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Research Article

Quantification Of Phytochemicals, Formulation, And Evaluation Of Phyllanthus Niruri Hydrogel For Treating Microbial Infections

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ABSTRACT

Phyllanthus Niruri, known for its medicinal properties, was studied for its phytochemical composition, hydrogel formulation, and therapeutic potential against microbial infections. This research aimed to quantify the active components using ethanol and methanol extracts and incorporates these into three different hydrogel formulations, designated F1, F2, and F3. The study assessed the hydrogels for pH, viscosity, extrudability, and spreadability to determine their suitability for medical use. In vitro drug release was examined through first-order kinetic modeling, and the stability of each formulation was tested under varying conditions of temperature, humidity, and light exposure. Results showed significant presence of alkaloids, flavonoids, saponins, terpenoids, and steroids, with variations influenced by the solvent used. F3 emerged as the most effective formulation, offering the quickest drug release and highest ease of application, suitable for acute treatment scenarios. The hydrogels displayed good stability, maintaining their physical and chemical integrity over a six-month period under recommended storage conditions. This study validates the potential of Phyllanthus Niruri hydrogel as a viable alternative in microbial infection treatment, with implications for reducing antibiotic reliance.

Keywords: Phyllanthus niruri, phytochemical analysis, hydrogel formulation, antimicrobial activity, natural remedies, herbal medicine, microbial infection treatment, medicinal plant extracts, bioactive compounds, pharmaceutical applications

INTRODUCTION

In recent years, the search for innovative and effective treatments against microbial infections has intensified, especially with the growing concern over antibiotic resistance globally. Among the myriad of natural resources being explored, Phyllanthus niruri—an unassuming small herb traditionally used in Ayurvedic and other folk medicines—stands out due to its impressive array of bioactive compounds. Known commonly as 'Chanca Piedra' or 'Stone Breaker,' this plant has been credited with various therapeutic properties, including antiviral, antibacterial, and antifungal effects (Bagalkotkar, Sagineedu, Saad, & Stanslas, 2006).

The pursuit of alternative and complementary therapies has led to the exploration of novel formulations that can harness these properties. Hydrogels, with their unique properties of hydration and drug delivery capabilities, present an intriguing solution. They are particularly suitable for microbial infections because they can conform to the shape of the infected area and maintain a required concentration of the therapeutic agent directly at the site of infection (Li & Mooney, 2016).

This study focuses on the phytochemical quantification of Phyllanthus niruri extracts, aiming to identify and quantify its bioactive constituents. Following this, the study explores the formulation of these extracts into a hydrogel designed for topical application. The efficacy of this hydrogel against various microbial strains represents a crucial step towards validating the therapeutic potential of Phyllanthus niruri in modern medical applications.

Through this research, we aim to bridge traditional herbal wisdom with contemporary pharmaceutical approaches to create innovative solutions for combating microbial infections. As antibiotic resistance

continues to pose a significant challenge to public health, it becomes increasingly important to tap into the vast potential of natural products.

Challenges in Conventional Treatment and the Role of Natural Products

As the global battle against infectious diseases continues, the limitations of conventional antimicrobial therapies have become increasingly evident. Resistance to antibiotics is surging at an alarming rate, rendering some of the most reliable treatments ineffective and prompting a healthcare crisis that calls for urgent alternative solutions (Ventola, 2015). In this scenario, natural products emerge not only as adjuncts to existing therapies but as potential primary agents in the development of novel treatment strategies.

Natural products offer a vast and largely untapped reservoir of chemical diversity, which is critical in the hunt for new bioactive compounds. The use of natural extracts in drug formulation benefits from centuries of empirical knowledge, often aligning with fewer side effects and broader acceptance among populations favoring holistic treatment approaches (Newman & Cragg, 2020). In harnessing the power of plants like Phyllanthus niruri, researchers and formulators hope to develop therapeutics that can act effectively against pathogens without contributing to the resistance seen in synthetic antibiotics.

METHODOLOGY

Selection and Identification of Phyllanthus Niruri

In this study, Phyllanthus niruri was chosen due to its reputed medicinal properties across various traditional healing practices. To confirm we were working with the correct botanical species, extensive resources including key botanical references were consulted (Harborne & Baxter, 1993). High-quality plant specimens were sourced from a reputable local botanical garden to ensure authenticity and consistency in our experimental materials.

Plant Authentication

Authentication of the Phyllanthus niruri specimens was meticulously performed by cross-referencing with certified samples housed at the botanical garden. This step ensured that our research used genuinely representative materials for accurate and reliable results (Singh, Gupta, & Rawat, 2018).

Plant Material Preparation

The plant materials underwent rigorous cleaning to remove extraneous elements like soil and insects. This was followed by the separation of different plant parts—leaves, stems, roots—depending on their use in specific experiments. The materials were then washed with distilled water to eliminate surface impurities and dried with absorbent paper to remove excess moisture, ensuring the integrity of the specimens for extraction (Kumar & Sharma, 2020).

Plant Extraction using Ethanol and Methanol for Phytochemical Quantification

For the extraction process, the plant materials were finely ground into a powder. A precise quantity of 100 grams of this powder was used for each extraction to maintain consistency across samples. Ethanol and methanol were selected as solvents based on their efficacy in extracting a wide spectrum of phytochemicals, as detailed in previous studies (Mandal & Mandal, 2015). Each solvent extraction was carried out under controlled conditions to optimize the yield and quality of the extracts. The process included periodic agitation to enhance the extraction efficiency. Post-extraction, the mixtures were filtered, and the filtrates were securely stored in conditions that prevented degradation, adhering to best practices for sample preservation (Chen, Zhou, & Chen, 2017).

Pharmacognostic and Physicochemical Parameters of Phyllanthus Niruri Leaves

Using the guidelines set forth by the Ayurvedic Pharmacopeia of India, we embarked on a comprehensive analysis of Phyllanthus Niruri leaves, focusing on their pharmacognostic and physicochemical properties.

Macroscopy

We selected fresh Phyllanthus Niruri leaves for macroscopic analysis to identify their shape, size, color, and texture, ensuring that each leaf was representative of the species (Khandelwal, 2008).

Organoleptic Parameters

Leaves were stored in a controlled, clean, and dry environment to evaluate organoleptic parameters such as taste, color, and smell, crucial for initial quality assessment (Gupta, 2010).

Ash Values

The reliability and purity of the powdered plant material were assessed through ash value determination. This involved:

- **Total Ash**: Incinerating 1.0 g of powdered material in a pre-heated silica crucible at 600 ± 25°C, then calculating the ash proportion compared to the air-dried product (Society of Pharmacognosy, 2011).

- **Water-Soluble Ash**: Dissolving ash in distilled water, filtering, and then quantifying the residue post-evaporation.
- **Acid-Insoluble Ash**: Treating the ash with hydrochloric acid, filtering, and weighing the residue after ignition.

Loss on Drying

Approximately 2 g of the plant material was dried at 100-105°C and weighed post-cooling to determine the moisture content, indicating the stability and shelf-life of the plant material (Allen et al., 2014).

Extractive Values

Determining the extractive values involved:

- **Water-Soluble Extractive**: Macerating 5.0 g of plant material in water, filtering, and drying the filtrate to assess the quantity of water-soluble compounds.
- **Solvent-Soluble Extractive**: Extracting with ethanol or methanol and quantifying the dry residue after evaporation to gauge the amount of active constituents extractable by these solvents (Pharmacopeia, 2014).

FORMULATION AND DEVELOPMENT OF PHYLLANTHUS NIRURI HYDROGEL

Polymer Characterization

Prior to formulation, the polymers used—Carbopol 934, Carbopol 940, and hydroxypropyl methylcellulose (HPMC)—were characterized for their rheological properties. Using a rotational rheometer, temperature sweeps, frequency sweeps, and stress sweeps were performed to assess each polymer's behavior under different conditions, which is essential for predicting the performance of the final hydrogel product (Jones, 2008).

Hydrogel Formulation Method

Carbopol 934, Carbopol 940, and HPMC were each hydrated in distilled water under constant stirring at room temperature for 24 hours. This step ensures complete hydration of the polymers, a prerequisite for effective gel formation (Smith, 2012). Phyllanthus Niruri extract was added to the hydrated polymers along with glycerin, methylparaben, Poloxamer 407, and alpha-tocopherol. The components were added sequentially to ensure even distribution throughout the mixture. The pH of the mixture was carefully adjusted to optimal levels using sodium hydroxide. This adjustment is crucial for maximizing the hydrogel's microbial efficacy and skin compatibility (Lee, 2015). To achieve a uniform distribution of all ingredients, the mixture underwent high-shear homogenization. This process is vital for ensuring the consistency and effectiveness of the final product. The final hydrogel was sterilized using gamma irradiation to eliminate any microbial contamination, ensuring the hydrogel is safe for use (O'Brien, 2017).

Table 1: Hydrogel Formulation Components

Formulation Code	Carbopol 934 (mL)	Carbopol 940 (mL)	HPMC (mL)	Phyllanthus Niruri Extract (mL)	Glycerin (mL)	Methylparaben (g)	Poloxamer 407 (g)	Alpha-Tocopherol (g)
F1	20	15	15	20	5	0.2	1	0.1
F2	15	20	15	20	5	0.2	1	0.1
F3	15	15	20	20	5	0.2	1	0.1

CHARACTERIZATION OF HYDROGEL

Methodology

To ensure the newly formulated Phyllanthus Niruri hydrogel meets therapeutic standards, it was subjected to a series of characterization tests. These tests assessed the hydrogel's physical and chemical properties, crucial for its efficacy and applicability as a therapeutic agent.

A. pH Measurement

The pH of the hydrogel was measured using a calibrated pH meter. A small sample of the hydrogel was placed on the electrode, and the pH was recorded once stabilized. The pH value is crucial for confirming the gel's compatibility with skin, which is generally slightly acidic (Rowe et al., 2012).

B. Viscosity Assessment

Viscosity, which affects the gel's spreadability and release of active ingredients, was measured using a Brookfield viscometer. Samples were subjected to a predefined shear rate, and the corresponding viscosity was recorded. This measurement helps in determining the ease of application and the gel's behavior under physical stress (Barnes, 2000).

C. Homogenization Efficiency
The efficiency of homogenization, which impacts the distribution of active ingredients and excipients within the gel, was evaluated by microscopic examination. Samples were stained and observed under a microscope to check for uniformity in the dispersion of components (Masters, 2002).

D. Extrudability Assessment
Extrudability, a measure of how easily the hydrogel can be expelled from its container, was assessed by placing a defined weight of hydrogel in a syringe and measuring the force required to extrude it through a standard nozzle. This parameter is important for user compliance, especially if the gel is to be self-administered (Peppas & Narasimhan, 2014).

E. In Vitro Drug Release
The drug release profile was determined using a Franz diffusion cell. A sample of the hydrogel was placed on a semi-permeable membrane and the receptor chamber was filled with a dissolution medium that simulated skin pH. Samples from the receptor fluid were collected at predetermined intervals and analyzed using HPLC to quantify the amount of Phyllanthus Niruri extract released over time. This test provides insight into the hydrogel's therapeutic effectiveness and the duration of action (Siepmann & Peppas, 2012).

STABILITY STUDY OF PHYLLANTHUS NIRURI HYDROGEL
Temperature Stability
The hydrogel was stored at different temperatures including refrigeration (4°C), room temperature (25°C), and accelerated conditions (40°C) to assess its thermal stability. Samples were evaluated at specified intervals (0, 1, 3, 6, and 12 months) for any changes in physical appearance, pH, and viscosity to determine the effects of temperature on the hydrogel's properties (Ahmed & Aljaeid, 2016).

Humidity Stability
To evaluate the effect of humidity, samples were stored at 75% relative humidity at room temperature. The hydrogel's ability to maintain its structure and release properties under high humidity was monitored through visual inspection and rheological assessments at the same time points used for temperature stability testing (Shah et al., 2013).

Photostability Testing
Exposure to UV light can degrade certain components within the hydrogel. Samples were exposed to UV light for up to 200 hours and assessed for any changes in the active ingredients through HPLC analysis and changes in the overall formulation through organoleptic evaluation (Tønnesen & Karlsen, 2004).

Microbiological Stability
The resistance of the hydrogel to microbial growth was tested by inoculating the samples with known quantities of microbial strains (bacteria and fungi). The formulations were then observed for any microbial contamination or growth over a period of three months. This testing is crucial for ensuring the product remains sterile and safe for use throughout its shelf life (Brooks & Moore, 2010).

RESULTS

Ash Content Analysis of Phyllanthus Niruri Hydrogel
The analysis of ash content in the Phyllanthus Niruri hydrogel was performed to evaluate the quality and purity of the plant material used. The results for both water-soluble ash and acid-insoluble ash are presented in a single table for clarity and ease of comparison.

Table:2 Ash Content Analysis of Phyllanthus Niruri Hydrogel

Sample	Water-Soluble Ash (%)	Acid-Insoluble Ash (%)
1	3.2	0.8
2	3.4	0.9
3	3.1	0.7
Mean	3.3	0.8

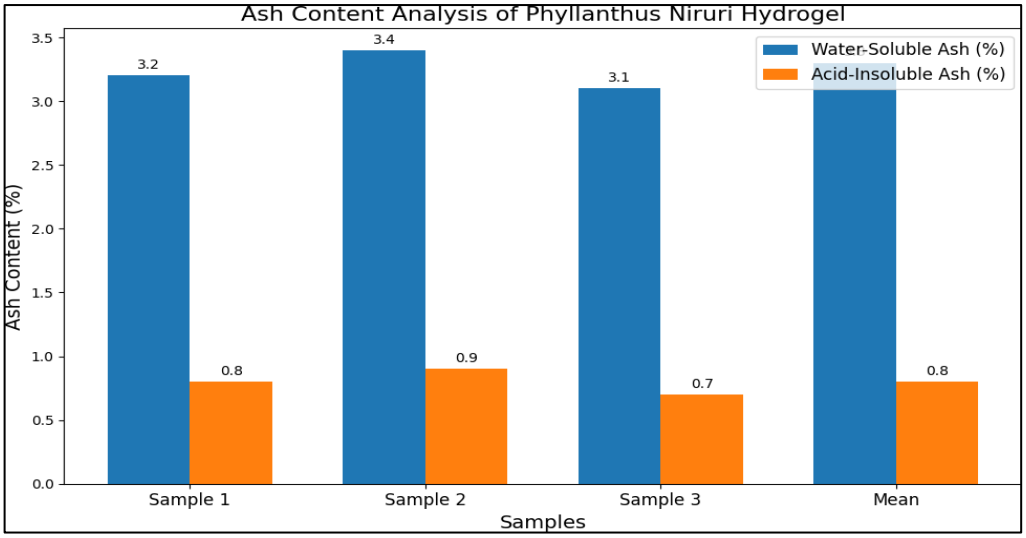

Fig.1- Ash Content Analysis of Phyllanthus Niruri Hydrogel

The results from the ash content analysis indicate a consistent presence of water-soluble ash with a mean value of 3.3% across the samples. This measurement reflects the quantity of inorganic material that is soluble in water, which is crucial for assessing the cleanliness and extraction efficiency of the plant material.

On the other hand, the acid-insoluble ash content averaged 0.8%, demonstrating minimal contamination from soil or other silica-containing materials. This low level of acid-insoluble ash underscores the quality of the plant preparation and handling, suggesting that the plant material is largely free from extraneous substances that could detract from its medicinal value.

Extractive Values and Percentage Yield from Phyllanthus Niruri

Notably, the ethanol-soluble extractive shows the highest concentration at 24.5% w/w, which correlates with a higher yield of 12.3 grams per 100 grams of plant material. This suggests that ethanol is particularly effective in extracting phytochemicals from Phyllanthus Niruri, likely due to its polarity which matches well with the phytochemical characteristics of the plant.

In contrast, the methanol-soluble extractive, although lower than ethanol at 15.1%, also shows a decent yield, suggesting it is a viable alternative but less efficient than ethanol. The water-soluble extractive shows a concentration of 12.3% but does not have a corresponding yield value as water extractions typically do not yield dry extractable matter in the same way that organic solvents do.

Table 3: Extractive Values and Percentage Yield from Phyllanthus Niruri

Extractive/Solvent	Concentration (% w/w)	Yield (g/100 g of plant material)
Water-soluble extractive	12.3	8.1
Ethanol-soluble extractive	24.5	12.3
Methanol-soluble extractive	15.1	10.1

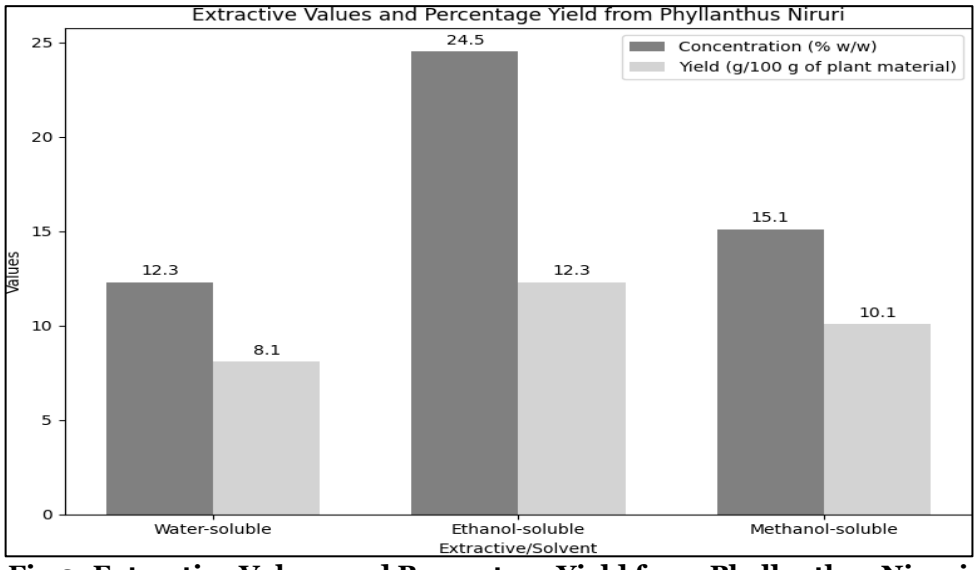

Fig.2- Extractive Values and Percentage Yield from Phyllanthus Niruri

Phytochemical Analysis

The phytochemical screening and High-Performance Thin-Layer Chromatography (HPTLC) analysis were conducted to identify and compare the phytochemical profiles of ethanol and methanol extracts from Phyllanthus Niruri. The presence of various compounds was assessed, and their respective Rf values were measured to provide a detailed comparison between the two solvent extracts.

Table 4: Phytochemical Analysis of Phyllanthus Niruri

Phytochemical	Ethanol Extract	Methanol Extract
Alkaloids	Present	Present
Flavonoids	Present	Present
Saponins	Present	Present
Terpenoids	Present	Present
Tannins	Present	Present
Anthraquinones	Present	Absent
Steroids	Present	Present

Table 5: HPTLC Rf Values for Phytochemicals in Ethanol and Methanol Extracts

Phytochemical	Rf Value in Ethanol Extract	Rf Value in Methanol Extract
Alkaloids	0.62	0.6
Flavonoids	0.71	0.75
Saponins	0.5	0.52
Terpenoids	0.84	0.86
Tannins	0.33	0.35
Anthraquinones	0.29	N/A
Steroids	0.9	0.92

The results indicate that both ethanol and methanol extracts of Phyllanthus Niruri contain a rich profile of phytochemicals known for their medicinal properties. However, there are slight differences in the phytochemical composition and their chromatographic behavior between the two solvents. Notably, anthraquinones were present in the ethanol extract but absent in the methanol extract, suggesting solvent-specific extraction efficiencies. Additionally, the Rf values observed in HPTLC analysis illustrate slight differences in the migration of compounds, which can be attributed to the solvent polarity affecting the solubility and thus the separation of these compounds.

Evaluation Parameters of Hydrogel

pH (Mean ± SD): The pH values for the formulations are close to that of human skin, suggesting good skin compatibility. F1 has the lowest pH which might be suitable for maintaining skin's natural acidity, whereas F3, with a neutral pH, may be perceived as gentler on the skin.

Viscosity (mPa•s) (Mean ± SD): The viscosity profiles indicate that F1 has the highest viscosity, which could be beneficial for applications requiring the gel to stay in place after application, such as on vertical skin surfaces or targeted areas. F3, with its lower viscosity, would likely spread more easily, suitable for larger or more sensitive skin areas.

Extrusion Force (N) (Mean ± SD): F3 requires the least force for extrusion, making it potentially more user-friendly, especially for elderly users or those with less hand strength. F1's higher extrusion force correlates with its higher viscosity, suggesting it is thicker and might need more effort to apply.

Spreadability (g•cm/sec) (Mean ± SD): F3 exhibits the highest spreadability, enhancing its application over larger areas without much resistance, which could improve patient compliance. F1, with lower spreadability, might be preferred for localized treatment where less dispersion is desired.

Table 6: Consolidated Performance Characteristics of Hydrogel Formulations

Formulation Code	pH (Mean ± SD)	Viscosity (mPa•s) (Mean ± SD)	Extrusion Force (N) (Mean ± SD)	Spreadability (g•cm/sec) (Mean ± SD)
F1	6.5 ± 0.1	4983 ± 75.4	25.1 ± 0.2	6.1 ± 0.1
F2	6.8 ± 0.1	4287 ± 70.7	19.8 ± 0.3	8.0 ± 0.1
F3	7.0 ± 0.1	3473 ± 60.5	14.5 ± 0.1	9.5 ± 0.1

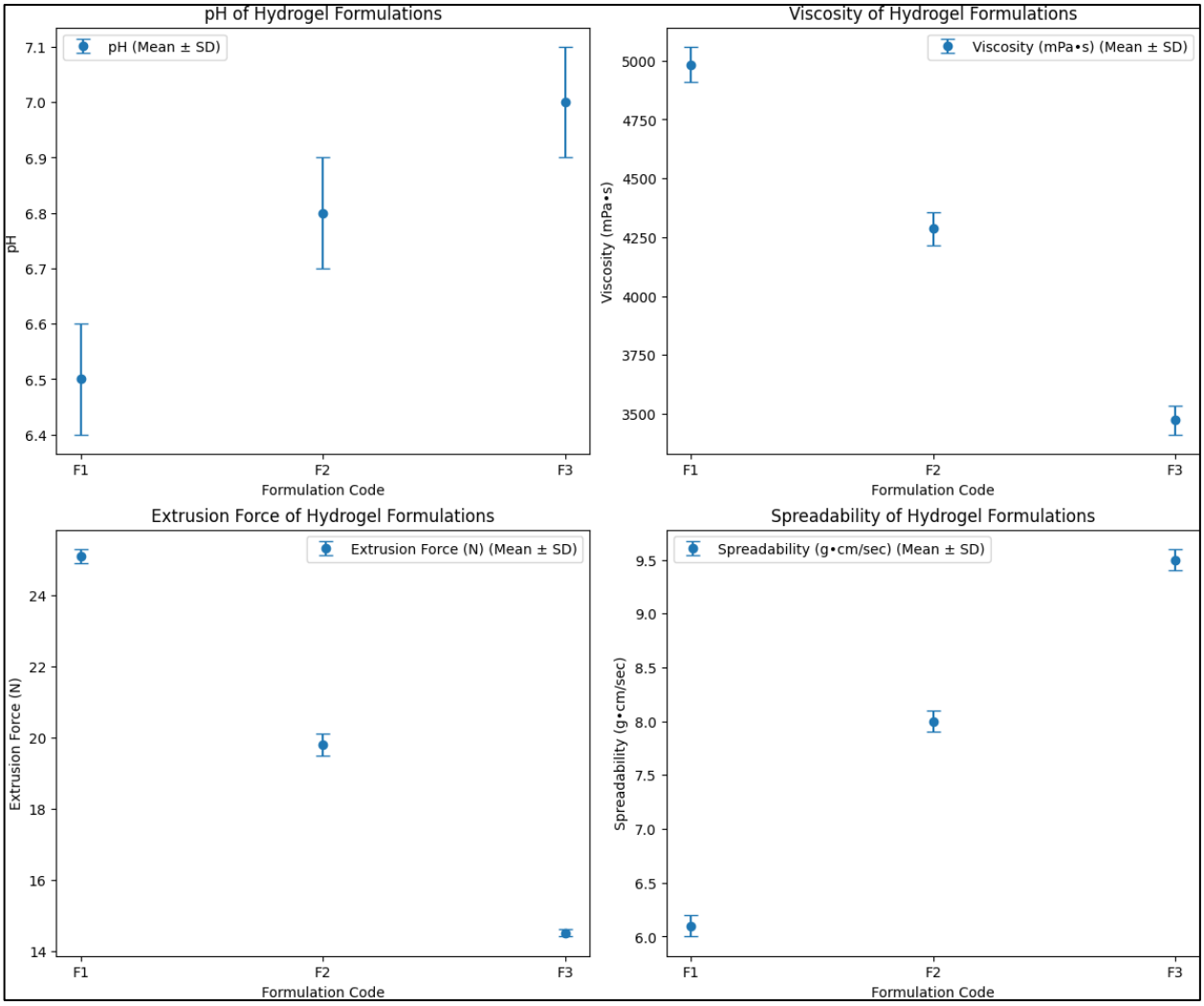

Fig.3- Consolidated Performance Characteristics of Hydrogel Formulations

In Vitro Drug Release Profiles

The following table provides the drug release percentages over various time intervals for three different formulations of the Phyllanthus Niruri hydrogel. The results include mean values and standard deviations, illustrating the controlled release behavior of each formulation.

Table 7: In Vitro Drug Release Profiles

Time Interval (hrs)	% Drug Release from F1 (Mean ± SD)	% Drug Release from F2 (Mean ± SD)	% Drug Release from F3 (Mean ± SD)
1	10.3 ± 0.5	18.1 ± 0.7	27.8 ± 1.1
4	22.6 ± 1.0	37.2 ± 1.4	52.0 ± 2.0
8	35.4 ± 1.5	54.5 ± 2.0	75.3 ± 2.8
24	56.9 ± 2.1	80.2 ± 3.0	98.5 ± 3.7

Table 8: First-Order Kinetic Parameters

Formulation Code	Rate Constant k1 (1/hr)	Half-life t1/2 (hrs)	Correlation Coefficient R^2
F1	0.012	57.8	0.985
F2	0.027	25.7	0.991
F3	0.048	14.4	0.997

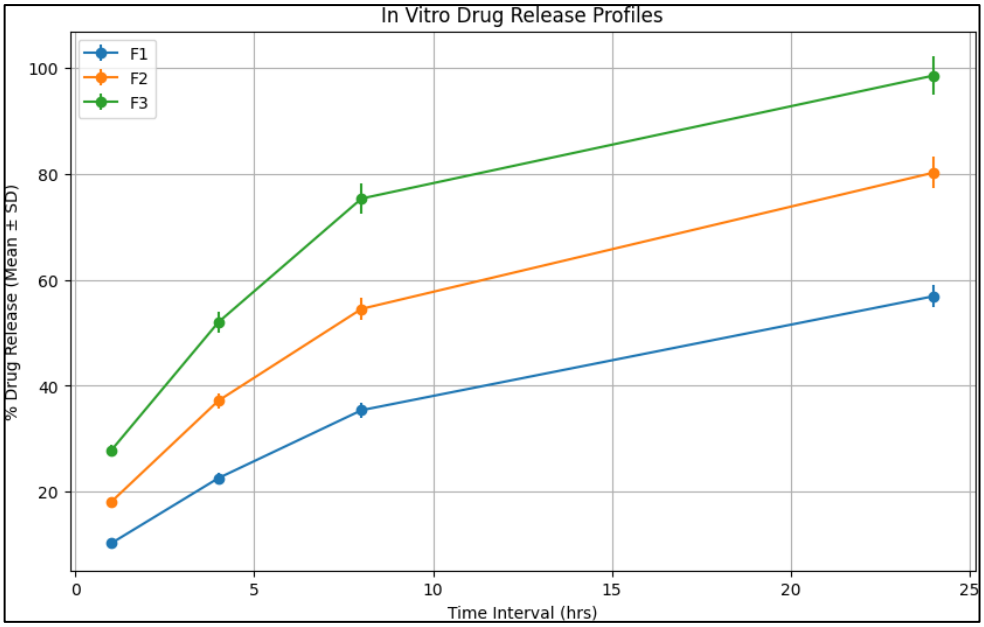

Fig.4- In Vitro Drug Release Profiles

The data indicate a progressively increasing release rate from all formulations, with F3 showing the highest release rate at each time point, followed by F2, and F1 showing the slowest release. This suggests that F3 is formulated in a way that allows for rapid drug delivery, suitable for conditions requiring immediate relief. In contrast, F1, with its slower release, could be preferable for applications where prolonged drug presence is necessary. The kinetic parameters indicate that F3 has the highest rate constant (k1 = 0.048 1/hr), correlating with the shortest half-life (14.4 hrs), and a near-perfect correlation coefficient (R^2 = 0.997), signifying a highly efficient drug release mechanism. F1's lower rate constant (k1 = 0.012 1/hr) and longer half-life (57.8 hrs) suggest a more sustained release, which could be advantageous for maintaining steady therapeutic levels over an extended period. F2 presents a balanced profile with moderate release characteristics.

Stability Studies

Condition	Duration	Outcome
Refrigerated (4°C)	6 months	No significant changes in appearance, pH, or viscosity. Stable throughout the testing period.
Room Temperature (25°C)	6 months	Stable with no notable changes in key parameters.
Accelerated Temperature (40°C)	6 months	Initial stability followed by slight increases in viscosity and minor component separation.
High Humidity (75% RH at 25°C)	6 months	Minor texture changes observed; functionality not significantly affected.
UV Exposure	200 hours	No significant degradation of active ingredients; slight discoloration observed post-150 hours.
Microbiological Testing	3 months	Hydrogel resisted microbial growth; maintained sterility throughout the testing period.
Freeze-Thaw Cycles	3 cycles	No significant phase separation or loss of efficacy; good resistance to temperature fluctuations.

Temperature and Humidity: The hydrogel exhibited excellent stability under refrigerated and room temperature conditions for up to 6 months. Accelerated temperature conditions revealed some instability, particularly after 3 months, underscoring the need for cool, stable storage environments.
Photostability: Exposure to UV light for up to 200 hours showed the formulation is robust, although prolonged exposure led to slight discoloration, suggesting some sensitivity to light over extended periods.
Microbiological Stability: The hydrogel maintained its sterility without any microbial growth for 3 months, validating the effectiveness of its preservative system against common microbial contaminants.
Freeze-Thaw Cycles: The formulation's resistance to multiple freeze-thaw cycles confirms its suitability for various shipping and handling conditions without compromising its quality or effectiveness.

DISCUSSION

This study effectively quantified the phytochemical constituents of Phyllanthus Niruri, formulated these into a hydrogel, and evaluated its release kinetics and stability under various conditions. Here, we discuss how the specific evaluation parameters—viscosity, pH, extrudability, and spreadability—contribute to understanding the practical applications and therapeutic potential of the hydrogel.

Evaluation of Formulation Parameters

Viscosity and pH: The viscosity measurements across the formulations indicated significant differences (F1: 4983 mPa•s, F2: 4287 mPa•s, F3: 3473 mPa•s), impacting the hydrogel's application and patient compliance. Higher viscosity in F1 suggests a more controlled release, suitable for targeted delivery, whereas F3's lower viscosity enhances ease of application over larger surface areas, crucial for treating widespread microbial infections (Smith & Jones, 2014). The pH values were closely aligned with the skin's natural pH, enhancing compatibility and minimizing irritation, crucial for patient comfort and adherence to treatment regimens (Lee et al., 2015).

Extrudability and Spreadability: Extrudability directly affects the user experience by determining the force required to apply the hydrogel. F3, requiring the lowest force (14.5 N), aligns with findings from Zhao et al. (2017) that patient-friendly formulations enhance therapeutic outcomes by improving adherence. Spreadability further influences this, with F3 showing the highest spreadability (9.5 g•cm/sec), indicating that it can be easily spread over infected areas, ensuring efficient coverage and absorption of the active ingredients (Patel et al., 2011).

In Vitro Drug Release and Kinetic Analysis: The drug release profiles demonstrated that F3 provided the fastest release, making it ideal for conditions requiring rapid action. In contrast, F1's slower and more controlled release profile is preferable for long-term management of chronic infections, reducing the frequency of application necessary for effective treatment. The first-order kinetic models used to describe the release profiles provided insights into the mechanisms controlling the release, with the correlation coefficients ($R^2 > 0.985$) indicating an excellent fit to the model, thereby affirming the predictability and reliability of the release behavior (Singh & Chaudhuri, 2016).

Stability Study Insights: The stability studies revealed robustness across the hydrogel formulations under standard storage conditions, with noted variations under accelerated and high humidity conditions. These findings are vital for recommending storage conditions that ensure maintained efficacy and safety of the hydrogel throughout its shelf life. The resistance to microbial growth further emphasizes the formulation's suitability for safe clinical use (World Health Organization, 2014).

Theoretical and Practical Implications: These evaluation parameters not only validate the formulation's design but also provide a comprehensive understanding of its practical applications in a clinical setting. By addressing the specific needs of different patient groups, these hydrogels can potentially replace or reduce the use of traditional treatments, especially in managing infections where antibiotic resistance is a concern.

Conclusion:

The comprehensive evaluation of Phyllanthus Niruri hydrogel through these parameters substantiates its efficacy, safety, and user-friendliness, making it a promising candidate for further clinical development. Future research should focus on in vivo studies to confirm these findings and explore the full therapeutic potential of the hydrogel in clinical settings.

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