RESEARCH ARTICLE

Assessment of intranasal in-situ gel efficacy of ethanolic extract of *Mimosa pudica* for anxiolytic and antidepressant activity: Formulation optimization and stability studies.

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ABSTRACT

This research delves into the therapeutic potential of *Mimosa pudica*, known as chuimui or lajwanti in Hindi, for alleviating anxiety and depression, capitalizing on its diverse pharmacological activities. Renowned for analgesic, antidiarrheal, anti-inflammatory, hepatoprotective, antiasthmatic, anti-ulcer, and antioxidant properties, the plant emerges as a promising herbal remedy for mental health. The study specifically accentuates its antidepressant and anxiolytic properties, aiming for minimal side effects compared to synthetic alternatives. Pre-formulation considerations involve optimizing the concentration of polymer for in-situ gelation. Preliminary phytochemical screening unveils the presence of bioactive compounds, including terpenoids, flavonoids, glycosides, alkaloids, quinines, phenols, tannins, and saponins, known for modulating neurotransmission and exhibiting anxiolytic effects. The concentration of deacetylated gellan gum for gelation is fine-tuned using simulated nasal fluid, emphasizing minimal viscosity. Compatibility studies using Fourier-transform infrared spectroscopy (FTIR) confirm the absence of significant drug-polymer interactions. Differential scanning calorimetry (DSC) provides insights into thermal behavior. Six trial batches with varying concentrations are prepared, and final formulations are evaluated for different physicochemical parameters. Stability studies are conducted under different conditions for three months. The comprehensive investigation aims to inspire advanced research into the manifold benefits of *Mimosa pudica*, particularly in mental health treatment, paving the way for potential herbal formulations with reduced side effects.

Keywords: Pre formulations, Antianxiety, Intranasal, Gel strength, Spreadability.

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INTRODUCTION

For centuries, different societies across the globe have tapped into nature's vast reservoir of medicinal compounds, utilizing the therapeutic properties of diverse plants in their daily lives to combat diseases. Herbal medicine rests on the premise that plants inherently possess natural substances capable of promoting well-being and alleviating illnesses.[1] Presently, a substantial segment of the worldwide population faces the challenges of depression and anxiety, as indicated by the prevalent occurrences of these conditions. According to the World Health Organization, global anxiety statistics highlight that around 264 million individuals, comprising 3.6 percent of the world's population, grapple with anxiety disorders. It is noteworthy that anxiety impacts 4.6 percent of females and 2.6 percent of males on a global scale.[2] Although synthetic drugs like Clomipramine, Imipramine, Desipramine, and Nortriptyline are frequently utilized as standard treatments for individuals suffering from clinical depression and anxiety, their effectiveness is frequently tempered by adverse effects, presenting challenges to the overall therapeutic process.[3] By giving the potential for drug-drug interactions and adverse effects linked to synthetic medications, an opportunity emerges to investigate alternative treatments for anxiety and depression, specifically through the use of medicinal plants or plant-based formulations possessing antianxiety and antidepressant properties. In the Ayurvedic system of Indian Medicine, intranasal administration has been long acknowledged as an accepted and effective form of therapy, offering self-administration by the patient and ensuring efficient drug delivery.[16] The plant *Mimosa pudica*, recognized for its dual

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anxiolytic and antidepressant qualities, presents itself as a promising herbal treatment, underscoring the importance of thoroughly documenting research conducted on traditional herbal medicines[4]

Plant Profile

Mimosa pudica, referred to as "chuimui" or "lajwanti" in Hindi and known for its distinctive characteristic of drooping or collapsing upon touch, *Mimosa pudica* is also recognized as "Lajjuki lota" in Assamese[4]

Scientific Classification

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Subfamily	Mimosoideae
Genus	Mimosa
Species	M. pudica

Polymer Used in *in-situ* Gel Drug Delivery System

For augmenting drug product performance, choosing the right polymer is essential. Polymers that can transition from a sol to a gel in water, enabling in-situ gelation, are vital. Polymers like pectin, gelrite, cellulose acetophthalate latex, gellan gum, alginate, matrigel, carbopol, and chitin exemplify materials that facilitate this process.[5] Gellan gum, a negatively charged deacetylated exopolysaccharide is used and by providing the presence of about 0.1 ml of mucus rich in sodium, potassium, and calcium ions on the nasal mucosa, a transition from liquid to gel is anticipated.[6] This research aims to develop a nasal in-situ gel combining temperature-sensitive and mucoadhesive polymers in a gel-forming solution, overcoming challenges like fatigue, diarrhea, nausea, and vomiting associated with oral drug delivery.[7]

MATERIAL AND METHODS

The *Mimosa pudica* plant was sourced from Dream Flower Nursery in Khanapara, Guwahati, Assam, and authenticated by Dr. Sourav Bora from the Botany Department of Guwahati University, with the authentication number Acc. No. GUBH20430 on 11.08.2023, and has since been preserved. Gellan gum, Polyethylene glycol 400 (PEG 400), mannitol, and methyl paraben were purchased from Zenith India Pvt. Ltd., Guwahati

Chemical Requirement

Table 1: Chemicals required

S. No.	*Chemical Name*	*Manufacture*
1	Ethanol	Assam Petrochemical Ltd.
2	Ferric chloride	Fisher Scientific
3	Lead acetate	ACS
4	Sodium Hydroxide	Fisher Scientific
5	Conc. Sulphuric acid	Fisher Scientific
6	Fehling's reagents A & B	Organo biotech labs
7	Benedict's reagent	Bullux laboratories
8	Alpha-napthol	Alpha chemika
9	Mayer's reagent	Bio rapid

Methods

Preparation of plant extract

The process entailed cleansing the raw *Mimosa pudica*, drying it in the shade, and grinding it to produce a dry, coarse powder. Around 500 grams of this powder was then subjected to ethanol extraction in a percolator apparatus.[18] After a 16-hour rest, the percolate was gathered, and this extraction step was replicated four times. The pooled extract was subsequently filtered and concentrated under vacuum at 40°C with a rotary evaporator, yielding a 1.5% extract.[8]

Phytochemical analysis:

The crude fractions have been subjected to different qualitative phytochemical screening to identify the presence of various phytoconstituents as described by Harborne.[17] Preliminary phytochemical screening was conducted on all extracts in accordance with established standard procedures.[9]

Preliminary investigations to ascertain the most effective strength of polymer for in-situ gel formation

Preliminary investigations involved testing various gellan gum concentrations to refine the optimal gelling concentration. Gelation studies were conducted in simulated nasal fluid, aiming for minimal viscosity at pH 6.4 ± 0.1 and 34 ± 1°C.[11]

Formulation of in situ gel systems

The formulation process comprised dissolving non-acetylated gellan gum in different concentrations in double distilled water, heating the mixture to 90°C, and subsequent cooling. The drug, *Mimosa pudica* extract, was then blended with PEG 400 and mixed with the solvent mixture. Sequential addition of an isotonic agent(mannitol) and preservative (methyl paraben) provides the resulting formulation underwent comprehensive evaluation.[12]

Preformulation Studies

FTIR spectral studies

FTIR analysis evaluated interactions in 1:1 w/w physical mixtures, including drug with gellan gum, drug with formulation, and gellan gum with nasal fluid components. After one month incubation at room temperature, ensuring complete interaction, samples were dried and subjected to the FTIR scanning process. Wavelength profiles were compared

by elucidating potential interactions within the formulations[13]

DSC spectral studies

DSC spectral studies was conducted to examine the thermal behavior of the pure drug. Precisely measured samples weighing 10 milligrams were enclosed in conventional aluminum containers and exposed to scanning across a temperature span from 60 to 290°C, utilizing a gradual rate of heat 10 degrees Celsius per minute.[13]

Formulation optimization

Six trial batches were prepared, maintaining a constant gellan gum concentration of 0.2% w/v while varying PEG 400 concentrations at 4, 5, and 6% w/v each. These trials indicated that the levels of PEG 400 and gellan gum significantly influenced consistency, Adhesion strength, and drug dispersion. Initial studies with polymer concentrations revealed optimal results at 0.2% w/v. Various in-situ gel formulations were then prepared and evaluated for different physio-chemical parameters and stability in Table 5.[19]

Spreadability

The spreadability of gel formulations F1 to F6 was assessed as the area traveled per unit time (cm^2/min). A 1-ml graduated pipette with a rubber bulb was vertically clamped to a stand, ensuring the tip was 2 cm above the horizontal surface of the filter paper. A 0.1-ml sol formulation was dropped at the center of the filter paper, and at fixed intervals (20 s), the surface area covered by the formulation was measured.[19]

pH

The digital pH meter underwent calibration, and for each preparation, 20 milliliters were taken in a beaker, and the glass electrode was immersed adequately into the samples. Subsequently, the pH of the solution was determined.[19]

Viscosity

Viscosity measurements were conducted with a Brookfield viscometer using a small volume adapter. The temperature sensing probe was immersed into the gel, and the gel temperature was recorded. Viscosity readings were then obtained at temperatures ranging from 32 to 34°C.[19]

Gel strength determination

A 50 g sample was placed in a 100-ml graduated measuring cylinder and gelled in a water bath at 32–34°C by adding simulated nasal fluid. A weight of 35 g was then applied to a disk (diameter: 2.3 cm, clearance: 0.4 cm, thickness: 0.5 cm) positioned on the gel surface. Gel strength is determined by the time to move the piston through the gel.[20]

Tissue adherence strength

Mucoadhesive force was assessed via a modified device on sheep nasal mucosa, determining detachment stress from the formulation by measuring the minimum water weight needed for separation.[21]

Pharmacokinetic testing of drug

Various formulations were evaluated for in vitro drug diffusion using a Franz diffusion cell, measuring drug release through a dialysis membrane.[21]

Stability study

A thorough stability assessment spanning three months, including varying temperature and humidity conditions, monitored sample parameters to evaluate stability profiles.[21]

RESULTS AND OBSERVATIONS

Drying

The plant material was air-dried in shade under controlled conditions at ambient temperature, following which it was pulverized into a dry coarse powder.[17]

Extraction

The composite extract underwent filtration and was subsequently concentrated under vacuum utilizing a rotary evaporator at a temperature of 40°C, and the mass of extract has been noted and, resulting in a 1.5% yield.[10]

Phytochemical Analysis

The initial phytochemical screening of Mimosa pudica extract revealed the existence of various bioactive components, including Terpenoids, Flavonoids, Glycosides, Alkaloids, Quinines, Phenols, Tannins, Saponins, etc. Notably, Flavonoids, Alkaloids, and Phenols were identified as key constituents responsible for modulating neurotransmission, thereby contributing to the observed anxiolytic effects.[14]

Preliminary exploration to ascertain the ideal concentration of gellan gum for *in-situ* gel formation

In the initial investigation phase, diverse gellan gum concentrations were tested in simulated nasal fluid, aiming to optimize gelation with minimal viscosity. Gelation studies conducted under specific pH and temperature conditions identified 0.2% w/v gellan gum as the most effective.[11]

FTIR Studies

FT-IR Data interpretation

Table 2: Tests were conducted to explore the gelation characteristics of deacetylated gellan gum

Formulations	*Gellan gum (GG) deacetylated concentration (%w/v)*	*Observation*
Gellan gum 1	0.1	Absence of gel development.
Gellan gum 2	0.2	Sturdy gel development.
Gellan gum 3	0.3	Intensely viscous gel
Gellan gum 4	0.4	Intensely viscous gel

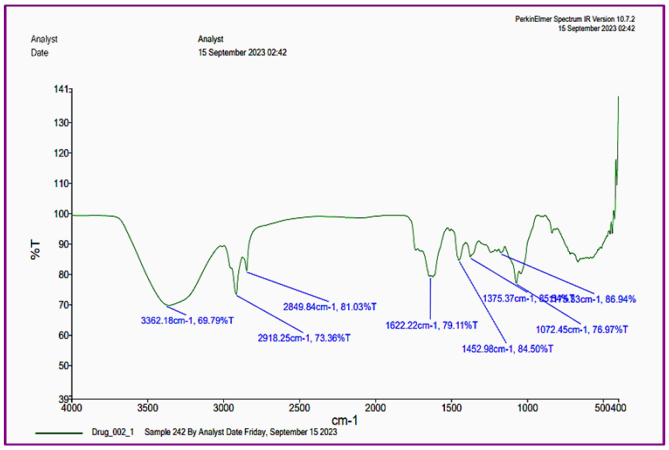

Figure 1: (Drug)

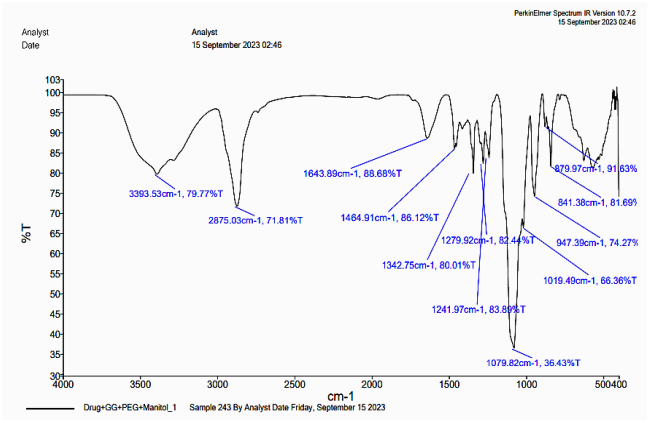

Figure 3: (Drug+ GG+ PEG+ Mannitol)

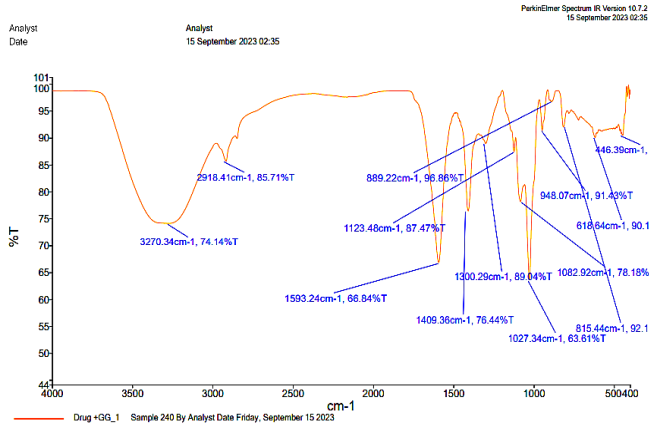

Figure 2: (Drug+ GG)

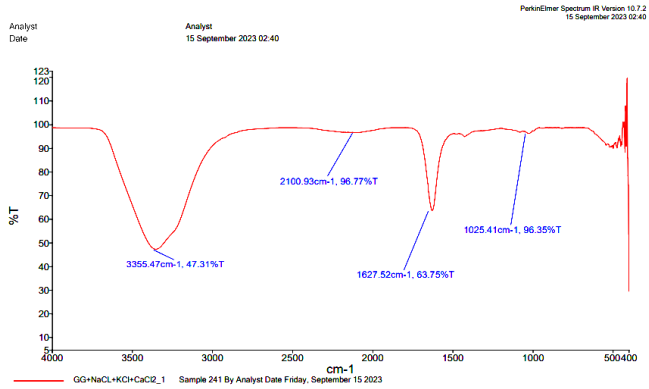

Figure 4: (GG+Nacl+Kcl+CaCl2)

Table 3: FT-IR Data interpretation

S. No.	*Component*	*Peak position (cm^{-1})*	*Functional group*
1	Ethanolic extract of Mimosa pudica (Drug)	3362.18	N-H stretching of secondary amine
		2918.25	C-H bending of aldehyde
		2849.84	C-H bending of aldehyde
		1622.22	Aromatic C=C stretching
2	Drug + GG	3270.34	N-H stretching of secondary amine
		2918.41	C-H bending
		1593.24	C=C aromatic stretching
3	Drug+ PEG+ GG+ Mannitol	3393.53	N-H stretching of secondary amine
		2875.03	C-H bending
		1643.89	C=C stretching
4	GG+NaCl+KCl+CaCl2	3355.47	N-H stretching of secondary amine
		2100.93	C–C Alkyne stretching
		1627.52	C=C aromatic stretching

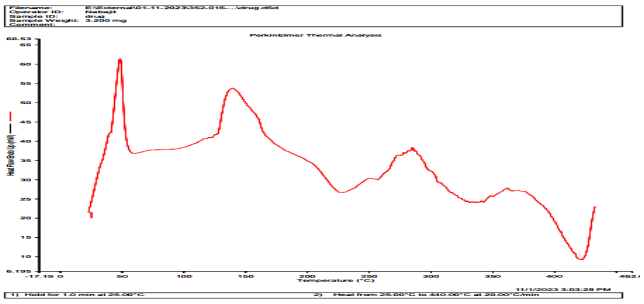

Figure 5: (Drug)

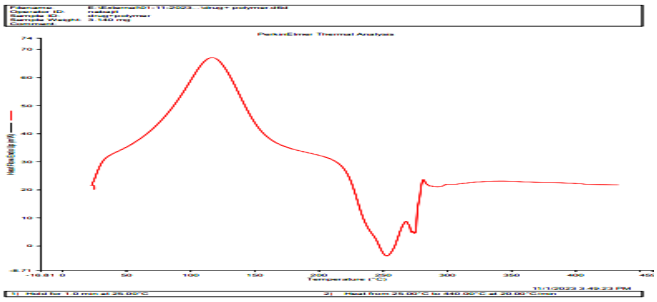

Figure 6: (Drug + Polymer)

DISCUSSION

The drug and polymer exhibit no significant interaction, as indicated by the absence of appreciable shifts in characteristic peaks in their respective spectra. Similarly, minimal interaction is observed between the formulation components and gellan gum in simulated nasal fluid.[13]

DSC Studies

DISCUSSION

DSC analysis revealed no significant peak shifts, indicating no interaction between amorphous drug and polymer, with melting points at 105.69°C and 102.86°C.[6]

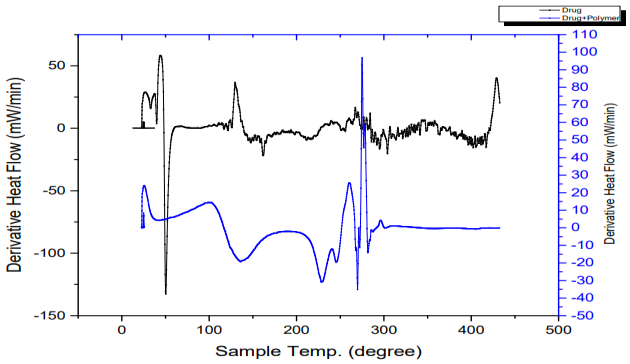

Figure 7: (Drug + Drug: Polymer)

Formulation of *in-situ* gel systems

The formulation began by dispersing nonacetylated gellan gum (0.2% w/v) in distilled water, heated to 90°C until complete dissolution, then cooled. Mimosa pudica extract was blended with water and PEG 400, added to the polymer solution, followed by mannitol and methyl paraben.[12]

DISCUSSION

All formulations maintained nasal pH range. F5, with 0.2% w/v gellan gum, demonstrated ideal viscosity and mucoadhesive strength. Increased PEG400 levels elevated viscosity. Effective spreadability is crucial for easy administration and mucosal adherence; F5 exhibited maximal spreadability, covering more surface area, while F4 and F5 showed optimal viscosity.

Stability Study

DISCUSSION

The stability study of formulation F5 revealed a notable viscosity increase after three months, attributed to vehicle evaporation and volume reduction, consistent across varied storage conditions. However, in vitro drug release studies indicated no significant alterations under the stated storage

Table 4: DSC Data interpretation

Data	*Sample Temperature (°C)*	*Derivative Heat Flow (mW/min)*	*Data*	*Sample Temperature (°C)*	*Derivative Heat Flow (mW/min)*
	101.2322	14.33333		104.026	2.265331
	101.5571	14.3009		104.3551	2.354953
	101.882	14.2284		104.6943	2.555714
	102.2169	14.20993		105.0235	2.893922
	102.5418	14.30317		105.3523	3.129408
Drug + Polymer	102.8668	14.38377	Drug	105.6911	3.138856
	103.1916	14.3073		106.0199	2.966056
	103.5263	14.07762		106.3587	2.685586
	103.8513	13.8142		106.6778	2.460447
	104.1864	13.61306		107.017	2.327645
	104.5116	13.41954		107.3463	2.275309

Table 5: Composition ion-activated mucoadhesive nasal *in-situ* gel formulations

Batch No	*Drug (Ethanolic extract of mimosa pudica) (mg)*	*Gellan gum (%w/v)*	*PEG 400 (%w/v)*	*Mannitol (%w/v)*	*Methyl paraben (%w/v)*	*Double distilled water (q. s.) (mL)*
F1	10	0.02 mg	4 mg	5 mg	0.018	10 mL
F2	10	0.02 mg	4 mg	5 mg	0.018	10 mL
F3	10	0.02 mg	5 mg	5 mg	0.018	10 mL
F4	10	0.02 mg	5 mg	5 mg	0.018	10 mL
F5	10	0.02 mg	6 mg	5 mg	0.018	10 mL
F6	10	0.02 mg	6 mg	5 mg	0.018	10 mL

Table 6: Result of measurement of pH, clarity, viscosity, spreadability, drug content, gel strength, mucoadhesive Strength

Formulation	*Clarity*		*Mucoadhesive Strength (dyne/cm2)*	*Drug content. (Ethanolic extract of Mimosa Pudica) mg.*	*Viscosity (cps)*	*Gel Strength (Sec)*	*P^H*	*Spreadability (cm2)*
	Visual Appearance	*Refractive Index*						
F1	Clear solution	1.357 ± 0.001	815.45	10mg	1090	285	4.79	15.195
F2	Clear solution	1.345 ± 0.002	965.43	10mg	1310	300	5	18.462
F3	Clear solution	1.359 ± 0.002	1250.73	10mg	1000	315	4.66	15.918
F4	Clear solution	1.327 ± 0.001	1150.45	10mg	1325	220	5.38	11.397
F5	Clear solution	1.337 ± 0.001	1275.35	10 mg	1325	345	4.9	21.462
F6	Clear solution	1.397 ± 0.001	1250.73	10 mg	1065	320	5.32	18.355

Table 7

Time Period	*Temperature and RH*	*Appearance*	*Viscosity (Cps)*	*In vitro drug release (%)*
	RT (25 ± 2°C) and RH 65 ± 5%	No visual change	1340	98.450
First One Month	(40 ± 2°C) RH 75 ± 5%	No visual change	1385	98.325
	(4 ± 2°C) RH 55 ± 5%	No visual change	1345	98.120
	RT (25 ± 2°C) and RH 65 ± 5%	No visual change	1378	98.572
Two Month	(40 ± 2°C) RH 75 ± 5%	No visual change	1365	97.355
	(4 ± 2°C) RH 55 ± 5%	No visual change	1388	97.465
	RT (25 ± 2°C) and RH 65 ± 5%	No visual change	1400	98.415
Three Month	(40 ± 2°C) RH 75 ± 5%	No visual change	1395	98.655
	(4 ± 2°C) RH 55 ± 5%	No visual change	1425	98.225

Time Period	*Temperature and RH*	*P^H*	*Gel Strength (Sec)*	*Spredebility (Cm2)*	*Mucoadhesive Strength (Dyne/Cm2)*
	RT (25 ± 2°C) and RH 65 ± 5%	4.60	315	21.245	1275.30
First One Month	(40°C ± 2°C) RH 75 ± 5%	5.35	285	20.462	1265.43
	(4 ± 2°C) RH 55 ± 5%	4.75	310	20.918	1250.73
	RT (25 ± 2°C) and RH 65 ± 5%	5.38	245	21.395	1250.45
Two Month	(40 ± 2°C) RH 75 ± 5%	4.32	320	21.462	1275.35
	(4 ± 2°C) RH 55 ± 5%	5.30	325	21.350	1245.73
	RT (25 ± 2°C) and RH 65 ± 5%	5.15	330	21.765	1215.45
Three Month	(40 ± 2°C) RH 75 ± 5%	4.85	340	20.145	1225.70
	(4 ± 2°C) RH 55 ± 5%	4.95	320	21.285	1215.30

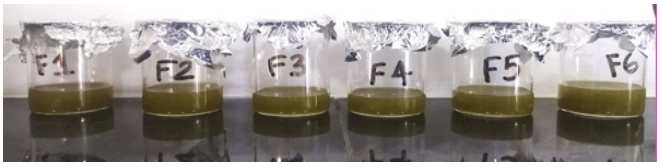

Figure 8: (Formulations F1, F2, F3, F4, F5, F6)

conditions. Throughout the study, there were no noteworthy changes in Spredebility, gel strength, mucoadhesive strength, and pH during the first, second, and third months, demonstrating the formulation's stability against the effects of temperature and time.

CONCLUSION

Following the collection, drying, and extraction of plant material, various phytochemical constituents, including flavonoids and tannins, were identified, providing evidence for potential anxiolytic and antidepressant activity. Preliminary investigations determined the optimal strength of nonacetylated gellan gum for gelation at 0.2% w/v in distilled water. Pre-formulation studies (FTIR, DSC) confirmed no significant interaction between the drug and polymer. Six trial batches with varying PEG 400 concentrations (F1-F6) were prepared, with F5 exhibiting optimal properties, including maximum drug release (98.57% in 5 h). Stability studies over three months showed no significant changes, prompting further evaluation through ex vivo permeation, histopathological, and in vivo studies.

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