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The Development and Assessment of *Senna tora* Phytosomal Gel for Dermatophytosis

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Abstract

A novel drug delivery system offers an innovative approach to overcoming the limitations of traditional drug formulations. The efficacy of Unani medicine depends on delivering an optimal concentration of therapeutically active compounds. Phytosomes, a vesicular drug delivery system, have been shown to enhance the absorption and bioavailability of herbal extracts, particularly in topical applications. *Senna tora* (Tukhm-e-Panwar) seeds contain anthraquinone glycosides, primarily Chrysophanic acid-9-anthrone, which exhibit significant antifungal activity. Dermatophytosis (Qooba) is a widespread public health concern, affecting 20-25% of the global population, as per WHO estimates. This study aimed to develop and evaluate a phytosomal gel containing *Senna tora* seed extract for its antifungal effectiveness in treating Dermatophytosis. Phytosomes were formulated using the anti-solvent precipitation method, with varying *Senna tora* seed extract-to-soya lecithin ratios. Among the three batches, Batch P1 (1:1 ratio) was optimized based on morphology, percentage yield, entrapment efficiency, and drug content. The optimized Batch P1 was further characterized for zeta potential (-55.3 mV), particle size (196.9 nm), entrapment efficiency (95.44%), and drug content (87.11% w/w). Additionally, Differential Scanning Calorimetry (DSC), Fourier Transform Infrared Spectroscopy (FTIR) for compatibility, Scanning Electron Microscopy (SEM), and in vitro drug diffusion study were conducted. Subsequently, four batches of phytosomal gel were prepared using 0.4% w/w of optimized Batch P1, with varying concentrations (1.25% w/w to 2% w/w) of Carbopol 934 as a gelling agent. These formulations were evaluated for organoleptic properties, homogeneity, pH, drug content, spreadability, extrudability, viscosity, and in vitro release. Batch PG1 was identified as the optimized formulation based on the results and was further assessed for antifungal activity against Trichophyton rubrum and stability. The optimized phytosomal gel Batch PG1 exhibited a visually appealing appearance, a pH of 6.5, a drug content of 96.45%, and an in-vitro drug release of 81.09%. It demonstrated enhanced skin permeability and strong antifungal activity against Trichophyton rubrum, reinforcing its potential as an effective topical treatment for dermatophytosis.

Keywords: Phytosomal gel, *Senna tora*, Dermatophytosis, Trichophyton rubrum, antifungal activity, skin permeability.

Introduction

Phytosomes are used as a new advanced modern dosage formulation technology for unani drugs, demonstrating superior absorption and, consequently, enhanced efficacy compared to traditional unani extracts (1). The term “phyto” means plant, while “some” means cell-like. Phospholipids play a crucial role in the development of phytopharmaceuticals due to their compatibility with phytochemicals. Typical-

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ly, phytosomes are prepared by combining an exact amount of phospholipid, such as soya lecithin, with herbal extracts in a solvent. Soya lecithin is a key component containing phosphatidylcholine, which exhibits both lipophilic and hydrophilic properties. The active constituents bind to the choline part, while the phosphatidyl portion forms an envelope around the choline and phytoconstituent complex. This process results in the formation of a lipid complex with improved stability and bioavailability.The entrapment of compounds with phospholipids leads to the formation of hydrogen bonds, resulting in high entrapment efficiency, an improved stability profile, enhanced bioavailability, and greater efficacy. Phytosomes have significant functional importance in skin-related applications and delivery. They enhance the absorption of phytoconstituents through the skin, regulate the physiology of skin components, and improve overall skin function (2-4). Tukhm-e-Panwar (*Senna tora*), commonly known as Purslane, is a well-regarded medicinal herb in the Unani system of medicine, traditionally used for its wide range of therapeutic properties, including its role in treating skin disorders such as Qooba (Dermatophytosis) (5,6). During the rainy season, *Senna tora*, a native annual herb of the Leguminosae family, grows throughout Maharashtra. It is known as Tarota in Marathi and Ponwar in Hindi. Dermatophytoses are becoming a major health concern worldwide, affecting children, adolescents, and adults. In India, 5 out of every 1000 people have a tinea infection. *Senna tora* exhibits a broad spectrum of therapeutic effects, including antiviral, antibacterial, antifungal, antitumor, anthelmintic, analgesic, hypotensive, wound healing, anti-inflammatory, anticancer, antioxidant, and immune-enhancing properties. Phytochemical investigations have revealed that the seeds contain anthraquinones such as chrysophanol, physcion, emodin, rhein, obtusifolin, obtusin, chyrso-obtusin, rubrofusarin, aurantio-obtusin, and chrysophanic acid-9-anthrone. Various studies have indicated that the plant possesses antifungal activity, with chrysophanic acid-9-anthrone against Trycophyton rubrum.This study set out to address the bioavailability, stability and efficacy issue by creating a phytosomal gel formulation of Tukhm-e-Panwar (*Senna tora*), which would increase its solubility and make it a more potent antifungal agent (7,8).

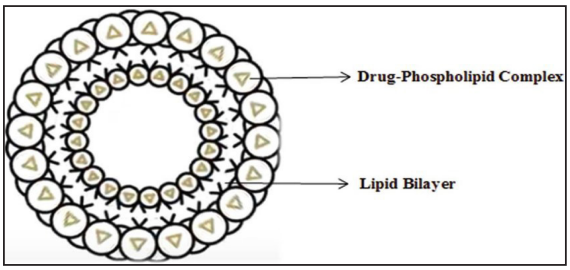


Figure 1: Structure of phytosome

Material and Methods

The *Senna tora* utilized in the study was collected from from local area of Dharmabad Dist Nanded, Maharashtra. The soya lecithin was obtained from SD Fine Chem Ltd., Indore and Dichloromethane, Ethanol, Chloroform, Carbopol 940, Triethanolamine, and PEG400 were obtained from Rankem ltd. The remaining chemicals employed in the experiment were of analytical quality.

Methods

Formulation of phytosomes of seena tora extract

Antisolvent precipitation technique

The Antisolvent Precipitation Technique was used to mix the Seena Tora extract and soya lecithin, at molar ratio of 1:1, 2:1 and 1:2, to prepare the phytosomes of Tukhem-e-panwar. A 100 ml round bottom flask was filled with the exact proportions of Seena Tora and soya lecithin, and it was rotate for two hours with 30 ml of dichloromethane at a temperature of no greater than 60°C. To make the precipitate, 20 ml of n-hexane were added to the mixture after it had been carefully concentrated to 5 ml, while being constantly stirred. Following filtering, collection, and overnight storage in vacum desiccators, the precipitate was then prepared. Phytosomes are packaged in a glass bottle with

an amber colour and kept in a refrigerator at (5-10°C) (9,10).

Uv spectroscopy study (determination of λ max)

To create a starting solution with a concentration of 1000 µg/ml, roughly 100mg of pure *Senna tora* extract was dissolved in 100ml of water. Next, 10ml of this stock solution was diluted with water to a total volume of 100ml. This diluted solution was then analyzed using a spectrophotometer within the 200-400nm wavelength range to determine the wavelength at which maximum light absorption occurred. The wavelength of maximum absorbance was identified as 280nm and selected for use in subsequent experiments.

Table 1: Formulation optimization table of *Senna tora* phytosome

Sr. No	Ingredients	Role	F1	F2	F3
1	Seena Tora (g)	Antidermatophytic Action	1	2	1
2	Soyalecithin (g)	Encapsulate drug substances	1	1	2
3	Dichloromethane (ml)	Dissolve phospholipid	30	30	30
4	n-Hexane (ml)	Non-Polar solvent (Rehydrate the thin film)	20	20	20

Table:2 Formulation of Phytosomal Gels (20g) (optimized batch)

Sr. No	Ingredients	Quantity in mg (%w/w)				
		Role of Ingredients	F1	F2	F3	F4
1	Phytosomes	Optimized Phytosomal batch	80 0.4%	80 0.4%	80 0.4%	80 0.4%
2	Carbopol 934	Gelling agent	250 1.25%	300 1.5%	350 1.75%	400 2%
3	Triethanolamine	PH adjuster	200 1%	200 1%	200 1%	200 1%
4	Propyl paraben	Preservative	2 0.01%	2 0.01%	2 0.01%	2 0.01%
5	Methyl paraben	Preservative	36 0.18%	36 0.18%	36 0.18%	36 0.18%
6	Polyethylene glycol	Humectant	2000 10%	2000 10%	2000 10%	2000 10%
7	Distilled Water	Solvent	Q.S to make 20g			

Formulation and optimization of phytosomal gels

Preparation of gel base

We made our gel bases by mixing Carbopol 934 into distilled water. We stirred it continuously at a medium speed using a mechanical shaker. Then, we used triethanolamine to adjust the pH of each mixture to be between 5.5 and 6.5.

Incorporation of phytosome into the gel base

The phytosome solution was separately prepared in a beaker and subsequently added to the Carbopol base. Various formulations were created by adjusting the concentration of the gelling agent. These formulated gels were then stored in appropriate containers at room temperature for future investigations (11).Ta-

ble:2 Formulation of Phytosomal Gels (20g) (optimized batch)

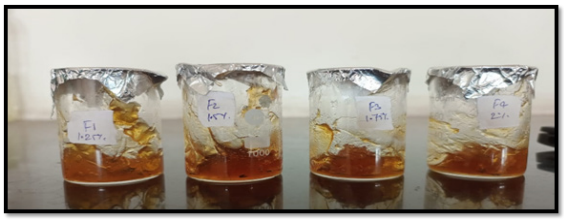


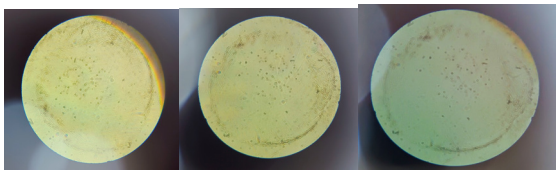
Figure 2: Phytosomal Gel formulation

Results and Discussion

Microscopic characterization phytosomes

Morphology analysis

Phytosomes were analyzed using optical microscopy. Specifically, a phytosome suspension in a buffer solution was prepared and a small drop was placed on a microscope slide, then covered with a coverslip. The resulting sample was observed under a microscope at 100x magnification (12).



P1 at 100x b) P2 at 100x c) P3 at 100x

Figure 3: Microscopic images of Phytosomes

UV spectroscopy study (determination of λ max)

To create a starting solution with a concentration of 1000 µg/ml, roughly 100mg of pure *Senna tora* extract was dissolved in 100ml of water. Next, 10ml of this stock solution was diluted with water to a total volume of 100ml. This diluted solution was then analyzed using a spectrophotometer within the 200-400nm wavelength range to determine the wavelength at which maximum light absorption occurred. The wavelength of maximum absorbance was identified as 280nm and selected for use in subsequent experiments.

Practical yield, drug content, entrapment efficiency

% Practical Yield of different formulations was shown in table no.3 P1 have higher % Practical yield of 37.05% than other formulations (14).

The drug content of Seena Tora extract in the phytosomes complexes was found to be in the range of 87.11% - 61.79% indicating the presence of an acceptable amount of *Senna tora* in the formulations. The percentage of *Senna tora* loading decreased with an increase in the concentration of lipid (Soyalecithin). The formulation P1 showed the maximum *Senna tora* content of 87.11% (15).

The formulation P1 exhibited the highest entrapment efficiency at 95.44%, indicating the optimal lipid concentration required for effective phytosome formation. With further increase in the lipid concentration (Soyalecithin), the entrapment efficiency decreased indicating that the lipid concentration did not help in entrapping the *Senna tora* into the phytosomal complex (16).

Zeta potential of optimized batch (P1)

For Seena Tora phytosomes zeta potential won found -55.3 mV with peak area 100 intensity as shown in fig. no.4 these values indicate that the formulated Seena Tora phytosomes are stable (17).

Table 3: Results of Practical Yield, Drug Content, & Entrapment Efficiency of phytosomes

Sr. No	Formulation	Practical Yield (% W/W) (Mean ± SD, n=3)	Drug Content (% W/W) (Mean ± SD, n=3)	Entrapment efficiency (%) (Mean ± SD, n=3)
1	P1	37.05 ±0.9	87.11 ±0.9	95.44±0.8
2	P2	33.33 ±1.24	34.72±1.07	92.49±1.1
3	P3	18.05 ±1.01	61.79±1.31	93.05±1.5

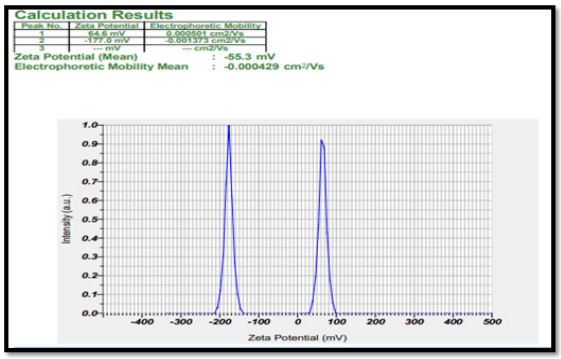


Figure 4: Zeta Potential of optimized of Tukhem-e-panwar phytosomes

Particle size characterization

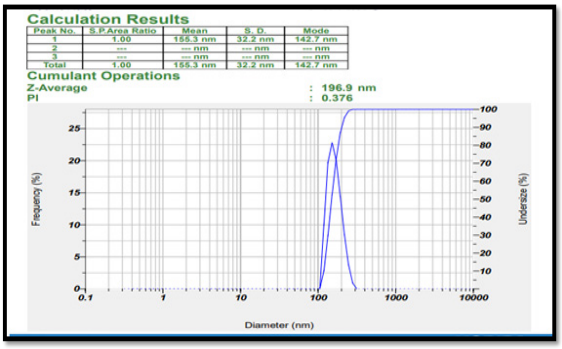


Figure 5: Partical size of optimized of Tukhem-e-panwar phytosomes

The average particle size (z-average) is found to be 196.9 nm. Particle size analysis showed the presence of phytosomes with polydispersity indices PDI value 0.376 as shown in Fig. no.8

Differential scanning calorimetry graph for optimized phytosome formulation (p1)

The formulated phytosome exhibits an endothermic peak at 135.61°C. The observed lower onset (133.29°C) and endset (139.60°C) temperatures, compared to the pure extract, suggest a potential interaction between the Seena Tora seed extract and phosphatidyl choline, resulting in a noticeable depression of the melting point and accounting for the enhanced entrapment efficiency. The enthalpy change of -0.53 J/g accurately represents the energy required for the melting transition in the formulated batch, indicating a potential enhancement in the thermal stability and homogeneity of the formulation (18,19).

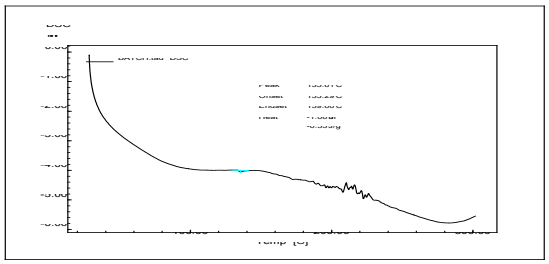


Figure 6: Thermal analysis of (Optimized Batch P1)

Compatibility study by using FT-IR spectroscopy

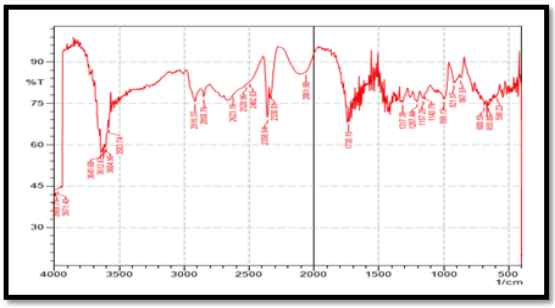


Figure 7: FT-IR spectra of Seena Tora pure extract

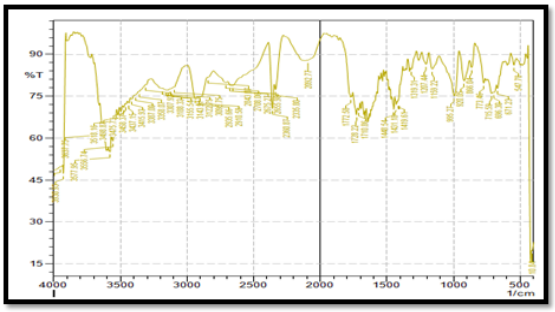


Figure 8: FT-IR spectra of optimized phytosome formulation (P1)

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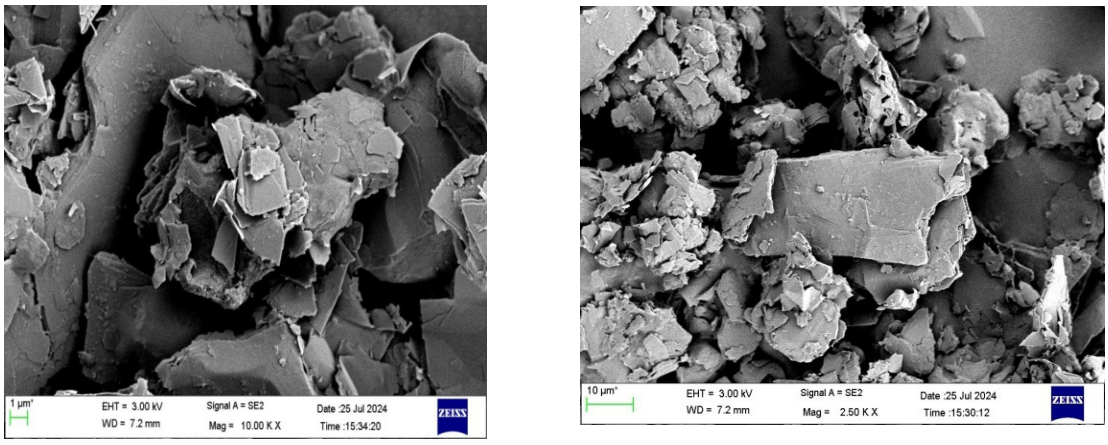
Table 4: FTIR Interpretation of optimized phytosome formulation (P1)

Material	Standard wave number (cm-1)	Test wave number (cm-1)	Functional group assessment
Optimized phytosome formulation (P1)	900-860	866.04	C-H bending
	1870-1650	1728.22	C=O stretching
	2970-2850	2910.58	C-H stretching
	3000-2800	2843.07	N-H stretching
	3700-3500	3358.07	O-H stretching
	1650-1500	1710.86	C=C stretching and
	1300-1000	1159.22	P=O stretching

The FTIR spectral analysis shows characteristic peaks of pure extract and soya lecithin in the FTIR spectra of optimized phytosome formulation which confirms the absence of any chemical interaction between pure extract and formulation excipients; hence pure extract was compatible with all formulation excipients (18,19).

Scanning electron microscopy imaging

The morphology study of optimized batch phytosomes (P1) was studied by scanning electron microscopy as shown in fig. no. 9 The SEM microgram shows smooth and spherical vesicles (20).



A B

Figure 9: Surface morphology study by SEM at (A) 10 K X, (B) 2.50 K X.

In-vitro drug diffusion study of phytosomes

The phytosome form of *Senna tora* demonstrated superior diffusion compared to the standard *Senna tora* extract. Over a 10-hour period, the standard extract only released 54.16% of its *Senna tora* content. In contrast, the phytosome formulations released between 82.56% and 92.57%. The formulation with a 1:1 ratio of *Senna tora* extract to soy lecithin (P1) achieved the highest release, reaching 92.57% at the 10-hour mark. The process of *Senna tora* particles diffusing out of its delivery method is complicated and depends on aspects like particle size, crystal structure, surface area, surface energies, and how well the particles get wet. The improved solubility and, consequently, the better diffusion of the phytosome complex, is due to the wetting and dispersing capabilities of phospholipids, which act as an amphiphilic surfactant(21).

Table 5: In-vitro percentage cumulative drug resealse

Time in hrs	Percentage cumulative drug release (Mean ± SD, n=3)			
	Pure drug extract	P1	P2	P3
0.25	2.51	4.61	3.75	3.04
0.5	9.16	12.49	11.45	9.12
1	14.16	20.19	21.89	19.35
2	17.89	32.09	28.65	20.54
3	24.06	41.34	39.76	35.19
4	32.67	52.56	45.09	43.78
5	37.68	59.66	51.34	50.75
6	43.94	67.43	58.32	55.11
7	48.00	76.15	65.56	63.61
8	56.38	85.89	73.23	69.65
9	61.49	90.85	83.78	76.31
10	66.45	92.57	87.84	82.56

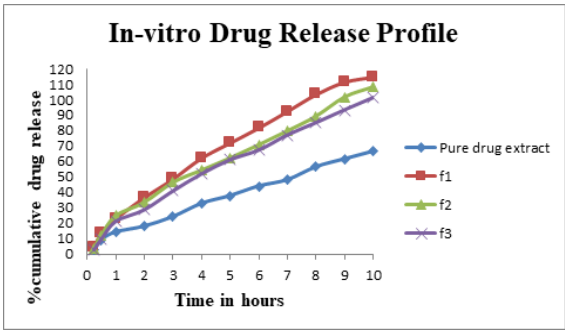


Figure 10: In-vitro Drug Diffusion Profile of Phytosomes Formulation

Evaluation of phytosomal gel

Organoleptic properties

The organoleptic characterization of prepared Gel shows the dark brown,semisolid gel with characteristic odor as shown in table no. 8.

Table 6: Organoleptic properties of Formulation

Sr. No.	Characteristics	Observation
1	Physical nature	Semisolid
2	Color	Dark Brown
3	Odor	Characteristics

Determination of pH & drug content

Table 7: Results of pH & Drug content of different gel formulations

Sr. No.	Formulation	pH	Drug content (%W/W)
1	P1	6.5	96.45
2	P2	6.3	95.56
3	P3	7.1	96.12
4	P4	6.9	93.92

The pH of the gel formulations was in the range of 6.3 to 7.1, which lies in the acceptable pH range (5-7) of the skin and would not produce any skin irritation (22,23).

The drug content of Tukhem-e-panwar in phytosomal gel was found to be in the range of 96.21% - 93.92% indicating the presence of an acceptable amount of drug in the formulations. The formulation P1 showed the maximum drug content of 96.45% (22,23).

Spreadability

The gel was weighed to be as high as 0.5 g and then placed on graph paper coated with plates. Then, we put another plate above the gel mass. Then we added an additional load of 250 g, allowed the gel to stand for 5 min, and measured the diameter of the circle after the spreading of the gels formulation was determined (n=3). The following equation was used to determine the percent spread (24,25).

Table 8: Results of Spredability & Extrudability of Different Gel Formulations

Sr. No.	Formulation	Spreadability (cm)	Spreadability (%)	Extrudability (gm/ cm^2)
1	P1	6.1	610	58.82
2	P2	5.2	520	66.66
3	P3	5.8	580	71.42
4	P4	4.1	410	83.33

The Spreadability test results of phytosomal gel formulation reveal a value between 6.1–4.1 cm, which indicates that the gel has a good spreadability.

Extrudability

In the present study, extrudability was determined by measuring the weight (in grams) required to extrude at least 0.5cm gel from lacquered aluminum collapsible tube in 10 sec. (26).

Rheological study

The measurements of viscosity of prepared gels were carried out with Brookfield Viscometer (spindle type RV-07 (7)) at 20 RPM after maintaining the sample at 30 ± 0.1°C. The readings of each formulation were taken (27).

In-vitro drug release study of phytosomal gel formulation

Preparation of egg membrane

An egg was bought from a nearby department store. The yolk was carefully extracted by creating a small hole in the eggshell. The shell was then submerged in a 0.1N hydrochloric acid (HCl) solution for two hours, under continuous stirring. This process allowed for the complete separation of the egg membrane. Finally, the membrane was rinsed with a phosphate buffer solution at a pH of 7.4, and prepared for use in the experiment.

Drug diffusion through egg membrane

To understand how well the drug in the phytosome gel could permeate a membrane, we used a Franz Diffusion Cell. We set up the experiment by placing an egg membrane (acting as a barrier) between two sections: a “donor” and a “receptor” compartment. The receptor compartment contained 5 ml of a pH 7.4 phosphate buffer, kept at a steady body temperature (37±0.1˚C), and constantly mixed with a magnetic stirrer. Then, 1 gram of the phytosome gel was applied directly onto the egg membrane. We drew samples from the receptor compartment every hour to measure how much drug had passed through the membrane. Each time we took a sample, we replaced it with the same amount of fresh buffer to keep the concentration gradient consistent. These samples were then analyzed using a spectrophotometer at a wavelength of 278 nm. This allowed us to calculate the cumulative percentage of the drug that was released over time (30).

Table 8 and Figure 11 illustrate the comparative percentages of drug permeation achieved by the different formulations over time. After 12 hours, the cumulative percentage of drug released from the gel formulations ranged from 67.33% to 81.09%. Formulation P1 exhibited the highest level of drug release in the in vitro study.

Table 9: Results of In-vitro drug release study of Gel Formulations

Time (Hours)	Cumulative % of drug release			
	P1	P2	P3	P4
0.5	10.25	9.12	8.46	7.22
1.5	14.69	12.89	12.04	10.67
1	15.57	17.65	15.75	13.77
2	27.31	24.68	20.7	18.61
3	35.64	29.62	24.78	22.57

4	41.28	32.11	27.26	25.42
5	45.39	36.57	29.72	29.84
6	51.63	42.0	38.24	36.47
7	61.39	47.18	43.29	41.91
8	67.71	56.71	52.38	49.99
9	80.01	67.88	63.92	57.64
10	88.02	78.80	69.73	68.10
11	91.92	88.13	82.67	77.84
12	99.47	97.26	91.11	82.89

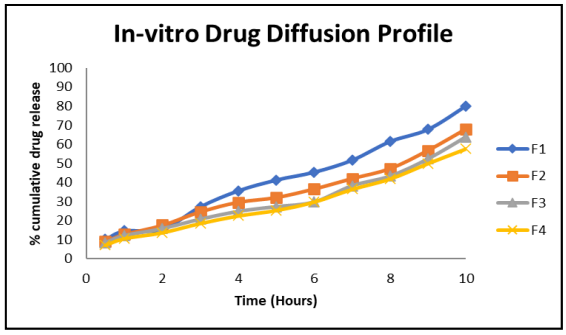


Figure 11: In-vitro Drug Diffusion Profile of Gel Formulations

In-vitro Antifungal Activity study of gel formulation [29,30,31]

The antifungal activity of the formulation was conducted at INFINITE BIOTECH, Sangli, Maharashtra, and evaluated against the fungal strain *Trichophyton rubrum* using the well diffusion method. The inoculum for microorganism was prepared from bacterial cultures, and 15 ml of Saubroad agar medium (HiMedia) was poured into sterilized Petri plates to solidify. A 100 µl broth of the fungal strain was evenly spread over the medium using a spreading rod. Wells of 6 mm diameter were created using a sterile cork borer, and solutions of the test compounds (100 µg/ml in DMSO) were added to the wells. Miconazole (1 mg/ml) served as positive control, and DMSO was used as the negative control. The Petri plates were incubated at 37°C for 24 hours. Antifungal activity was assessed by measuring the diameters of the zones of inhibition, with all determinations performed in triplicate. The antifungal activity of optimized phytosomal gel was evaluated by measuring the zone of inhibition against fungal strain Trycophyton rubrum via well diffusion method. The compounds optimized phytosomal gel exhibited good activity as compared to the standard Miconazole as shown in fig. no. 11 (28).

Table 10: In-vitro antifungal activity of optimized gel formulation 29,30,31

Sr. No	Samples	Concentration (µl/ml)	Zone in diameter (mm) Trycophyton rubrum
1	Control	-	-
2	Standard	100	28
3	P1	100	16

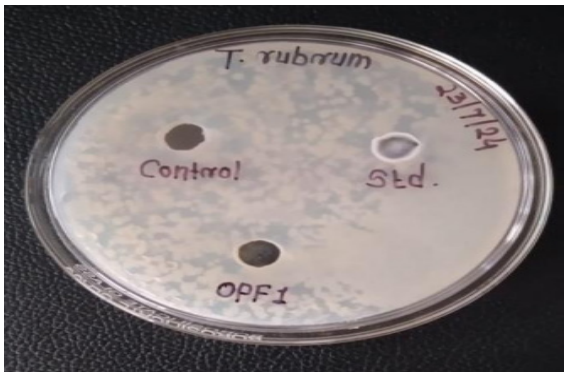


Figure 12: Zone of inhibition against Trycophyton rubrum

Stability study of optimized batch

Stability study at 4^0C±2 &25^0C±2 for optimized batch P1 were subjected to stability study as per ICH guidelines and results were obtained during the stability studies, the appearance of formulation was clear and no significant variations in the organoleptic properties, pH, spreadability and viscosity of optimized formulation for a period of 1 month. Batch P1 was observed to remain stable for a period of 1 month.

Conclusion

Unani medicine is venturing into novel

drug delivery systems, focusing on plant-derived compounds with therapeutic and cosmetic potential, especially those rich in flavonoids and polyphenols. However, these compounds often struggle to be absorbed by the body due to their poor ability to dissolve in fats and their large size, hindering their passage through cell membranes. Phytosomes offer a promising solution, presenting an advanced form of herbal products that are more easily absorbed and utilized, leading to improved results compared to traditional herbal extracts. By complexing plant compounds with phospholipids, phytosomes enhance the stability and fat-solubility of these compounds. This allows for better penetration into cells, higher concentrations in the body, sustained therapeutic effects, and enhanced cosmetic benefits. Studies have shown that a phytosomal gel of *Senna tora* demonstrated superior diffusion and stability, highlighting its potential as a delivery system for various plant compounds. Therefore, phytosomal formulations hold great promise as topical pharmaceutical and cosmetic agents, offering improved safety and effectiveness for herbal drugs, ultimately leading to more cost-effective pharmaceutical products.

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Statements and Declarations

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The authors declare that no funding was received for conducting this study.

Conflicts of interest

The authors do not have any conflicts of interest.

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