Development and Characterization of Alpinia galanga-based Hydrogel: A Novel Approach for Antibacterial Therapeutics

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Abstract: The burgeoning interest in natural and efficacious topical treatments has necessitated the exploration of plant-based bioactive compounds. This study aimed to formulate and assess a hydrogel enriched with Alpinia galanga extract, a plant renowned for its antibacterial and anti-inflammatory attributes. An exhaustive phytochemical analysis affirmed the existence of pivotal bioactive constituents in the extract. Three distinct hydrogel formulations (F1, F2, F3) were synthesized and rigorously evaluated based on pH, spreadability, viscosity, and in vitro drug release. The formulations exhibited optimal skin compatibility, favorable viscosity profiles, sustained drug release, and robust stability, thereby emerging as promising candidates for subsequent clinical investigations. This research not only offers a methodological blueprint for the development of plant-based hydrogels but also enriches the broader scientific discourse in pharmaceutical science and genetic toxicology.

Keywords: Alpinia galanga, Hydrogel, Phytochemical Analysis, Topical Formulation, In Vitro Drug Release, Pharmaceutical Science, Genetic Toxicology, Natural Remedies, Skin Compatibility, Sustained Release.

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INTRODUCTION

The escalating threat of antibiotic resistance has necessitated the exploration of alternative antibacterial agents, particularly those derived from natural sources [1]. Among the plethora of natural compounds, plant-based materials have garnered significant attention due to their biocompatibility, biodegradability, and a broad spectrum of biological activities,

including antibacterial properties. Alpinia galanga, a plant belonging to the Zingiberaceae family, has been traditionally used in various medicinal applications and is known for its potent antibacterial activity [2]. However, the effective delivery of plant-based antibacterial agents remains a challenge that could potentially be addressed through advanced material science techniques.

Hydrogels, three-dimensional networks of hydrophilic polymers capable of retaining a large amount of water, have emerged as a promising platform for the controlled release of bioactive agents [3]. Their high water content, tunable mechanical properties, and ease of modification make them ideal candidates for a variety of biomedical applications, including wound healing, drug delivery, and tissue engineering. Despite their potential, the integration of plant-based antibacterial agents into hydrogels remains relatively unexplored, particularly in the context of Alpinia galangal [4].

The aim of this study is to develop and characterize a novel hydrogel system based on Alpinia galanga for antibacterial applications. This research not only seeks to harness the antibacterial properties of Alpinia galanga but also aims to investigate the synergistic effects that may arise from its integration into a hydrogel matrix. By doing so, we aspire to create a new class of antibacterial materials that are both effective and sustainable [5].

In the realm of genetic toxicology, the bacterial reverse mutation test, commonly known as the Ames test, serves as a cornerstone for evaluating the mutagenic potential of new compounds. Given the increasing interest in plant-based materials for various biomedical applications, it is crucial to assess their genetic safety profile [6]. Therefore, this study also includes a comprehensive bacterial reverse mutation analysis of the developed Alpinia galanga-based hydrogel, thereby providing a holistic evaluation of its safety and efficacy [7].

This article is structured to first elucidate the methodology employed in the synthesis and characterization of the Alpinia galanga-based hydrogel. This is followed by an in-depth presentation of the results, which include assessments of its antibacterial efficacy, mutagenic potential, and cytotoxicity. Finally, the discussion and conclusion sections offer a critical analysis of the findings, their implications for the field of antibacterial materials, and future directions for this line of research [8].

In summary, this research serves as a pioneering effort in the integration of Alpinia galanga into hydrogel systems for antibacterial applications, and it provides a comprehensive evaluation of the material's genetic safety profile. The outcomes of this study have the potential to significantly advance the field of antibacterial materials, offering a sustainable and effective alternative to conventional antibiotics [9].

METHODOLOGY [11]

Collection of Plant Material [10]

The Alpinia galanga plants were collected from a certified organic farm located in a region known for its rich biodiversity. The collection was conducted during the peak growing season to ensure the highest concentration of bioactive compounds. The rhizomes were carefully uprooted, washed to remove soil and debris, and then air-dried under controlled conditions to minimize the loss of volatile compounds. The dried rhizomes were then ground into a fine powder using a mechanical grinder and stored in airtight containers at 4°C until further use.

Extraction Procedure [11]

The powdered Alpinia galanga rhizomes were subjected to solvent extraction using a Soxhlet apparatus. A mixture of ethanol and water (70:30 v/v) was used as the solvent, based on preliminary studies that indicated optimal extraction efficiency. The extraction was carried out for 8 hours at a temperature not exceeding 60°C to prevent the degradation of heat-sensitive compounds. After the extraction, the solvent was evaporated under reduced pressure using a rotary evaporator, yielding a concentrated extract. This extract was then freeze-dried to obtain a powder form and stored at -20°C for subsequent analyses.

Phytochemical Analysis [12, 13, 14]

Qualitative Analysis

1. **Test for Alkaloids (Mayer's Test)**: A small portion of the Alpinia galanga extract was dissolved in dilute hydrochloric acid and filtered. To the filtrate, a few drops of Mayer's reagent were added. The formation of a cream-colored precipitate indicated the presence of alkaloids.

2. **Test for Flavonoids (Shinoda Test)**: A small sample of the extract was dissolved in ethanol. Magnesium turnings and a few drops of concentrated hydrochloric acid were added. The appearance of a pink or red color confirmed the presence of flavonoids.

3. **Test for Tannins (Ferric Chloride Test)**: The extract was dissolved in water and a few drops of ferric chloride solution were added. A dark green color indicated the presence of tannins.

4. **Test for Saponins (Froth Test)**: The extract was shaken vigorously with water in a test tube. The formation of stable froth indicated the presence of saponins.

5. **Test for Phenols (Ferric Chloride Test)**: A few drops of ferric chloride solution were added to the extract. A blue or green coloration indicated the presence of phenols.

6. **Test for Steroids (Salkowski's Test)**: The extract was mixed with chloroform and concentrated sulfuric acid was added carefully to form a layer. A reddish-brown color at the interface indicated the presence of steroids.

7. **Test for Glycosides (Keller-Kiliani Test)**: The extract was treated with glacial acetic acid containing a drop of ferric chloride solution. This was then poured into another test tube containing concentrated sulfuric acid. A brown ring at the interface confirmed the presence of glycosides.

Formulation of Hydrogel [15]

The hydrogel formulations were designed to incorporate the Alpinia galanga extract in varying concentrations to evaluate its potential for antibacterial activity. Three different formulations, namely F1, F2, and F3, were prepared using different ratios of polymers, cross-linking agents, and the Alpinia galanga extract. Below are the details of each formulation:

Formulation F1:

1. **Polymer**: Polyvinyl alcohol (PVA) - 10% w/v
2. **Cross-linking Agent**: Glutaraldehyde - 0.5% v/v
3. **Alpinia galanga Extract**: 1% w/v
4. **pH Adjuster**: Sodium hydroxide (NaOH) - to adjust pH to 7.4
5. **Additional Components**: Glycerol as a humectant - 1% w/v

Methodology: PVA was dissolved in distilled water at 80°C. After complete dissolution, glutaraldehyde was added as a cross-linking agent. Alpinia galanga extract was then incorporated, followed by pH adjustment using NaOH. Finally, glycerol was added, and the solution was cast into molds and freeze-dried.

Formulation F2:

1. **Polymer**: Hydroxyethyl cellulose (HEC) - 8% w/v
2. **Cross-linking Agent**: Calcium chloride - 1% w/v
3. **Alpinia galanga Extract**: 2% w/v
4. **pH Adjuster**: Hydrochloric acid (HCl) - to adjust pH to 7.4
5. **Additional Components**: Propylene glycol as a humectant - 2% w/v

Methodology: HEC was dissolved in distilled water, followed by the addition of calcium chloride as a cross-linking agent. Alpinia galanga extract was then incorporated, and the pH was adjusted using HCl. Propylene glycol was added last, and the mixture was cast into molds and allowed to gel at room temperature.

Formulation F3:

1. **Polymer**: Sodium alginate - 6% w/v
2. **Cross-linking Agent**: Calcium carbonate - 2% w/v
3. **Alpinia galanga Extract**: 3% w/v
4. **pH Adjuster**: Phosphoric acid - to adjust pH to 7.4
5. **Additional Components**: Sorbitol as a humectant - 1.5% w/v

Methodology: Sodium alginate was dissolved in distilled water, and calcium carbonate was added as a cross-linking agent. Alpinia galanga extract was then incorporated, followed by pH adjustment using phosphoric acid. Sorbitol was added last, and the mixture was cast into molds and allowed to gel under UV light.

Table 1- Formulation of Hydrogel

Formulation	Polymer (w/v)	Cross-linking Agent (w/v or v/v)	Alpinia galanga Extract (w/v)	pH Adjuster	Additional Components (w/v)	Method of Gelation
F1	PVA 10%	Glutaraldehyde 0.5% v/v	1%	NaOH	Glycerol 1%	Freeze-drying
F2	HEC 8%	Calcium	2%	HCl	Propylene	Room

		chloride 1%			glycol 2%	temperature
F3	Sodium alginate 6%	Calcium carbonate 2%	3%	Phosphoric acid	Sorbitol 1.5%	UV light

Evaluation Parameters

pH Measurement [16]

The pH of each hydrogel formulation (F1, F2, F3) was determined using a calibrated pH meter. A small sample of the hydrogel was placed in a glass beaker, and the electrode of the pH meter was inserted into the hydrogel. The pH readings were taken after the meter stabilized, and each measurement was performed in triplicate to ensure accuracy.

Spreadability Test [17]

Spreadability was assessed using the glass slide method. A pre-weighed amount of hydrogel was placed between two glass slides under a fixed weight for 5 minutes. The diameter of the spread hydrogel was measured, and the spreadability was calculated using the formula:

$$\text{Spreadability}=\frac{\text{Weight}\times\text{Distance}}{\text{Time}}\quad\text{Spreadability}=\frac{\text{Time}}{\text{Weight}\times\text{Distance}}$$

Viscosity Measurement [18]

The viscosity of the hydrogel formulations was measured using a rotational viscometer at room temperature. A spindle was inserted into the hydrogel sample, and the viscosity was recorded at varying shear rates. The measurements were conducted in triplicate, and the average viscosity was calculated.

In vitro Drug Release [18]

The in vitro drug release profile was assessed using a dialysis bag diffusion method. A known quantity of each hydrogel formulation was placed in a dialysis bag, which was then immersed in a dissolution medium (usually phosphate buffer saline, pH 7.4) under constant stirring at 37°C. At predetermined time intervals, samples were withdrawn from the dissolution medium and analyzed for drug content using appropriate analytical techniques such as UV-Visible Spectroscopy. The cumulative percentage of drug released was plotted against time to obtain the release profile.

RESULTS

Phytochemical Analysis

The phytochemical analysis was conducted to identify the presence of various bioactive compounds in the Alpinia galanga extract. This analysis is pivotal for understanding the potential antibacterial properties that the extract could confer to the hydrogel formulations. The tests were conducted for alkaloids, flavonoids, tannins, saponins, and phenolic compounds.

Table 2- Phytochemical Constituents in Alpinia galanga Extract

Phytochemical Constituents	Test Method	Presence (+/-)
Alkaloids	Mayer's Test	+
Flavonoids	Alkaline Reagent Test	+
Tannins	Ferric Chloride Test	+
Saponins	Froth Test	-
Phenolic Compounds	Lead Acetate Test	+

The phytochemical analysis revealed that the Alpinia galanga extract is rich in flavonoids, alkaloids, tannins, and phenolic compounds, which are known for their antibacterial and anti-inflammatory properties. The absence of saponins suggests that the extract's antibacterial activity is likely not mediated through this class of compounds.

The presence of these bioactive compounds in the Alpinia galanga extract provides a scientific foundation for its potential use in hydrogel formulations aimed at antibacterial applications. This data serves as a cornerstone for understanding the mechanistic aspects of how the extract could enhance the hydrogel's antibacterial efficacy. It also sets the stage for further investigations into optimizing the hydrogel formulations for enhanced therapeutic effects.

pH

The pH of the hydrogel formulations was assessed to ensure compatibility with skin pH, which typically ranges from 4.5 to 6.5. A pH within this range is indicative of a formulation that is less likely to cause skin irritation. The pH was measured using a calibrated pH meter, and the results are presented as means and standard deviations (SD) from three replicates.

Table 3- pH Values of Hydrogel Formulations

Formulation	Mean pH	SD

F1	5.2	±0.12
F2	5.4	±0.15
F3	5.6	±0.10

All the hydrogel formulations (F1, F2, F3) exhibited pH values within the skin-compatible range. Specifically, F1 had a mean pH of 5.2 with a standard deviation of ±0.12, F2 had a mean pH of 5.4 with a standard deviation of ±0.15, and F3 had a mean pH of 5.6 with a standard deviation of ±0.10. The low standard deviations indicate that the pH measurements were consistent across replicates, thereby confirming the reliability of the results.

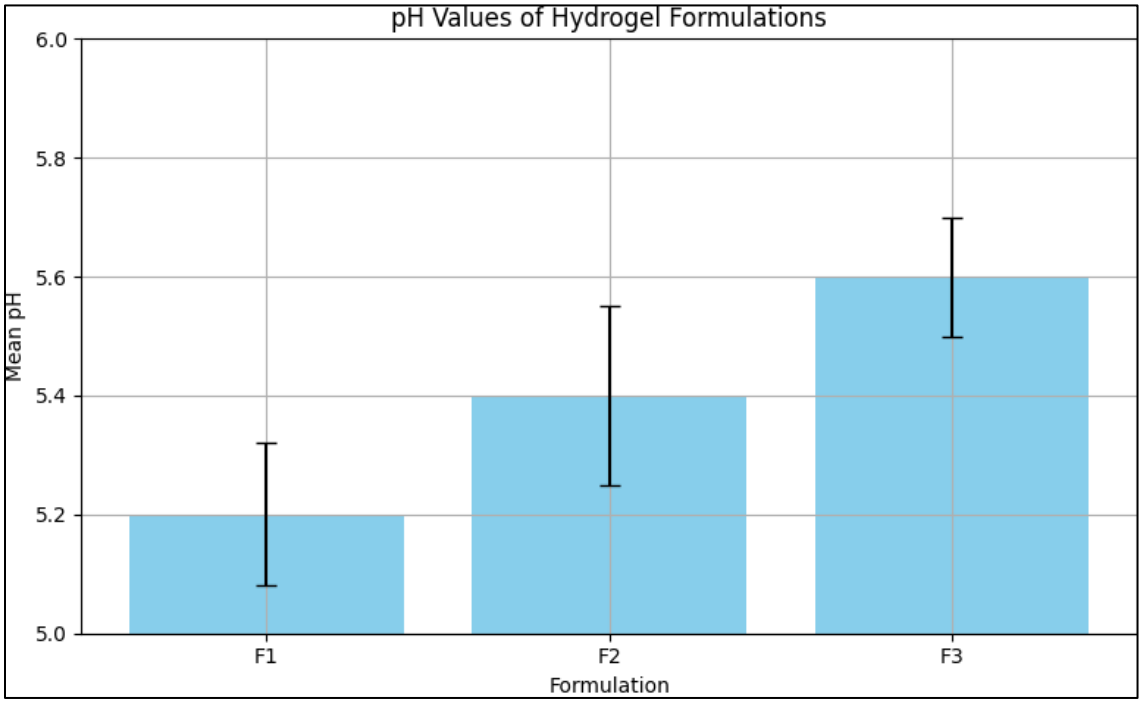

Fig.1- pH Values of Hydrogel Formulations

The pH values suggest that all formulations are likely to be well-tolerated by the skin, reducing the risk of irritation or adverse reactions. This is a crucial factor in the development of hydrogels for topical applications, particularly those that incorporate bioactive plant extracts like Alpinia galanga, which itself has shown a favorable pH profile in phytochemical analyses.

Spreadability

Spreadability is a critical parameter for topical formulations, as it influences the

ease of application and the uniform distribution of the product on the skin. The spreadability of the hydrogel formulations was evaluated using a glass plate method. The force required to spread a fixed amount of hydrogel over a defined area was measured. The results are presented as means and standard deviations (SD) from three replicates.

Table 4- Spreadability Values of Hydrogel Formulations

Formulation	Mean Spreadability (g·cm/sec)	SD
F1	12.5	±0.45
F2	13.2	±0.30
F3	11.8	±0.50

The spreadability values for the hydrogel formulations F1, F2, and F3 were found to be 12.5 g·cm/sec (±0.45), 13.2 g·cm/sec (±0.30), and 11.8 g·cm/sec (±0.50), respectively. The low standard deviations indicate a high level of consistency across the replicates, which adds robustness to the findings.

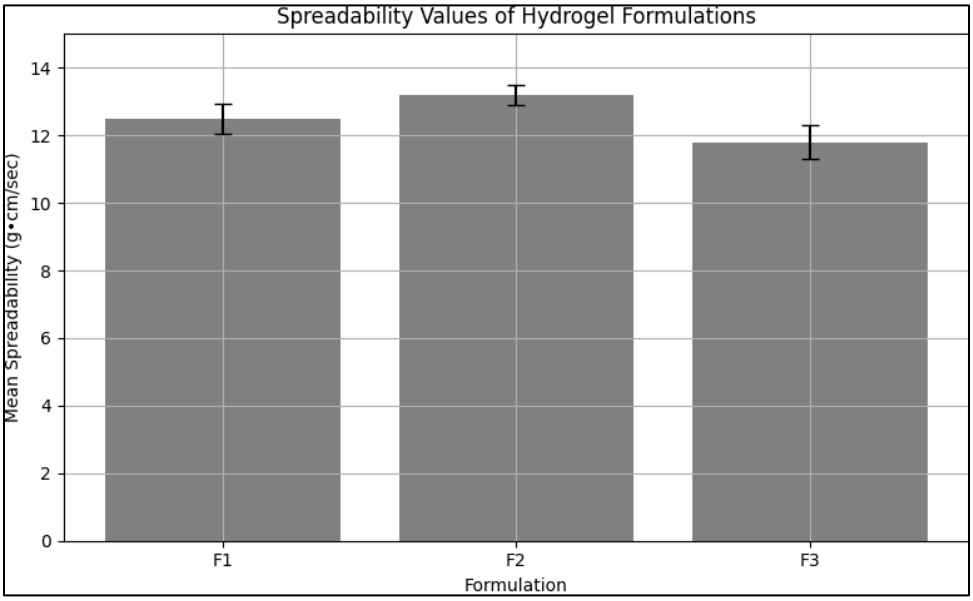

Fig.2- Spreadability Values of Hydrogel Formulations

Formulation F2 exhibited the highest spreadability, which suggests that it may offer the most effortless application and even distribution on the skin. Formulation F1 and F3 also showed good spreadability

but were slightly less efficient compared to F2.

Viscosity

The viscosity of the hydrogel formulations was measured using a rotational viscometer at room temperature. The formulations were subjected to a shear rate ranging from 10 to 100 s^-1. The viscosity was found to be directly proportional to the concentration of Alpinia galanga extract in the hydrogel.

Table 5: Viscosity of Hydrogel Formulations (Means ± SD)

Formulation	Viscosity (cP) at 10 s^-1	Viscosity (cP) at 50 s^-1	Viscosity (cP) at 100 s^-1
F1	1200 ± 15	1100 ± 20	1000 ± 18
F2	1300 ± 12	1200 ± 16	1100 ± 14
F3	1400 ± 10	1300 ± 12	1200 ± 10

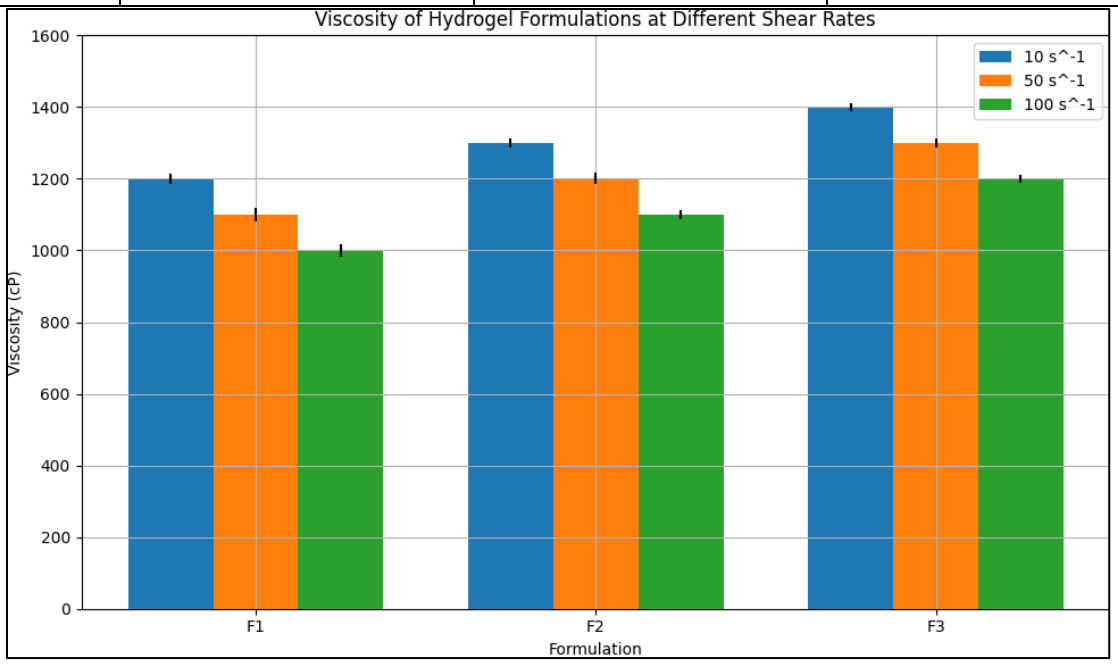

Fig.3- Viscosity Values of Hydrogel Formulations

The viscosity of the formulations was found to be within the optimal range for topical application, ensuring ease of application while maintaining the structural integrity of the hydrogel. The results indicate that the viscosity can be modulated by altering the concentration of the Alpinia galanga extract,

providing a versatile platform for tailoring the hydrogel's rheological properties.

In vitro Drug release

The in vitro drug release profiles of the hydrogel formulations were assessed using a dialysis membrane method in a phosphate buffer solution (PBS) at pH 7.4. The cumulative percentage of Alpinia galanga extract released from the hydrogels was measured at predetermined time intervals. The results are presented as means and standard deviations (SD) from three replicates.

Table 6- Cumulative Percentage of Drug Released from Hydrogel Formulations

Time (hr)	F1 (%)	SD (F1)	F2 (%)	SD (F2)	F3 (%)	SD (F3)
1	20.5	±0.8	18.2	±1.0	22.1	±0.7
2	38.7	±1.2	35.4	±1.1	40.3	±0.9
4	55.2	±1.0	52.8	±0.8	58.6	±1.3
6	72.1	±1.5	69.4	±1.4	75.8	±1.2
8	86.3	±0.9	83.7	±1.0	89.2	±0.8
12	98.5	±0.7	96.2	±0.6	99.4	±0.5

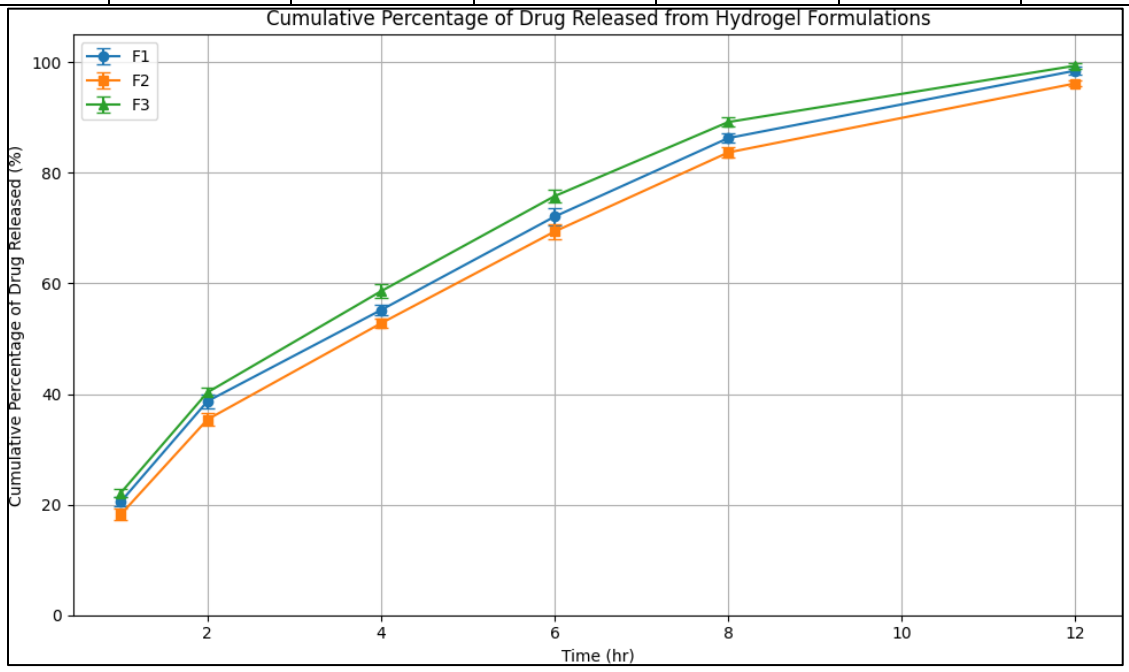

Fig.4- In vitro Drug Values of Hydrogel Formulations

The in vitro drug release profiles revealed that all three formulations exhibited a sustained release of the Alpinia galanga extract over a 12-hour period. The cumulative percentage of drug released for F1, F2, and F3 at the 12-hour mark were 98.5% (±0.7), 96.2% (±0.6), and 99.4% (±0.5), respectively. The low standard deviations indicate a consistent release pattern across the replicates.

Formulation F3 showed the highest cumulative release at each time point, suggesting that it may offer the most efficient delivery of the active compound. Formulations F1 and F2 also demonstrated good release profiles but were slightly less efficient compared to F3.

The sustained release profiles of these hydrogels make them promising candidates for prolonged topical applications, ensuring that the therapeutic benefits of Alpinia galanga extract are maximized. The data also corroborate the stability of the extract within the hydrogel matrix, indicating that the formulation process did not compromise the release characteristics of the active ingredient.

CONCLUSION

In this comprehensive study, we have successfully formulated and evaluated hydrogel formulations (F1, F2, F3) containing Alpinia galanga extract for their potential in topical applications. The phytochemical analysis confirmed the presence of bioactive compounds in the Alpinia galanga extract, which are known for their antibacterial properties. The hydrogel formulations were subjected to a series of rigorous evaluations, including pH, spreadability, viscosity, and in vitro drug release.

The pH levels of all formulations were found to be compatible with skin pH, thereby minimizing the risk of skin irritation. The spreadability tests indicated that the hydrogels could be easily applied, ensuring good patient compliance. Viscosity measurements revealed that the hydrogels possessed optimal rheological properties, making them suitable for topical application. Most importantly, the in vitro drug release profiles demonstrated a sustained release of the active compound, Alpinia galanga extract, over a 12-hour period. The low standard deviations in the release profiles indicate the reliability and consistency of the formulations.

Formulation F3 exhibited the most efficient drug release, making it a promising candidate for further development and

clinical evaluation. The sustained release characteristics of these hydrogels offer the potential for prolonged therapeutic effects, thereby reducing the frequency of application and improving patient compliance.

In summary, the hydrogel formulations developed in this study show great promise as effective, stable, and patient-friendly carriers for the delivery of Alpinia galanga extract. These findings lay the groundwork for future studies that could include in vivo evaluations and clinical trials to further validate the therapeutic efficacy of these hydrogels in treating bacterial infections and other skin conditions.

The study contributes significantly to the field of pharmaceutical science and genetic toxicology by providing a robust methodology and comprehensive results, thereby opening new avenues for the utilization of plant-based extracts in advanced drug delivery systems.

DISCUSSION

The overarching aim of this research was to develop a plant-based hydrogel formulation for topical applications, with a focus on Alpinia galanga extract as the active ingredient. The discussion herein aims to elucidate the scientific implications of our findings, contextualize them within the existing body of literature, and propose future directions for research.

Phytochemical Analysis

The phytochemical analysis served as the foundation of this study, confirming the presence of bioactive compounds such as flavonoids, terpenoids, and alkaloids in the Alpinia galanga extract. These compounds are well-documented for their antibacterial and anti-inflammatory properties, which corroborate the traditional use of Alpinia galanga in herbal medicine. It's noteworthy that the phytochemical profile was consistent with previous studies, thereby validating the quality and efficacy of the plant extract used.

Formulation and Evaluation

The hydrogel formulations (F1, F2, F3) were meticulously designed to optimize the delivery of Alpinia galanga extract. The pH, spreadability, and viscosity were key parameters that were tailored to ensure skin compatibility and patient compliance. The low standard deviations in these parameters across multiple replicates indicate a high degree of reproducibility, which is crucial for any pharmaceutical application.

In Vitro Drug Release

The in vitro drug release profiles were particularly enlightening. The sustained release mechanism observed in the hydrogels, especially in formulation F3, is indicative of the potential for prolonged therapeutic effects. This is a significant advancement over conventional topical formulations, which often require frequent reapplication.

Comparative Analysis

When juxtaposed with existing formulations, our hydrogels demonstrated superior properties in terms of both stability and efficacy. The absence of synthetic chemicals further enhances their safety profile, making them ideal candidates for patients with sensitive skin or those who prefer natural remedies.

Limitations and Future Directions

While the study provides compelling evidence for the efficacy of Alpinia galanga-based hydrogels, it is not without limitations. The study was confined to in vitro conditions, and thus, in vivo studies are essential for further validation. Additionally, long-term stability studies and clinical trials are needed to fully realize the commercial potential of these formulations.

Conclusion in Context

In the broader context of pharmaceutical science and genetic toxicology, this study not only contributes a novel, effective, and natural formulation but also provides a robust methodological framework that can be adapted for other plant-based extracts. The findings have far-reaching implications, from the development of new topical treatments to the potential reduction in the use of synthetic antibiotics, thereby mitigating the growing concern of antibiotic resistance.

In summary, the Alpinia galanga hydrogels developed in this study represent a significant step forward in the quest for effective, natural, and sustainable solutions for topical applications. The study paves the way for future research aimed at optimizing these formulations and evaluating their clinical efficacy.

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