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PHYTOPHARMACEUTICAL DEVELOPMENT AND CHARACTERIZATION OF GYMNEMIC ACID LOADED HYDROGEL FOR IMPROVED WOUND CARE

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ABSTRACT

This study aims to develop and characterize a phytopharmaceutical hydrogel containing isolated gymnemic acid from *Gymnema sylvestre* leaves for improved wound care. Gymnemic acid, a triterpenoid saponin, was extracted using ethanol (12.6% w/w yield) and isolated via acid precipitation (1.21% w/w yield). Its identity and purity were confirmed using thin-layer chromatography (Rf 0.7) and Fourier-transform infrared (FT-IR) spectroscopy. A hydrogel formulation was developed utilizing Carbopol 940 as the gelling agent. The prepared hydrogel was evaluated for its physicochemical properties, rheological behavior, and in-vitro drug release. The formulation exhibited a skin-compatible pH of 6.5, excellent homogeneity, and complete washability. Rheological assessment revealed a desirable pseudoplastic (shear-thinning) flow, and spreadability testing demonstrated a spreadability factor of 20.0 g.cm/sec, ensuring pain-free application. In-vitro drug release studies, uniquely performed using an eggshell membrane sac method to simulate a multidirectional wound bed environment, demonstrated a biphasic and sustained release

profile, achieving a cumulative drug release of 21.44 mg over 5 hours. These findings suggest that the gymnemic acid-loaded Carbopol hydrogel is a promising, stable, and effective topical delivery system for sustained wound management.

KEYWORDS: Gymnemic acid, *Gymnema sylvestre*, Hydrogel, Wound healing, Carbopol 940, Eggshell membrane.

INTRODUCTION

A wound is defined as a disruption of the anatomical continuity and physiological function of the skin and its underlying tissues. The immediate pathophysiological consequences include the loss of the protective barrier, exposure to potential microbial contamination, and the initiation of the wound healing cascade, comprising hemostasis, inflammation, proliferation, and remodeling. Chronic wounds often fail to proceed through this normal healing cascade and become arrested in the inflammatory phase.

The therapeutic application of plant-based preparations is a foundational element in accelerating tissue repair. *Gymnema sylvestre*, a perennial woody vine rich in triterpenoid saponins known collectively as gymnemic acids, exhibits potent anti-inflammatory, antimicrobial, and antioxidant properties. While the therapeutic potential of crude *Gymnema sylvestre* extracts in wound healing has been documented, there is a lacuna in the formulation and evaluation of its isolated principal active constituent, gymnemic acid, within a functionally optimized topical delivery system. Hydrogels provide an optimal microenvironment for wound healing by maintaining moisture and enabling the controlled, sustained release of active pharmaceutical ingredients. Therefore, this study aims to develop and characterize a phytopharmaceutical hydrogel containing isolated gymnemic acid to achieve improved wound care through sustained drug delivery.

MATERIALS AND METHODS

Materials: *Gymnema sylvestre* leaves were collected and authenticated. Carbopol 940, Triethanolamine (TEOA), glycerol, ethanol, and methylparaben were sourced for the formulation process.

Extraction of the plant material

Shade-dried leaves of *Gymnema sylvestre* were coarsely powdered and passed through a 40-mesh sieve to ensure uniform particle size. A known quantity of the powdered material was

packed into a Soxhlet thimble and subjected to continuous extraction using 90% v/v ethanol as the solvent. The extraction process was maintained for 6 to 8 hours at a strictly regulated reflux temperature of 50-60°C using a heating mantle, achieving approximately 10 to 15 siphoning cycles per hour. Upon completion, the combined ethanolic extracts were concentrated into a semisolid mass via controlled evaporation at 45°C to prevent the thermal degradation of thermolabile constituents. The final crude extract was weighed, and the percentage yield was calculated relative to the initial weight of the dried plant material.

Isolation of Gymnemic Acid

A weighed portion of the concentrated ethanolic extract was dissolved in a minimal volume of ethanol. To facilitate the conversion of gymnemic acid into its soluble salt form, the solution was gradually alkalinized to a pH of 9-10 by the slow addition of 1% w/v aqueous sodium hydroxide under continuous stirring. The alkaline mixture was subsequently filtered to remove insoluble impurities. The resulting clear filtrate was carefully acidified using dilute hydrochloric acid to adjust the pH to 2-3, which induced the precipitation of gymnemic acid. The collected precipitate was dried naturally at ambient laboratory conditions for three days until a constant weight was achieved. The final dried isolate was weighed, and the percentage yield was calculated relative to the weight of the crude extract.

Preliminary Phytochemical Screening

The isolated compound was subjected to qualitative phytochemical evaluation to confirm the presence of triterpenoid saponins.

- **Foam Test for Saponins**: A small quantity of the isolate was dissolved in distilled water and agitated vigorously in a test tube. The development and persistence of a stable froth for more than ten minutes was recorded as a positive indication of saponins.

- **Salkowski Test for Triterpenoids**: To verify the triterpenoid nature of the compound, the isolate was dissolved in chloroform and carefully treated with concentrated sulfuric acid, added dropwise along the inner wall of the test tube to form a separate lower layer. The appearance of a distinct reddish-brown coloration at the interface was taken as positive evidence for the presence of triterpenoids.

Determination of melting point

A small portion of the dried isolate was introduced into a capillary tube sealed at one end and placed in a calibrated digital melting point apparatus. The temperature of the heating block

was gradually increased at a controlled rate of approximately 1-2 °C per minute as the expected melting region was approached. The onset and completion temperatures corresponding to the melting or thermal decomposition of the sample were carefully recorded. The observed melting point served as a preliminary indicator of the isolate's purity and was subsequently compared with literature-reported values for gymnemic acid to confirm its identity.

Chromatographic evaluation (TLC)

Thin Layer Chromatography (TLC) was performed to qualitatively identify the isolated gymnemic acid.

- Preparation of Stationary Phase: Silica Gel G plates were prepared utilizing the pouring method. A slurry was formulated by mixing Silica Gel G with distilled water and poured onto glass slides to form a uniform thin layer. The coated plates were subsequently activated by heating in a hot air oven at 110°C for 30 minutes.

- Mobile Phase and Chamber Saturation: A solvent system consisting of chloroform and methanol in a 6:5 (v/v) ratio was selected as the mobile phase. The solvent mixture was placed in a TLC chamber, securely covered, and allowed to saturate for 20 minutes to ensure the equilibrium of solvent vapors.

- Sample Application and Development: A small quantity of the isolated gymnemic acid was dissolved in ethanol. The sample was applied as a spot on the baseline of the activated TLC plate using a capillary tube. The plate was then placed into the saturated chamber and allowed to develop until the solvent front reached approximately three-quarters of the plate height.

- Detection: The developed plate was removed, dried, and placed in an iodine chamber containing iodine crystals. The iodine vapours were allowed to react with the separated spots to facilitate visualization.

Fourier transform infrared (FT-IR) spectroscopy

To confirm the presence of characteristic functional groups in the isolated gymnemic acid, a small portion of the dried sample was finely triturated with dry potassium bromide (KBr) and subsequently compressed into a transparent pellet using a hydraulic press. The prepared pellet was analysed using an FT-IR spectrophotometer, with spectra recorded over the range of

4000-400 cm^{-1}. Major absorption bands corresponding to key functional groups, such as O-H stretching, C=O stretching, and C-O stretching vibrations, were identified. The obtained spectral data were then compared with literature reference values to corroborate the structural identity of the gymnemic acid.

Determination of λmax (UV-Visible) and calibration curve

A stock solution of the isolated gymnemic acid was prepared by accurately weighing 10 mg of the dried compound and dissolving it in ethanol, followed by dilution to 10 mL to obtain Stock A (1000 µg/mL). From this, 1 mL was further diluted to 10 mL with ethanol to yield Stock B (100 µg/mL). Serial dilutions in the range of 5-100 µg/mL were subsequently prepared from Stock B to establish the working solutions. These solutions were scanned in a UV-Visible spectrophotometer over the wavelength range of 200-400 nm to determine the absorption maximum λmax of gymnemic acid. The identified λmax was employed for quantitative estimation, and a calibration curve plotting absorbance against concentration was constructed. The calibration curve was further validated for linearity, precision, and accuracy to ensure the reliability of the analytical measurements.

Formulation development of gymnemic acid hydrogel

The hydrogel base was prepared by gradually dispersing Carbopol 940 into an appropriate volume of distilled water under gentle stirring to prevent lump formation. The dispersion was allowed to hydrate at room temperature for 12-24 hours to ensure complete swelling of the polymer. Separately, methyl paraben was dissolved in a small volume of warm water or ethanol and incorporated into the hydrated Carbopol dispersion. The required quantity of isolated gymnemic acid was dissolved in a minimal volume of ethanol and subsequently incorporated into the hydrated polymer matrix under mechanical stirring at approximately 300-500 RPM, achieving uniform distribution while minimizing vortex formation. Glycerol was then added as a humectant and mixed for 10-15 minutes to ensure formulation homogeneity. Finally, triethanolamine was introduced dropwise to adjust the pH to approximately 6.4-6.6, thereby neutralizing the Carbopol dispersion and promoting complete gelation. The final volume of the formulation was adjusted with distilled water.

Table 1: formulation of gymnemic acid loaded hydrogel.

S.No	Ingredients	Working Quantity(100gm)	USES
01.	Isolated gymnemic acid	1gm	API
02.	Carbopol 940	1gm	Gelling agent(polymer)

03.	Glycerol	7.5gm	Humectant, Plasticizer
04.	Ethanol	1.5gm	Co-solvent
05.	Methyl paraben	0.1gm	Preservative
06.	TEOA(Triethanolamine)	Q.S	pH adjuster
07.	Distilled water	Q.S to 100gm	Vehicle

Evaluation Tests

Physicochemical Evaluation

The formulated hydrogel was subjected to the following physicochemical evaluations to assess its organoleptic properties and compatibility for topical application.

1. Physical Appearance (Colour, Texture, and Odour): The hydrogel was inspected visually against a white background to determine its colour and clarity. The texture was evaluated by rubbing a small quantity between the thumb and index finger to check for grittiness or smoothness. The odour was evaluated by smelling the formulation.

2. Homogeneity: The formulation was tested for homogeneity by visual inspection after the gel was set in the container. It was checked for the presence of any aggregates, lumps, or phase separation.

3. pH Measurement: The pH of the hydrogel was determined using a digital pH meter. One gram of the hydrogel was dispersed in 100 ml of distilled water and allowed to stand for 2 hours to ensure complete dissolution. The electrode was immersed in the solution, and the reading was recorded in triplicate at room temperature.

4. Washability: A small amount of hydrogel was applied to the dorsal surface of the hand. After drying, the site was washed with tap water to evaluate the ease of removal.

Rheological evaluation

The rheological properties of the optimized gymnemic acid-loaded hydrogel were evaluated using a digital rotational viscometer (Model LMDV-60, Labman Scientific Instruments) to assess the flow behavior and consistency of the formulation under varying shear rates. A sufficient quantity of the hydrogel was placed into the sample holder, ensuring the absence of entrapped air bubbles to maintain measurement accuracy. Due to the high viscosity of the Carbopol-based matrix, Spindle #4 was selected to ensure that torque values remained within the valid instrument acceptance range (10-100%). Measurements were conducted at a controlled ambient temperature of approximately 24.9°C. Viscosity (in mPa·s) was recorded at low shear speeds (1.5 RPM and 3.0 RPM) to evaluate the non-Newtonian behavior of the

formulation. Readings were recorded only after the display value stabilized to ensure that dynamic equilibrium was reached.

Spreadability studies

Spreadability was determined using the parallel plate method. Two glass slides of standard dimensions were selected, with the weight of the upper glass plate recorded as 309.97 g. One gram of the formulated hydrogel was placed on the center of the lower glass plate. The upper plate was carefully placed over the gel, and a standard weight of 500 g was placed on top of it, exerting a total downward force of 809.97 g. After 5 minutes, the spread of the gel was measured along three different axes (d1, d2, d3) to calculate the mean spread diameter.

In-Vitro Drug Release Studies

- **Preparation of Biological Membrane:** To mimic a biological permeation barrier, a semi-permeable eggshell membrane was isolated. A fresh chicken egg was emptied of its yolk and albumin through a small hole and washed thoroughly with distilled water. The empty shell was then immersed in 1N hydrochloric acid (HCl) for approximately 2-3 hours to dissolve the hard calcium carbonate layer. The exposed soft membrane was carefully peeled off and washed repeatedly with distilled water to remove any residual acid. The membrane was inspected for integrity and stored in phosphate-buffered saline (PBS, pH 7.4) for 24 hours prior to the diffusion study to ensure complete equilibration.

- **Egg Shell Membrane Sac Method:** A 10 g quantity of the formulated gymnemic acid hydrogel was filled directly into the prepared membrane sac. The opening was tightly secured with a non-reactive thread to form a leak-proof pouch, effectively utilizing the entire surface area of the membrane for multidirectional diffusion. The receptor compartment consisted of a borosilicate beaker containing 75 mL of PBS (pH 7.4). The gel-loaded membrane sac was suspended into the receptor medium, ensuring complete immersion without contacting the bottom or sides of the beaker. The assembly was placed on a magnetic stirrer, and the receptor medium was stirred at 50 RPM at ambient temperature (28°C ± 2°C) to maintain sink conditions.

- **Sampling and Analysis:** Sample aliquots (5 mL) were withdrawn from the receptor compartment at predetermined time intervals (15, 30, 45, 60, 120, 180, 240, and 300 minutes). Each withdrawn sample was transferred to a 10 mL volumetric flask, and the volume was made up to the mark with PBS (pH 7.4), resulting in a dilution factor of 2. After

each withdrawal, an equal volume (5 mL) of fresh buffer was immediately added to the receptor compartment to maintain a constant sink volume. The diluted samples were analysed spectrophotometrically at 205 nm to quantify the cumulative drug release.

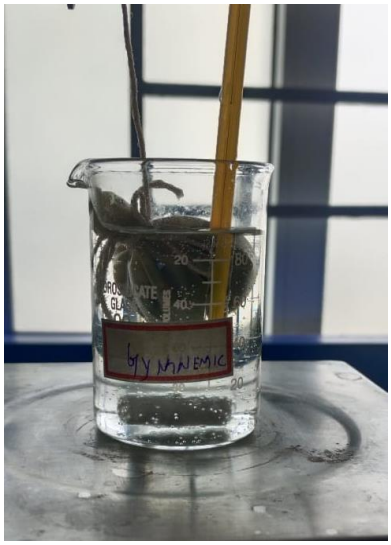

Fig. 1: in-vitro drug release setup utilizing isolated egg shell membrane sac.

RESULT AND DISCUSSION

Extraction and isolation yield

The Soxhlet extraction of 50 g of shade-dried *Gymnema sylvestre* leaves yielded 6.3 g of concentrated ethanolic extract, corresponding to a percentage yield of 12.6% w/w. Subsequent acid precipitation from the 6.3 g of crude extract produced 0.604 g of dried gymnemic acid isolate. This represents a 9.6% w/w yield relative to the crude extract and a 1.21% w/w yield relative to the initial dried plant material.

Melting point

The dried, isolated gymnemic acid decomposed at approximately 167-177°C (onset at 167°C; end at 177°C). This fairly high melting region is consistent with a relatively pure triterpene-type acid, aligning with the established literature reports for various gymnemic acid derivatives.

Preliminary phytochemistry

The isolated compound was subjected to qualitative phytochemical evaluation, yielding the following results

fig. 2: foam test for saponins.

fig. 3: salkowski test for triterpenoids.

Foam test (saponins): A positive result was observed, characterized by the development of a stable froth that persisted for more than 10 minutes, indicating the presence of saponins. (fig.2)

- **Salkowski test (triterpenoids):** A positive result was recorded, indicated by the appearance of a distinct reddish-brown coloration at the interface, confirming the presence of triterpenoids. (fig. 3)

Interpretation of extraction and phytochemical data

The initial extraction yield of 12.6% falls well within an acceptable range for the standard ethanol extraction of leaf saponins. Furthermore, the isolation step successfully produced a modest but pure yield of the target compound. The positive results from both the saponin and triterpenoid qualitative tests serve as strong preliminary confirmation that the obtained isolate is indeed a gymnemic-type saponin fraction.

TLC Profiling

The chromatographic evaluation of the isolated sample confirmed the presence of the phytoconstituent. Upon exposure to iodine vapours, a distinct **yellowish-brown** spot was observed. **(fig. 4)**

Fig. 4: spot on tlc plate indicating isolated gymnemic acid.

Retardation Factor (*Rf*) Value

The distance travelled by the solute and the solvent front was measured, and the Rf value was calculated using the formula

$$Rf = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent front}}$$

The isolated Gymnemic acid exhibited a single spot with an Rf value of **0.7.**

DISCUSSION

The appearance of a distinct spot with a specific Rf value confirms the purity and identity of the isolated compound. The use of Iodine as a detecting agent is specific for organic compounds, where the accumulation of iodine on the solute spot results in a yellowish-brown coloration. The specific Rf value of 0.7 in the Chloroform: Methanol (6:5) system serves as a qualitative fingerprint for the isolated Gymnemic acid.

Structural confirmation (FT-IR)

FT-IR (KBr pellet) major absorption bands

- **3420 cm^{-1}** → Broad O-H stretching (hydroxyl groups in alcohols/phenols, common in saponins and triterpenoid glycosides).
- **2950 cm^{-1}** → C-H stretching vibrations of alkanes (methyl/methylene groups in triterpenoid backbone).
- **1740–1710 cm^{-1}** → Strong C=O stretching (carboxylic acid group of gymnemic acid).
- **1630 cm^{-1}** → C=C stretching (olefinic bonds in triterpenoid skeleton, or conjugated C=O).

- **1260–1050 cm⁻¹** → C-O stretching (glycosidic linkages, ester/alcohol groups).
- **890–750 cm⁻¹** → Fingerprint region, often indicating characteristic bending vibrations of glycosidic units.

Interpretation: The observed bands are consistent with triterpenoid glycosides (saponins) - broad OH, carbonyl stretch and C-O bands support presence of gymnemic acid moiety and sugar residues. FT-IR therefore supports the identity of the isolate as a gymnemic acid containing fraction.

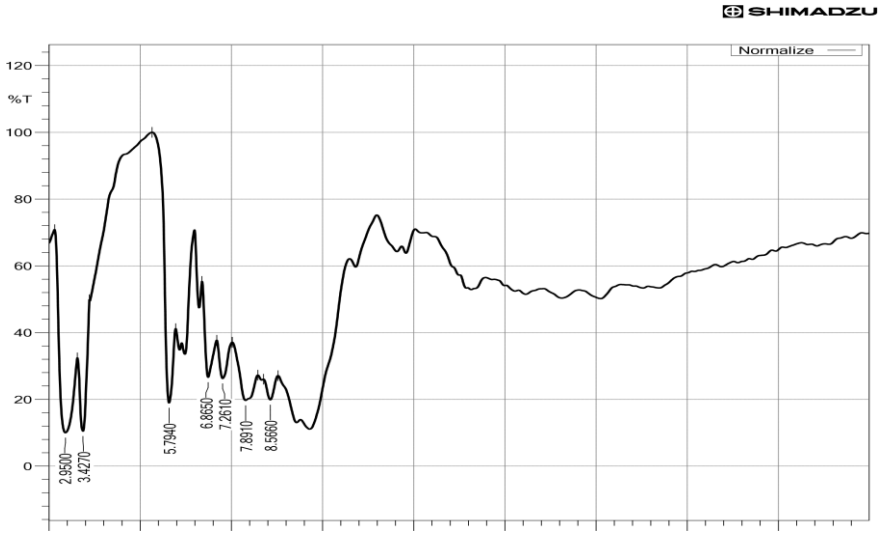

Fig. 5: ft-ir spectra of the isolated gymnemic acid.

UV-Visible Analysis and Calibration

λmax: Serial scans (200–400 nm) of working solutions revealed a prominent absorption peak at **205 nm**, which was selected as λmax for quantification.

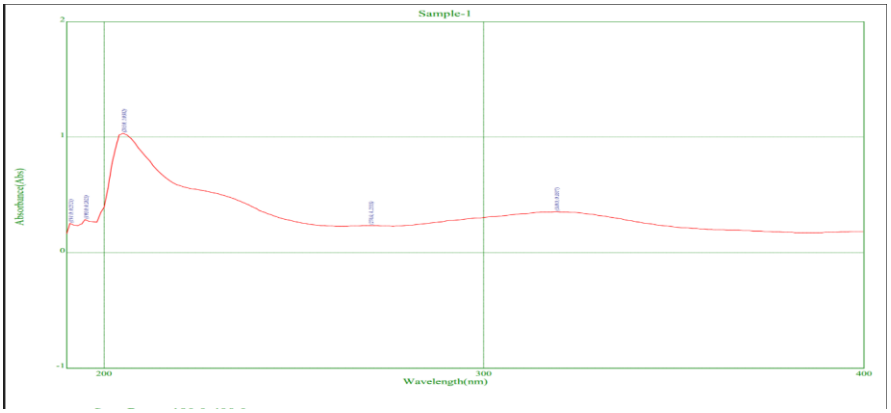

Fig. 6: uv-visible spectra of isolated gymnemic acid.

Calibration curve: A calibration curve prepared over **10–100 µg/mL** (ethanol) showed linearity with the regression equation: **A = 0.0147C + 0.0655** and **R² = 0.9786**.

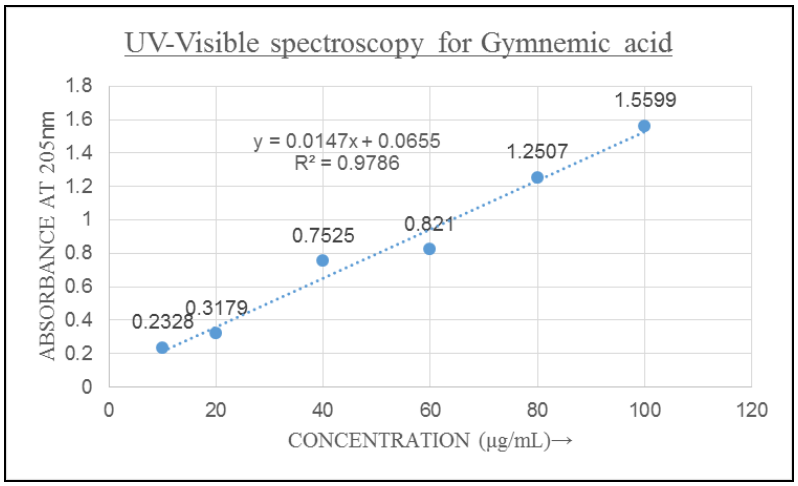

Fig 7: calibration curve of gymnemic acid.

Interpretation: The good linearity ($R^2 \approx 0.979$) demonstrates that the UV assay at 205 nm is reliable for gymnemic acid quantification in ethanolic solutions within the tested concentration range.

Evaluation Tests

Physicochemical Properties

The results of the physicochemical evaluation confirm that the formulated Gymnemic acid-loaded hydrogel possesses suitable characteristics for topical application.

- **Organoleptic Properties**: The prepared hydrogel was found to be **Translucent brownish-green** in colour, which is characteristic of the phytoconstituent. It possessed a **smooth, homogeneous texture** with no evidence of grittiness or lumps, indicating excellent compatibility between the API and the Carbopol base. The formulation had a characteristic odour.

- **pH Value:** The pH of the formulation was found to be 6.5. This is a critical result, as the pH of a wound bed and healthy skin typically ranges from 5.5 to 6.8. A pH of 6.5 ensures that the hydrogel is non-irritating and compatible with the skin environment, minimizing the risk of stinging upon application to open wounds.

- **Homogeneity:** The formulation showed no signs of phase separation or aggregation, confirming its physical stability.

- **Washability:** The gel demonstrated excellent washability; it was easily removed with water without leaving a sticky or greasy residue, which is a significant advantage for patient compliance and wound hygiene.

Table 2: evaluation tests of physicochemical properties.

Parameters	Observations	Inference
Colour	brownish-green	Characteristic of API
Appearance	Translucent	Good gel formation
Texture	Smooth & Homogeneous	No Grittiness/Lumps
Odour	Characteristic	Acceptable
pH	6.5	Skin Compatible
Washability	Good	Easily washable with water

Rheological Properties

The formulated hydrogel exhibited distinct non-Newtonian, pseudoplastic (shear-thinning) flow behaviour when analysed using the Labman LMDV-60 viscometer. The viscosity measurements indicated a clear inverse relationship between shear rate and viscosity. At a lower speed of **1.5 RPM**, the formulation demonstrated a high viscosity of **184,743 mPa·s** (with a torque of 46.2%), indicating a strong gel structure at rest. When the speed was increased to **3.0 RPM**, the viscosity significantly decreased to **119,650 mPa·s** (with a torque of 59.6%).

Table 03: rheological evaluation of gymnemic acid loaded hydrogel.

Spindle No.	Speed (RPM)	Temperature(°C)	Torque(%)	Viscosity(mPa·s)
#4	1.5	24.9	46.2	184,743
#4	3.0	24.9	59.6	119,650

Discussion: The observed pseudoplastic behaviour is highly desirable for topical wound care formulations. The high viscosity at low shear rates (1.5 RPM) ensures that the hydrogel possesses sufficient structural integrity to remain at the application site without running off or dripping. Conversely, the reduction in viscosity at higher shear rates (simulating the mechanical force of application or rubbing) facilitates easy spreadability over the wound bed.

The choice of Spindle #4 was validated by the torque percentages obtained (46.2% and 59.6%), which fall well within the optimal sensitivity range of the LMDV-60 instrument, confirming the reliability of the data.

Spreadability

Weight Applied (M):

- Weight of Upper Plate = 309.97 g, Weight Added = 500 g
- Total Weight (M)= 309.97 + 500 = 809.97 g.

Trial Calculations

- Trial 1**:** (8.3 + 8.7 + 8.8) / 3 = 8.60 cm
- Trial 2: (7.0 + 7.2 + 6.6) / 3 = 6.93 cm
- Trial 3: (6.9 + 6.6 + 6.6) / 3 = 6.70 cm

Overall Average Diameter**:** (8.60 + 6.93 + 6.70) / 3 **= 7.41 cm**

Table 04: mean diameter of the circle.

Trial No.	Diameter 1 (cm)	Diameter 2 (cm)	Diameter 3 (cm)	Average Diameter (cm)
1	8.3	8.7	8.8	**8.60**
2	7.0	7.2	6.6	**6.93**
3	6.9	6.6	6.6	**6.70**
Mean				**7.41**

Spreadability Factor (S) Calculation

$$S = \frac{M \times L}{T}$$

M- Total Weight,

L- Mean Diameter,

T- Time.

$$S = \frac{809.97 \times 7.41}{300} = \frac{60001.82}{300} = 20.00 \text{ g.cm/sec}$$

Discussion: The formulation demonstrated excellent spreadability with a mean spread diameter of **7.41 cm** under a total weight of 809.97 g. The calculated Spreadability Factor (S) was found to be **20.0 g.cm/sec**. This value indicates that the gel spreads easily upon application of minimal shear force, ensuring good coverage of the wound area without requiring painful rubbing. The high spreadability is attributed to the pseudoplastic nature of the Carbopol 940 matrix.

In-Vitro Drug Release Profile

The release of Gymnemic acid from the Carbopol-based hydrogel was quantified using the standard calibration curve equation (y = 0.0147x + 0.0655). A correction factor was applied to account for the dilution of samples (DF=2) and the volume of the receptor medium (75 mL).

The study revealed a progressive and sustained release profile. In the initial phase (15 minutes), the formulation released **1.46 mg** of the drug. This release gradually increased to **6.62 mg** by the end of the first hour. A steady-state release pattern was observed over the subsequent hours, reaching **9.75 mg** at 2 hours and **17.02 mg** at 3 hours. By the end of the 5-hour study period (300 minutes), the formulation achieved a maximum cumulative drug release of **21.44 mg**.

Table 05: in-vitro drug release data.

Time (min)	Absorbance	Actual Conc. (µg/mL)	Cumulative Release (mg)	% Cumulative Release
15	0.2084	19.44	1.46	1.46%
30	0.3389	37.20	2.89	2.89%
45	0.4269	49.18	3.97	3.97%
60	0.6620	81.16	6.62	6.62%
120	0.9300	117.62	9.75	9.75%
180	1.5841	206.62	17.02	17.02%
240	1.7704	231.96	19.95	19.95%
300	1.8031	236.40	21.44	21.44%

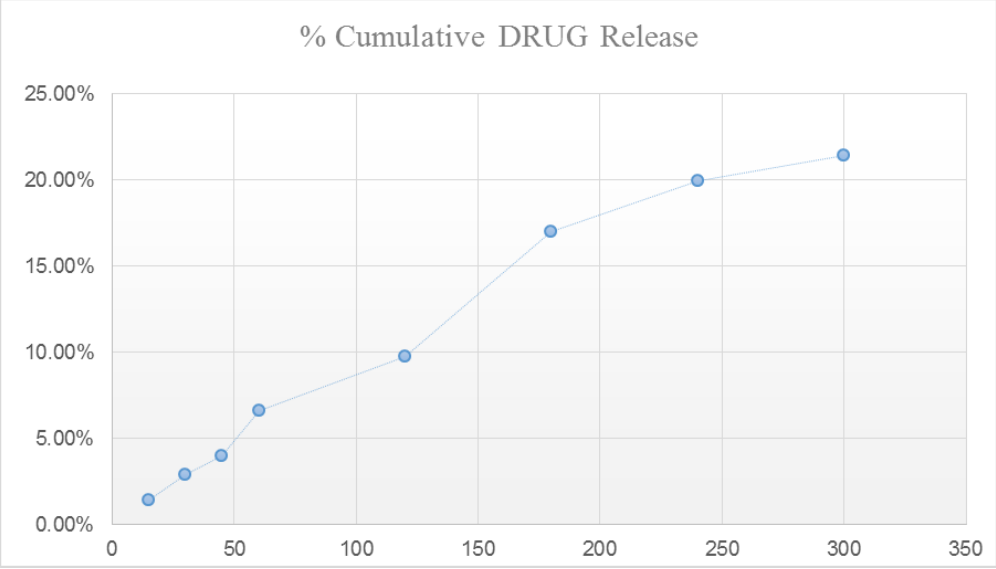

Fig. 8: in-vitro cumulative drug release profile of gymnemic acid hydrogel.

DISCUSSION

In-vitro Release Kinetics and Mechanism The drug release study was conducted using the modified egg shell membrane sac method, which offers a distinct advantage over conventional unidirectional diffusion cells. The effective surface area for diffusion was defined by the total outer surface area of the isolated egg membrane sac. This large surface

area facilitated **multidirectional drug release**, simulating the three-dimensional environment of a deep wound bed more accurately than the limited flat surface area of a standard Franz diffusion cell.

The formulation demonstrated a **biphasic release pattern**. The initial phase showed a controlled release (1.46 mg in 15 mins), likely due to the drug present at the periphery of the gel matrix adjacent to the membrane. This was followed by a sustained release phase, reaching 21.44 mg over 5 hours. This sustained profile is attributed to the cross-linked Carbopol 940 matrix, which swells upon contact with the buffer (75 mL sink volume), creating a tortuous path for the drug to diffuse from the core of the 10 g sample mass. This release behaviour is clinically beneficial for wound management, as it ensures a prolonged therapeutic effect, minimizing the frequency of dressing changes require.

CONCLUSION

The present research successfully fulfilled its primary objective of developing and characterizing a novel phytopharmaceutical hydrogel containing isolated gymnemic acid for improved wound care. The study began with the effective ethanolic extraction of *Gymnema sylvestre* leaves, yielding 12.6% w/w of crude extract, followed by the isolation of the active triterpenoid saponin, gymnemic acid (1.21% w/w). The identity of the isolate was authenticated via thin-layer chromatography (Rf 0.7) and FT-IR spectroscopy, which confirmed the presence of the characteristic triterpenoid structure. The formulation, developed using Carbopol 940, demonstrated ideal physicochemical properties for topical application, including a skin-compatible pH of 6.5, excellent homogeneity, and superior washability. Rheological evaluation revealed desirable pseudoplastic (shear-thinning) flow behavior, where viscosity decreased from 184,743 mPa.s to 119,650 mPa.s under shear, ensuring structural stability at rest and ease of spreading during application (spreadability factor: 20.0 g.cm/sec). Furthermore, the in-vitro release study utilizing the novel eggshell membrane sac method established a biphasic release pattern, achieving a sustained cumulative drug release of 21.44 mg over 5 hours. These findings scientifically validate the potential of the gymnemic acid-loaded hydrogel as a stable, non-toxic, and effective topical delivery system for sustained wound healing.

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REFERENCES

1. Szulc-Musioł B, Siemiradzka W, Dolińska B. (Formulation and evaluation of hydrogels based on sodium alginate and cellulose derivatives with quercetin for topical application). Appl. Sci., 2023; 13(13): 7826.
2. Vats S, Dey A, Bhandari N, et al. (Gymnema sylvestre R. Br.: Phytochemicals and medicinal properties). In: Medicinal and Aromatic Plants of India Vol. 2. Springer, 2023; 125-151.
3. Gavan A, Colobatiu L, Hanganu D, et al. (Development and evaluation of hydrogel wound dressings loaded with herbal extracts). Processes, 2022; 10(2): 242.
4. Tyagi Y, Madhav NVS. (Inbuilt novel bioretardant feature of biopolymer isolated from Cucumis sativa for designing drug loaded bionanosuspension). J Drug Assess, 2020; 9(1): 31-42.
5. Bashir S, Hina M, Iqbal J, et al. (Fundamental concepts of hydrogels: Synthesis, properties, and their applications). Polymers, 2020; 12(11): 2702.
6. Khan F, Sarker M, Ming LC, et al. (Comprehensive review on phytochemicals, pharmacological and clinical potentials of Gymnema sylvestre). Front Pharmacol, 2019; 10: 1223.
7. Gunasekaran V, Srinivasan S, Rani SS. (Potential antioxidant and antimicrobial activity of Gymnema sylvestre related to diabetes). J Med. Plants Stud., 2019; 7(2): 05-11.
8. Srinivasan K, Perumal K. (Study on extraction and purification of gymnemic acid from Gymnema sylvestre R.Br). Int. J Pharm. Biol. Sci., 2019; 9(1): 305-311.
9. Upadhyay B, Sisodiya D. (Extraction, analytical study, formulation and evaluation of herbal transdermal patch of gymnemic acid). Sch. Acad J Pharm., 2019; 8(11): 514-519.

10. Dongre P, et al. (A Simple Egg Membrane Model for Understanding Diffusion Characteristics of Nanoparticles and Amino Acids). Curr. Sci., 2017; 112(7): 1576-1580.
11. Dantas MGB, et al. (Development and evaluation of stability of a gel formulation containing the monoterpene borneol). Sci. World J, 2016; 2016: 7394685.
12. Lal VR. (Isolation and characterization of gymnemic acid from Gurmar (Gymnema sylvestre R. BR.) for control of diabetes in Jharkhand). Int. J Exch. Knowl., 2015; 2(1): 117-123.
13. Subashini MS, Rajendran P, Ashok G, Kanthesh BM. (TLC, FTIR and GCMS analysis of leaves of Gymnema sylvestre R.Br from Kolli Hills, Tamil Nadu, India). Int. J Curr. Microbiol. App. Sci., 2015; 4(7): 757-764.
14. Gupta A, Kumar P. (Assessment of the histological state of the healing wound). Plast. Aesthet. Res., 2015; 2(5): 239-242.
15. Kurahashi T, Fujii J. (Roles of antioxidative enzymes in wound healing). J Dev. Biol., 2015; 3(2): 57-70.
16. Tiwari P, Mishra BN, Sangwan NS. (Phytochemical and pharmacological properties of Gymnema sylvestre: An important medicinal plant). Biomed. Res. Int., 2014; 2014: 830285.
17. Vediyappan G, Dumontet V, Pelissier F, d'Enfert C. (Gymnemic acids inhibit hyphal growth and virulence in Candida albicans). PLoS One, 2013; 8(9): e74189.
18. Rathore RPS, Nema RK. (Formulation and evaluation of topical gels of Carbopol 940). IOSR J Pharm Biol. Sci., 2012; 1(1): 70-73.
19. Kiranmai M, Kazim SM, Ibrahim M. (Combined wound healing activity of Gymnema sylvestre and Tagetes erecta Linn). Int J Pharm. Appl., 2011; 2(2): 135-140.
20. Guo S, DiPietro LA. (Factors affecting wound healing). J Dent. Res., 2010; 89(3): 219-229.
21. Chowdhary F, Rasool MH. (Isolation and characterization of gymnemic acid from indigenous Gymnema sylvestre). J Appl. Pharm., 2010; 3(2): 60-65.
22. Velnar T, Bailey T, Smrkolj V. (The wound healing process: An overview of the cellular and molecular mechanisms). J Int. Med. Res., 2009; 37(5): 1528-1542.
23. Broughton G II, Janis JE, Attinger CE. (The basic science of wound healing). Plast Reconstr Surg, 2006; 117(7S): 12S-34S.
24. Kirsner RS, Eaglstein WH. (The wound healing process). Dermatol. Clin., 1993; 11(4): 629-640.