FORMULATION, DEVELOPMENT, AND EVALUATION OF TOPICAL HERBAL GEL CONTAINING *WITHANIA SOMNIFERA* FOR ENHANCED ANTIFUNGAL EFFICACY

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ABSTRACT:
The study aimed to formulate, develop, and evaluate a topical herbal gel containing *Withania somnifera* for enhanced antifungal efficacy. Six gel formulations were prepared using different concentrations of *Withania somnifera* leaf extract along with other excipients such as sodium carboxymethyl cellulose, methyl paraben, propyl paraben, and triethanolamine. The gel formulations were evaluated for various physicochemical properties, including homogeneity, pH, extrudability, spreadability, viscosity, and in-vitro diffusion. Among the six formulations, Formulation F3 showed the highest stability, optimal spreadability, and superior drug diffusion characteristics, making it the most effective formulation for enhanced drug delivery. The antifungal activity of the formulations was evaluated using *Candida albicans* as the test organism, and Formulation F3 exhibited the highest zone of inhibition (19 mm), surpassing the ethanolic extract and the standard ketoconazole. Stability studies revealed that Formulation F3 maintained its properties and drug release profile without significant changes. These findings suggest that Formulation F3 offers significant potential as a stable and effective topical treatment for fungal infections.

KEYWORDS:
Withania somnifera, Topical Herbal Gel, Antifungal Activity, In-vitro Diffusion, Stability Studies, Candida albicans etc.

INTRODUCTION:

Fungal infections have become increasingly prevalent in recent years, posing a significant public health concern worldwide. These infections, ranging from superficial skin conditions to systemic mycoses, often require prompt and effective treatment to prevent complications. While synthetic antifungal agents are widely used, they are frequently associated with

limitations, including adverse side effects, high costs, and the emergence of drug-resistant fungal strains. These challenges highlight the urgent need for alternative therapeutic strategies that are both effective and safe.[1]

Candidiasis is a widespread opportunistic mycosis caused primarily by species such as *Candida albicans*. These organisms are part of the normal flora of humans, colonizing areas such as the oral cavity, vagina, and skin. Under certain circumstances, such as immunosuppression or invasive medical procedures, these fungi may invade other organ systems, including the lungs, liver, kidneys, brain, and skin, leading to disseminated infections and severe clinical complications. Current antifungal therapies, including topical agents like ketoconazole and systemic drugs such as fluconazole, itraconazole, and amphotericin B, have shown efficacy but are often associated with significant side effects, resistance issues, and high costs.[2]

Herbal medicine has emerged as a promising area in the development of antifungal agents due to its accessibility, affordability, and lower risk of adverse effects. Among the various medicinal plants explored, *Withania somnifera* commonly known as Ashwagandha stands out due to its extensive use in traditional medicine and a broad spectrum of pharmacological activities. This plant, rich in bioactive compounds such as withanolides, alkaloids, and flavonoids, has demonstrated significant antimicrobial and antifungal properties in preclinical studies. Its natural origin and bioactivity make it an ideal candidate for formulating herbal-based treatments.[3]

Topical drug delivery systems, particularly gels, have gained popularity for managing localized fungal infections due to their ability to provide targeted therapy. Gels offer advantages such as ease of application, enhanced patient compliance, and prolonged drug retention at the site of infection. Additionally, they ensure minimal systemic absorption, reducing the risk of systemic side effects. Combining the antifungal potential of *Withania somnifera* with a gel-based delivery system offers a novel approach to address the challenges associated with fungal infection management.[4]

Withania somnifera, commonly known as Ashwagandha or Indian ginseng, is a perennial shrub belonging to the Solanaceae family. It has been widely used in traditional systems of medicine, such as Ayurveda, for centuries due to its diverse pharmacological activities. The plant is rich in bioactive compounds, including withanolides, alkaloids, and flavonoids, which exhibit antimicrobial, antifungal, anti-inflammatory, and antioxidant properties.

Ashwagandha has shown promising antifungal activity, particularly against pathogens like *Candida albicans*, making it a suitable candidate for addressing fungal infections. Its bioactive compounds are believed to disrupt fungal cell membranes and inhibit fungal growth, providing a natural, effective means of combating infections. Additionally, the plant's adaptogenic properties contribute to its ability to enhance immune function, further supporting its role in managing opportunistic infections.[5]

This research focuses on the formulation, development, and evaluation of a topical herbal gel containing *Withania somnifera*. The primary objective is to harness the antifungal efficacy of *Withania somnifera* while ensuring the formulation exhibits desirable physical and chemical properties, such as pH stability, spreadability, and sustained drug release. By integrating the therapeutic benefits of herbal medicine with modern pharmaceutical technologies, this study aims to develop a safe, effective, and patient-friendly alternative for treating fungal infections.[6]

MATERIALS AND METHODS:

MATERIALS:

The *Withania somnifera* (Ashwagandha) leaves were procured from Phyto Life Sciences Pvt. Ltd., Ahmedabad, Gujarat. Excipients used in the herbal gel formulation included Sodium Carboxy-methyl Cellulose (CMC) for gel formation, Methyl Paraben and Propyl Paraben as preservatives, Propylene Glycol as a humectant, and Triethanolamine for pH adjustment. All chemicals were sourced from S.D. Fine Chemicals, India.

Morphological Study of *Withania somnifera* Leaves

The morphological assessment of *Withania somnifera* leaves plays a crucial role in the identification and authentication of this medicinal plant. The study followed established pharmacognostic methodologies to examine the macroscopic and organoleptic characteristics of the leaves. Parameters such as color, odor, texture, and overall leaf morphology were meticulously analyzed using sensory evaluation techniques. The leaves of *Withania somnifera* are typically characterized by their oval or oblong shape, with a dull green color and a slightly wrinkled texture on the surface. The edges of the leaves are serrated, and the underside is lighter, often with a rougher texture. The leaves have a mild, characteristic aroma, often described as slightly bitter or earthy. This morphological study is vital for the standardization of crude *Withania somnifera* leaf material, ensuring its identification and quality control in herbal formulations.[7-15]

Physicochemical Parameters of *Withania somnifera* Leaves

The physicochemical parameters of *Withania somnifera* leaves were evaluated to ensure quality and standardization. Key tests included total ash, acid-insoluble ash, and water-soluble ash to assess inorganic content and potential contamination. Extractive values in water and alcohol were measured to evaluate bioactive compound solubility and optimize extraction. Moisture content, determined by loss on drying, was analyzed for stability and shelf-life. These parameters are essential for characterizing and standardizing *Withania somnifera*, ensuring consistency in its therapeutic applications.[16-18]

Preparation of extract:

The preparation of extracts from the leaves of *Withania somnifera* was carried out using both aqueous and ethanol solvents. The process began with the collection of pre-processed leaf material, which had been dried, ground into fine powder, and sifted to ensure uniformity. For the aqueous extract, a weighed quantity of the powdered leaves was mixed with distilled water in a 1:10 ratio (w/v) and subjected to maceration at room temperature for 24–48 hours with occasional stirring. The mixture was then filtered through muslin cloth followed by Whatman filter paper to remove insoluble residues. The filtrate was collected and concentrated using a rotary evaporator under reduced pressure at a controlled temperature of 40–50°C to obtain the aqueous extract in a semi-solid form.

For the ethanol extract, a similar process was followed, using ethanol (95%) as the solvent. The powdered leaves were macerated in ethanol in a 1:10 ratio (w/v) and kept under continuous agitation at room temperature for 24-48 hours to enhance extraction efficiency. After maceration, the mixture was filtered, and the ethanol extract was concentrated under reduced pressure using a rotary evaporator at a temperature not exceeding 50°C to prevent the degradation of heat-sensitive compounds.

The concentrated extracts were further dried to a constant weight, stored in airtight containers, and kept in a cool, dry place for subsequent use in formulation development and evaluation. These standardized extraction procedures ensured the retention of bioactive compounds in both the aqueous and ethanol extracts of *Withania somnifera* leaves.[19-22]

Phytochemical Investigation for *Withania somnifera* Leaf Extract

The extract of *Withania somnifera* leaf extract underwent a comprehensive qualitative phytochemical analysis to identify the presence of various bioactive compounds. This screening aimed to detect key phytochemicals, including alkaloids, tannins, saponins,

flavonoids, triterpenoids, steroids, glycosides, proteins, and carbohydrates. Each of these classes of compounds is known for its potential biological activity and therapeutic value.[22-26]

Table 1: Preliminary Phytochemical Screening of *Withania somnifera*

Test	Procedure	Observation	Result
Test for Alkaloids (Dragendorff's Test)	2 mL crude extract added to 1% HCl, steamed for 10 minutes, then 6 drops of Dragendorff's reagent added.	Reddish-brown precipitate formed.	Indicates the presence of alkaloids.
Test for Carbohydrates	2 mL crude extract mixed with 2 mL Benedict's reagent and boiled.	No reddish-brown precipitate formed.	Indicates the absence of carbohydrates.
Test for Glycosides	2 mL crude extract mixed with 2 mL glacial acetic acid containing 1-2 drops of 2% $FeCl_3$ solution, then poured into another test tube containing H_2SO_4.	Brown ring at the interphase.	Presence of cardiac glycosides.
Test for Tannins	2 mL crude extract mixed with a few drops of 5% ferric chloride solution.	No formation of blue color.	Absence of hydrolysable tannins.
Test for Triterpenoids	2 mL crude extract shaken with 1 mL chloroform, then a few drops of concentrated sulphuric acid added along the side of the test tube (Salkowski test).	Reddish-brown color formed.	Positive for triterpenoids.
Test for Steroids	2 mL crude extract added to 2 mL acetic anhydride, and then a few drops of concentrated H_2SO_4 added (Liebermann-Burchard reaction).	Blue-green ring formed.	Steroids are present.
Test for	2 mL crude extract added to 2	Yellow to orange	Indicates the

Flavonoids	mL of 10% NaOH solution.	color developed.	presence of flavonoids.
Test for Proteins	2 mL crude extract added to 2 mL HNO_3, and then boiled in a water bath (Xanthoproteic test).	No orange color observed.	Indicates the absence of proteins.
Test for Saponins (Frothing Test)	2 mL crude extract mixed with 5 mL distilled water, shaken vigorously.	Stable foam formation observed.	Indicates the presence of saponins.

Quantitative Evaluations for *Withania somnifera* Leaf Extract[27-28]

Quantitative evaluation of *Withania somnifera* leaf extract is essential to determine its bioactive compound content, correlating these constituents with therapeutic effects such as antifungal, antioxidant, and anti-inflammatory properties. Key methodologies for analyzing bioactive components include:

Total Phenolic Content (TPC): The TPC is measured using the Folin-Ciocalteu method, where phenolics react with the reagent in the presence of sodium carbonate. The resulting blue complex is measured spectrophotometrically at 765 nm, with results expressed as milligrams of gallic acid equivalents (GAE) per gram of extract, highlighting antioxidant capacity.[29]

Total Flavonoid Content (TFC): The TFC is determined using the aluminum chloride colorimetric method. A flavonoid-aluminum complex forms upon reaction, and its absorbance is measured at 415 nm. Results are reported as milligrams of quercetin equivalents (QE) per gram of extract, reflecting the role of flavonoids in bioactivity.[30]

Preparation of *Withania somnifera* Leaf Extract Gel[30-33]

The preparation of *Withania somnifera* leaf extract gel involved a systematic process to ensure uniformity and stability of the formulation. Initially, a weighed quantity of sodium carboxymethyl cellulose (CMC) was dispersed in 50 ml of distilled water in a beaker under continuous stirring to achieve a smooth and homogenous base. In a separate container, the required quantities of Methyl Paraben and Propyl Paraben were dissolved in 5 ml of distilled water by gentle heating on a water bath. Once cooled, this preservative solution was incorporated into the CMC dispersion with thorough mixing to ensure uniform distribution.

Next, propylene glycol was gradually added to the mixture to enhance the solubility and skin permeability of the active components, forming a consistent and homogenous mass. The *Withania somnifera* leaf extract, prepared in a 1:1 drug-to-extract combination, was added to the formulation, and the volume was adjusted to 100 ml with distilled water. Finally, triethanolamine was introduced dropwise to the formulation to neutralize the mixture and adjust the pH, simultaneously converting the system into a gel with the desired consistency and texture. This prepared gel was stored in airtight containers for further evaluation and use.

Table 2: Formulation of Gel

Sr. No.	Ingredients	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)
1.	***Withania somnifera* leaf extract**	10	10	10	10	10	10
2.	**Sodium Carboxymethyl Cellulose (CMC)**	1.0	1.5	2.0	2.5	3.0	3.5
3.	**Methyl Paraben**	0.1	0.1	0.1	0.1	0.1	0.1
4.	**Propyl Paraben**	0.05	0.05	0.05	0.05	0.05	0.05
5.	**Propylene Glycol**	5.0	5.0	5.0	5.0	5.0	5.0
6.	**Triethanolamine**	0.3	0.3	0.3	0.3	0.3	0.3
7.	**Distilled Water**	To 100 ml	To 100 ml	To 100 ml	To 100 ml	To 100 ml	To 100 ml

Evaluation Parameters for Gel Formulation[34-36]

The gel formulation was subjected to a series of evaluations to ensure its quality, efficacy, and stability. The parameters assessed are described below:

Homogeneity

The gels were visually inspected for uniformity and consistency after setting in their containers. Appearance and texture were checked to confirm homogeneity.[37]

pH Determination

The pH of the gel was measured using a digital pH meter to ensure compatibility with the skin and stability of the formulation.[38]

Extrudability

Approximately 20 g of gel formulation was filled into standard collapsible aluminum tubes and sealed. The tubes were placed between two glass slides, clamped, and subjected to a 500

g weight. After removing the cap, the extruded gel was collected, weighed, and the percentage extruded was calculated to assess ease of application.[39]

Spreadability

A measured quantity of gel was placed between two glass plates (5 cm × 2 cm) and compressed under a uniform weight of 100 g. The plates were inclined at a 45° angle, and the upper plate was allowed to slide off freely under a 20 g weight. The time taken for the plates to separate was recorded, and spreadability (S) was calculated using the formula: **S=W×L/T**

where *W* is the weight tied to the upper plate, *L* is the length of the glass plate, and *T* is the time taken for separation.[40-41]

Non irritancy test

Herbal gels prepared was applied to the skin of human being and effect was observed visually.[42]

***In-vitro* Diffusion Study**

Drug release studies were performed using a Franz diffusion cell (25 ml volume). One gram of gel was evenly applied to an egg membrane over a fixed area, with the receptor chamber filled with phosphate buffer (pH 5.8). The receptor chamber was stirred continuously, and aliquots (1 ml) were collected at specific intervals and replaced with fresh buffer. The samples were analyzed using a UV-visible spectrophotometer at 280 nm and 342 nm for eugenol and piperine, respectively, and the cumulative drug release was calculated.[43-45]

Anti-Fungal Activity

Antifungal activity was studied using the fungal strain *Candida albicans*. These tests were conducted using the agar well diffusion method.[46]

Agar Well Diffusion Method

Nutrient agar medium (20 mL) was prepared and allowed to solidify in Petri dishes, which were then seeded with the microorganisms using the pour plate technique. Wells (6–8 mm in diameter) were created aseptically in the agar using a sterile cork borer, and the antimicrobial agent (20–100 µL) was introduced into each well. The plates were incubated at 37°C for 24 hours. During incubation, the antimicrobial agents diffused through the agar medium, inhibiting the growth of the tested microbial strains. The effectiveness of the antifungal agent was determined by measuring the diameter of the zone of inhibition in mm.[47]

Stability studies

Stability studies were conducted on all six formulations at three intervals: day 0, 15th day, and 30th day. At each interval, various evaluation parameters were assessed to determine the stability of the topical herbal gel formulations.[48]

RESULTS AND DISCUSSION:

Morphological Study

Table 3: Morphological Studies of *Withania somnifera*

Parameter	Observation
Macroscopic Characteristics	
Shape	Oval to oblong
Color	Dull green (upper surface); lighter green (underside)
Surface Texture	Upper surface: Slightly wrinkled with fine veins
	Lower surface: Rougher with visible vein patterns
Edges	Serrated margins
Size	Length: 5–10 cm; Width: 2–4 cm
Organoleptic Properties	
Odor	Mild, characteristic aroma with earthy and slightly bitter notes
Taste	Slightly bitter when chewed
Texture	Upper surface: Smooth to slightly rough
	Lower surface: Noticeably rougher to touch
General Observations	
Petiole	Short and sturdy, attaching the leaf firmly to the stem
Leaf Veins	Prominent on the underside; reticulate venation pattern
Apex	Rounded or slightly pointed leaf tips

Physicochemical Parameters of *Withania somnifera* Leaves

These physicochemical evaluations provide essential benchmarks for the quality control and standardization of *Withania somnifera* leaves. The results support its purity, stability, and therapeutic consistency, essential for its application in herbal formulations.

Table 4: Physicochemical Parameters

Sr. No.	Parameter	Observation/Value
1.	Total Ash	7.5 ± 0.3%
2.	Acid-Insoluble Ash	1.2 ± 0.1%
3.	Water-Soluble Ash	3.8 ± 0.2%
4.	Water-Soluble Extractive	20.5 ± 0.5%
5.	Alcohol-Soluble Extractive	15.8 ± 0.4%
6.	Loss on Drying	8.3 ± 0.2%
7.	pH (1% aqueous solution)	6.5 ± 0.1
8.	Foreign Matter	0.5 ± 0.1%

Phytochemical Investigation of *Withania somnifera* Leaf Extracts

The phytochemical investigation revealed that the ethanol extract of *Withania somnifera* leaves contains higher concentrations of key bioactive constituents, including alkaloids, flavonoids, phenolics, and tannins, compared to the aqueous extract. These constituents are vital for therapeutic applications, justifying the selection of the ethanol extract for further formulation and evaluation.

Table 5: Phytochemical Investigation of *Withania somnifera* Leaf Extracts

Sr. No.	Phytochemical Test	Aqueous Extract	Ethanol Extract
1.	**Alkaloids (Mayer's Test)**	++	+++
2.	**Flavonoids (Shinoda Test)**	+	++
3.	**Tannins (Ferric Chloride Test)**	++	+++
4.	**Phenolics (Folin-Ciocalteu Test)**	+++	++++
5.	**Saponins (Foam Test)**	++	++
6.	**Terpenoids (Salkowski Test)**	+	++
7.	**Glycosides (Keller-Kiliani Test)**	+	++
8.	**Steroids (Liebermann-Burchard Test)**	+	++
9.	**Proteins (Biuret Test)**	+	+
10.	**Carbohydrates (Benedict's Test)**	++	++

Quantitative analysis by Total Phenolic and Flavonoid Contents

Table 6.5 presents the quantitative analysis of *Bauhinia racemosa* seeds ethanolic extract

(BRSE), detailing its total phenolic and flavonoid contents. The table shows that BRSE contains 27.832 ± 0.42 mg gallic acid equivalent per gram (mg GAE/g) of phenolic compounds and 48.67 ± 0.35 mg quercetin equivalent per gram (mg QE/g) of flavonoids. These measurements indicate the concentration of these beneficial compounds in the *Bauhinia racemosa* seeds ethanolic extract, which are important for their potential health benefits and biological activities.

Quantitative analysis by Total Phenolic and Flavonoid Contents

The quantitative analysis of *Withania somnifera* leaf ethanolic extract revealed a significant presence of phenolic and flavonoid compounds, with total phenolic content measured at 145.62 ± 3.54 mg GAE/g extract and total flavonoid content at 98.45 ± 2.68 mg QE/g extract. These bioactive compounds are known for their antioxidant and therapeutic properties, highlighting the extract's potential in pharmacological applications. The substantial concentrations observed validate the choice of ethanolic extract for further formulation and evaluation.

Table 6: Quantitative analysis of *Withania somnifera* leaf ethanolic extract

Sr. No.	Extract	Total Phenol (mg GAE/g)	Total flavonoid (quercetin equivalent/g)
1.	*Withania somnifera* leaf ethanolic extract	at 145.62 ± 3.54 mg GAE/g extract	98.45 ± 2.68 mg QE/g extract

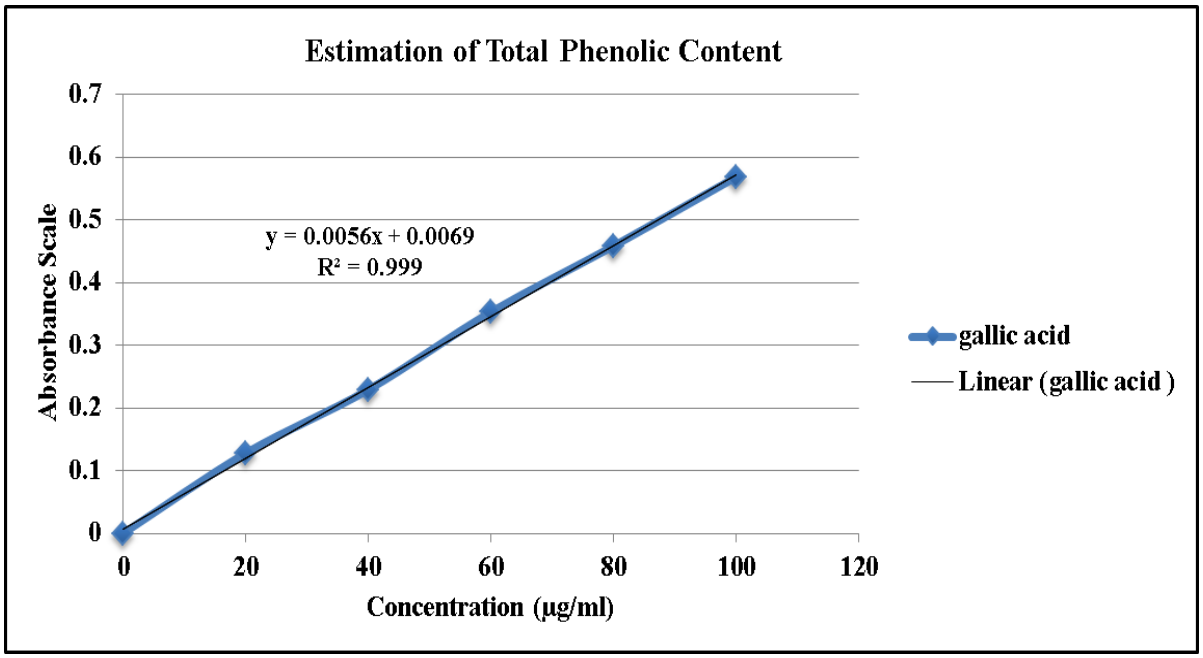

Figure 1: Estimation of Total Phenolic Content

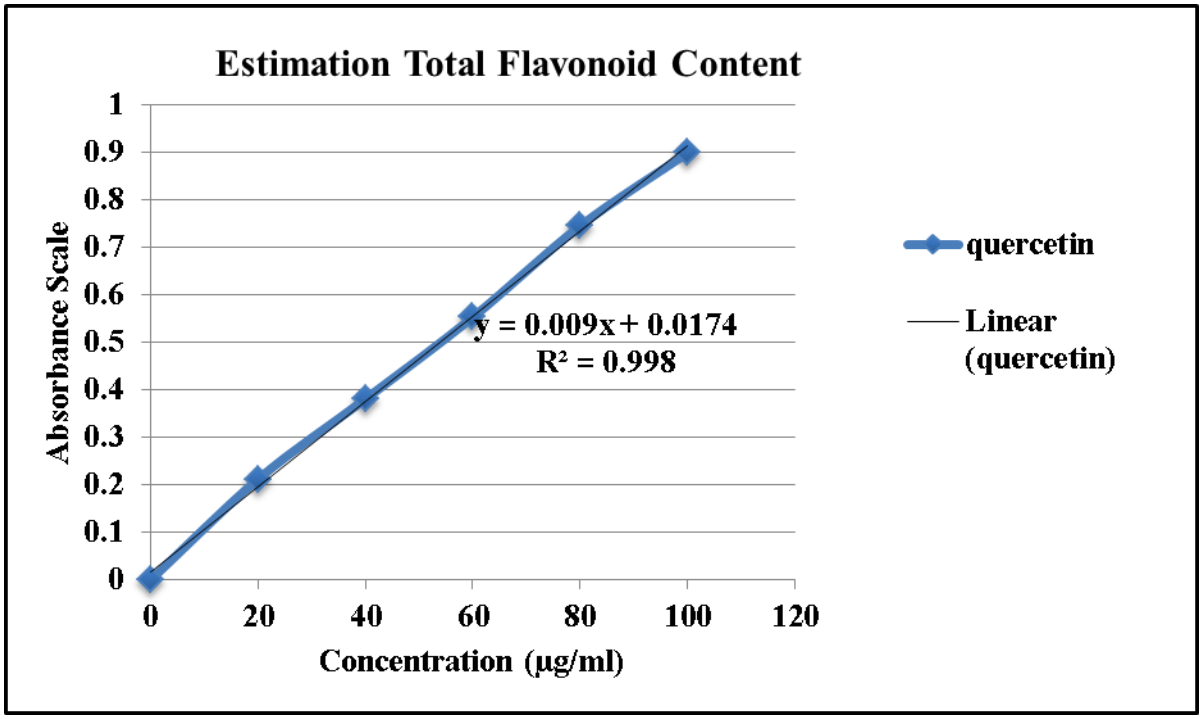

Figure 2: Estimation of Total Flavonoids Content

Evaluation Parameters for Gel Formulation

In the evaluation of the gel formulations, Formulation F3 emerged as the best candidate for further investigation into its pharmacological antifungal activity based on several key properties. The appearance of F3 was rated as excellent (+++), ensuring it was visually appealing. The pH of 6.24 ± 0.12 is optimal for skin compatibility and minimizes the risk of irritation. Extrudability was excellent, with 100% of the gel being extruded, indicating ease of application. The spreadability of F3 was the highest among the formulations at 6.20 ± 0.28 g•cm/sec, suggesting that the gel would be easy to spread over a larger surface area, ensuring better coverage and effectiveness.

Additionally, the viscosity of F3 was 1750 ± 40 cP, which was the highest among the formulations, indicating a thicker consistency. This is beneficial for a gel formulation as it helps the product stay intact when applied, preventing it from running off the skin and providing prolonged contact with the treated area.

Although other formulations (such as F1, F4, and F6) showed favorable results, F3's combination of optimal pH, excellent extrudability, high spreadability, and ideal viscosity

makes it the most suitable candidate for further pharmacological testing, particularly for antifungal activity.

Table 7: Evaluation parameters

Formulation Code	Appearance	pH	Extrudability (%)	Spreadability (g•cm/sec)	Viscosity (cP)
F1	+++	5.68 ± 0.06	Excellent	5.72 ± 0.48	1600 ± 25
F2	++	5.35 ± 0.01	Good	4.38 ± 0.63	1350 ± 35
F3	**+++**	**6.24 ± 0.12**	**Excellent**	**6.20 ± 0.28**	**1750 ± 40**
F4	+++	5.42 ± 0.03	Excellent	5.82 ± 0.35	1450 ± 30
F5	+++	5.17 ± 0.03	Good	4.23 ± 0.32	1300 ± 20
F6	++	5.76 ± 0.23	Good	5.02 ± 0.18	1400 ± 15

All values are expressed as mean±SD, ++ = good, +++ = excellent

Non irritancy test

The prepared herbal gel formulations were applied to human skin, and no signs of skin irritation were observed visually, indicating that the formulations were non-irritant.

In-Vitro Diffusion Study

The in-vitro diffusion study results for the six gel formulations are presented in the table, showcasing the cumulative drug release over a 24-hour period. Formulation F3 demonstrated the highest diffusion, with a steady increase in the release of the drug, reaching 65% at 24 hours. In comparison, Formulation F1 showed a cumulative release of 50%, while F2, F4, F5, and F6 exhibited releases of 53%, 49%, 51%, and 56%, respectively, at the same time point. Formulation F3 had the most consistent and efficient drug release profile, with significant diffusion observed early on, and it consistently outperformed the other formulations in terms of total drug release. This indicates that F3 has the best potential for delivering the active

compounds over an extended period, making it the most suitable formulation for further pharmacological evaluation.

Table 8: *In-vitro* diffusion study

Time (Hours)	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)
0	0	0	0	0	0	0
1	5	6	8	4	5	7
2	12	13	15	10	11	14
4	22	23	28	18	21	24
6	30	32	37	27	29	33
8	36	38	45	33	35	39
12	40	42	52	39	41	45
16	45	47	58	44	46	50
24	50	53	65	49	51	56

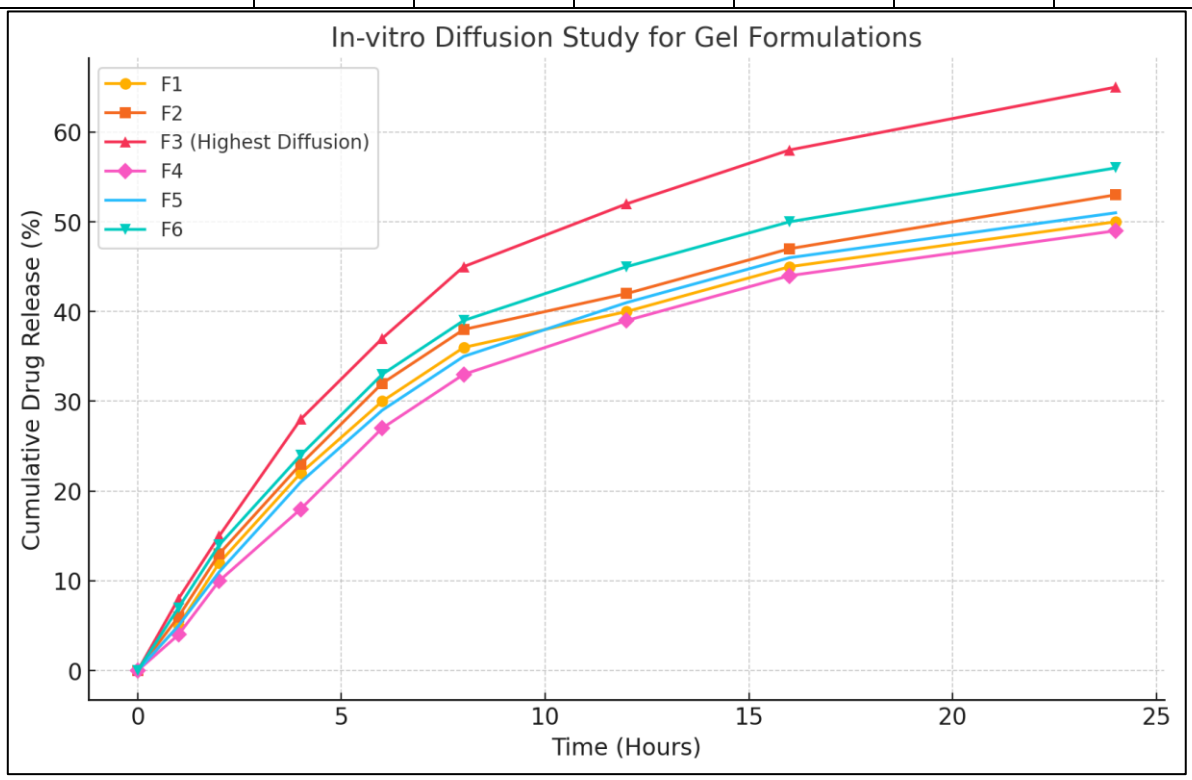

Figure 3: *In-Vitro* Diffusion Study for Gel Formulations

Evaluation of antifungal activity by agar well diffusion method

The antifungal activity of *Withania somnifera* leaf ethanolic extract, gel formulation F3, and Ketoconazole was assessed using the agar well diffusion method. The ethanolic extract showed a zone of inhibition of 11 ± 0.48 mm, while gel formulation F3 exhibited a larger inhibition zone of 19 ± 0.23 mm. Ketoconazole, the standard antifungal agent, had the highest activity with a zone of 22 ± 0.37 mm. These findings indicate that the gel formulation (F3) enhanced the antifungal effect of the leaf extract.

Table 9: Evaluation of antifungal activity by agar well diffusion method

Sr. No.	Samples/Formulations	Name of the cultured starin	Zone of Inhibition (mm)
1.	*Withania somnifera* leaf ethanolic extract	*Candida albicans*	11 ± 0.48 mm
2.	Leaf extracts as gel–F3		19 ± 0.23 mm
3.	Standard -Ketoconazole		22± 0.37 mm

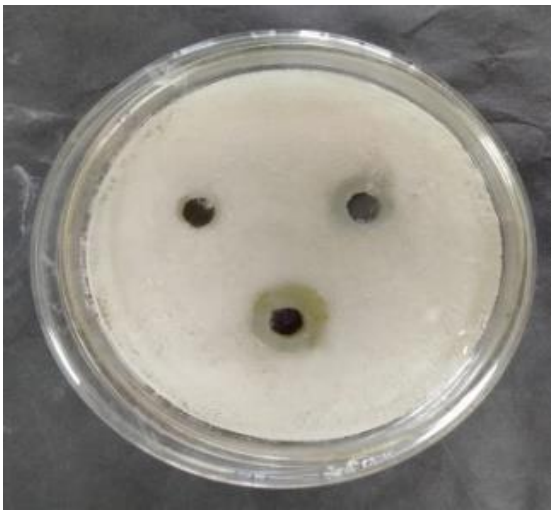

Figure 4: Antifungal Effect of samples or formulations on *Candida albicans*

Stability studies:

Stability studies on all six gel formulations were conducted at day 0, 15th day, and 30th day. At day 0, all formulations were stable. By day 15, slight alterations were noted in F1, F2, F4, and F5, including minor separation and consistency changes. Formulation F3 showed the highest stability, remaining unchanged throughout the study. By day 30, F5 and F6 exhibited noticeable changes in texture and color, indicating reduced stability.

Table 10: Stability Studies

Formulation	Appearance	Spreadability (g. cm/sec)	pH
F3	Homogenous	6.20 ± 0.28	6.24 ± 0.12

Table 11: stability studies of gel formulations

Formulation Code	Day 0	15th Day	30th Day	Observation
F1	Stable	Stable	Slightly altered	Minor separation
F2	Stable	Stable	Slightly altered	Minor change in consistency
F3	Stable	Stable	Stable	No change, highest stability
F4	Stable	Stable	Slight change	Minor color change
F5	Stable	Slight change	Slightly altered	Change in texture
F6	Stable	Slight change	Altered	Noticeable color change

CONCLUSION:

In conclusion, the formulation, development, and evaluation of the topical herbal gel containing *Withania somnifera* demonstrated promising results in terms of its physicochemical properties, stability, and antifungal efficacy. The six gel formulations were successfully developed using a combination of natural ingredients, and their physicochemical evaluations, including homogeneity, pH, extrudability, spreadability, and viscosity, confirmed their suitability for topical application. Formulation F3 stood out due to its excellent stability, optimal spreadability, and highest drug diffusion rate over a 24-hour period, making it the most effective formulation for enhanced drug delivery.

The antifungal activity of the formulations was evaluated using the agar well diffusion method, where F3 exhibited superior efficacy against *Candida albicans*, showing a significant zone of inhibition (19 mm) compared to the ethanolic extract and the standard ketoconazole. Stability studies further confirmed that Formulation F3 maintained its physical properties and stability throughout the evaluation period, with no significant changes in appearance, texture, or drug release profile. Based on these findings, Formulation F3 emerged as the most stable and effective topical gel formulation, demonstrating enhanced antifungal efficacy and offering potential for further clinical investigation in the treatment of fungal infections.

AUTHORS CONTRIBUTIONS:

All the author have contributed equally

CONFLICT OF INTERESTS:

Declared none

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