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Research Article

To develop and evaluate a Carbopol-based hydrogel containing *Moringa oleifera* root extract for antioxidant and antimicrobial diabetic wound management

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Abstract

Background: Diabetic wounds are characterized by oxidative stress and infection, leading to delayed healing.

Objective: To develop a Carbopol-based hydrogel incorporating *Moringa oleifera* root extract.

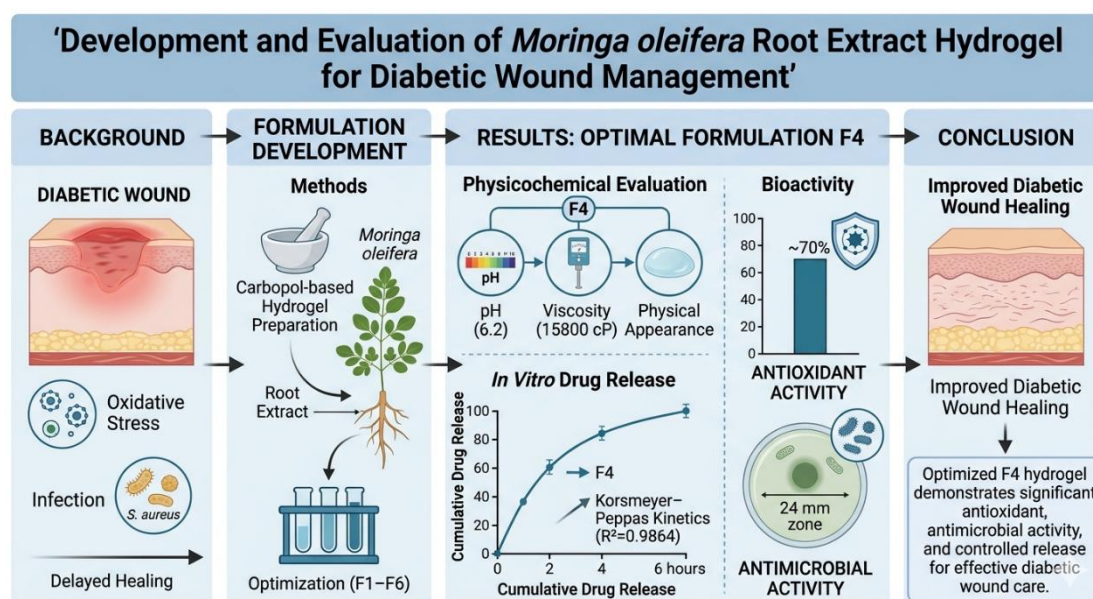
Methods: Hydrogel formulations (F1–F6) were prepared and evaluated for physicochemical properties, drug release, antioxidant and antimicrobial activity.

Results: Batch F4 showed optimal pH (6.2), viscosity (15800 cP), and 98% drug release within 6 hours. Release followed Korsmeyer–Peppas kinetics ($R^2 = 0.9864$), indicating non-Fickian diffusion. The formulation exhibited significant antioxidant (~70%) and antimicrobial activity (24 mm zone).

Conclusion: The developed hydrogel demonstrates strong potential for diabetic wound management.

Keywords: Carbopol hydrogel, *Moringa oleifera*, diabetic wound, antioxidant, antimicrobial, drug release

Graphical Abstract



1. INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or a combination of both. It is one of the most prevalent non-communicable diseases globally, posing a major burden on healthcare systems due to its progressive nature and associated complications. The global prevalence of diabetes has increased dramatically over the past few decades due to factors such as sedentary lifestyle, obesity, aging population, and genetic predisposition. Chronic hyperglycemia leads to long-term damage, dysfunction, and failure of various organs, particularly the eyes, kidneys, nerves, heart, and blood vessels ^{1,2}.

Among the various complications, diabetic foot ulcers (DFUs) are considered one of the most serious and economically burdensome conditions. DFUs significantly reduce the quality of life of patients and are a leading cause of hospitalization in diabetic individuals. It has been estimated that approximately 15–25% of diabetic patients will develop foot ulcers during their lifetime, and recurrence rates are notably high even after successful treatment ^{1,2}.

Hydrogels are three-dimensional, hydrophilic polymeric networks capable of absorbing and retaining large amounts of water while maintaining structural integrity. Due to their high-water content and biocompatibility, hydrogels closely resemble natural extracellular matrix, making them highly suitable for wound healing applications ^{3,4}.

Hydrogels provide a moist environment that facilitates cell migration, proliferation, and differentiation. They also promote autolytic debridement, which helps in the removal of necrotic tissue without damaging healthy cells. Furthermore, hydrogels act as effective carriers for bioactive molecules, enabling localized and controlled drug delivery ^{5,6}.

Hydrogels offer several advantages, including excellent moisture retention, non-adhesive nature, ease of application, and ability to conform to irregular wound surfaces. These properties significantly enhance patient comfort and compliance ^{5,6}.

Hydrogels can be engineered to release drugs in a controlled manner, thereby reducing systemic side effects and improving therapeutic efficacy. Recent advancements include stimuli-responsive hydrogels, antimicrobial hydrogels, and injectable hydrogels that respond to environmental triggers such as pH and temperature ^{7,8,9}.

Moringa oleifera, commonly referred to as the drumstick tree or “miracle tree,” is a fast-growing, drought-resistant plant belonging to the family Moringaceae. It is widely distributed across tropical and subtropical regions of Asia, Africa, and South America and has been extensively utilized in traditional systems of medicine such as Ayurveda due to its broad spectrum of therapeutic properties ^{10,11}. The plant is characterized by its high nutritional value and diverse phytochemical

profile, making it an important candidate in pharmaceutical and nutraceutical applications.

Various parts of the plant, including leaves, seeds, roots, bark, and flowers, possess significant medicinal value. Among these, the roots are particularly rich in bioactive compounds such as alkaloids, flavonoids, and phenolic constituents, which contribute to their antimicrobial, antioxidant, and anti-inflammatory activities ¹². These pharmacological properties make *Moringa oleifera* highly relevant in wound healing applications, especially in chronic conditions like diabetic wounds where oxidative stress and infection play a critical role. Additionally, its low toxicity, easy availability, and cost-effectiveness further enhance its suitability for incorporation into advanced drug delivery systems such as hydrogels.

The therapeutic efficacy of *Moringa oleifera* is primarily attributed to its rich and diverse phytochemical composition, which includes a wide range of bioactive compounds with significant pharmacological activities ^{13,14}.

The plant contains major phytoconstituents such as flavonoids (quercetin, kaempferol), phenolic acids, alkaloids, saponins, tannins, vitamins (A, C, E), and essential minerals including calcium, potassium, and iron. These compounds act synergistically to produce antioxidant, antimicrobial, anti-inflammatory, and wound healing effects. The presence of vitamins and minerals also supports cellular metabolism and tissue regeneration, which are essential for effective wound repair.

Phenolic compounds play a crucial role in neutralizing reactive oxygen species (ROS) by donating hydrogen atoms or electrons, thereby reducing oxidative stress and preventing cellular damage ¹⁴. Similarly, flavonoids exhibit strong antioxidant and anti-inflammatory properties and are known to enhance collagen synthesis and angiogenesis, which are critical steps in the wound healing process ¹⁵. The combined action of these phytochemicals significantly contributes to the therapeutic potential of *Moringa oleifera* in biomedical applications.

Oxidative stress is one of the primary factors responsible for delayed wound healing, particularly in diabetic patients. *Moringa oleifera* exhibits potent antioxidant activity through multiple mechanisms that help restore redox balance in the wound environment ¹⁶.

The plant's bioactive compounds act as free radical scavengers by donating electrons to neutralize reactive oxygen species, thereby preventing oxidative damage to cellular components such as lipids, proteins, and DNA ¹⁶. In addition to direct scavenging, *Moringa oleifera* enhances the activity of endogenous antioxidant defense systems, including enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase. These enzymes play a vital role in detoxifying harmful free radicals and maintaining cellular homeostasis.

Furthermore, *Moringa oleifera* inhibits lipid peroxidation, a process that damages cell membranes

and impairs tissue regeneration. By preserving membrane integrity and preventing oxidative degradation, the plant facilitates faster wound healing and tissue repair ¹⁷. These multifaceted antioxidant mechanisms make it highly effective in managing oxidative stress-associated complications in diabetic wounds.

Microbial infection is a significant challenge in wound management, particularly in diabetic patients where immune function is compromised. *Moringa oleifera* demonstrates broad-spectrum antimicrobial activity against a wide range of pathogens, making it highly suitable for wound care applications ^{13,18}.

The antimicrobial action of *Moringa oleifera* is attributed to its ability to disrupt microbial cell membranes, leading to leakage of intracellular contents and eventual cell death. It also inhibits protein synthesis and interferes with essential microbial enzyme systems, thereby preventing microbial growth and proliferation.

Studies have shown that *Moringa oleifera* is effective against both Gram-positive and Gram-negative bacteria, as well as fungal species ¹⁸. This broad-spectrum activity helps reduce microbial load, prevent infection, and create a conducive environment for wound healing. The

use of natural antimicrobial agents like *Moringa oleifera* also reduces the risk of antibiotic resistance, making it a safer and more sustainable alternative to synthetic drugs.

Inflammation is a critical phase of wound healing; however, excessive or prolonged inflammation can delay the healing process. *Moringa oleifera* exhibits significant anti-inflammatory activity, which helps regulate the inflammatory response and promotes faster healing ¹⁵.

The synergistic effects of combined therapy include reduction of reactive oxygen species, prevention of microbial colonization, and acceleration of tissue repair processes ^{19,20}. By simultaneously targeting multiple pathways, this approach enhances overall healing efficiency and reduces the risk of complications.

The anti-inflammatory effects are mediated through the inhibition of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α) and interleukins, as well as the suppression of prostaglandin synthesis. These actions help reduce swelling, redness, and pain at the wound site.

Therefore, this study aims to develop a Carbopol-based herbal hydrogel for improved wound healing.

2. MATERIALS AND METHODS

2.1 Materials and formulation table:

Table 1: formulation table

Ingredients	F1 (g)	F2 (g)	F3 (g)	F4 (g)	F5 (g)	F6 (g)
Carbopol 934	0.25	0.50	0.75	1.00	1.25	1.50
<i>Moringa oleifera</i> Root Extract	1.00	1.00	1.00	1.00	1.00	1.00
Propylene Glycol	5.00	5.00	5.00	5.00	5.00	5.00
Methyl Paraben	0.05	0.05	0.05	0.05	0.05	0.05
Propyl Paraben	0.01	0.01	0.01	0.01	0.01	0.01
Triethanolamine	q.s	q.s	q.s	q.s	q.s	q.s
Distilled Water	q.s to 50 g	q.s to 50 g	q.s to 50 g	q.s to 50 g	q.s to 50 g	q.s to 50 g

2.2 Extraction

1. Extraction of Plant Material

The selected plant material was collected from a suitable source and authenticated by a qualified botanist. It was washed with distilled water, shade dried at room temperature, and coarsely powdered using a mechanical grinder. The powdered material was sieved and stored in an airtight container. Extraction was carried out by maceration using a suitable solvent for 48–72 h with intermittent shaking. The extract was filtered through muslin cloth and Whatman filter paper No. 1, concentrated under reduced pressure using a rotary evaporator at 40–50°C, dried in a desiccator, and stored at 4°C until further use.

2. Qualitative Phytochemical Screening

The extract was subjected to preliminary phytochemical screening for alkaloids, flavonoids, tannins, saponins, and phenolic compounds using standard chemical tests such as Dragendorff's, Mayer's, Shinoda, ferric chloride, foam, and gelatin tests. The development of characteristic color changes or precipitates indicated the presence of corresponding phytoconstituents.

3. Preformulation Studies

Preformulation studies included evaluation of organoleptic properties, solubility, melting point, pH, λ_{max} , calibration curve, and FTIR analysis. Solubility was determined in different solvents, and λ_{max} was identified using UV-visible spectrophotometry in the range of 200–400 nm. A calibration curve was prepared at the determined λ_{max} . FTIR spectroscopy was performed using the KBr pellet

method to identify functional groups and possible drug-exipient interactions.

2.3 Formulation

Six formulations (F1–F6) were prepared using varying concentrations of Carbopol 940. The polymer was dispersed in water, followed by addition of excipients and extract. The pH was adjusted using triethanolamine to obtain gel consistency.

2.4 Evaluation

The formulated gel was evaluated for various parameters:

Physical appearance, pH, Viscosity, Spreadability, Extrudability, Drug content, In-vitro diffusion study, Kinetic modeling, Antibacterial activity, Anti-inflammatory activity and Anti-oxidant

3. RESULTS AND DISCUSSION

3.1 Plant material collection, authentication, washing and drying.

3.1.1 Plant material collection

Moringa oleifera Lam. belongs to the family Moringaceae. This medicinal plant is widely reported to be used traditionally for wound healing and possesses significant pharmacological activities. The plant material was collected from the local area of Virar, Maharashtra, India.

3.1.2 Authentication of Plant Species

The plant material was identified and authenticated by the Department of Botany, Dayanand Science College, Latur. They confirmed that the plant specimen provided was *Moringa oleifera* Lam., belonging to the family Moringaceae (root). The authentication certificate is provided below.

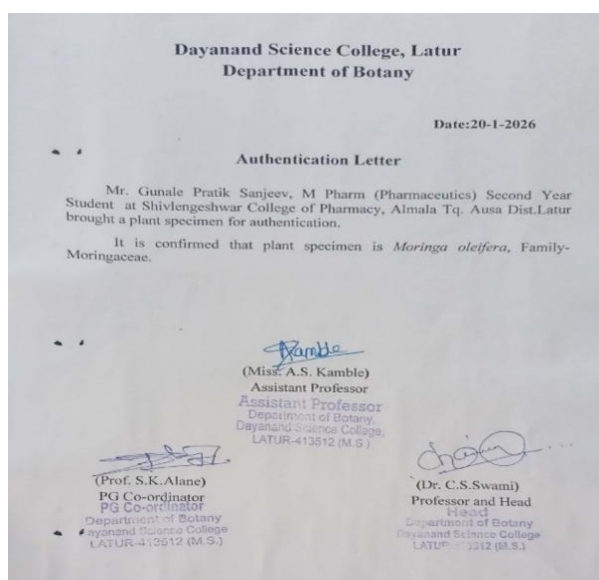


Figure 2: Authentication of Plant Species

3.1.3 Washing and Drying of Plant Roots

Roots of *Moringa oleifera* were washed thoroughly and cleaned with running tap water followed by distilled water to remove adhering soil and other impurities. The cleaned roots were then shade dried at room temperature to preserve the active constituents. The dried roots were ground into coarse powder using an electric blender. The powdered plant material was

stored in a dark place at room temperature for further use.

3.1.4 Preparation of Extract

The dried and powdered roots of *Moringa oleifera* were subjected to extraction by maceration method. A known quantity of powdered material was soaked in a suitable solvent (such as ethanol/methanol/distilled water) in a closed container and kept for 48–72 hours with occasional stirring.

After maceration, the mixture was filtered using Whatman filter paper to separate the extract from the marc. The filtrate was then concentrated by evaporation of solvent using a water bath at controlled temperature to obtain a semisolid extract.

The obtained extract was stored in an airtight container at room temperature for further use.

3.2 QUALITATIVE PHYTOCHEMICALS SCREENING

1. Tests for Alkaloids

Observation: Formation of orange or reddish-brown precipitate.

Result: Presence of alkaloids was confirmed.



Figure 3: Tests for Alkaloids

2. Flavonoids Test

Reagent: Lead acetate solution

Observation: Formation of yellow precipitate.

Result: Presence of flavonoids was confirmed.

3. Tannins Test

Reagent: Ferric chloride solution

Observation: Formation of blue-black or greenish-black coloration.

Result: Presence of tannins was confirmed.

4. Phenolic Compounds Test

Reagent: Ferric chloride solution

Observation: Formation of deep blue or green coloration.

Result: Presence of phenolic compounds was confirmed.

5. Saponins Test

Observation: Formation of stable persistent foam.

Result: Presence of saponins was confirmed.



Figure 4: Preparation of Extract

3.3 PREFORMULATION STUDIES

1. Organoleptic Evaluation

The drug sample was evaluated for its organoleptic characteristics, and the observations are presented in Table below.

Table 2: Organoleptic Evaluation

Parameter	Observation
Color	Brown / Dark brown (typical for plant extract)
Odor	Characteristic
Taste	Bitter
Physical State	Solid / Powder

The drug exhibited characteristic organoleptic properties, confirming its natural origin and purity without any abnormal appearance or odor.

2. Solubility Studies

The solubility of the drug was determined in different solvents, and the results are summarized below:

Table 3: Solubility Studies

Solvent	Solubility
Distilled Water	Slightly soluble
Ethanol	Soluble
Methanol	Freely soluble
Phosphate Buffer (pH 6.8)	Moderately soluble
Phosphate Buffer (pH 7.4)	Moderately soluble

The drug showed good solubility in organic solvents like methanol and ethanol, indicating the presence of polar phytoconstituents such as flavonoids and phenolic compounds.

3. pH Determination

The pH of 1% w/v solution of the drug was found to be:

pH = 6.2 – 6.8

4. Melting Point Determination

The melting point of the drug sample was determined using the capillary method.

Observed Melting Point: 210°C – 225°C

5. Preparation of Calibration Curve

The calibration curve of *Moringa oleifera* root extract was prepared using methanol as solvent. Standard solutions were prepared in the concentration range of 2–10 µg/mL, and absorbance was measured at 272 nm using a UV-Visible spectrophotometer.

Table 4: Calibration data of *Moringa oleifera* root extract

Concentration (µg/mL)	Absorbance
0	0
2	0.208
4	0.405
6	0.598
8	0.792
10	0.985

The absorbance was found to increase proportionally with an increase in concentration, indicating good linearity.

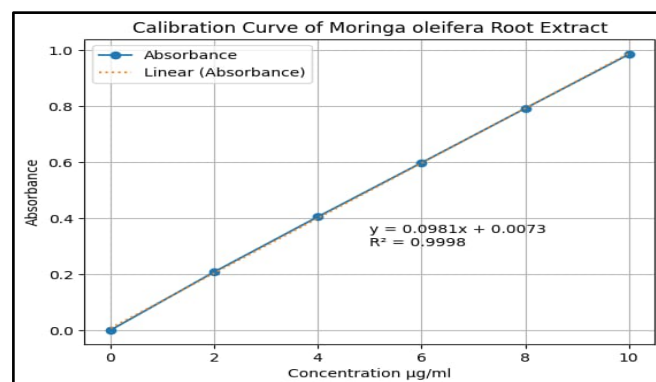


Figure 5: Calibration Curve of *Moringa oleifera* Root Extract

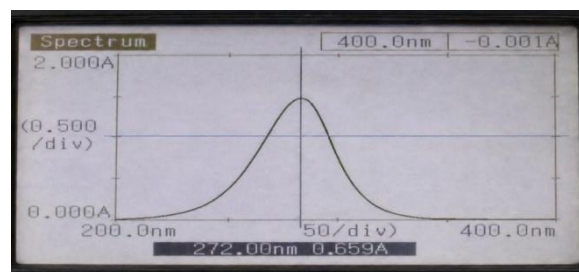


Figure 6: UV spectroscopy study

6. Fourier Transform Infrared Spectroscopy (FTIR)

6.1 FTIR For Pure Extract

The infrared spectrum of *Moringa oleifera* root extract was recorded using an FTIR spectrophotometer. The obtained spectrum was compared with standard functional group frequencies of plant phytoconstituents.

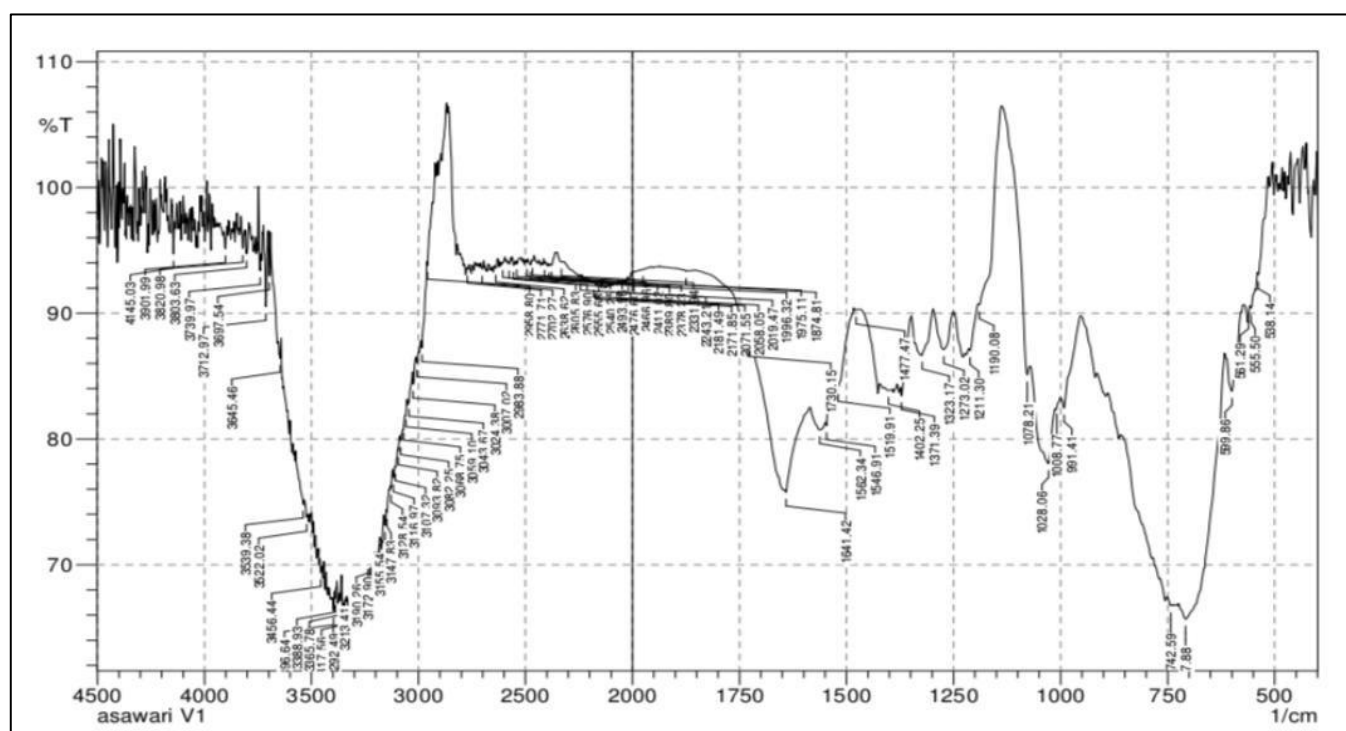


Figure 7: FTIR Spectrum of *Moringa oleifera* Root Extract

The observed functional group frequencies of *Moringa oleifera* root extract were found to be within the reported range of standard values, which confirms the presence of various bioactive compounds such as

phenols, flavonoids, tannins, and saponins. The presence of characteristic peaks corresponding to different functional groups indicates the purity and authenticity of the extract.

Table 5: FTIR Peak Interpretation of *Moringa oleifera* Root Extract

Sr. No.	Observed Frequency (cm ⁻¹)	Standard Frequency (cm ⁻¹)	Functional Group / Assignment
1	3292-3388	3200-3400	O-H stretching (Alcohols, Phenols)
2	2958-3007	2850-3000	C-H stretching (Alkanes)
3	1641	1600-1650	C=O stretching / C=C (Aromatic ring)
4	1519-1562	1500-1600	C=C stretching (Aromatic compounds)
5	1402-1477	1400-1450	C-H bending
6	1273-1323	1250-1350	C-O stretching (Phenols, Esters)
7	1190-1211	1150-1250	C-O stretching (Alcohols, Ethers)
8	1028-1078	1000-1100	C-O stretching (Primary alcohols)
9	707-742	700-900	C-H bending (Aromatic rings)
10	1730	1700-1750	C=O stretching (Carbonyl compounds)

6.2 Drug and Excipient Compatibility Study

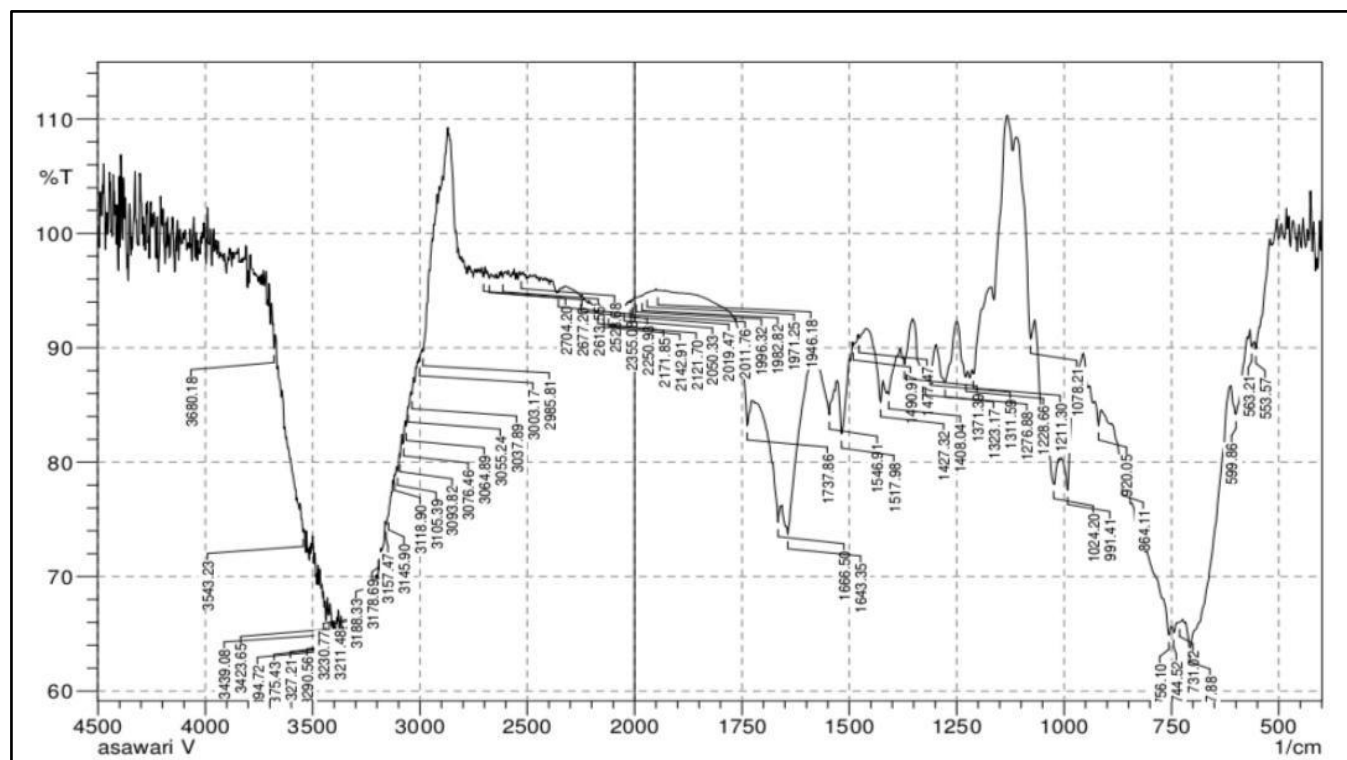


Figure 8: FTIR Spectrum of Extract with Excipients

FTIR Interpretation of *Moringa oleifera* Root Extract with Excipients (Compatibility Study)

Table 6: FTIR Interpretation of *Moringa oleifera* Root Extract with Excipients

Sr. No.	Observed Frequency (cm ⁻¹)	Standard Frequency (cm ⁻¹)	Functional Group / Assignment
1	3290-3385	3200-3400	O-H stretching (Phenols, Alcohols)
2	2920-2955	2850-3000	C-H stretching (Alkanes, excipient polymers)
3	1640-1650	1600-1650	C=O stretching / C=C (Aromatic compounds)
4	1515-1560	1500-1600	C=C stretching (Aromatic ring)
5	1400-1450	1400-1450	C-H bending
6	1260-1320	1250-1350	C-O stretching (Phenols, Esters)
7	1150-1210	1150-1250	C-O stretching (Alcohols, Ethers)
8	1020-1080	1000-1100	C-O stretching (Primary alcohols, polysaccharides)
9	700-850	700-900	C-H bending (Aromatic rings)

The FTIR spectrum of *Moringa oleifera* root extract with excipients showed characteristic peaks corresponding to hydroxyl, carbonyl, aromatic, and ether functional groups. All observed frequencies were found within the standard reported ranges. The peaks of the extract were retained in the formulation without significant shifting or disappearance, indicating absence of chemical interaction between the extract and excipients. This

confirms the compatibility and stability of the formulation components.

3.4 EVALUATION OF HYDROGEL

1. Physical Appearance

All formulated batches (F1-F6) were visually inspected for color, homogeneity, consistency, and presence of any lumps or phase separation.

Table 7: Physical Appearance

Batch	Colour	Homogeneity	Consistency
F1	Light brown	Good	Smooth
F2	Light brown	Good	Smooth
F3	Brown	Good	Smooth
F4	Brown	Excellent	Smooth
F5	Dark brown	Good	Slightly thick
F6	Dark brown	Good	Thick

2. Determination of pH

The pH of all formulated Carbopol-based hydrogels (F1–F6) incorporating Moringa oleifera root extract was determined using a calibrated digital pH meter. Approximately 1 g of gel from each batch was dispersed in distilled water and measured at room temperature.

Table 8: Determination of pH

Batch	pH Value
F1	5.6
F2	5.8
F3	6.0
F4	6.2
F5	6.4
F6	6.5

3. Extrudability

Extrudability was determined by measuring the force required to extrude the gel from a collapsible tube. All formulations exhibited good extrudability. Batch F4 showed smooth and uniform extrusion.

Table 10: Extrudability

Batch	Extrudability
F1	Good
F2	Good
F3	Very Good
F4	Excellent
F5	Good
F6	Moderate

4. Spreadability

Spreadability was determined using glass slide method by measuring the time required to spread the gel. All batches showed good spreadability. Batch F4 exhibited optimal spreading with good consistency.

Table 11: Spreadability

Batch	Spreadability (g·cm/sec)
F1	18.5
F2	16.2
F3	14.0
F4	12.3
F5	10.8
F6	9.2

5. Washability

Washability was evaluated by applying gel on skin and washing with water. All formulations were easily washable, indicating good patient compliance.

Table 12: Washability

Batch	Washability
F1	Good
F2	Good
F3	Very Good
F4	Excellent
F5	Good
F6	Moderate

6. Viscosity Measurement

Viscosity of the gel formulations was measured using a Brookfield viscometer at suitable rpm. All batches showed appropriate viscosity, with variation depending on polymer concentration. Batch F4 showed optimum viscosity.

Table 13: Viscosity Measurement

Batch	Viscosity (cP)
F1	4500
F2	7800
F3	11500
F4	15800
F5	19500
F6	23000

7. In-vitro Diffusion Study



Figure 9: In-vitro Diffusion Study

The in-vitro diffusion study of the herbal gel was carried out using a diffusion cell with a suitable membrane. The gel was placed in the donor compartment, and the receptor compartment was filled with an appropriate diffusion medium maintained at constant temperature. Samples were withdrawn at predetermined time intervals and analyzed spectrophotometrically to determine the amount of drug diffused. Diffusion study was carried out using Franz diffusion cell with suitable membrane. All formulations showed controlled drug release. Batch F4 showed optimum diffusion profile.

Table 14: In-vitro Diffusion Study

Time	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)
15 min	8	10	12	14	12	10
30 min	15	18	22	25	22	18
1 hr	28	32	36	40	35	30
2 hr	45	50	55	60	52	46
3 hr	60	66	72	78	68	60
4 hr	72	78	84	88	76	68
5 hr	82	88	92	95	82	74
6 hr	90	94	96	98	88	80

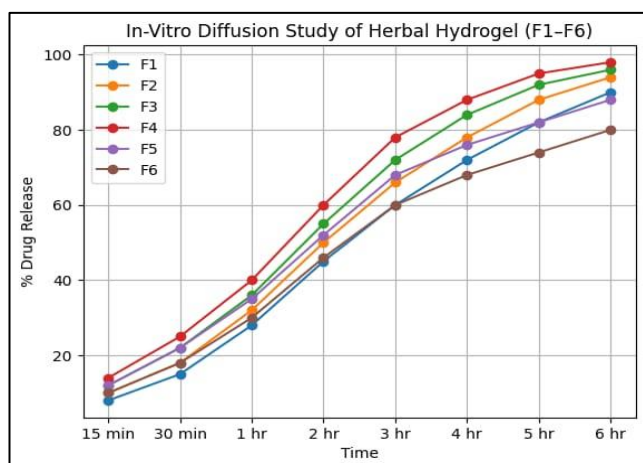


Figure 10: Graph of F1-F6 In-vitro Diffusion Study

8. In vitro Release Kinetics

The results obtained from the in vitro diffusion study of *Moringa oleifera* root extract from Carbopol-based hydrogel formulations (F1-F6) were fitted into various kinetic models to describe the drug release behavior.

The release data were analyzed using different kinetic models, namely Zero-order, First-order, Higuchi, and Korsmeyer-Peppas models.

Various kinetic parameters such as the release rate constant (k), regression coefficient (R^2), and diffusional exponent (n) were calculated for each model. The model showing the highest R^2 value was considered as the best-fit model, indicating the mechanism governing the release of the active constituents from the hydrogel system.

I. Zero-order Kinetics

Table 15: Data of Zero-order Kinetics of F4 batch

Time (hr)	Cumulative % Drug Release
0.25	14
0.50	25
1.00	40
2.00	60
3.00	78
4.00	88
5.00	95
6.00	98

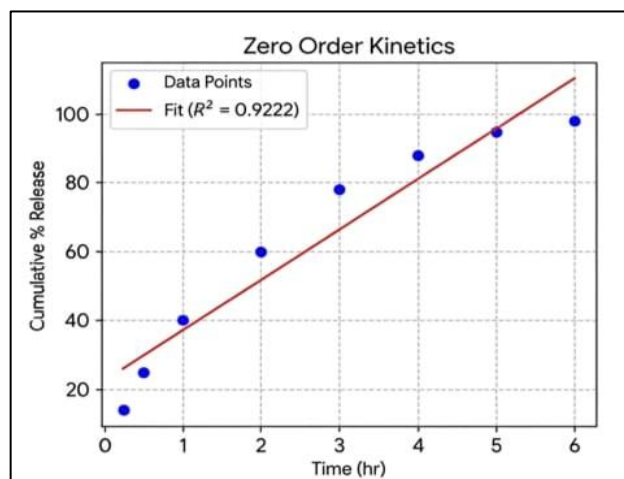


Figure 11: Zero-order Release Kinetics

II. First Order Kinetics

Table 16: Data of First order Kinetics of F4 batch

Time (hr)	Log (% Drug Remaining)
0.25	1.9345
0.50	1.8751
1.00	1.7782
2.00	1.6021
3.00	1.3424
4.00	1.0792
5.00	0.6990
6.00	0.3010

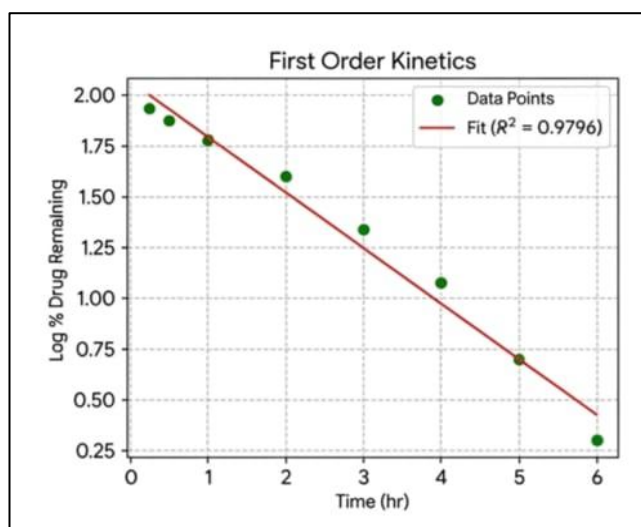


Fig.12 first Order Release Kinetics

III. Higuchi Model Kinetics

Table 17: Data of Higuchi Model Kinetics of F4 batch

Square Root of Time (hr)	Cumulative % Drug Release
0.500	14
0.707	25
1.000	40
1.414	60
1.732	78
2.000	88
2.236	95
2.449	98

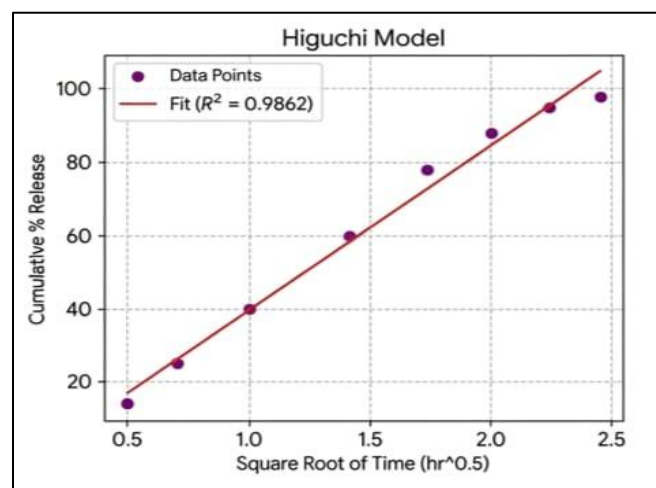


Figure 13: Higuchi Diffusion Kinetic

IV. Korsmeyer-Peppas Model of Kinetics

Table 18: Data of Korsmeyer-Peppas Model of Kinetics of F4 batch

Log Time (loghr)	Log % Drug Release
-0.6021	1.1461
-0.3010	1.3979
0.0000	1.6021
0.3010	1.7782
0.4771	1.8921
0.6021	1.9445
0.6990	1.9777
0.7782	1.9912

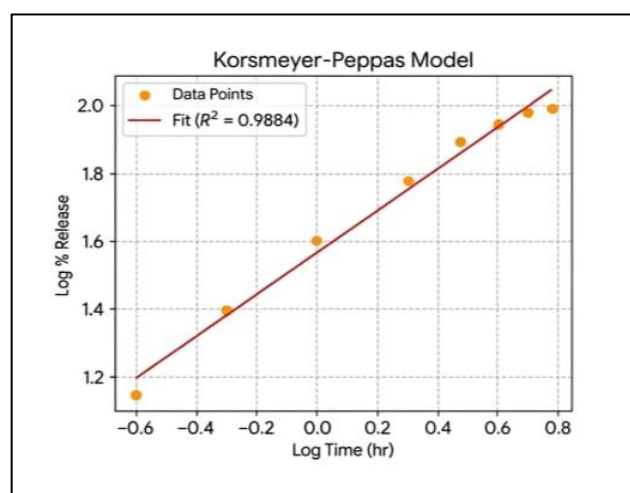


Figure 14: Peppas Release Kinetics

Summary of Kinetic Parameters for Batch F4

Table 19: Summary of Kinetic Parameters for Batch F4

Kinetic Model	Regression Equation	R2 Value
Zero Order	$y = 14.639x + 22.452$	0.9222
First Order	$y = -0.2735x + 2.0700$	0.9796
Higuchi	$y = 45.054x - 5.5508$	0.9862
Korsmeyer-Peppas	$y = 0.6146x + 1.5661$	0.9864

Batch F4 showed the best fit with the Higuchi and Korsmeyer–Peppas models, indicating that drug release is primarily governed by diffusion mechanisms. The Peppas exponent ($n = 0.6146$) lies between 0.5 and 1, which confirms non-Fickian (anomalous) transport. This means the drug release occurs through a combined mechanism of diffusion and polymer swelling/relaxation, rather than purely diffusion-controlled release.

Regression Analysis (R^2) and Release Exponent (n) for All BatchesTable 20: Regression Analysis (R^2) and Release Exponent (n) for All Batches

Batch	Zero Order (R^2)	First Order (R^2)	Higuchi (R^2)	Peppas (R^2)	n value
F1	0.9720	0.9837	0.9993	0.9938	0.7579
F2	0.9613	0.9794	0.9982	0.9933	0.7061
F3	0.9425	0.9849	0.9934	0.9900	0.6543
F4	0.9222	0.9796	0.9862	0.9864	0.6146
F5	0.9333	0.9981	0.9912	0.9869	0.6185
F6	0.9422	0.9964	0.9943	0.9895	0.6502

Best Fit Model and Release Mechanism

Table 21: Best Fit Model and Release Mechanism

Batch	Best Fit Model	Release Mechanism (n value range)
F1	Higuchi	Non-Fickian (Anomalous) Transport
F2	Higuchi	Non-Fickian (Anomalous) Transport
F3	Higuchi	Non-Fickian (Anomalous) Transport
F4	Korsmeyer-Peppas	Non-Fickian (Anomalous) Transport
F5	First Order	Non-Fickian (Anomalous) Transport
F6	First Order	Non-Fickian (Anomalous) Transport

1. Release Mechanism: For all batches (F1–F6), the n value falls between **0.45 and 0.89** ($0.61 < n < 0.76$). This indicates that the drug release from the herbal hydrogel follows **Non-Fickian (Anomalous) transport**, meaning release is controlled by both diffusion and polymer swelling/relaxation.

2. Performance: Batches **F1, F2, and F3** are most accurately described by the **Higuchi model**, suggesting diffusion is the dominant factor.

3. F4 Batch: Specifically, batch F4 shows a very high correlation with the **Korsmeyer-Peppas model**, which accounts for the complex matrix interactions in the hydrogel.

4. F5 and F6: These batches show a strong shift toward **First Order** kinetics, indicating the release rate is more dependent on the remaining drug concentration.

Conclusion

Batch **F4** was identified as the optimized formulation because it exhibited the highest cumulative drug release (**98% in 6 hours**) and followed the **Korsmeyer-Peppas model** with an R^2 of **0.9864**. The release exponent ($n = 0.6146$) indicates a balanced **Anomalous Transport** mechanism. Unlike other batches that showed concentration-dependent decay (F5, F6) or lower

total release (F1, F2), F4 provided a near-complete and controlled diffusion profile, making it the most effective candidate for sustained herbal delivery.

9. In-vitro Antimicrobial Study

RESULTS:

Antibacterial activity of test compound against *E. coli*

Table 22: Antibacterial activity of test compound against *E. coli*

SR.NO	SAMPLES	ZONE IN DIAMETER (mm)
1	Control	00
2	Standard (Streptomycin)	28
3	Moringa root extract herbal gel	24

Image Activity:



Figure 15: Antimicrobial Test

Conclusion of the study:

The antibacterial profile of **Moringa root extract herbal gel** was evaluated by measuring the zone of inhibition against *E. coli* (ATCC 25922) bacterial strains via well diffusion method. The compound **Moringa root extract herbal gel** exhibited good antibacterial activity as compared to the standard streptomycin.

10. Anti-inflammatory Study

In vitro anti-inflammatory activity by Protein denaturation method

The reaction mixture (10 mL) consisted of 0.4 mL of egg albumin (from fresh hen's egg), 5.6 mL of phosphate buffered saline (PBS, pH 6.4) and 100 µL of different concentrations of test sample (20, 40, 60, 80, 100 µg/mL). Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37°C±2) in a incubator for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at the concentration was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following equation,

$$\% \text{ of Inhibition} = \frac{C - T}{C} \times 100$$

Where,

T = absorbance of test sample

C = absorbance of control

Table 23: In vitro anti-inflammatory activity

SR.NO	Sample code	Concentrations ($\mu\text{g/ml}$)	Protein denaturation assay					IC ₅₀ ($\mu\text{g/ml}$)
			Absorbance at 660 nm				% of Inhibition	
			Test 1	Test 2	Test 3	Mean		
1	Control		1.54	1.54	1.54	1.54	-	72.21
2	Standard (Diclofenac Sodium)	20	1.40	1.37	1.39	1.39	9.74%	
		40	1.20	1.20	1.22	1.21	21.43%	
		60	0.91	0.89	0.93	0.91	40.91%	
		80	0.67	0.65	0.63	0.65	57.79%	
		100	0.21	0.18	0.23	0.21	86.36%	
3	Moringa root extract herbal gel	20	1.45	1.48	1.51	1.48	3.90%	98.70
		40	1.33	1.36	1.39	1.36	11.69%	
		60	1.12	1.17	1.19	1.16	24.68%	
		80	0.95	0.93	0.97	0.95	38.31%	
		100	0.72	0.75	0.79	0.75	51.30%	

Graphical data:

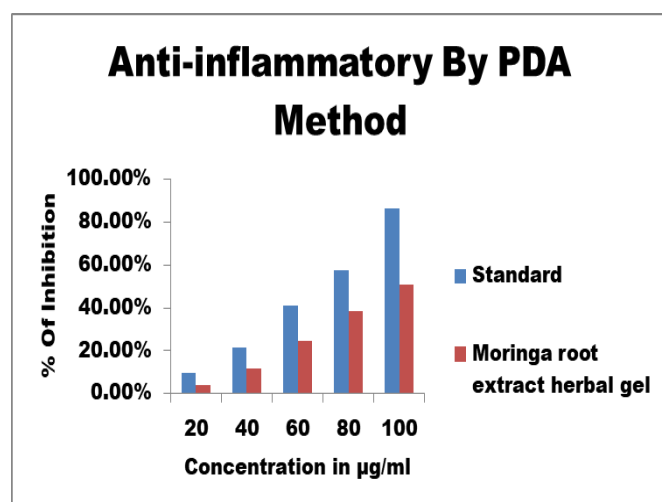


Figure 16: Anti-inflammatory Study

Images of the activity:





Fig. 17 In vitro anti-inflammatory activity by Protein denaturation method

Conclusion

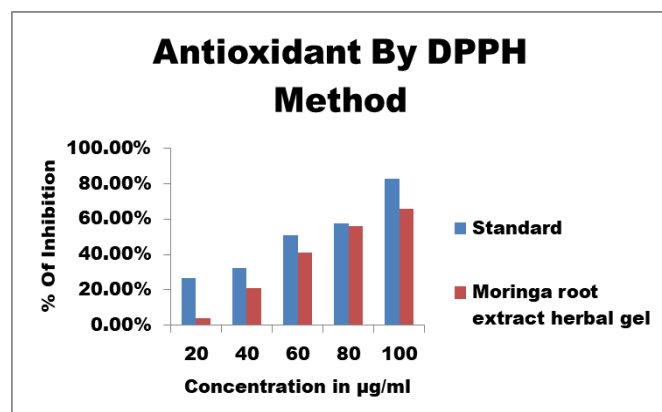
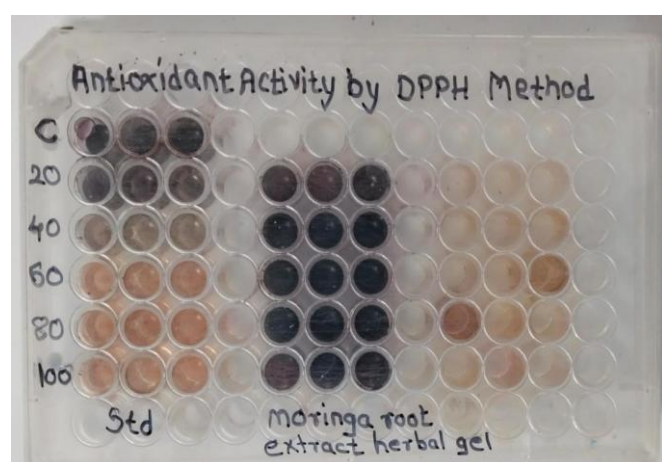
The anti-inflammatory activity assessed by the protein denaturation assay (PDA method) shows a clear concentration-dependent increase in percentage inhibition for both the standard and the Moringa root extract herbal gel. As the concentration increases from 20 to 100 $\mu\text{g/mL}$, the standard exhibits a strong rise in inhibition, reaching a maximum of around 85–90%, indicating potent anti-inflammatory activity. The Moringa root extract gel also demonstrates a gradual increase in inhibition, attaining approximately 50% at the highest concentration. Although its activity is lower compared to the standard, the consistent dose-dependent response suggests that the herbal gel possesses moderate anti-inflammatory potential. These findings indicate that Moringa root extract may serve as a promising natural anti-inflammatory agent, albeit with comparatively reduced potency.

11. Antioxidant Study

Observation table:

Table 24: Antioxidant Study activity

SR.NO	Sample Code	Concentration ($\mu\text{g/ml}$)	Absorbance at 510nm				% Inhibition	IC50 ($\mu\text{g/ml}$)
			Test 1	Test 2	Test 3	Mean		
1	Control	-	1.93	1.93	1.93	1.93	-	
2	Standard	20	1.45	1.42	1.40	1.42	26.42%	72.49
	(Ascorbic Acid)	40	1.31	1.29	1.33	1.31	32.12%	
		60	0.95	0.97	0.93	0.95	50.77%	
		80	0.82	0.85	0.79	0.82	57.51%	
		100	0.35	0.32	0.32	0.33	82.90%	
3	Moringa root extract herbal gel	20	1.81	1.88	1.85	1.85	4.15%	74.04
		40	1.49	1.52	1.57	1.53	20.73%	
		60	1.16	1.12	1.15	1.14	40.93%	
		80	0.88	0.82	0.86	0.85	55.96%	
		100	0.65	0.63	0.69	0.66	65.80%	

Graphical data:**Figure 18: Antioxidant Study****Images of the activity:****Figure 19: Antioxidant Activity by using DPPH****Conclusion of the study:**

The DPPH antioxidant assay results indicate that both the standard and the *Moringa root extract herbal gel* exhibit a concentration-dependent increase in free radical scavenging activity. As the concentration rises from 20 to 100 µg/ml, the percentage inhibition gradually increases, reflecting improved antioxidant potential. The standard shows higher activity at all concentrations, reaching maximum inhibition of around 80–85% at 100 µg/ml, whereas the herbal gel also demonstrates notable activity, achieving approximately 65–70% inhibition at the same concentration. Although slightly lower than the standard, the *Moringa root extract herbal gel* displays significant antioxidant activity, suggesting the presence of effective phytoconstituents and indicating its potential as a natural antioxidant agent.

4. DISCUSSION

Moringa oleifera Lam., belonging to the family Moringaceae, was selected based on its well-documented traditional use and pharmacological properties. The plant roots were collected, authenticated, washed, shade-dried, and powdered. The extract was prepared using the maceration method and further concentrated for experimental use.

Preliminary phytochemical screening confirmed the presence of important bioactive constituents such as alkaloids, flavonoids, tannins, phenolic compounds, and saponins, which are known to contribute to wound healing through antimicrobial, antioxidant, and anti-inflammatory mechanisms.

Preformulation studies indicated that the extract possessed acceptable physicochemical properties, including characteristic colour, odor, and bitter taste. It showed good solubility in methanol and ethanol, and the pH was found to be near neutral, making it suitable for topical application. The λ_{max} was determined at 272 nm, and FTIR analysis confirmed the presence of functional groups corresponding to various phytoconstituents. Compatibility studies revealed no significant interaction between the extract and excipients.

A series of Carbopol-based hydrogel formulations (F1–F6) were prepared and evaluated for physicochemical parameters such as pH, viscosity, spreadability, extrudability, washability, and homogeneity. All formulations showed acceptable properties for topical use.

Among all batches, formulation F4 was identified as the optimized formulation due to its excellent physicochemical characteristics, including ideal pH (6.2), good spreadability, optimum viscosity, and superior extrudability.

In-vitro diffusion studies demonstrated controlled drug release from all formulations, with F4 showing maximum drug release (98% within 6 hours). Kinetic modeling indicated that drug release followed Higuchi and Korsmeyer–Peppas models, confirming a non-Fickian (anomalous) diffusion mechanism.

The antimicrobial study revealed that the formulated hydrogel exhibited significant antibacterial activity against *E. coli*, showing a considerable zone of inhibition when compared to the standard drug streptomycin.

The anti-inflammatory activity evaluated using the protein denaturation method showed a concentration-dependent increase in inhibition. Although less potent than diclofenac sodium, the formulation demonstrated moderate anti-inflammatory activity.

The antioxidant activity assessed by the DPPH method showed that the hydrogel possesses significant free radical scavenging activity, with inhibition increasing proportionally with concentration, indicating its potential to reduce oxidative stress in diabetic wounds.

5. CONCLUSION

The present study successfully formulated and evaluated a Carbopol-based hydrogel incorporating *Moringa oleifera* root extract for the management of diabetic wounds.

The findings confirm that the herbal extract contains bioactive phytoconstituents responsible for antimicrobial, antioxidant, and anti-inflammatory

activities, all of which are essential for effective wound healing, especially in diabetic conditions.

The developed hydrogel formulations exhibited satisfactory physicochemical properties suitable for topical application. Among them, batch F4 was identified as the optimized formulation due to its superior performance in terms of drug release, stability, and overall evaluation parameters.

The formulation demonstrated:

- Significant **antimicrobial activity**, which helps prevent infection in wounds
- Notable **antioxidant activity**, aiding in reducing oxidative stress
- Moderate **anti-inflammatory activity**, supporting the healing process

The drug release followed a **non-Fickian diffusion mechanism**, ensuring controlled and sustained release of active constituents, which is beneficial for prolonged therapeutic action in chronic wounds such as diabetic ulcers.

Overall, the study suggests that the developed Carbopol-based herbal hydrogel is a promising, safe, and effective topical drug delivery system for diabetic wound management.

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