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Development and Characterization of Hydrogel Based Formulation of *Holarrhena antidysenterica* for the Antimicrobial Activity

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ABSTRACT

Hydrogels are promising carriers for topical delivery of herbal extracts due to their biocompatibility, ability to provide controlled drug release, and potential to enhance local therapeutic efficacy. The present study aimed to develop and characterize hydrogel formulations of *Holarrhena antidysenterica* bark extract for antimicrobial applications. The extract was prepared using Soxhlet extraction with ethanol, yielding a dark brown, greasy, and water- and alcohol-soluble extract with a percentage yield of 5.12%. Twelve hydrogel formulations were developed using HPMC K-100M and Carbopol 934 at different concentrations, and evaluated for physicochemical properties including appearance, pH, spreadability, viscosity, extrudability, in-vitro drug release, and stability. The hydrogels exhibited transparent, yellowish-green appearances, suitable pH (6.05–6.73), good spreadability (14.23–21.76 g·cm/s), and pseudoplastic rheological behavior. Extrudability studies confirmed convenient topical application, while in-vitro drug release demonstrated sustained diffusion of phytoconstituents, with HPMC-based formulations showing superior release profiles. Stability studies indicated minimal variations in pH, viscosity, and spreadability over three months. Antimicrobial evaluation against Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans revealed significant inhibitory effects, with selected formulations (HF2 and CF2) exhibiting the highest activity. The results suggest that *H. antidysenterica* hydrogel formulations are stable, effective, and suitable for topical delivery, offering a promising plant-based alternative for managing microbial infections.

Keywords: Hydrogels, *Holarrhena antidysenterica*, Antimicrobial activity

INTRODUCTION

Hydrogels are three-dimensional, hydrophilic polymeric networks capable of absorbing and retaining large amounts of water while maintaining structural integrity. Their biocompatibility, ability to provide controlled drug release, and suitability for topical administration make them ideal carriers for delivering herbal extracts with therapeutic potential. [1, 2] In recent years, herbal hydrogels have gained significant attention as an effective platform for enhancing the stability, bioavailability, and local action of plant-derived bioactives. [3] Such formulations allow sustained diffusion of antimicrobial compounds at the site of infection, improving therapeutic efficacy and minimizing systemic side effects. As microbial resistance to conventional antibiotics continues to rise, the development of novel plant-based antimicrobial formulations is becoming increasingly important. [4, 5]

Holarrhena antidysenterica (Family: Apocynaceae), commonly known as Kutaja, Kurchi, or Indrajav, is a well-established medicinal plant in Ayurvedic, Unani, and traditional systems of medicine. The plant is widely known for its potent antimicrobial, antidiarrheal, anti-inflammatory, and antiprotozoal properties. [6, 7] Its bark and seeds contain several pharmacologically active constituents, including conessine, holarrhenine, holarrhimine, and various alkaloids, which have demonstrated strong activity against bacterial, fungal, and protozoal pathogens. [8] Despite its rich medicinal value, the therapeutic potential of H. antidysenterica is limited by issues such as low aqueous solubility and instability in conventional dosage forms. Formulating the extract into a hydrogel system offers a promising strategy to enhance its delivery, prolong its antimicrobial action, and improve patient compliance. [9]

Given the increasing demand for natural, safe, and effective antimicrobial agents, there is a strong need to develop novel phytopharmaceutical formulations that enhance the functional efficacy of medicinal plant extracts. The present study focuses on the development and characterization of a hydrogel-based formulation of *Holarrhena antidysenterica* to evaluate its physicochemical properties, stability, and antimicrobial activity. By integrating the plant extract into a hydrogel matrix, this research aims to improve

its therapeutic performance against pathogenic microorganisms and establish a scientifically validated platform for future herbal antimicrobial formulations.

MATERIALS AND METHODS

Materials and Chemicals: The bark of *H. antidysenterica* was collected from side of Local region of Bareilly, Uttar, Pradesh India. The polymers and chemicals required for the formulation were procured from reputed suppliers. HPMC K100M, triethanolamine, and sodium hydroxide were obtained from Loba Chem. Pvt. Ltd., Mumbai, while Carbopol 934, methanol, ethanol, chloroform, and buffer solutions were supplied by E. Merck (India) Ltd., Mumbai. Hydrochloric acid, sulfuric acid, and propylene glycol were purchased from Bodal Chemicals Limited, Vadodara. Distilled water used throughout the study was prepared within the college campus. All chemicals and reagents were of analytical grade and were used without further purification.

Extraction and preliminary phytochemical study: The barks used in this study were carefully chosen, cleaned of contaminants, and allowed to dry in the shade. In the mechanical grinder, the dry material was ground into a fine powder. After passing through filter number 43, the fine powder was put away for later use in an airtight container. Using a Soxhlet apparatus and ethanol as a solvent, about 100 grammes of powdered material were extracted using a hot extraction process. After the extraction process was finished and the solvent in the thimble turned clear, a few drops of the solvent were collected in the test tube and the solvent was subjected to a chemical test. The extract was dried in a rotating vacuum evaporator after each extraction. Additionally, a portion of the extract was saved for first phytochemical screening in order to identify different plant components, while the remainder was utilised to create gel batches. [10] A qualitative chemical analysis was performed on the alcoholic extract. To check for the presence of different phytochemical elements in the extract, the following methods were used. Steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins, saponins, and glycosides are the most significant of these bioactive plant components. [11]

Formulation And Development: Two gelling agents were utilised in the formulation process at two different concentrations, producing six distinct batches of bark extract gels—a total of twelve batches were made. The gelling agents HPMC K100M and Carbopol 934 were used in this instance. The following is how the gelling agents were used:

- HPMC K 100 M (at concentration 1% 1.5%, and 2%)
- Carbopol 934 (at concentration 1% 1.5%, and 2%)

After several trials and errors, the final gel composition was determined. And this is a description of the finished composition. A total of twelve batches of gel formulations were produced using the same experimental strategy for the various extract types. Every batch was made in compliance with the experimental plan. [12, 13]

Preparation of gel with HPMC K-100 M: One gramme of extract, precisely measured, was put into a beaker and dissolved in ten millilitres of propylene glycol before preservatives were added. After dispersing in distilled water, HPMC K 100M was heated to 80–90 °C while being constantly stirred, and it was then allowed to cool. After adding 1 and 2% w/v extract-loaded propylene glycol solution to the HPMC K-100M preparation and violently stirring to mix in a cold environment, water was added to bring the volume up to 100 ml and mechanically agitated well to create a homogenous gel.

Preparation of gel with Carbopol 934: 50 millilitres of distilled water were used to dissolve precisely weighed Carbopol 934 in a beaker. Set aside the beaker to let the carbopol to swell for 30 minutes. After that, stir with a mechanical or lab stirrer for 30 minutes at 1200 rpm. Take the necessary amount of extract and 5 millilitres of propylene glycol. Put 5 millilitres of propylene glycol in a different beaker, then weigh out the propyl and methyl parabens and mix well. With continuous stirring, 1% and 2% extract and preservative solutions were added once all of the carbopol had been distributed. Finally, volume made upto 100 ml by adding remaining distilled water and Triethanolamine was added drop wise to the formulations for adjustment of required skin pH (6.8-7) and to obtain the gel at required consistency.

Table 1. Herbal gel formulation (HPMC-K100M)

Ingredients	Quantity taken					
	HF1 (1%)	HF2 (1%)	HF3 (1%)	HF4 (2%)	HF5 (2%)	HF6 (2%)
Extract (gm)	1.0	1.0	1.0	2.0	2.0	2.0
HPMC-K100M (gm)	1	1.5	2	1	1.5	2
Propylene glycol (gm)	10	10	10	10	10	10
Glycerin (ml)	1.0	1.0	1.0	1.0	1.0	1.0
Methyl paraben (ml)	0.2	0.2	0.2	0.2	0.2	0.2

Triethanolamine	qs	qs	qs	qs	qs	qs
Propyl paraben (ml)	0.1	0.1	0.1	0.1	0.1	0.1
Water	qs	qs	qs	qs	qs	qs

Table 2. Herbal gel formulation (Carbopol® 934)

Ingredients	Quantity taken					
	CF1 (1%)	CF2 (1%)	CF3 (1%)	CF4 (2%)	CF5 (2%)	CF6 (2%)
Extract (gm)	1.0	1.0	1.0	2.0	2.0	2.0
Carbopol® 934 (gm)	1.0	1.5	2.0	1.0	1.5	2.0
Propylene glycol (gm)	10	10	10	10	10	10
Glycerin (ml)	1.0	1.0	1.0	1.0	1.0	1.0
Methyl paraben (ml)	0.2	0.2	0.2	0.2	0.2	0.2
Triethanolamine	qs	qs	qs	qs	qs	qs
Propyl paraben (ml)	0.1	0.1	0.1	0.1	0.1	0.1
Water	qs	qs	qs	qs	qs	qs

Physicochemical Evaluations: The physicochemical evaluation of the hydrogel formulations prepared using *Holarrhena antidysenterica* extract involved assessment of various parameters including physical appearance, pH, spreadability, homogeneity, viscosity, extrudability, drug diffusion, and stability. The physical appearance—such as colour, consistency, homogeneity, and phase separation—was examined visually to ensure uniformity. The pH of each formulation was determined using a digital pH meter after dispersing 1 g of gel in 100 mL of distilled water and allowing it to stand for two hours, with each sample measured in triplicate. Spreadability was assessed using a wooden block–pulley apparatus based on slip and drag characteristics. Approximately 2 g of gel was placed between two glass slides, compressed with a 1 kg weight for five minutes, and the time required for the upper slide to move 7.5 cm under an 80 g load was recorded. Spreadability was then calculated using the formula $S = M \times L/T$, where shorter times indicated better spreadability. Rheological behavior, which influences stability and drug release, was examined using a Brookfield viscometer at controlled spindle speeds (10–50 rpm) and room temperature (25 ± 1°C), ensuring accuracy through multiple readings. [14, 15]

Extrudability was measured by filling formulations into collapsible aluminum tubes, clamping them between glass plates, and applying a 500 g weight to determine the percentage of gel extruded, with >90% rated excellent, >80% good, and >70% fair. In-vitro drug diffusion was performed using a Franz diffusion cell with 0.5 g of hydrogel placed on a cellulose nitrate membrane and phosphate buffer (pH 6.8) as the diffusion medium at 37 ± 1°C. Samples were withdrawn at predetermined intervals up to 24 hours and analyzed using UV spectrophotometry to determine drug release profiles. Stability studies were conducted following ICH Q1A(R2) guidelines to evaluate the formulation's performance under accelerated and real-time storage conditions. [16] The hydrogels were stored at 4°C ± 2°C and 25°C ± 2°C/60% ± 5% RH for three months, during which changes in appearance, viscosity, and spreadability were monitored. These evaluations ensured that the hydrogel formulations maintained their integrity, performance, and antimicrobial potential throughout storage.

Antimicrobial Activity: The antimicrobial evaluation of the hydrogel formulation required the use of clinical strains of *Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Candida albicans*, all obtained as pure isolate cultures. Additional materials, including Mueller-Hinton broth (single and double strength), 0.5 McFarland standard, diethyl ether, ethanol, and distilled water, were procured from the Department of Pharmaceutics, BIU College of Pharmacy, Bareilly International University, Bareilly. Preparation of the culture media involved dissolving 2.1 g of Mueller-Hinton broth in 100 mL of sterile water, dispensing it into 10 mL test tubes, and autoclaving at 121°C for 30 minutes to obtain single-strength broth. Double-strength broth was prepared similarly by dissolving 4.2 g of the medium in 100 mL of sterile water and sterilizing it under the same conditions. These broths served as essential growth media for bacterial and fungal propagation during antimicrobial testing. [17]

Microorganisms were subcultured by aseptically transferring 1 mL of each pure isolate into sterile Mueller-Hinton broth under a laminar flow cabinet, with the rim of each test tube flamed before and after inoculation to ensure sterility. The cultures were then incubated at 37°C for 24 hours to obtain actively growing cells. For preparation of pure isolates, sterile Mueller-Hinton agar was autoclaved, poured into petri dishes, cooled, and streaked using a sterile inoculating loop with the subcultured microorganisms, followed by incubation for 24 hours. Estimation of the minimum inhibitory concentration (MIC) of the hydrogel was carried out by preparing and sterilizing agar plates in the same manner; the subcultured microorganisms were streaked onto the agar surface to allow microbial growth, after which the hydrogel samples were introduced to determine the lowest concentration capable of inhibiting visible microbial growth.[18]

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RESULTS AND DISCUSSION

The extract was prepared from the stem bark of *Holarrhena antidysenterica* using the Soxhlet extraction method with ethanol as the solvent. The resulting extract appeared dark brown in colour with a greasy texture and an indistinct odour. Solubility analysis showed that the extract was soluble in both water and alcohol but insoluble in acidic solutions. The extraction process yielded a practical output of 5.12 g, corresponding to a percentage yield of 5.12%. These characteristics indicate the successful extraction of phytoconstituents into the ethanolic medium.

Preliminary Phytochemical Study: Following the findings of the phytochemical screening, it was determined if the alkaloids were present because there was no sign of turbidity or precipitate development. In several samples, the lack of steroids was indicated by the hue not changing from violet to blue or green. In the absence of terpenoids, a reddish brown interface did not emerge, which is a good outcome. Flavonoids are shown by red colouration (Shinado's test). Two observations below showed that saponins were present. The test was validated by the formation of stable foam. The test was validated by the development of a soluble emulsion. The test was validated by the acetic acid layer becoming blue. The test was validated by the formation of the red colour. The two observations mentioned suggested that glycosides were present.

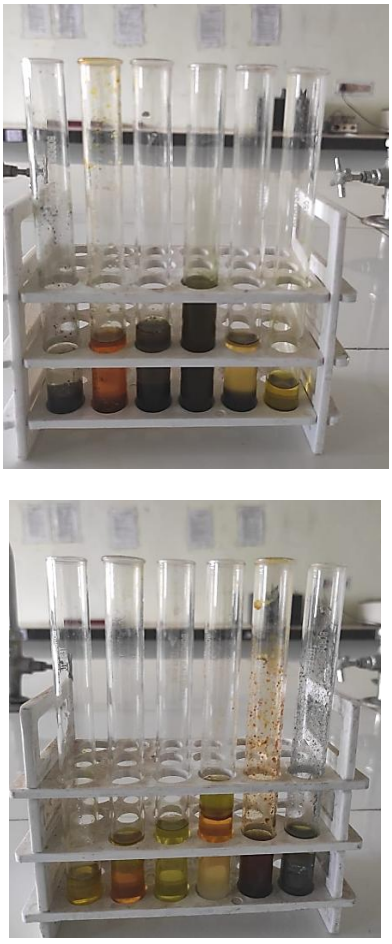

Figure 1: Preliminary phytochemical investigation of extract

Formulation And Development: Twelve distinct batches of hydrogels for extract were produced as a consequence of the employment of two gelling agents at three different concentrations during formulation. The gelling agents HPMC K 100 M and Carbopol 934 were used in this instance. The following two gelling agents were used: a. HPMC K-100M (at 1% 1.5% and 2.0%) b. Carbopol 934 (at 1% 1.5% and 2.0%). Every batch was made in compliance with the experimental plan. The same experimental design was employed for the extract, yielding a total of 12 hydrogel batches, of which 6 batches had extract concentrations of 1% and 2%.

Characterization and Evaluation of Hydrogel: The prepared hydrogels were evaluated for their physicochemical properties, including appearance, pH, spreadability, and viscosity. Visual inspection revealed that the formulations possessed a transparent

appearance with a characteristic yellowish-green hue, indicating uniform dispersion of the extract within the gel matrix. The pH of the hydrogels ranged from 6.05 to 6.73, which falls within the acceptable range for topical application and minimizes the risk of skin irritation; no significant variation in pH was observed over the evaluation period. Spreadability measurements showed values between 14.23 and 21.76 g·cm/s, with formulations containing HPMC K-100M exhibiting superior spreadability compared to those prepared with Carbopol 934, suggesting better ease of application similar to commercial gels. Viscosity determination using a Brookfield viscometer indicated that the hydrogels had viscosities ranging from 2453.69 to 3321.65 cps. Furthermore, a decrease in viscosity with increasing shear rate was observed, confirming the pseudoplastic or shear-thinning behavior of the formulations, which is desirable for topical preparations as it enhances spreadability during application.

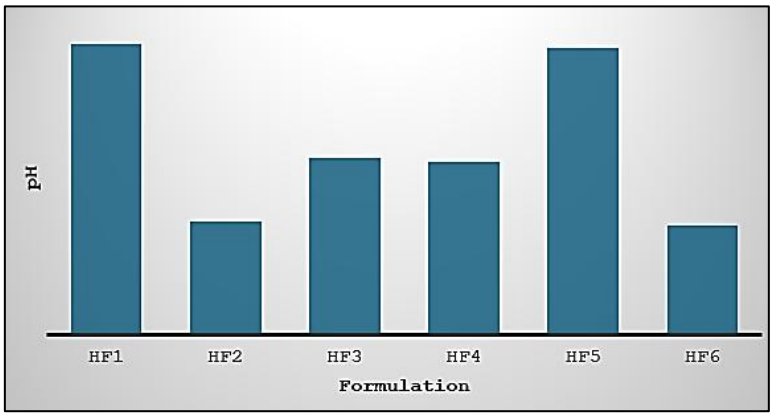

Figure 2: pH of formulated hydrogel (HF1-HF6)

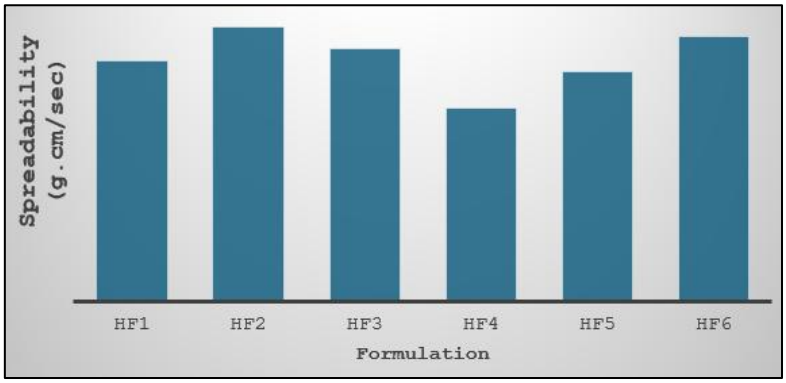

Figure 3: Spreadability of formulated hydrogel (HF1-HF6)

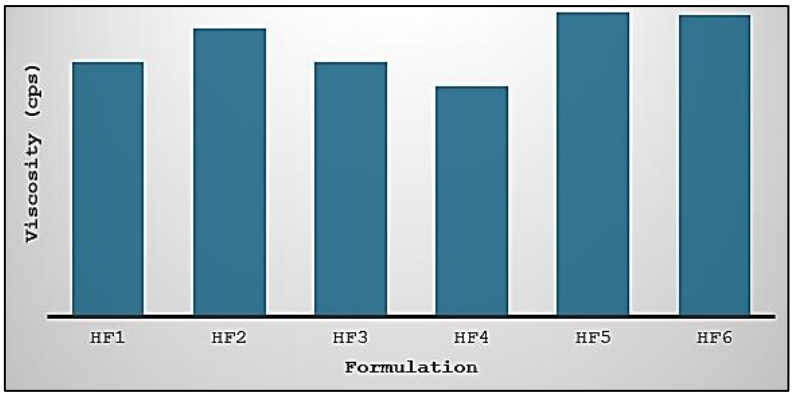

Figure 4: Viscosity of formulated hydrogel (HF1-HF6)

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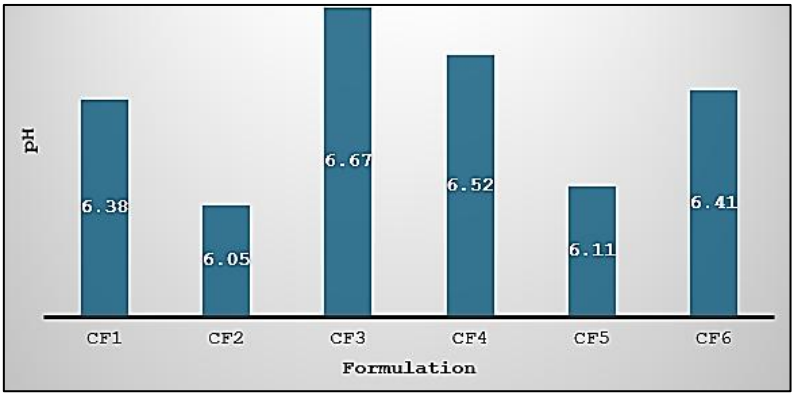

Figure 5: pH of formulated hydrogel (CF1-CF6)

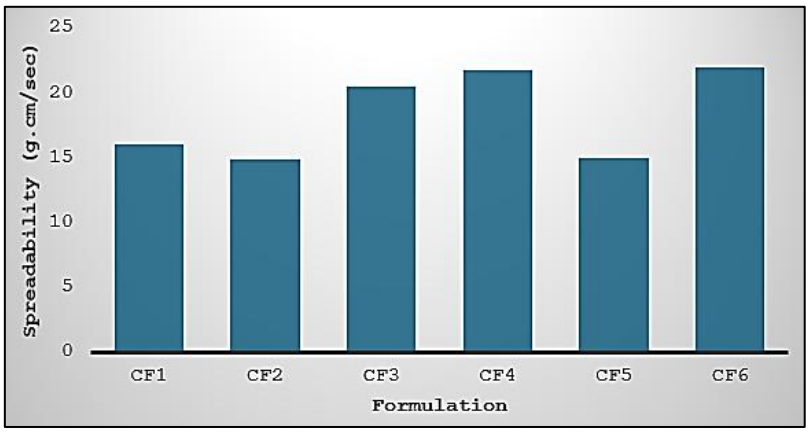

Figure 6: Spreadability of formulated hydrogel (CF1-CF6)

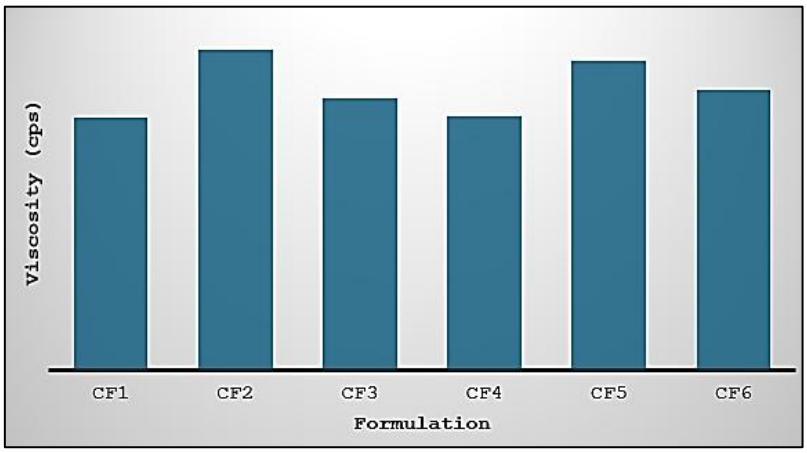

Figure 7: Viscosity of formulated hydrogel (CF1-CF6)

The extrudability of the hydrogel formulations was evaluated to determine their ease of removal from collapsible tubes, a parameter essential for patient compliance and effective topical application. Hydrogels with excessively low viscosity tend to flow too rapidly, whereas those with very high consistency may not extrude efficiently; therefore, an optimal consistency is required. The HPMC K-100M–based formulations (HF1–HF6) demonstrated high extrudability, with extrusion percentages ranging from 84.12% to 93.91%, all of which were graded as "Good." Specifically, HF6 exhibited the highest extrudability at 93.91%, followed closely by HF4 and HF5 with values of 91.17% and 90.11%, respectively. Similarly, Carbopol 934–based formulations (CF1–CF6) also showed good extrudability, with values between 82.29% and 87.72%. Among these, CF1 displayed the highest extrusion percentage at 87.72%,

while CF5 exhibited the lowest at 82.29%, though still rated as "Good." Overall, both polymer types produced hydrogels with satisfactory extrusion properties, indicating their suitability for convenient and consistent topical application.

***In-vitro* drug release study:** The in-vitro drug release studies were carried out using a cell membrane diffusion system to evaluate the release characteristics of the hydrogel formulations containing *Holarrhena antidysenterica* extract. The cumulative drug release data for HPMC K-100M–based formulations (HF1–HF6) and Carbopol 934–based formulations (CF1–CF6). Among the HPMC formulations, HF2 demonstrated the highest cumulative drug release over an 8-hour period, reaching 90.24%, followed by HF3 (87.24%) and HF1 (84.24%), indicating that HPMC K-100M effectively supported sustained and enhanced drug diffusion. In contrast, Carbopol-based hydrogels exhibited comparatively slower release, with CF2 showing the greatest drug release at 89.25% after 8 hours, followed closely by CF3 (88.24%) and CF5 (85.24%). Overall, both polymer systems demonstrated controlled drug release profiles; however, HPMC K-100M formulations displayed faster and more efficient drug release compared to Carbopol 934, suggesting a superior diffusion-enhancing capability. The results confirm that polymer type significantly influences the drug release behavior of hydrogel formulations.

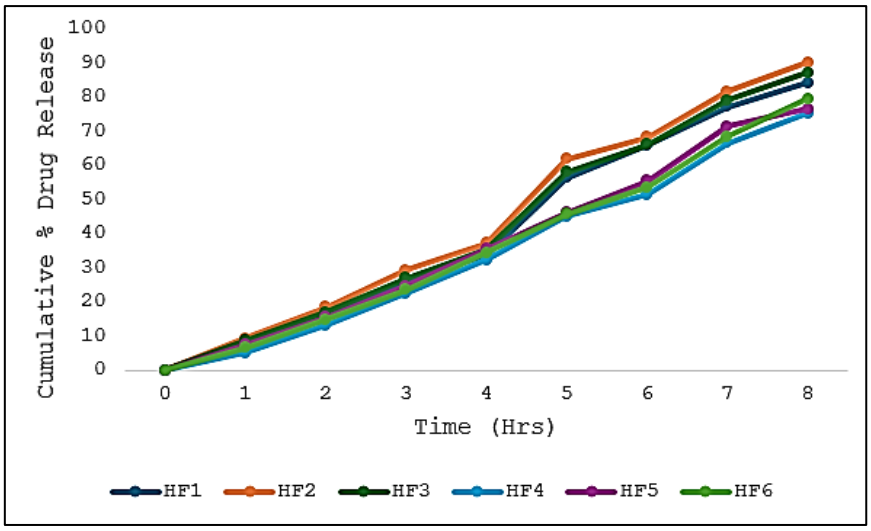

Figure 8: In-vitro drug release study of (HF1-HF6)

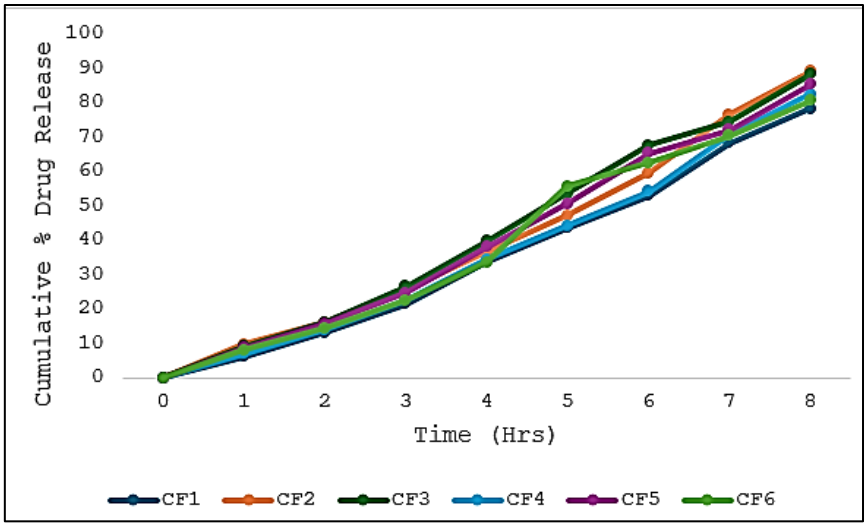

Figure 9: In-vitro drug release study of (CF1-CF6)

Stability Studies: Stability studies conducted on the selected hydrogel formulations stored at different temperatures showed minimal variations in pH, viscosity, and spreadability throughout the three-month evaluation period. For the HPMC-based formulation HF2, the pH exhibited a slight increase from 6.29 at one month to 6.55 at three months, while viscosity showed a gradual decrease from 3064.32 cps to 3011.56 cps. Similarly, spreadability decreased marginally from 20.11 to 18.34 g·cm/sec over

the same period. The Carbopol-based formulation CF2 demonstrated comparable trends, with pH increasing from 6.05 to 6.27, viscosity decreasing steadily from 3153.33 cps to 3011.56 cps, and spreadability reducing from 14.89 to 12.34 g·cm/sec across three months. These slight fluctuations remained within acceptable limits, indicating that both hydrogel formulations maintained their structural integrity and performance characteristics under the tested storage conditions. Overall, the results confirm that the hydrogels were physically and chemically stable throughout the stability study.

Microbiological Evaluation Formulated Hydrogels: To determine the dosage in the formula, the MIC of the extracts against the organisms under test must be known. The dosage, which was subsequently used to determine how much extract was used in the gel formulations, was created by increasing this MIC by 10. Results for the MIC are displayed in Tables 3 and 4. The microbroth dilution method was used to examine the plant extracts' antibacterial qualities. The study's conclusions demonstrated the plant extracts' ability to effectively combat the test microbes. At 6.25, 12.5, and 25 mg/mL, the various hydrogel formulations demonstrated inhibitory effects against Staphylococcus aureus and Staphylococcus epidermidis. Only formulations that contained Carbopol 934 (CF2) and HPMCK-100M (HF2) alone had the most antibacterial activity against Staph epidermidis.

Tables 3 and 4 further showed that, with regard to the test organisms, some formulations were bacteriostatic and others were bactericidal. The formulations' therapeutic potential in terms of antibacterial characteristics is revealed by the current investigation. The findings of the aforementioned studies indicate that the extracts significantly restrict the organisms' ability to develop. These formulations' effectiveness, as shown by MIC values, lends credence to their application in the prevention and management of bacterial infections brought on by a range of pathogenic bacteria that are resistant to antibiotics, most notably acne.

Table 3: Mean MICs (mg/mL) of the different gel formulation against test organisms

Gel formulations	*Staphylococcus epidermidis*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*
HF1	6.25±0.02	-	12.50±0 05	-	25.00±0 05
HF2	6.25±0.21	6.25±0.31	3.13±0.15	25.00±0 22	12.50±0.22
HF3	12.50±0.10	25.00±0.22	6.25±0.21	25.00±0.10	12.50±0.08
HF4	12.50±0.06	25.00±0.20	-	25.00±0.13	6.25 ± 0.21
HF5	25.00 ± 0.13	25.00 ± 0.08	25.00±0.31	-	6.25 ± 0.43
HF6	6.25±0.06	25.00 ± 0.20	-	12.50±0.21	-

Key: (-) no inhibition.

Table 4: Mean MICs (mg/mL) of the different gel formulation against test organisms

Gel formulations	*Staphylococcus epidermidis*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*
CF1	6.25±0.06	25.00±0.20	-	12.50±0.6	25.00 ± 0.43
CF2	25.00 ± 0.13	6.25 ± 0.03	25.00±0.31	-	6.25 ± 0.43
CF3	6.25±0.06	-	25.00±0.13	12.50±0.21	-
CF4	12.50±0.10	25.00±0.22	6.25±0.21	25.00±0.10	12.50±0.08
CF5	25.00±0.13	-	25.00±0.31	12.25±0.11	6.25±0.43
CF6	6.25±0 06	25.00±0.20	-	-	12.50 ± 0.21

Key: (-) no inhibition.

CONCLUSION

The present study successfully developed and characterized hydrogel-based formulations of *Holarrhena antidysenterica* bark extract using HPMC K-100M and Carbopol 934 as gelling agents. These findings validate the traditional medicinal use of *H. antidysenterica* and support its incorporation into modern topical phytopharmaceutical preparations. Overall, the study establishes that hydrogel-based delivery of *H. antidysenterica* extract is a promising strategy to enhance therapeutic efficacy, stability, and patient acceptability, with significant potential as a natural, safe, and effective topical antimicrobial agent, warranting further in-vivo studies and clinical evaluations for therapeutic and commercial application.

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