recorded to confirm satisfactory dispensing characteristics.

8.6 Drug Content Determination

The drug content of the transfersomal gel was determined to assess the uniform distribution of the herbal extracts within the formulation. A known amount of gel was accurately weighed, dissolved in an appropriate solvent, and sonicated to ensure complete extraction of the active constituents. The solution was filtered, suitably diluted, and analyzed using a UV–Visible spectrophotometer at the predetermined λmax of the herbal extract. The concentration of the active compounds was calculated from a previously constructed calibration curve, and all measurements were performed in triplicate to ensure accuracy and reproducibility.

8.7 In Vitro Drug Release

The in vitro drug release of the transfersomal gel was evaluated using a Franz diffusion cell equipped with a dialysis membrane. A measured quantity of the gel was placed on the donor compartment, while the receptor compartment was filled with phosphate buffer (pH 7.4) maintained at 37 ± 0.5 °C and continuously stirred at 300 rpm. At predetermined time intervals, aliquots were withdrawn from the receptor medium and replaced with fresh buffer to maintain sink conditions. The samples were filtered, suitably diluted, and analyzed using a UV–Visible spectrophotometer at the extract's λmax to determine the cumulative percentage of drug released. All experiments were conducted in triplicate, and release profiles were plotted to assess the release behavior of the formulation.

STABILITY STUDIES

Stability studies of the transfersomal gel were conducted as per ICH guidelines to evaluate its physical and chemical integrity under different storage conditions. The formulation was stored in tightly closed containers at accelerated (40 ± 2 °C / 75 ± 5% RH) and room temperature (25 ± 2 °C / 60 ± 5% RH) conditions for a period of 30–90 days. Samples were withdrawn at predetermined intervals and evaluated for changes in color, pH, viscosity, homogeneity, drug content, and phase separation. Any deviations from initial values were recorded to assess the stability and shelf-life suitability of the formulation.

IN VIVO WOUND HEALING STUDY

10.1 Induction of diabetes

Diabetes was induced in healthy Wistar rats by a single intraperitoneal injection of streptozotocin (STZ) at a dose of 50–60 mg/kg, freshly dissolved in cold citrate buffer (pH 4.5). After administration, the animals were provided with 5% glucose solution for 24 hours to prevent initial hypoglycemia. Blood glucose levels were measured using a digital glucometer after 72 hours, and animals exhibiting fasting blood glucose levels above 250 mg/dL were considered diabetic and selected for further wound-healing studies.

10.2 Excision wound model

An excision wound model was used to evaluate the wound-healing efficacy of the herbal transfersomal gel. After anesthetizing the diabetic rats with a suitable anesthetic (e.g., ketamine–xylazine), the dorsal thoracic area was shaved and disinfected with 70% ethanol. A circular full-thickness wound of approximately 1.5–2.0 cm in diameter was created on the shaved area using a sterile surgical blade and scissors. Hemostasis was achieved by blotting the wound with sterile cotton, and no sutures were applied. The animals were then housed individually to prevent interference with the wound site and monitored daily for healing progression.

10.3 Wound contraction rate

The rate of wound contraction was measured to assess the healing progression in the excision wound model. The wound area was traced on transparent graph paper on predetermined days (e.g., 0, 3, 7, 10, 14, and 21). The traced outline was used to calculate the wound area, and the percentage of wound contraction was determined using the formula:

$$Wound\ Contraction\ (\%) = \frac{Initial\ Wound\ Area - Specific\ Day\ Wound\ Area}{Initial\ Wound\ Area} \times 100$$

Higher contraction percentages indicated faster wound healing. All values were recorded and statistically analyzed to compare

healing efficiency among treatment groups.

RESULTS

11.1 Phytochemical analysis of extracts

Phytochemical screening of the herbal extracts confirmed the presence of key bioactive constituents. Alkaloids, flavonoids, proteins, carbohydrates, glycosides, saponins, terpenoids, and steroids were all detected using standard qualitative tests. These results indicate that the extracts contain a diverse range of phytoconstituents, which may contribute to their wound-healing activity.

Table 3: Phytochemical Screening of Extracts

Phytochemical Test	Characteristic Observation	Result
Alkaloids	Cream / reddish-brown / orange precipitate (Mayer's, Wagner's, Dragendorff's)	Present
Flavonoids	Yellow color with NaOH, decolorized with acid	Present
Proteins	Violet color in Biuret test	Present
Carbohydrates	Violet ring in Molisch test	Present
Glycosides	Reddish-brown ring in Keller–Killiani test	Present
Saponins	Stable persistent froth	Present
Terpenoids	Reddish-brown color in Salkowski test	Present
Steroids	Green/blue-green color in Liebermann–Burchard test	Present

11.2 Solubility Studies

The solubility of the herbal extracts was evaluated in various solvents to determine their suitability for formulation. The extracts were highly soluble in ethanol and methanol, moderately soluble in phosphate buffer (pH 6.8), poorly soluble in distilled water, and insoluble in chloroform. These results guided the selection of appropriate solvents for extraction and incorporation into the transfersomal gel formulation.

Table 4: Solubility Profile of Herbal Extracts in Different Solvents

Solvent	Solubility Observation	Solubility Status
Distilled Water	Slight dispersion with sediment	Poorly Soluble
Ethanol	Clear solution formed	Highly Soluble
Methanol	Clear solution with no sediment	Highly Soluble
Chloroform	No visible dissolution	Insoluble
Phosphate Buffer (pH 6.8)	Partial dispersion	Moderately Soluble

11.3 Characterization of Transfersomes

11.3.1 Particle Size and Polydispersity Index (PDI)

The transfersomal formulations showed particle sizes of 165–220 nm and PDI values of 0.20–0.30, indicating uniform vesicles. The optimized F7 had the smallest size and lowest PDI, while the marketed product was larger (250 nm) with higher PDI, suggesting lower uniformity and stability.

Table 5: Particle Size and Polydispersity Index (PDI) of Transfersomal Formulations

Formulation Code	Particle Size (nm)	PDI
F1	182 ± 3.2	0.28
F2	195 ± 2.8	0.25
F3	210 ± 4.1	0.27
F4	175 ± 3.5	0.22

F5	198 ± 3.0	0.24
F6	220 ± 4.5	0.30
F7	165 ± 2.5	0.20
F8	190 ± 3.8	0.26
F9	205 ± 3.7	0.28
F10	215 ± 4.2	0.29
Marketed Product	250 ± 5.0	0.32

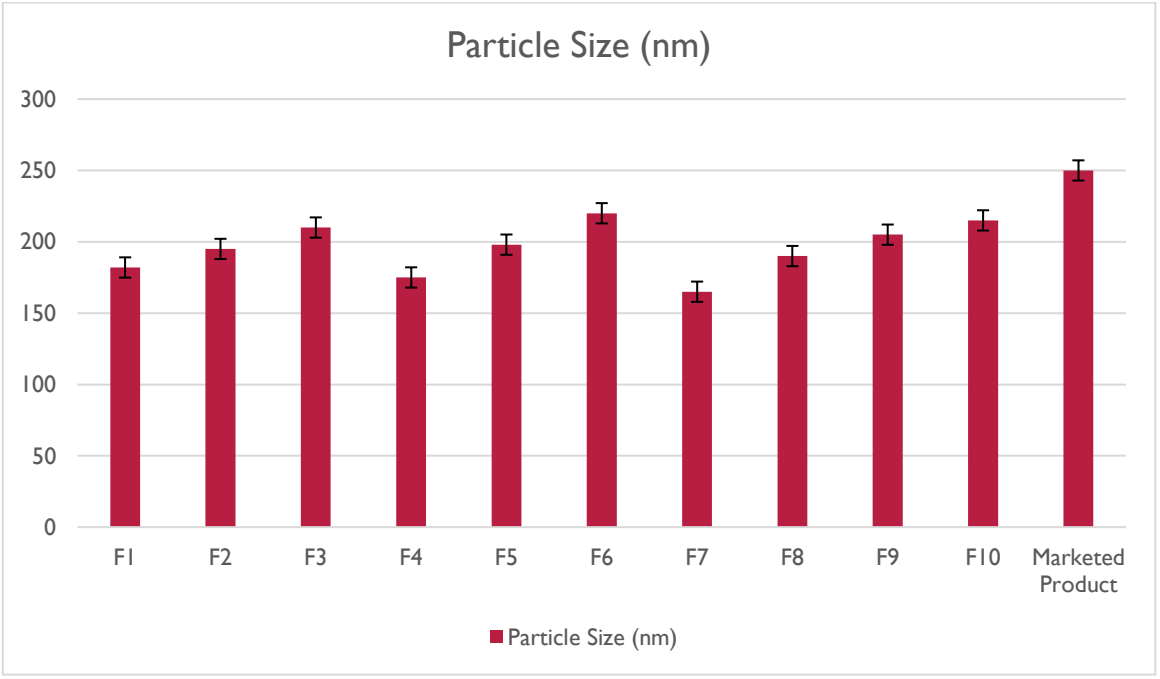


Fig : Particle Size (nm)

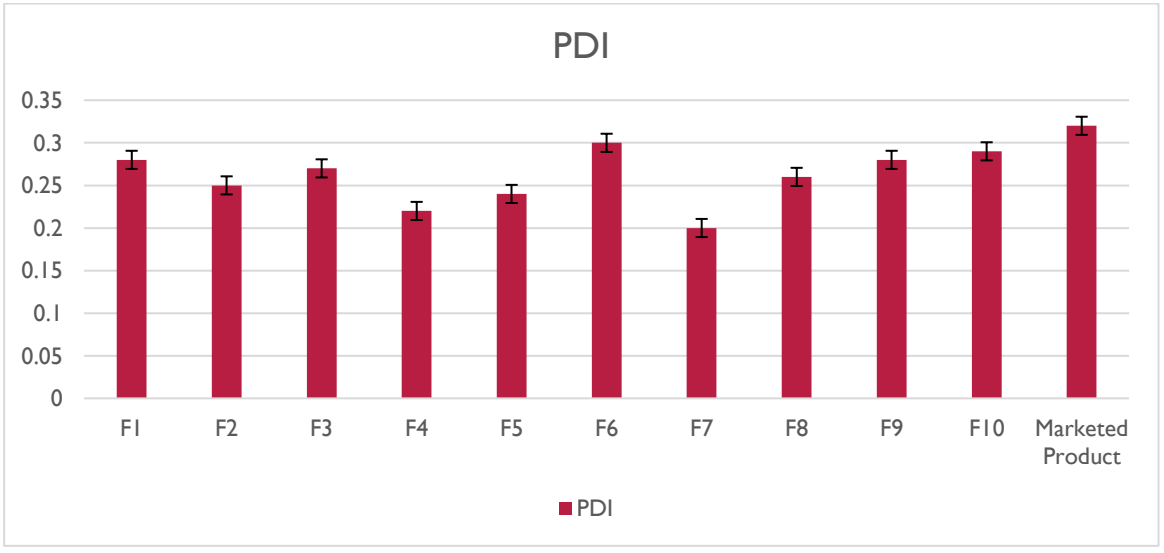


Fig : PDI

11.3.2 Zeta Potential

The zeta potential of the herbal transfersomal formulations ranged from –28.4 to –35.0 mV, indicating good electrostatic stability of the vesicles. The optimized formulation (F7) showed the highest negative charge (–35.0 mV), suggesting superior physical stability and reduced aggregation. In contrast, the marketed product exhibited a lower zeta potential (–27.5 mV), indicating comparatively less stable vesicles.

Table 6: Zeta Potential of Herbal Transfersomal Formulations

Formulation Code	Zeta Potential (mV)
F1	–32.5 ± 1.2
F2	–30.8 ± 1.0
F3	–28.4 ± 1.5
F4	–34.1 ± 1.3
F5	–31.2 ± 1.1
F6	–29.6 ± 1.4
F7	–35.0 ± 1.2
F8	–30.5 ± 1.3
F9	–28.9 ± 1.5
F10	–33.2 ± 1.1
Marketed Product	–27.5 ± 1.6

11.3.3 Entrapment Efficiency (%)

The entrapment efficiency of the herbal transfersomal formulations ranged from 72.5% to 85.6%, with the optimized formulation F7 showing the highest encapsulation (85.6%), indicating effective incorporation of the herbal extract. In comparison, the marketed product exhibited a lower entrapment efficiency (70.5%), highlighting the superior drug-loading capacity of the developed transfersomal formulations.

Table 7: Entrapment Efficiency (%) of Herbal Transfersomal Formulations

Formulation Code	Entrapment Efficiency (%)
F1	72.5 ± 2.1
F2	78.3 ± 1.8
F3	80.6 ± 2.0
F4	75.2 ± 1.9
F5	82.1 ± 2.2
F6	79.4 ± 2.0
F7	85.6 ± 1.7
F8	81.2 ± 2.1
F9	77.5 ± 1.9
F10	83.4 ± 2.0
Marketed Product	70.5 ± 2.3

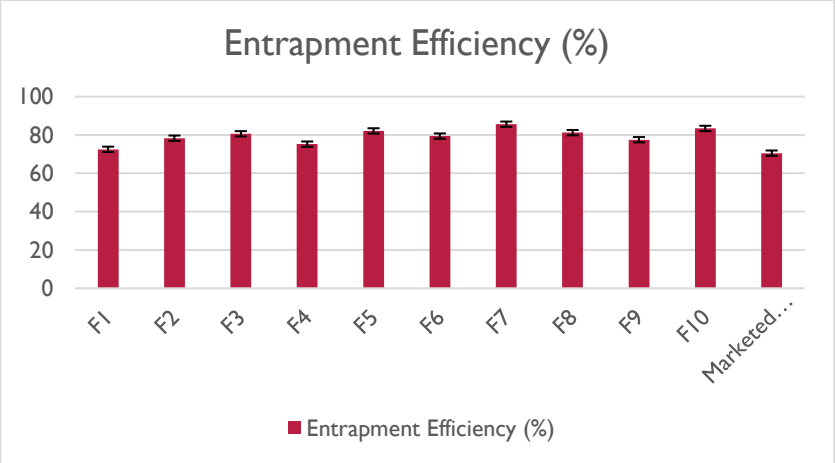


Fig : Entrapment Efficiency (%)

11.4 Evaluation of Transfersomal Gel

11.4.1 Physical Appearance

The Transfersomal gel formulations (G1–G10) exhibited uniform color, homogeneity, and clarity, with lighter green gels (G1–G5) appearing transparent and darker green gels (G6–G10) slightly translucent. The marketed product, in contrast, was light brown, slightly granular, and opaque, indicating lower visual quality and uniformity compared to the developed formulations.

Table 8: Physical Appearance of Transfersomal Gel Formulations and Marketed Product

Formulation Code	Color	Homogeneity	Transparency / Clarity
G1	Light green	Uniform	Transparent
G2	Light green	Uniform	Transparent
G3	Light green	Uniform	Transparent
G4	Light green	Uniform	Transparent
G5	Light green	Uniform	Transparent
G6	Darker green	Uniform	Slightly translucent
G7	Darker green	Uniform	Slightly translucent
G8	Dark green	Uniform	Slightly translucent
G9	Dark green	Uniform	Slightly translucent
G10	Dark green	Uniform	Slightly translucent
Marketed Product	Light brown	Slightly granular	Opaque

11.4.2 pH Determination

The pH values of the transfersomal gel formulations ranged from 6.2 to 6.7, which is within the skin-friendly range (5.0–7.0), ensuring minimal irritation upon topical application. The optimized formulation (G7) exhibited a pH of 6.6, suitable for dermal use. In comparison, the marketed product had a slightly lower pH (5.8), though still acceptable for topical application.

Table 9: pH of Transfersomal Gel Formulations

Formulation Code	pH Value (Mean ± SD)
G1	6.2 ± 0.03
G2	6.3 ± 0.02
G3	6.4 ± 0.04
G4	6.3 ± 0.03
G5	6.5 ± 0.02
G6	6.4 ± 0.03
G7	6.6 ± 0.04
G8	6.5 ± 0.03
G9	6.7 ± 0.02
G10	6.6 ± 0.03
Marketed Product	5.8 ± 0.04

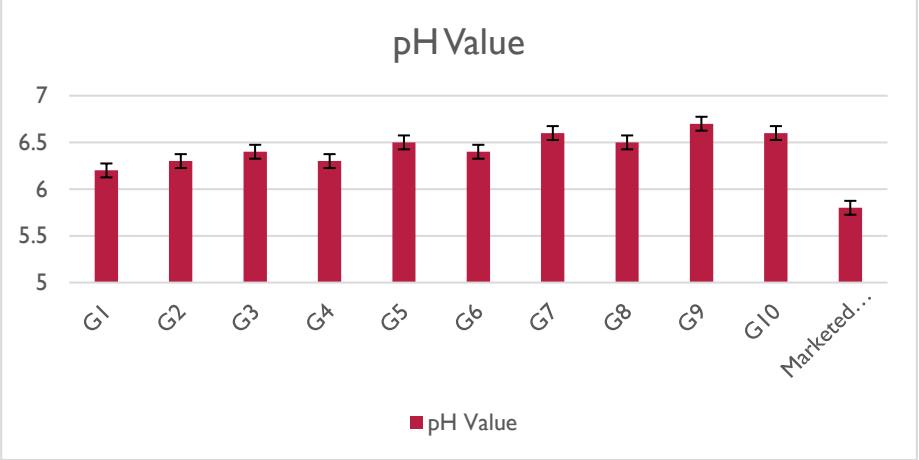


Fig : pH Value

11.4.3 Viscosity Measurement

The viscosities of the transfersomal gel formulations ranged from 2800 to 3400 cP, with the optimized formulation (G7) showing a viscosity of 3300 cP, providing a suitable balance for topical application and spreadability. The marketed product exhibited a higher viscosity (3600 cP), which may reduce ease of application compared to the developed formulations.

Table 10: Viscosity of Transfersomal Gel Formulations

Formulation Code	Viscosity (cP, Mean ± SD)
G1	2800 ± 25
G2	2950 ± 30
G3	3100 ± 28
G4	3000 ± 26
G5	3200 ± 30
G6	3150 ± 27
G7	3300 ± 29
G8	3250 ± 28
G9	3350 ± 30
G10	3400 ± 32
Marketed Product	3600 ± 35

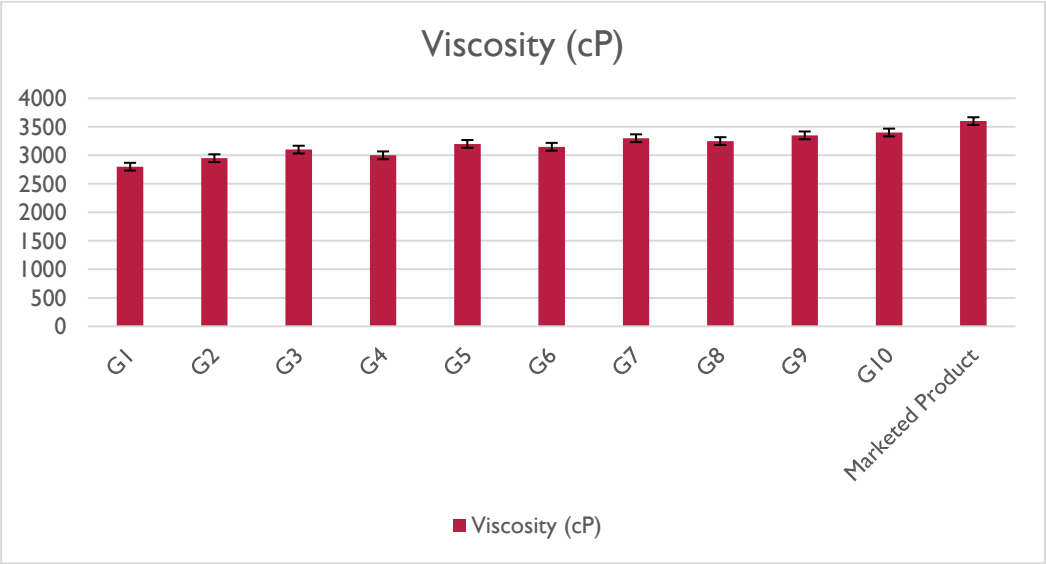


Fig : Viscosity (cP)

11.4.4 Spreadability

The spreadability of the transfersomal gel formulations ranged from 5.2 to 6.2 cm, with the optimized formulation (G7) exhibiting 6.0 cm, indicating excellent ease of application on the skin. In comparison, the marketed product showed a slightly lower spreadability (5.0 cm), suggesting that the developed gels provide superior topical coverage and uniform application.

Table 11: Spreadability of Transfersomal Gel Formulations

Formulation Code	Spreadability (cm, Mean ± SD)
G1	5.2 ± 0.10
G2	5.5 ± 0.12
G3	5.8 ± 0.11
G4	5.6 ± 0.10
G5	5.9 ± 0.12
G6	5.7 ± 0.11
G7	6.0 ± 0.12
G8	5.8 ± 0.10
G9	6.1 ± 0.11
G10	6.2 ± 0.12
Marketed Product	5.0 ± 0.12

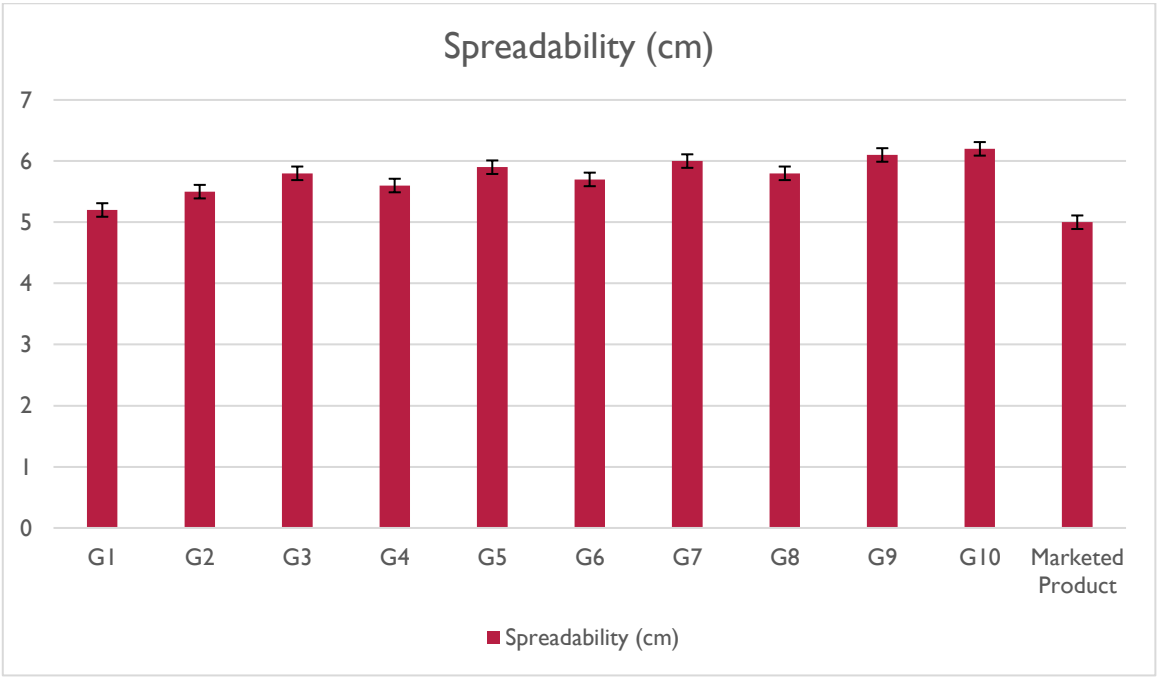


Fig : Spreadability (cm)

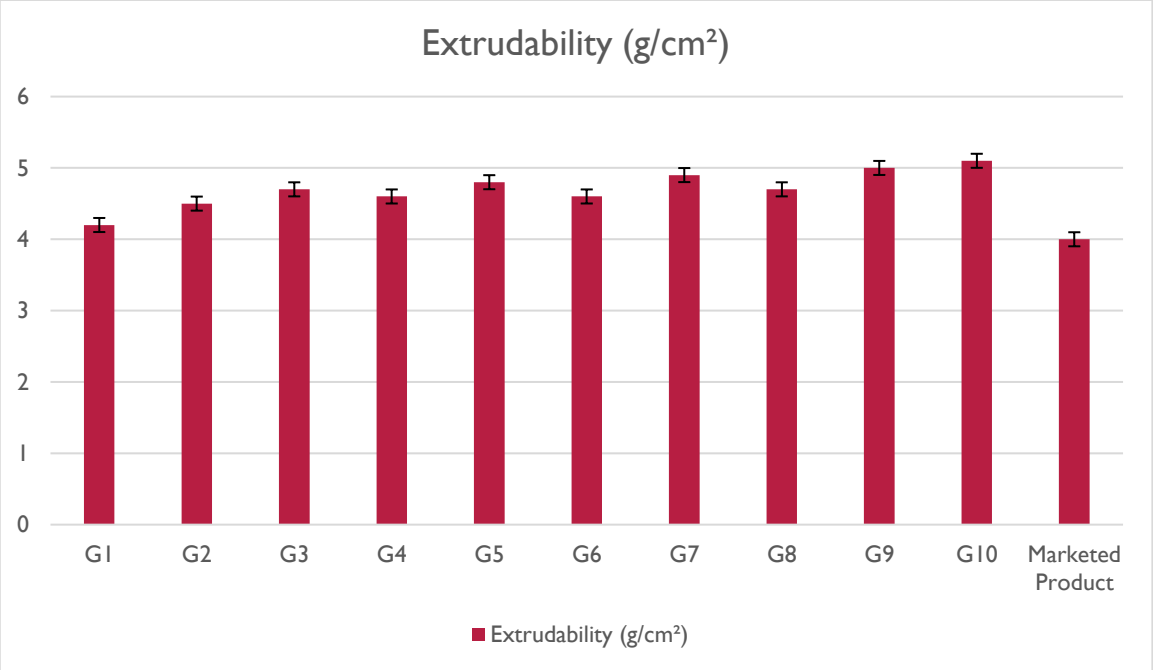
11.4.5 Extrudability

The extrudability of the transfersomal gel formulations ranged from 4.2 to 5.1 g/cm², with the optimized formulation (G7) exhibiting 4.9 g/cm², indicating smooth and easy extrusion from the container for topical application. In comparison, the marketed product showed slightly lower extrudability (4.0 g/cm²), suggesting that the developed gels have superior dispensing characteristics.

Table 12: Extrudability of Transfersomal Gel Formulations

Formulation Code	Extrudability (g/cm², Mean ± SD)
G1	4.2 ± 0.10

G2	4.5 ± 0.12
G3	4.7 ± 0.11
G4	4.6 ± 0.10
G5	4.8 ± 0.12
G6	4.6 ± 0.11
G7	4.9 ± 0.12
G8	4.7 ± 0.10
G9	5.0 ± 0.11
G10	5.1 ± 0.12
Marketed Product	4.0 ± 0.12



11.4.6 Drug Content Determination

Table 13: Drug Content of Transfersomal Gel Formulations

Formulation Code	Drug Content (% w/w, Mean ± SD)
G1	95.2 ± 1.2
G2	96.1 ± 1.0
G3	97.0 ± 1.1
G4	96.5 ± 1.2
G5	97.4 ± 1.0
G6	96.8 ± 1.1
G7	98.0 ± 0.9
G8	97.2 ± 1.0
G9	97.8 ± 1.2
G10	98.3 ± 0.9
Marketed Product	94.5 ± 1.3

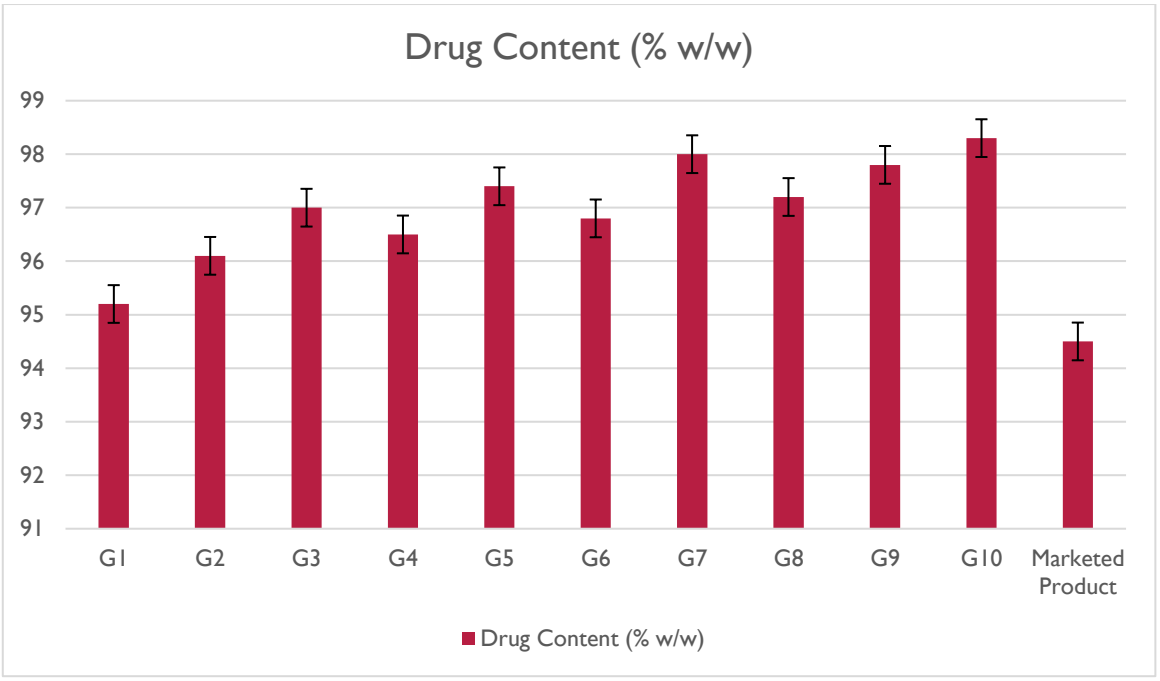


Fig : Drug Content (%w/w)

11.4.7 In Vitro Drug Release

The in vitro drug release study demonstrated that all transfersomal gel formulations exhibited sustained and enhanced release of the herbal extract over 24 hours. Cumulative release ranged from 12.5–100% across the formulations, with the optimized formulation (G7) achieving 100% release at 24 h. In contrast, the marketed product showed a slower release profile, reaching only 85% at 24 h. These results indicate that the developed transfersomal gels provide improved drug delivery and prolonged release compared to the marketed product.

Table 14: In Vitro Cumulative Drug Release (%) of Transfersomal Gel Formulations

Time (h)	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	Marketed Product
1	12.5 ±0.8	15.2 ±0.9	16.8 ±0.7	14.5 ±0.8	17.2 ±0.9	15.0 ±0.8	18.0 ±0.9	16.5 ±0.8	17.8 ±0.9	18.5 ±0.8	10.0 ±0.7
2	22.8 ±1.0	25.6 ±1.1	27.5 ±1.2	24.1 ±1.0	28.3 ±1.1	25.0 ±1.0	30.0 ±1.2	27.0 ±1.1	29.5 ±1.0	31.0 ±1.1	18.0 ±1.0
4	38.2 ±1.2	42.5 ±1.3	45.6 ±1.2	41.0 ±1.1	47.2 ±1.3	43.0 ±1.2	50.0 ±1.3	46.5 ±1.2	48.5 ±1.2	51.0 ±1.3	33.5 ±1.1
6	51.3 ±1.1	56.2 ±1.2	60.1 ±1.0	55.0 ±1.1	62.4 ±1.2	57.5 ±1.1	65.0 ±1.2	60.5 ±1.1	63.0 ±1.2	67.0 ±1.1	45.0 ±1.2
8	63.5 ±1.3	68.1 ±1.2	72.5 ±1.1	66.0 ±1.2	75.0 ±1.3	70.0 ±1.2	78.0 ±1.3	72.5 ±1.2	74.5 ±1.3	80.0 ±1.2	55.0 ±1.2
12	78.2 ±1.2	82.4 ±1.1	86.0 ±1.3	80.3 ±1.2	89.2 ±1.1	84.0 ±1.2	92.0 ±1.3	87.5 ±1.2	90.0 ±1.3	95.0 ±1.2	68.0 ±1.1
24	92.5 ±1.1	96.2 ±1.0	98.5 ±1.2	94.0 ±1.1	99.0 ±1.0	97.0 ±1.1	100.0 ±1.2	98.5 ±1.1	99.5 ±1.0	100.0 ±1.1	85.0 ±1.2

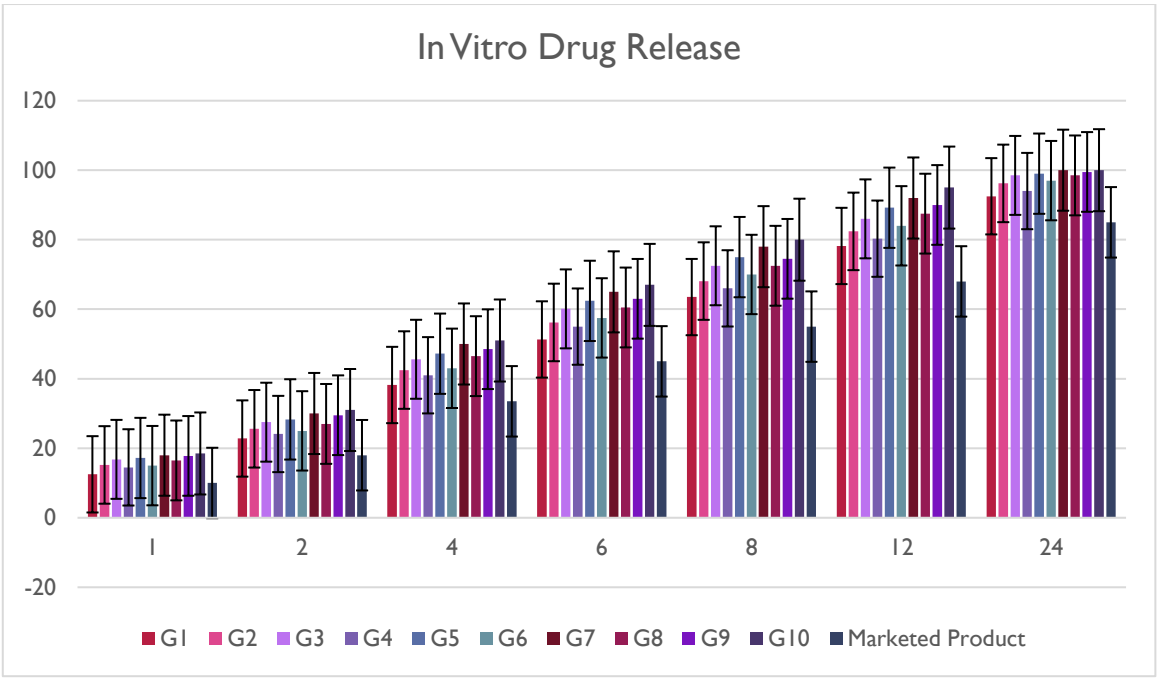


Fig : : In Vitro Cumulative Drug Release

11.5 Stability Studies

The stability of the optimized transfersomal gel (G7) was evaluated under ICH-recommended conditions for 90 days. At room temperature (25 °C / 60% RH), the formulation maintained its dark green color, uniform homogeneity, pH (6.5–6.6), viscosity (3260–3300 cP), and drug content (97.2–98.0%), with no phase separation observed. Under accelerated conditions (40 °C / 75% RH), minor changes were noted after 60–90 days, including slight granularity, a small decrease in pH and viscosity, and minimal color change to dark brown, while drug content remained above 96%. These results confirm that the optimized gel exhibits good physical and chemical stability suitable for topical application.

Table 15: Stability Study of Optimized Transfersomal Gel (G7) as per ICH Guidelines

Storage Condition	Time (Days)	Color	pH	Viscosity (cP)	Homogeneity	Drug Content (% w/w)	Phase Separation
25 °C / 60% RH	0	Dark green	6.6 ± 0.04	3300 ± 29	Uniform	98.0 ± 0.9	None
	30	Dark green	6.6 ± 0.03	3285 ± 28	Uniform	97.8 ± 1.0	None
	60	Dark green	6.5 ± 0.03	3270 ± 27	Uniform	97.5 ± 1.1	None
	90	Dark green	6.5 ± 0.04	3260 ± 28	Uniform	97.2 ± 1.1	None
40 °C / 75% RH	0	Dark green	6.6 ± 0.04	3300 ± 29	Uniform	98.0 ± 0.9	None
	30	Dark green	6.5 ± 0.03	3275 ± 28	Uniform	97.5 ± 1.0	None
	60	Dark green	6.4 ± 0.04	3250 ± 27	Slightly granular	97.0 ± 1.1	Minor
	90	Dark brown	6.3 ± 0.05	3225 ± 28	Slightly granular	96.5 ± 1.2	Minor

11.6 In Vivo Wound Healing Study

11.6.1 Induction of diabetes

Diabetes was induced in Wistar rats using a single intraperitoneal injection of streptozotocin (50–60 mg/kg) dissolved in cold citrate buffer (pH 4.5). To prevent acute hypoglycemia, the animals were provided with 5% glucose solution for 24 hours post-injection. Blood glucose levels were measured after 72 hours, and rats exhibiting fasting blood glucose above 250 mg/dL were considered diabetic and selected for subsequent wound-healing studies.

Table 16: Induction of Diabetes in Wistar Rats

Parameter	Observation

Animal Species	Wistar rats
Method of Induction	Single intraperitoneal injection of STZ
Dose of STZ	50–60 mg/kg
Solvent	Cold citrate buffer (pH 4.5)
Post-injection Care	5% glucose solution for 24 h to prevent hypoglycaemia
Blood Glucose Confirmation	Measured at 72 h using glucometer
Criteria for Diabetes Selection	Fasting blood glucose > 250 mg/Dl

11.6.2 Excision wound model

An excision wound of 1.5–2.0 cm diameter was created on the dorsal thoracic region of diabetic Wistar rats under ketamine–xylazine anesthesia. Wounds were disinfected with 70% ethanol, hemostasis was achieved with sterile cotton, and no sutures were applied. Rats were housed individually and monitored daily for healing progression.

Table 17: Excision Wound Model Parameters

Parameter	Observation
Animal Model	Streptozotocin-induced diabetic Wistar rats
Anesthesia	Ketamine–xylazine
Wound Site	Dorsal thoracic region
Wound Type	Circular full-thickness excision
Wound Size	1.5–2.0 cm diameter
Disinfection	70% ethanol
Hemostasis	Achieved by sterile cotton blotting
Sutures	Not applied
Housing	Individually to prevent wound interference
Monitoring	Daily for healing progression

11.6.3 Wound contraction rate

The wound contraction study demonstrated progressive healing in all groups over 21 days. The herbal transfersomal gel exhibited the fastest wound closure, reaching 92.3% by day 21, followed by the herbal gel (78.5%) and positive control (60.8%). The gel base and untreated control showed slower healing, indicating the enhanced efficacy of the transfersomal formulation in accelerating diabetic wound healing.

Table 18: Wound Contraction (%) in Diabetic Rats Treated with Transfersomal Gel

Day	Control (%)	Positive Control (%)	Gel Base (%)	Herbal Gel (%)	Herbal Transfersomal Gel (%)
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
3	5.2 ± 0.5	10.8 ± 0.6	6.1 ± 0.5	12.5 ± 0.7	15.6 ± 0.8
7	12.5 ± 0.8	22.4 ± 1.0	14.2 ± 0.9	28.5 ± 1.2	36.8 ± 1.5
10	18.8 ± 1.0	32.5 ± 1.2	22.0 ± 1.1	42.6 ± 1.4	55.2 ± 1.7
14	26.5 ± 1.2	45.6 ± 1.3	32.1 ± 1.5	58.7 ± 1.6	72.4 ± 1.8
21	35.2 ± 1.3	60.8 ± 1.4	44.5 ± 1.6	78.5 ± 1.7	92.3 ± 1.9

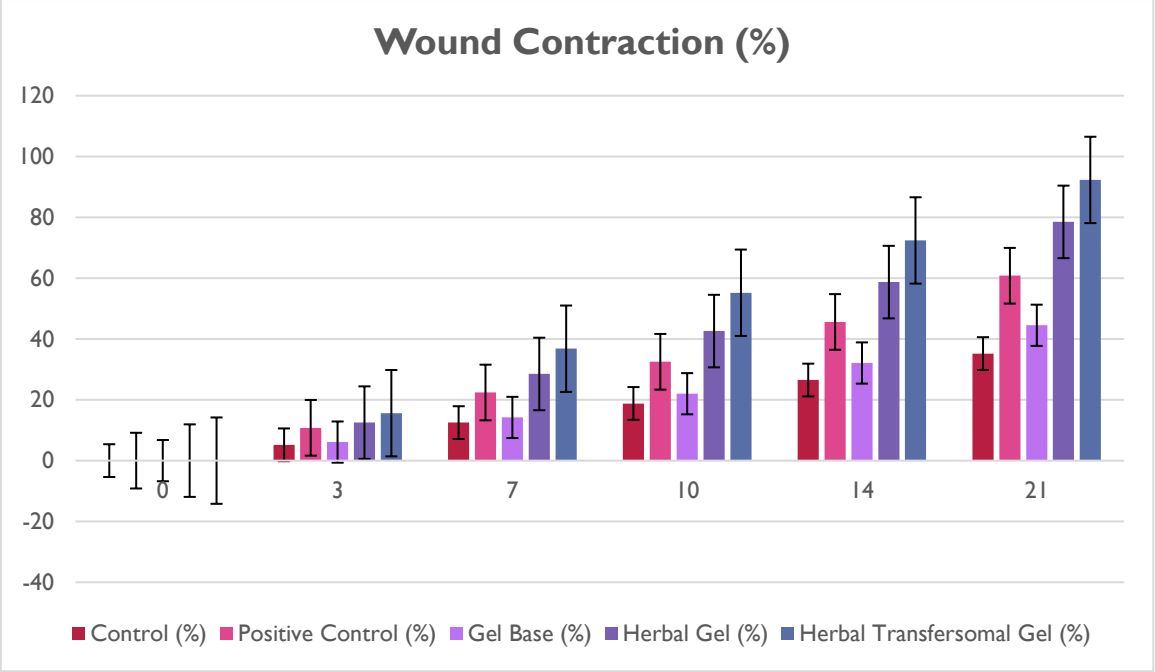


Fig :Wound Contraction (%)

CONCLUSION

The present study successfully developed and evaluated a **herbal transfersomal gel containing *Azadirachta indica*, *Ocimum sanctum*, and *Allium sativum*** for diabetic wound healing. The optimized formulation (G7) demonstrated favorable physicochemical properties, including uniform particle size, low polydispersity, high zeta potential, excellent entrapment efficiency, and stable drug content. The gel exhibited suitable pH, viscosity, spreadability, and extrudability for topical application. In vitro release studies confirmed sustained and enhanced drug delivery compared to conventional formulations, while stability studies under ICH conditions demonstrated good physical and chemical integrity. Furthermore, in vivo wound-healing studies revealed that the herbal transfersomal gel significantly accelerated wound contraction and closure in diabetic rats, outperforming both the herbal gel and positive control.

Overall, the findings indicate that the transfersomal gel effectively combines the therapeutic potential of herbal extracts with nanocarrier-mediated delivery, offering a promising, safe, and efficient strategy for the treatment of diabetic wounds. This formulation has the potential to improve patient compliance, accelerate healing, and reduce complications associated with chronic diabetic ulcers.

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CONFLICT OF INTEREST

No authors declared Conflict of Interest.

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