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Development And Characterization Of Phytosomes Of Shivlingi Extract (*Bryonia Laciniosa*) For Topical Fungal Infection

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ABSTRACT- The effectiveness of phytosome-loaded with Shivlingi extraxt in treating a topical fungal infection was evaluated and established. The formulation was characterized by physicochemical methods. Methodology: Shivlingi seed extract loaded phytosome gels were prepared, and various analyses were done, including antifungal activity. A number of factors were optimized, including drug content, solubility tests, particle size estimation, FTIR analysis, entrapment effectiveness, *in vitro* release, and stability studies. Result: These studies indicated the successful formation of Shivlingi extracts loaded phytosomes and the optimum and sustained release of drug from topical preparation. Conclusion-The study concludes that topical gel loaded with Shivlingi seed extract phytosome is a promising formulation for topical medication delivery to treat topical infections.

KEYWORDS: Phytosomes, Shivlingi, phytochemicals, *Bryonia laciniosa*, fungal infection.

1. INTRODUCTION-

Plant-based medicines have long been employed in the treatment of human illness on a global scale.[1] Traditional knowledge, knowledge of medical science, clinical experience and scientific research on herbal medicine all contribute to the foundation of modern herbal therapy. People are gradually converting to alternative medical treatments.[2] Herbal products have attracted a lot of interest and access to the global medical markets as it is safer and more competent alternatives to contemporary synthetic medications, which are thought to be full of harmful and unfavorable interactions.[3] Researchers are now concerned about the bioavailability of plant phytoconstituents because most of them are of poor oral bioavailability, particularly the compounds with polyphenolic rings in their structures that are soluble in water, like flavonoids, terpenoids, and tannins. These medications' low bioavailability is brought about by factors such as low water or lipid solubility, poor plasma membrane permeability, high molecular weight/size, etc.[4] In recent years, novel delivery strategies have been tested in an effort to get around these difficulties and increase the potency of herbal medicine. Liposomes, niosomes, transfersomes, and phytosomes are some types of these carriers. In addition to offering scientific evidence to support the standardization of herbal medicine, an innovative herbal medicine delivery system also enables the targeted administration of herbal pharmaceuticals to the right place, at the right dose, for the right amount of time. Along with science and technology, dosage forms have developed,

moving from straightforward tablets to novel carriers that demand a significant amount of ingenuity and cutting-edge technology (NDDS). In recent decades, there has been a lot of research and development into novel approaches to giving herbal medications.[5]

Novel structures called phytosomes contain the active parts of the plant inside a phospholipid membrane. Due to the presence of two fat-soluble tails and a water-soluble head in their molecular structure, phospholipids serve as effective emulsifiers.[4] A chemical known as an emulsifier can mix two liquids which are immiscible. The bioavailability and intestinal absorption of phytosome forms are significantly boosted by combining the phospholipid's emulsifying function with the standardized plant extracts.[6] The phytosome technology has been used to process a variety of well-known herbal extracts, including grape seed, milk thistle, hawthorn, Ginkgo biloba, green tea, and ginseng. These plant extracts' flavonoid and terpenoid components form a direct interaction with phosphatidylcholine. The phosphatidylcholine molecule's choline head specifically attaches to these molecules, and the fat-soluble phosphatidyl part that surrounds the body and tail encloses the choline-bound substance. Consequently, a tiny micro sphere or cell is created.[7] A fungal infection or mycosis, is a skin disease which occurs due to the fungus. The fourth-highest incidence of fungi-related fatalities is brought on by a skin infection.[8] The two most prevalent fungi are mould and yeast.[9] Fungal skin infections can occur anywhere on our body. However, due to potential side effects, long-term systemic antifungal medication in large doses is not recommended. In order to prevent these negative effects and the worrisome possibility of drug resistance, topical therapy should be taken into consideration the first-line therapeutic choice for first or recurrent occurrences of topical fungal infections.[10] Shivlingi, also known as *Bryonia laciniosa* Linn. (Cucurbitaceae), is categorized by Ayurveda as Vrishyarasayana, which means "sustaining fertility and sexual performance." The Ayurvedic prescription "Striravallabhpugpak," illustrated in ancient scriptures to improve sexual behavior, contains the Shivlingi seed as a key ingredient. They have anti-inflammatory, anti-diabetic, anti-microbial, antifungal, analgesic, antipyretic qualities and traditionally have been employed as an aphrodisiacs and fertility boosters.[11-12] The bioavailability of numerous well-known herbal extracts has been improved with the use of phytosome technology. In this study, phytosome-loaded with Shivlingi extraxt was prepared and evaluated for effective treatment of topical fungal infection.

2. MATERIALS AND METHODS

2.1 Materials: The seeds of Shivlingi were obtained from Sangam Seed farm, Bhopal, India and preserved at room temperature for future research. Purchases of the additional chemicals and solvents were made at Rankem (Sugandha Enterprises PaontaShahib), H.P., and S D Fine Chem. Ltd., Bhopal, India.

2.2 Methods

2.2.1 Preparation of plant extract: Approximately 10 gm of seeds were ground into a fine powder and cooked in 100 ml of purified water for two hrs. For additional trials, the extract was filtered and placed in a refrigerator (4°C) for storage.

2.2.2 Phytochemical Screening of crude extract: Shivlingi seed extracts were screened phytochemically, for that we qualitatively analyzed the following compounds: (triterpenoids, glycosides, volatile oil, alkaloids, flavonoids, phenols, anthraquinones, tannins, proteins and amino acids).[13] Briefly, Fehling's test, Iodine test, Biuret test, Ninhydrin test were used to detect the Carbohydrate, starch, Proteins and Amino acids. Dragondroff's reagent was used to detect the alkaloids. Borntragers test, Shinoda test, Legal test, Foam test, Liebermann-Burchard's test, Salkowski's test were used to detect Anthraquinones, Flavonoids, Glycosides, Saponins, Steroids, Sterols, Triterpenoids, Tannins and Phenolic Compounds.

2.2.3 Antifungal activity of Shivlingi seeds extract:

Each substance was diffused in DMSO at concentrations of 5 mg/ml and 10 mg/ml for an antifungal investigation, and the mixture was kept chilled until it was employed. Agar well diffusion assay was used to evaluate the sample's antifungal properties.[14]

2.2.4 Thin layer chromatographic (TLC) analysis: The extract was split with petroleum ether, put to a silica gel 60 GF254 TLC plate that had been coated with toluene: methanol (96:04), and then developed in that solvent system. To show the dots, Anisaldehyde-sulfuric acid reagent was sprayed onto the plate, which was then heated at 110°C for 3–6 minutes.

2.2.5 Determination of λmax and preparation of calibration curve[15]

In order to create the stock solution, 100 mg of pure Shivlingi seed extract was liquified in 100 ml phosphate buffer having pH 7.4. Then the solution was scanned between 200 and 400 nm to determine its maximum absorbance. The obtained maximum wavelength was 271 nm, which was applied to further study. A UV spectrometer was used to measure the serially diluted solutions at the drug's 271nm wavelength. The calibration curve's slope was calculated by plotting the absorbance on the Y-axis and the concentration in g/ml on the X-axis.

2.2.6 Formulation of phytosomes of Shivlingi seeds extract by antisolvent precipitation technique (Table 1)

Shivlingi extract and soy lecithin were combined in a vacuum rotary evaporator flask at molar ratios of 1:1, 1:2, and 1:3 to create the phytosomes of seed extract.[16-17]

Table 1: Formulation table of Shivlingi phytosomes

Ingredients	F1	F2	F3
Seed extract: Soyalecithin	1:1	1:2	1:3
Dichloromethane (ml)	20	20	20
Ethanol (ml)	5	5	5

2.2.7 Characterization of the prepared phytosomes

I. Percentage Practical Yield

In order to determine the percent yield or efficiency of any process, percentage practical yield was determined; this helped producers choose the best way of production.[18] To calculate the practical yield using the following equation, phytosomes were produced, collected and weighed.

(%) Yield =

II. Entrapment efficiency

The formula used to determine the percentage of drug entrapment is given as:

$$\text{Entrapment efficiency}(\%) = \frac{(\text{Total amount of drug}) - (\text{Amount of free drug})}{\text{Total amount of drug}} \times 100$$

III. Drug content

After the appropriate dilutions, the drug content was calculated spectrophotometrically by using a UV spectrophotometer.[18]

IV. *In-vitro* diffusion study through egg membrane

The Franz Diffusion Cell through egg membrane was taken in use for the *in-vitro* diffusion study. Samples that had been withdrawn were examined spectrophotometrically at 271nm.[19]

V. *Ex-vivo* Skin Permeation Study

An *in-vitro* Franz Diffusion Cell was used to examine the permeation of Shivlingi seeds extract from phytosomes. Samples that had been withdrawn were examined spectrophotometrically at 271nm.[20]

VI. Scanning Electron Microcopy (SEM) Analysis

For the determination of surface structure of the phytosome, SEM of complex was carried out by Scanning Electron Microscope at MANIT, Bhopal. The sample was examined using a secondary electron detector linked to scanning electron microscopy to characterize its morphology.[20]

2.2.8 Formulation of gels of phytosome

I. Experimental design

Carbopol 940 (X1) and PEG 400 (X2) concentrations, two independent variables, were investigated at three levels each. The study was conducted at the origin (0, 0). According to the software design expert, Table 2 summarized information about the nine experimental runs that were investigated, their factor combinations, and the conversion of the coded levels to the experimental units used in the study. Cumulative Drug permeation percentage (% CDP) was used as the response variable.[21] (Table 2)

Table 2: Formula given for the formulation of carbopol gels according to design expert

S. No.	Formulation code	Carbopol 940 (gm)	PEG 400 (gm)
1.	G1	0.5	2.5
2.	G2	0.5	6.25
3.	G3	0.5	10
4.	G4	2	2.5
5.	G5	2	6.25
6.	G6	2	10
7.	G7	1.25	2.5
8.	G8	1.25	6.25
9.	G9	1.25	10

II. Preparation of gel

Distilled water was added to a weighed amount of carbopol 940, along with PEG 400, and the mixture was agitated to dissolve it. The subsequent addition of triethanolamine neutralized the solution and made it viscous. Until a clear gel formed, the liquid was constantly agitated for one hour.[21-22]

III. Incorporation of Phytosomes into the gel

In another beaker, the phytosomes were dissolved in 0.1 ml of ethanol, and the ethanolic phytosome solution was slowly poured into the polymer gel that had already been formed. Oleic acid was added to help with permeation (0.03 ml). With distilled water, the final amount was brought up to 100 gm. The prepared gels were kept at room temperature in suitable containers for further research.[21-22]

2.2.9 Evaluation of gels of prepared phytosomes

The drug concentration, spreadability, extrudability, in vitro diffusion, pH, and viscosity of the phytosome topical gel preparation were also examined. For the determination of the drug concentration, absorbance at 271 nm was used to evaluate viscosity, weight (in grams) was used to calculate extrudability, and *in-vitro* drug release tests were conducted by using the modified Franz diffusion (FD) cell. The spectrophotometric analysis of the samples taken out was done at 271 nm, and the cumulative% drug release was determined. The pH of numerous gel formulations was assessed with the use of digital pH meter respectively.[22-23]

2.2.10 Stability studies

The gel formulation was stored at 5±1^{0}C (refrigerated condition),37±2^{0}C, 60±5% and 45±2^{o}C, 70% RH 5% for 45 days in order to undertake stability tests. The samples were taken during the initial, 30th, and 45th days and appropriately examined for their drug content, physical properties and cumulative drug release.[24]

3. RESULTS

3.1Evaluation of crude extract

3.1.1 Phytochemical Screening of crude extract: The results on the phytochemical screening of seeds extract of Shivlingi drug presented in Table 3. The presence of Carbohydrates, alkaloids, proteins and amino acids, glycosides, phenols and tannins, triterpenoids, flavonoids, anthraquinones, steroids as well as sterols and are revealed in extracts. Seed extract showed negative response to saponin and volatile oils.

Table 3: Phytochemical Analysis of Different Extracts of Shivlingi seeds

S.No.	Constituents	Present/Absent
1.	Carbohydrates	+
2.	Proteins &Amino acids	+
3.	Alkaloids	+
4.	Flavonoids	+
5.	Glycosides	+
6.	Phenol &Tannins	+
7.	Saponins	-
8.	Steroids & Sterols	+
9.	Anthraquinones	+
10.	Triterpenoids	+
11.	Volatile oil	-

3.1.2 Antifungal activity of Shivlingi seeds extract: Shivlingi seeds extract shown good antifungal activity against selected test *Candida albicans*, in concentration A1 (5mg/ml) have the potential to make inhibition zone against *Candida albicans* (IZ=17mm), at concentration A2(10mg/ml) make inhibition zone (IZ=27mm), while for fluconazole inhibition zone was IZ=30mm. These results showed that the test extracts have good antifungal activity against *Candida albicans* in all concentration range.

3.1.3 TLC analysis: After derivatization with the anisaldehyde sulphuric acid reagent, TLC analysis of the extract revealed the existence of six main spots in the range of 0.2-0.91 Rf values, with colors ranging from green to brown to purple to violet (Figure 1). It verifies whether the extract contains sterols, triterpenoids, or other similar chemicals.

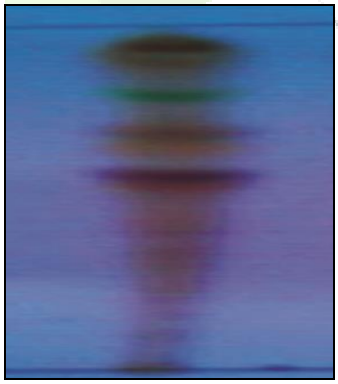

Figure 1: TLC of Shivlingi seeds extract

3.2 Determination of λmax and preparation of calibration curve

By examining the produced solution in the 200–400 nm wavelength range, the maximum amount of Shivlingi seeds extract was found. It was discovered that the maximum wavelength was 271nm. By solubilizing the medication in pH 7.4 phosphate buffer, the calibration curve for Shivlingi seeds extract was created. The concentration range of 2–10 gm/ml was where the curve was determined to be linear. The regression coefficient (R2) value obtained was 0.9994. (Figure 2A and 2B, Table 4).

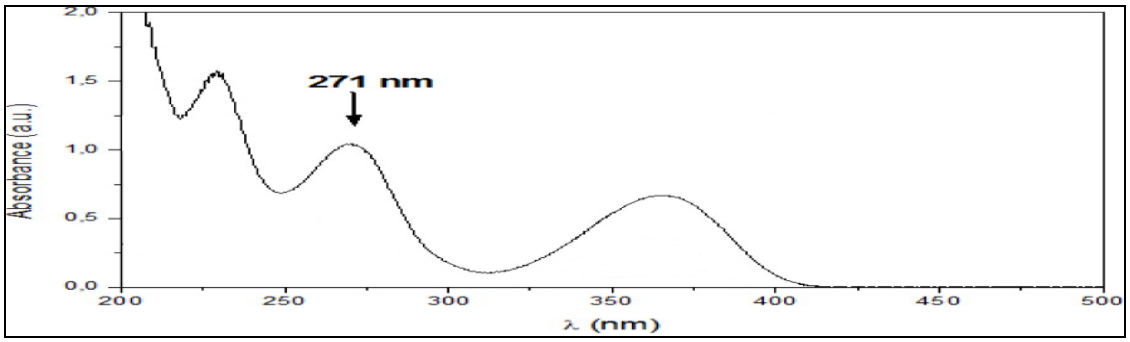

Figure 2A: UV/Vis absorption spectra of Shivlingi seeds extract

Table 4: Calibration curve data of Shivlingi seeds extract

Concentration(µg/ml)	Absorbance
2	0.0824
4	0.1684
6	0.2553
8	0.3345
10	0.4125

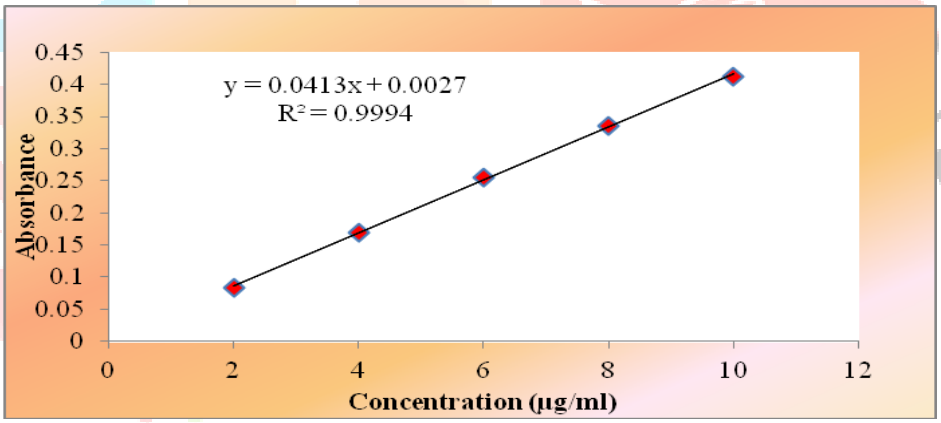

Figure 2B: Calibration curve of Shivlingi seeds extract

3.3 Evaluation of prepared phytosomes

3.3.1 Percentage Practical Yield: % Practical Yield for various formulations was given in table 5. F1 have higher % Practical yield of 91.24% as compared to the other formulations. (Figure 3, Table 5)

Table 5: Results of Percentage Practical Yield (SD±, n=3)

Formulation	Percentage Practical Yield
F1	91.24±2.45
F2	88.57±1.56
F3	86.21±2.08

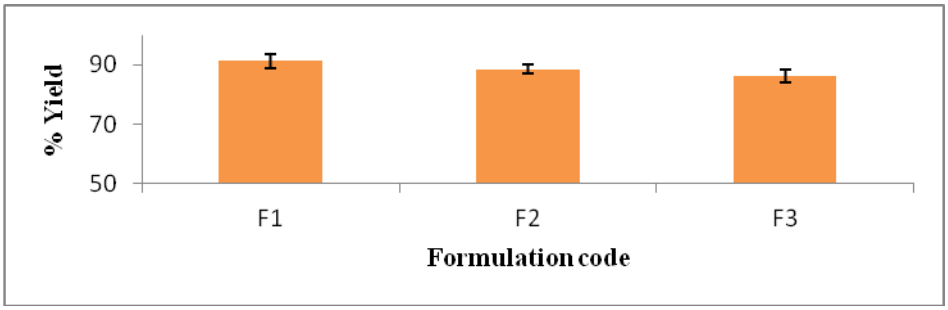

Figure 3: % Practical Yield of different phytosomes formulations (SD±, n=3)

3.3.2 Entrapment Efficiency: Based on the absorbance measured from the supernatant solution, the entrapment efficiency was estimated. The formulation F1 demonstrated the highest level of release entrapment effectiveness (89.75%), demonstrating the ideal level of lipid required for the production of phytosomes. The entrapment efficiency dropped as the lipid concentration was raised higher, demonstrating that this did not aid in trapping the medication in the matrix. (Figure 4 Table 6)

Table No: 6- Results of % Entrapment Efficiency (SD±, n=3)

Formulation	% Entrapment Efficiency
F1	89.75±1.45
F2	85.62±1.86
F3	82.88±1.24

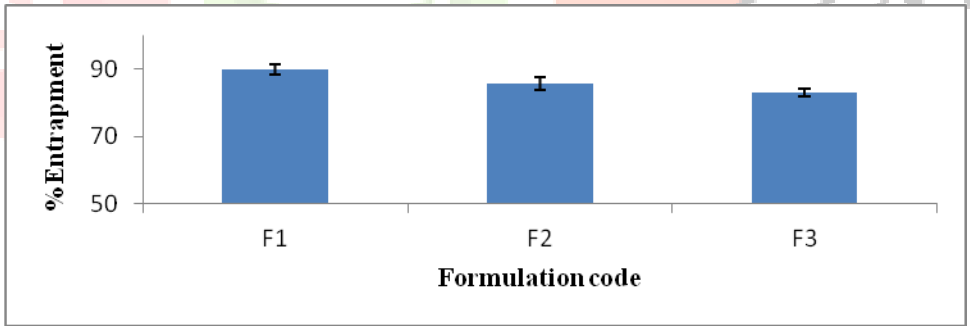

Figure 4: % Entrapment Efficiency of different phytosomes formulations (SD±, n=3)

3.3.3. Drug Content: The drug content of Shivlingi seeds extract in the phytosomes was found to be in the range of 88.43% - 84.14% which indicate the presence of an allowable amount of drug in the formulations. With an increase in lipid concentration, the percentage of medication loading dropped. Formulation F1 contained the highest amount of medication at 88.43 percent. (Table 7, Figure 5)

Table 7: Results of % Drug Content (SD±, n=3)

Formulation	Drug Content (%W/W)
F1	88.43±1.63
F2	86.81±1.06
F3	84.14±1.14

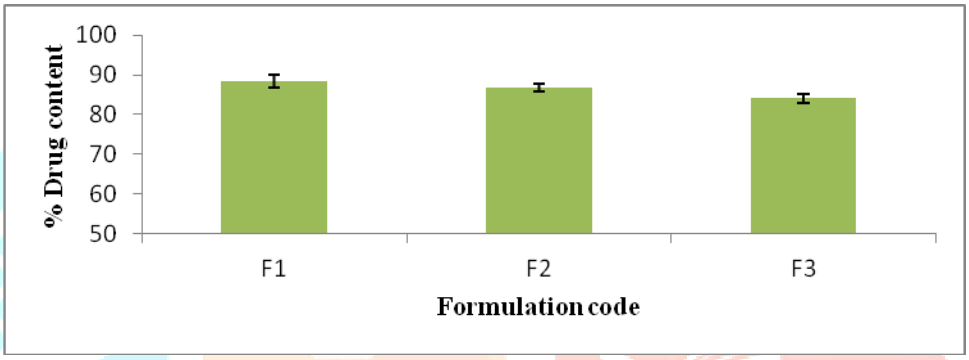

Figure 5: % Drug Content of different phytosomes formulations (SD±, n=3)

3.3.4 *In-vitro* Drug Diffusion Study of Phytosomes: Shivlingi seeds extract's phytosomes displayed a superior diffusion profile as compared to the substance's pure extract. All of the formulations displayed cumulative drug release percentages between 85.31 and 91.23%. At the 10th hour, the formulation F1 with a drug extract: soy lecithin ratio of 1:1 demonstrated the greatest release of 91.23%. Particle size, surface area, crystal habit, surface energy, and permeability are only a few of the complicated factors that affect how quickly drug particles diffuse from their dosage form. By making the medication more soluble, the wetting and dispersing properties of phospholipids (an amphiploid surfactant) enhanced the diffusion profile of the combination. (Figure 6, Table 8)

Table 8: Results of *In-vitro* Drug Diffusion Study (SD±, n=3)

Time in hrs	F1	F2	F3
0.25	4.6±1.4	3.61±0.8	3.24±1.3
0.5	11.56±2.2	10.22±2.3	9.67±2.8
1	22.35±3.7	20.51±3.4	18.96±3.8
2	33.48±3.4	27.68±3.2	26.82±2.
4	51.63±1.3	44.47±5.5	42.34±3.5
6	65.77±1.2	56.92±3.8	52.67±2.7
12	91.23±5.6	88.78±5.2	85.31±2.6

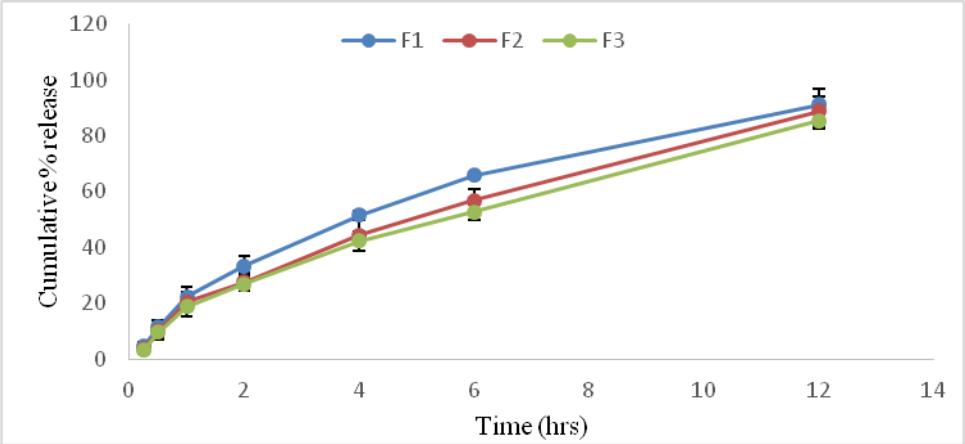

Figure 6: Cumulative % drug release from prepared phytosomes (SD±, n=3)

3.3.5 *Ex-vivo* Skin Permeation Study: by using a Franz diffusion cell, the drug penetration of the formulation F1 through the chicken's abdomen skin was tested. The results are shown in table 9. The permeation profile was determined by plotting % cumulative drug permeation vs. time. Over a period of 12 hrs, the formulation demonstrated an optimal release of 92.57%. (Figure 7, Table 9)

Table 9: Results of *Ex-vivo* Skin Permeation Study (F1)

Time in hrs	Cumulative % permeation
0.25	2.4
0.5	8.62
1	18.05
2	723.42
4	41.38
6	56.23
12	84.85

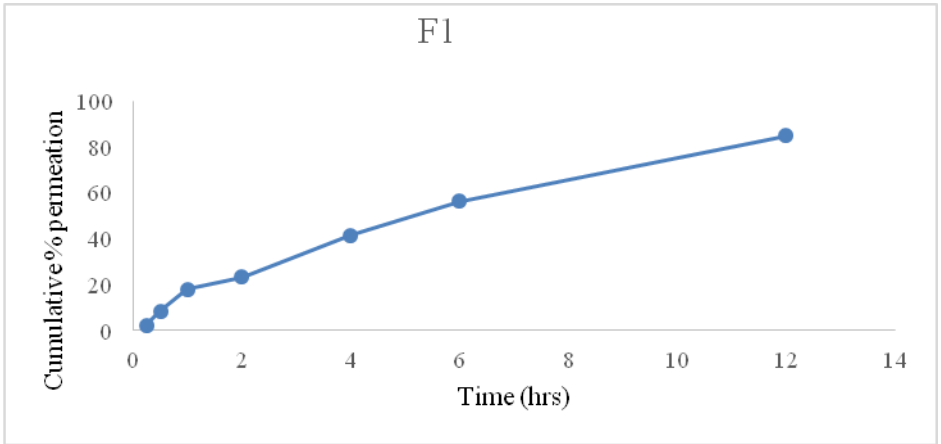

Figure 7: Drug Permeation Profile of F1

3.3.6 Scanning Electron Microcopy (SEM) Analysis: By using scanning electron microscopy, the surface structure of the synthesized phytosome (F1) was verified. The vesicles are round and smooth in appearance. (Figure 8)

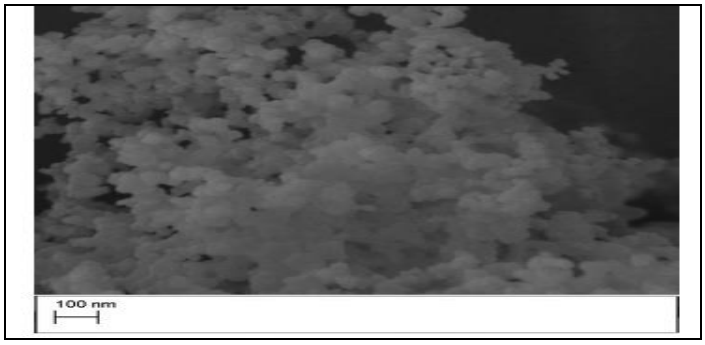

Figure 8: SEM image of prepared phytosomes

3.4 Evaluation of gels of Shivlingi seeds phytosomes (Table 10)

3.4.1 Drug Content: Spectrophotometric analysis at 271nm was used to determine the drug concentration of the gel formulations. The drug content of all formulations ranged from 96.95% to 98.92%, with G8 containing the highest concentration of drug at 98.92%. (Figure 9)

3.4.2 Viscosity Measurements: This study demonstrates that as the polymer concentration increases, the formulation viscosity increases proportionally. Viscosity was found to be greatest at the highest polymer content. (Figure 10)

3.4.3 Spreadability: The spreadability of a gel refers to the extent to which it can be applied to skin or the afflicted area without difficulty. The spreadability of the developed gel formulations was assessed, and it was found that each formulation had a good spreadability. The spreadability coefficient of the gel formulation G8 was found to be best which was 4.1cm. The spreadability rating shows that a small amount of shear might quickly spread the gel. (Figure 11)

3.4.4 Extrudability: It was discovered that the concentration of the gelling ingredients affected the extrudability of the manufactured gel formulation. With an increase in gelling agent concentration, extrudability decreased. The gel formulation G8 was found to have the optimum extrudability. of 9.74 gm/cm^2. As a result, the prepared gel has the best extrudability. (Figure 12)

3.4.5 pH: The pH of the gel formulations was between 6.3 and 6.9, which is within the skin's normal pH range and won't irritate the skin. There should be no allergic reaction to the skin at this pH level. (Figure 13)

Table 10: Results of prepared phytosomal gel (SD±, n=3)

Run	Drug Content (%)	Viscosity (cp)	Spreadability (cm)	Extrudability (gm/cm^2)	pH
G1	97.48±1.0	799±30	2.9±0.8	9.70±1.2	6.9±0.9
G2	97.53±1.4	789±48	3.9±0.7	8.71±1.1	6.2±0.6
G3	97.30±1.2	891±52	3.9±0.9	8.32±1.2	6.5±0.8
G4	96.95±1.6	512±36	3.8±0.8	9.46±1.6	6.3±0.8
G5	97.82±1.6	751±35	3.3±0.8	9.23±1.4	6.5±0.6
G6	97.02±1.4	751±28	3.6±0.6	9.72±1.6	6.6±0.8
G7	97.29±1.8	678±37	3.5±0.8	9.11±1.2	6.3±0.4
G8	98.92±1.6	751±44	4.1±0.9	9.74±1.4	6.5±0.6

G9	97.74±1.4	751±38	3.8±0.8	9.36±1.8	6.4±0.6

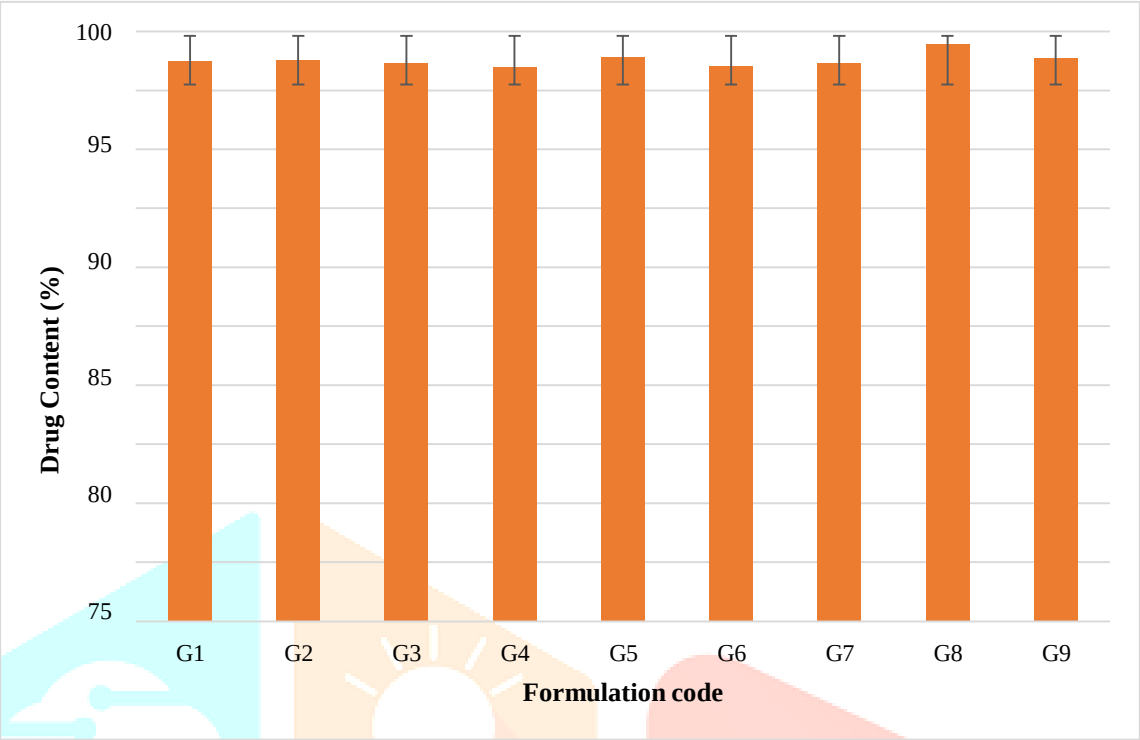

Figure 9: Drug Content (%) of prepared phytosomal gel (SD±, n=3)

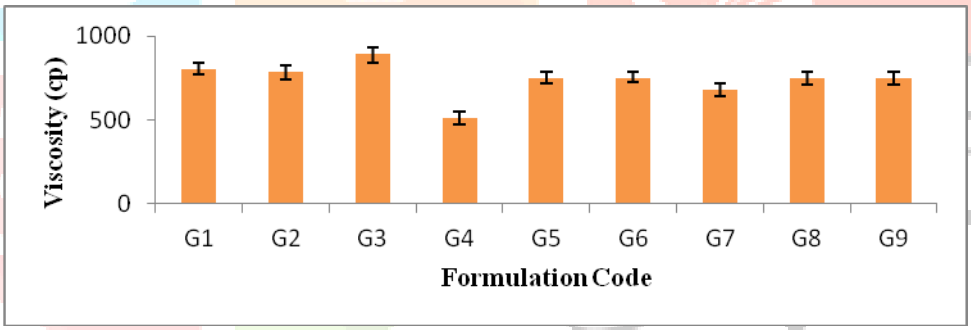

Figure 10: Viscosity (cp) of prepared phytosomal gel (SD±, n=3)

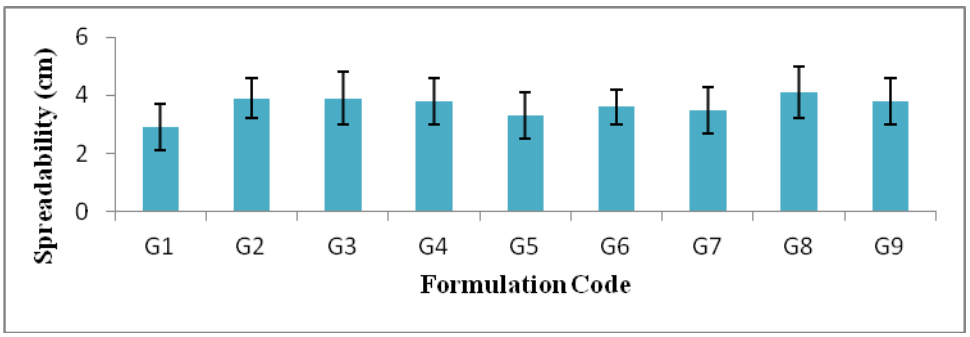

Figure 11: Spreadability (cm) of prepared pytosomal gel (SD±, n=3)

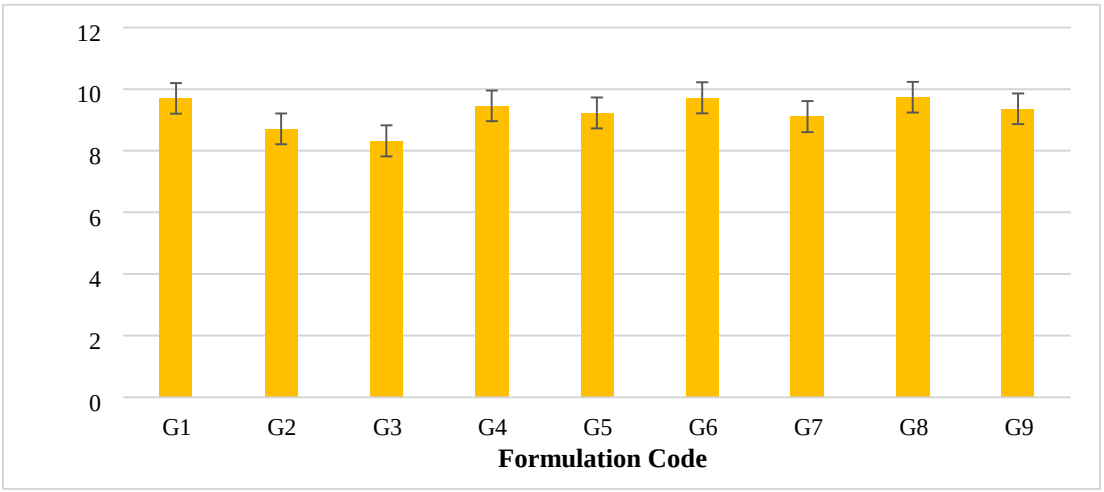

Figure 12: Extrudability (gm/cm^2) of prepared phytosomal gel (SD±, n=3)

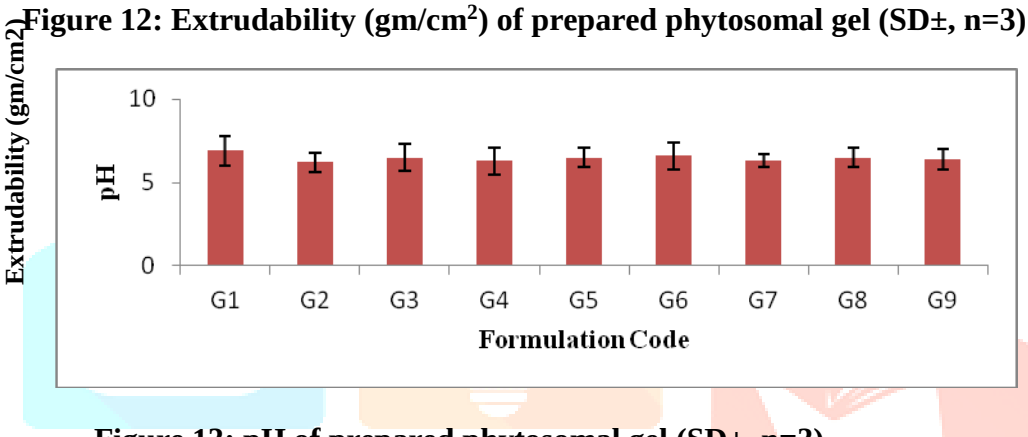

Figure 13: pH of prepared phytosomal gel (SD±, n=3)

3.4.6 In vitro diffusion studies: The result shows comparative in vitro performance of various batches of phytosomal gel. The % Cumulative drug release from phytosomal gel in 8 hrs was found to be in range of 70.48 to 95.58% respectively. The result indicates that, the phytosomal gel (G8) having higher conc. of polymer has given the highest drug release (Table 11 and Figure 14,15 and 16).

Table 11: Results of *In-vitro* drug release study of Gel Formulations (SD±, n=3)

Time (hrs)	G1	G2	G3	G4	G5	G6	G7	G8	G9
0	0	0	0	0	0	0	0	0	0
0.5	10.5±0.5	11.25±0.8	8.9±0.7	8.74±0.6	8.32±0.8	7.64±0.8	17.81±1.2	17.93±1.4	15.48±1.1
1	19.36±1.2	19.09±1.4	18.49±1.6	22.07±1.8	21.55±1.6	21.02±1.6	34.21±1.8	34.57±2.0	30.71±1.4
2	34.36±1.6	32.06±1.2	31.55±2.0	37.12±2.1	36.55±2.1	35.88±2.0	45.27±2.2	50.14±3.0	45.29±2.2
4	64.71±1.2	59.25±1.8	54.72±2.2	56.18±2.8	54.23±2.6	51.17±2.2	75.25±2.6	78.83±3.0	69.91±2.8
6	68.23±1.8	65.39±1.6	69.25±1.2	69.28±2.8	65.23±2.4	60.92±2.8	84.74±3.4	88.24±3.8	79.81±3.6
8	96.34±3.2	98.19±4.2	94.52±4.0	79.18±4.1	71.64±3.2	70.48±3.0	95.41±4.8	99.58±4.4	88.22±3.2

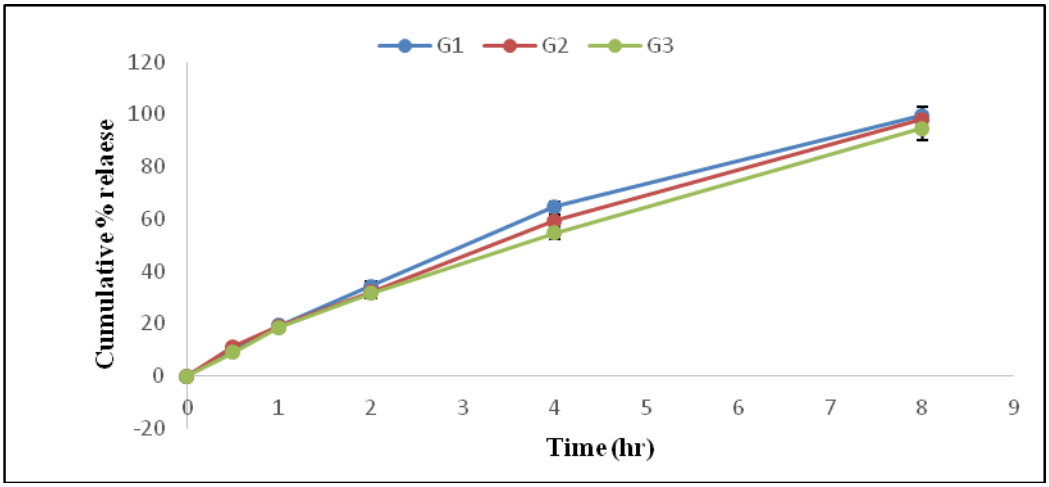

Figure 14: % CDP for formulation G1 to G3 (SD±, n=3)

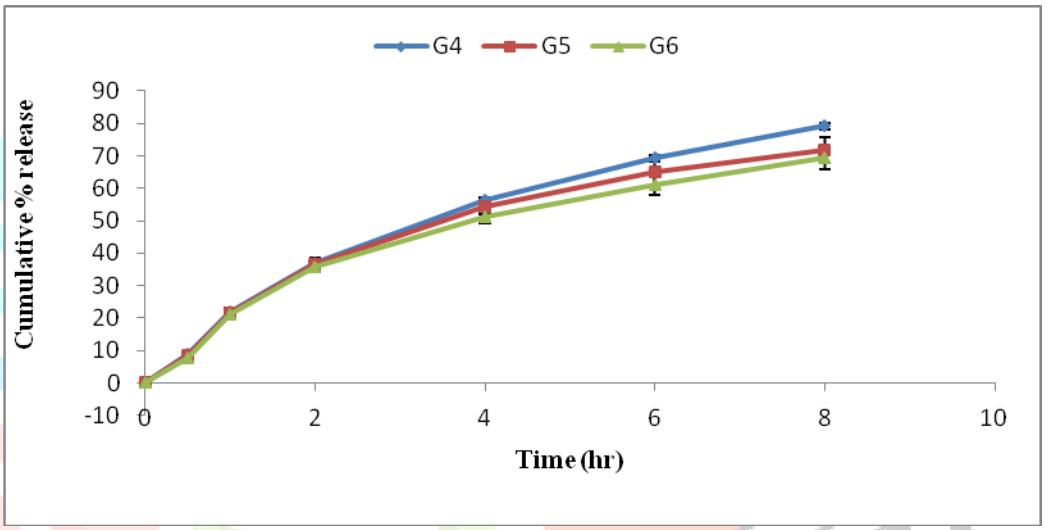

Figure 15: % CDP for formulation G4 to G6 (SD±, n=3)

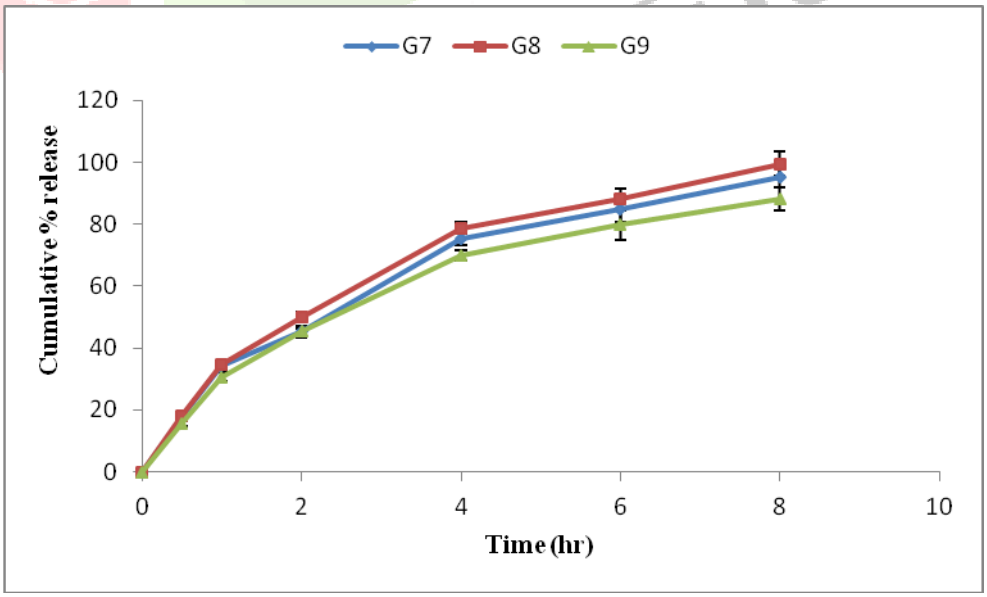

Figure 16: % CDP for formulation G7 to G9 (SD±, n=3)

As the evaluation of phytosomal gel was performed, it was found that the gel formulation G8 was more stable and effective because it was with more drug content, highest drug release, optimized viscosity, highest spreadability and extruadability and lies between the normal pH range. For further authentication of the obtained gel formulation G8, the optimization was carried out by using the Face Centered Central Composite Design (FCCCD).

3.5 Optimization of formulations using face centered central composite design (FCCCD)

3.5.1 Response surface methodology (RSM) for carbopol gel: By showing how the independent variables contribute, response surface approach enables comprehension of the behavior of the system. In order to acquire the necessary information as quickly and accurately as feasible, an experimental design arranges the trials. The tests done in accordance with the chosen experimental design are known as runs or trials.

3.5.2 ANOVA- Table 12 anticipates the data for the analysis of variance.

Table 12: ANOVA for response surface quadratic model

Response factor	Model F-value	p-value Prob>F	Lack of fit	
			F-value	p-value
% CDP	47.84	< 0.0001	2.90	0.165

3.5.3 Mathematical Modeling: For the investigated response variables, a mathematical relationship created using multiple linear regression analysis is expressed in the equation below:

$$\% \text{ CDP} = + 24.05 - 1.21 X_1 + 5.9X_2 + 0.12 X_1 X_2$$

The higher-order effect coefficient, the interaction term, the intercept coefficient, and the first-order main effect coefficient were all included in the polynomial equation. Each variable's proportionate influence on the result was indicated by the main effect's sign and degree.

3.5.4 Response surface analysis: The relationship between a few chosen independent variables and the response was demonstrated by a 3-dimensional response surface plot and a corresponding contour plot for the studied response parameter, % CDP (8 hrs).

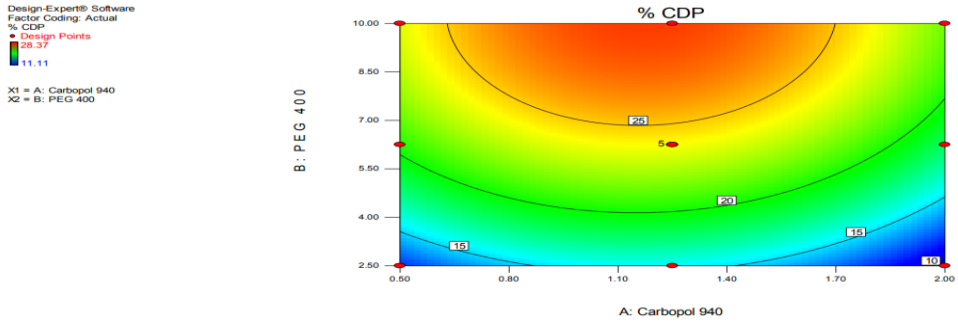

Figure 17: Contour plot showing the influence of two factors on %CDP

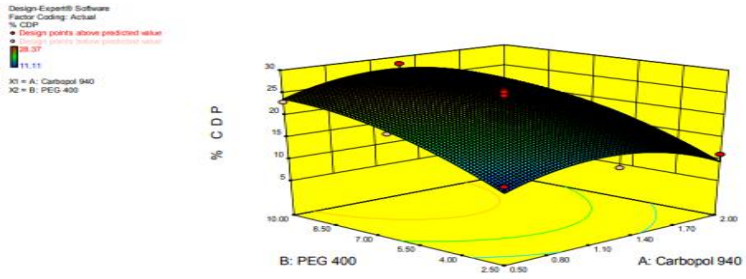

Figure 18: 3D response surface plot showing the relationship between various levels of two factors on % CDP

3.5.5 Cumulative drug permeation (%CDP): According to the polynomial equation (1) for %CDP, the coefficients X1 and X2 have opposite signs. As a result, it was anticipated that increasing the concentration of PEG 400 would raise the% CDP while increasing the concentration of Carbopol 940 would lower the% CDP. The reaction surface plots further demonstrated this. (Figure 17 and 18)

By conducting the *in vitro* optimization studies and *in silico* optimization studies, the prepared gel formulation G8 was confirmed as optimized phytosomal gel preparation of Shivlingi extract amongst all the 9 formulations developed in the laboratory. The composition of best optimized formulation is given in the Table 13

Table 13: Selected Optimized formulation parameters

Run	Drug Content (%)	Viscosity (cp)	Spreadability (cm)	Extrudability (gm/cm^2)	pH
G8	98.92±1.6	751±44	4.1±0.9	9.74±1.4	6.5±0.6

3.6 Stability Studies

Here, the gel is filled in collapsible aluminum tubes and was loaded in a stability chamber under accelerated conditions at 5-10°C (refrigerated condition), 37-20°C, 60-5°C, and 45-52°C, 70% RH. The drug concentration, spreadability, extrudability, viscosity, and *in-vitro* diffusion profile of the samples were assessed at the initial, 30 and 45-day withdrawal points. The findings demonstrated that these circumstances had no impact on those parameters during storage. Formulation G8 was found to be most stable at refrigerated conditions while it was found to be less stable at 45±2°C, 70% RH. This may be due to opening of phytosomal structure and leakage of entrapped extract. (Table 14

Table 14: Stability study of optimized batch of gel (G8)

Parameters	5±1°C			37±2°C, 60±5%			45±2°C, 70% RH		
	Initial	30th Day	45th Day	Initial	30th Day	45th Day	Initial	30th Day	45th Day
pH	6.5	6.5	6.5	6.5	6.5	6.4	6.5	6.4	6.2
Drug Content(%)	98.92	98.78	98.75	98.92	97.46	97.05	98.92	97.08	95.15
Spreadability(cm)	4.1	4.0	3.8	4.1	4.1	4.0	4.1	4.1	4.0
Extrudability(gm/cm^2)	9.74	9.74	9.70	9.74	9.73	9.73	9.74	9.72	9.72
Viscosity (cps)	751	750	754	751	750	748	751	745	741
% cumulative release	96.34	96.12	95.22	96.34	96.08	95.35	96.34	96.04	95.82

The qualitative phytochemical screening of plant extracts often gives the crucial information with reference to the chemical ingredients for the pharmacological as well as pathological discovery of novel medications. In our study, the phytochemical investigation of the Shivlingi extract revealed the presence of significant amounts of various chemical components (carbohydrates, proteins and amino acids, flavonoids, anthraquinones. glycosides, alkaloids, triterpenoids, phenols and tannins, steroids and sterols) with a history of pharmacological effects. Shivlingi seed extract's phytosomal gel was shown to have an improved diffusion and stability profile, making it a suitable delivery system for the different phytoconstituents it contains. In conclusion, the use of phytosomal formulations as topical pharmacological agents with enhanced safety and efficacy leads in the appropriate utilization of herbal medications and cost-effective pharmaceutical products.

CONCLUSION-

Shivlingi extract phytosome formulation showed a potential topical antifungal activity. It was determined that there is a lot of promise for the topical distribution of antifungal medicines against fungal infection in the production of phytosomes of Shivlingi extract. Herbal gel could perhaps be the finest method for treating fungus-related skin disorders.

REFERENCES-

1. Cott J. Natural Product Formulations Available in Europe for Psychotropic Indications. Psychopharmacol Bull, 1995; 31: 745.
2. Sumathi A, Senthamari R: A Review on Herbal Medicines and Herbal Drug Technology, Asia J. Pharm. Health Sci. 2015;5(1):1172-1173.
3. Bhokare Suvarna G., Dongaonkar Chaitali C., Lahane Surekha V., Salunke Pushpa B., Sawale Vilas S., Thombare Madhuri S.: Herbal Novel Drug Delivery A Review, WJPPS.2016; 12(8):595-596
4. Atram Seema: Recent Development of Herbal Formulation- A Novel Drug Delivery System, Int Ayurveda and Medicinal Journal. 2014; 2(6):953-954.
5. Patel Amit, Tanwar YS, Suman Rakesh, Patel pooja: Phytosome: - Phytolipid Drug Delivery System for Improving Bioavailability of Herbal Drug, J Pharma Sci and Bio Sci Research.2013;3(2):51-52
6. Joseph A. Kareparamban, Nikam Pravin H, Jadhav Aruna P, Kadam Vilasrao J: Phytosome A Novel Revolution in Herbal Drugs, Int J Res Pharma Chemistry. 2012; 2(2):299-300.
7. Patil PS, Salunkhe VR, Magdum CS, Mohite SK. Phytosome: Increasing Bioavailability of Phytoconstituents, Int J Uni Pharma Bio Sci. 2016;5(4):82-90.
8. Ma H, Guo D, Fan Y, Wang J, Cheng J, Zhang X. Paeonol-loaded ethosomes as transdermal delivery carriers: design, preparation and evaluation. Molecules. 2018 Jul;23(7):1756
9. Karpuz M, Gunay MS, Ozer AY. Liposomes and phytosomes for phytoconstituents. In Advances and Avenues in the Development of Novel Carriers for Bioactives and Biological Agents. Academic Press. 2020 (pp. 525-553).
10. Fathalla D, Youssef EM, Soliman GM. Liposomal and ethosomal gels for the topical delivery of anthraline: preparation, comparative evaluation and clinical assessment in psoriatic patients. Pharmaceutics. 2020 May; 12(5):446.
11. Fatima Z. and Shahidula SM, Formulation, Optimization and Evaluation of Hexadecanoic acid phytosomal gel for anti-fungal activity. International Journal of Pharmaceutical Sciences and Research.2023;14(1):519-529
12. Naik BJ, Patil SV, Ghotane RB and Patil PB Review article on view on *Bryonia laciniosa* (Shivlingi Beej). World Journal of Pharmaceutical Research .2022; 11:340-352.
13. Rowe RC, Shesky PJ and Owen CS. Pharmaceutical excipients; 6th edn; 2009: pp 916-925, 2180-2188, 341-352, 486-495.
14. Kokate CK, Khandelwal KR, Pawar AP and Gohaiz SB 1995. Practical Pharmacognosy, Vallabh Prakasham, New Delhi, 4th Ed. P-107.
15. Raimundo GOJ, Christiane AAF, Grasielly RS, Amanda LG. Phytochemical analysis and evaluation of antioxidant and photoprotective activities of extracts from flowers of *Bromelia laciniosa* (Bromeliaceae), Biotechnology & Biotechnological Equipment. 2017; 31:3, 600-605.
16. Rajashekar K., Prasuna Sundari PJ, Srinivas P. Development of A Topical Phytosomal Gel of *Woodfordia Fruticosa*, WJPPS. 2015;4(11):919-931.
17. Awasthi R., Kulkarni GT, Pawar VK, Phytosomes: An approach to increase the bioavailability of plant extracts; International Journal of Pharmacy and Pharmaceutical Sciences. 2011. 3(2); 1-3
18. Alharbi WS, Almughem FA, Almehmady AM, Jarallah SJ, Alsharif WK, Alzahrani NM, Alshehri AA. Phytosomes as an Emerging Nanotechnology Platform for the Topical Delivery of Bioactive Phytochemicals. Pharmaceutics. 2021; 13(9):1475.
19. Naik SR, Pilgaonkar VW, Panda V. Evaluation of antioxidant activity of Ginkgo biloba phytosomes in rat brain. Phytother. Res. 2006; 20:1013–1016.
20. Sbrini G., Brivio P., Fumagalli M. et al. Phytosome Improves Cognitive Performance by Promoting Bdnf Expression in Rat Prefrontal Cortex. Nutrients. 2020; 12:355.

21. Alsarra I., Bosela AA, Ahmed SM and Mahrous GM. Proniosomes as a drug carrier for transdermal delivery of ketorolac. European Journal of Pharmaceutics and Biopharmaceutics 2005; 59: 485-490.
22. Kumar L. and Verma R. In vitro evaluation of topical gel prepared using natural polymer. International Journal of Drug Delivery 2010; 2: 58-63.
23. Raissi S. and Farsani RE. Statistical Process Optimization through Multi-Response Surface Methodology, World Academy of Science, Engineering and Technology 2009; 51: 267-271.
24. Yang JH, Kim TY, Lee JH, Yoon SW, Yang KH and Shin SC. Antihyperalgesic and anti-Inflammatory effects of ketorolac tromethamine gel using pulsed ultrasound in inflamed rats. Archives of pharmacal research 2008; 31 suppl 4: 511-517.