"Formulation And Characterization of Phytosomes Based Gel of *Nigella Sativa* L. Seed Extract for Management of Acne Vulgaris"

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Abstract:
The aim of this study was to develop a transdermal gel formulation of *Nigella Sativa* L. seed extract using phytosomes for the management of acne vulgaris. The optimized formulation was prepared and evaluated for various parameters. *Nigella Sativa*-loaded phytosomes were created using the rotary vacuum evaporator method by mixing *Nigella Sativa* L. extract and lecithin in a 1:1 ratio, with a stirring speed ranging from 25 to 150 rpm. Design Expert software was employed for optimization, utilizing a central composite factorial design. Spreadability and viscosity were selected as dependent variables, while Carbopol 934P and PEG 400 were independent variables. The optimized gel formulation was further characterized through *in vitro* skin permeation studies, skin irritation tests, antimicrobial assays and stability studies. The *Nigella Sativa*-loaded phytosomal formulation had a particle size of 202.5 nm with a zeta potential of -2.43. Scanning electron microscopy revealed a uniform structure, and confocal laser microscopy demonstrated enhanced penetration of the phytosomes into deeper skin layers. MTT assay results confirmed that the optimized gel is non-irritant to the skin. Antibacterial studies showed that the *Nigella Sativa* phytosomal gel effectively reduced the growth of *Propionibacterium acnes*, an acne-causing bacterium, in both MIC and MBC tests.

Keywords: *Nigella sativa* L., Phytosomes, Gel, Particle size, MTT assay, *In vitro* skin permeation.

Introduction:

The effectiveness of herbal medications relies on the efficient delivery of therapeutically active compounds. However, these compounds often face significant bioavailability limitations when administered orally or topically. Phytosomes, a recently introduced herbal formulation, offer improved absorption compared to traditional extracts. The term "phyto" refers to plants, while "some" refers to cell-like structures[1]. Phytosomes are vesicular drug delivery systems where the phytoconstituents of herbal extracts are surrounded and bound by lipids, with each phytoconstituent molecule linked to at least one phospholipid molecule. Phospholipids have a unique molecular structure comprising a water-soluble head and two fat-soluble tails, making them highly effective emulsifiers due to their dual solubility[2-3].

Nigella sativa is native to a wide region, spanning northern Africa, the eastern Mediterranean, the Indian subcontinent, and southwest Asia. It is cultivated in several countries, including Egypt, Greece, India, Saudi Arabia, and Pakistan[4]. Beyond its culinary uses, *Nigella sativa* has been widely utilized in traditional medicine in various forms—such as essential oil, extract, powder, and paste to treat conditions like asthma, rheumatism, headaches, back pain, eczema, and amenorrhea[5]. Modern research has extensively explored *Nigella sativa* for its antioxidant, anti-inflammatory, antidiabetic, antihypertensive, and antimicrobial properties[6]. Its key active constituents include terpenes, phenols, and flavonoids. The antibacterial properties of black seed oil, particularly due to its active compound thymoquinone, inhibit the bacteria responsible for acne by disrupting their cell walls. Additionally, the linoleic, oleic, and stearic acids in the oil help unclog pores and regulate sebum production. A 2010 clinical study involving 62 acne patients demonstrated that a 20% black seed oil lotion was more effective in reducing acne lesions and scars than a 5% benzoyl peroxide lotion. Furthermore, the phenolic compounds in black seed oil act as antioxidants, reducing oxidative stress and neutralizing free radicals on the skin, thereby preventing acne. These compounds also assist in treating hypopigmentation and fading acne scars[7].

Acne vulgaris is a common dermatological condition that affects individuals globally, particularly during adolescence and early adulthood. Studies show that approximately 85% of people aged 12 to 24 will experience acne at some point, regardless of ethnicity or gender. The condition is caused by a combination of genetic, environmental, and lifestyle factors, with hormonal changes during puberty, especially increased androgen levels, playing a key role in stimulating sebaceous glands and sebum production. This, along with abnormal follicular keratinization and the growth of *Propionibacterium acnes* bacteria, leads to the formation of comedones and inflammatory lesions such as papules, pustules, nodules, and cysts. Acne vulgaris can have significant social and psychological effects during adolescence, making it essential to understand its causes and develop effective prevention and treatment strategies[8,9].

In recent decades, significant attention has been directed toward the development of innovative drug delivery systems for herbal medicines[10]. Herbal medicines are gaining popularity in the modern world due to their ability to treat various diseases with lower toxicity and improved therapeutic outcomes[11,12]. Based on these advantages and supported by existing literature, the objective of the present study was to develop a phytosomal gel formulation loaded with *Nigella sativa* extract using a systematic optimization approach. The optimized formulation was then characterized through *in vitro* skin permeation studies, skin irritation tests, antimicrobial assays, and stability studies.

Material and Methods

Materials Collection and Authentication of Plant

The plant material was sourced from the local market in Gadhinglaj, District Kolhapur, Maharashtra, India. The taxonomic authentication of the plant was performed by Dr. Sanket S. Patil, Department of Botany, Shivraj College, Gadhinglaj, India. Seeds of *Nigella sativa* was collected, thoroughly cleaned by washing with tap water, followed by distilled water, and then dried. The dried seeds were finely powdered and stored in an airtight container for further experimental studies. Lecithin was procured from Vav Life Science, Mumbai, while Carbopol 934 and PEG were obtained from Research-Lab Fine Chem Industries, Mumbai, Maharashtra. All other chemicals used in the formulation were of analytical grade and sourced from SD Fine Chemical, Mumbai, India.

Methods

Preparation of *N. sativa* seed extract

The seeds were dried and ground into a powder. They were then sequentially extracted with ethanol using a Soxhlet apparatus. The solvent was evaporated and concentrated using a water bath. The resulting extracts were stored for further studies[13].

Preparation of *Nigella sativa*loaded Phytosomes

The *N. sativa*phytosomes were prepared by refluxing *N. sativa*and lecithin in ratios of (1:1).Briefly, accurately weighed amounts of *N. sativa*and lecithin were placed into a 100 mL round bottom flask and dissolved in 20 mL of dichloromethane. The reaction temperature of the reflux was controlled at 40 °C using a water bath for 3 h. The resultant clear solution was dried at 40°C under vacuum to remove traces of solvents in order to obtain the *N. sativa*phytosomes. The prepared thin layer had been kept overnight in room temperature prior to hydration. This dried film was hydrated with 10ml phosphate buffer pH 5.5 in a rotary at 40°C. The phytosomes were finally sonicated for 4 minutes in a probe sonicator, with 60% amplitude and 5 seconds on-off interval. All phytosomes batches were stored in the refrigerator[14,15].

Characterization of *N. sativa* -loaded Phytosomes

Determination of Particle Size

The particle size of *N. sativa*-loaded phytosomes was determined using a particle size analyzer[16].

Determination of Zeta Potential

Zeta potential is a key parameter in determining the physical stability of phytosomes. A 5 mL aliquot of the diluted sample was transferred to a cuvette, and the zeta potential was measured[16].

Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) is employed to examine the shape and size of the formulated *N. sativa*-loaded phytosomes. After the samples are cleaned, fixed, rinsed, dehydrated, and dried following the appropriate protocol, they are mounted on a holder and inserted into the scanning electron microscope for analysis[17].

Formulation of*N. sativa* Loaded Phytosomal Gel

The preparation of the *Nigella sativa* phytosomal gel involved pre-made *Nigella sativa*-loaded phytosomes and Carbopol 934P as the gel base due to its excellent gelling properties. First, Carbopol 934P was dispersed in water and left to swell overnight. Preservatives (methyl paraben and propyl paraben) were dissolved in ethanol, slightly heated, and then slowly

mixed into the Carbopol934P dispersion while stirring. Next, PEG 400 was added as a plasticizing agent to improve the gel's application. The *Nigella sativa*-loaded phytosomes were incorporated under continuous stirring for uniform distribution. Finally, triethanolamine was gradually added to neutralize the Carbopol and achieve a clear, viscous gel. The resulting *Nigella sativa* L. phytosomal gel was ready for further evaluation[18].

Optimization by Design of Experiment

The impact of various process factors on gel preparation was evaluated using a Design of Experiment (DoE) approach. The independent variables chosen were Carbopol 934P and PEG 400, while the dependent variables included spreadability (gm.cm/sec) and viscosity (cps). The screening study indicated that other ingredients and parameters did not significantly affect the gel's properties and were thus kept constant in subsequent experiments. The experimental conditions were optimized using the Central Composite Design (CCD), as guided by Design Expert® Software. Table 1 presents the selected factors and their levels, while Table 2 outlines the 9 generated trial runs for the gel formulation.

Table 1. Independent and dependent factors selected and levels for the experiment

Independent Factors	Name	Level (-1)	Level (0)	Level (+1)
1	Carbopol934P (%w/w)	0.5	1	1.5
2	PEG400 (%w/w)	1	1.5	2.5

Table 2. 3^2 Levels of factorial randomized central composite design experimental trial batches for gel.

Formulation Code	Factor 1	Factor 2
	Carbopol934P (%w/w)	PEG400 (%w/w)
HF1	-1	-1
HF2	0	-1
HF3	+1	-1
HF4	-1	0
HF5	0	0
HF6	+1	0
HF7	-1	+1
HF8	0	+1
HF9	+1	+1

Evaluation of *N. sativa* Loaded Phytosomal Gel

pH -

The pH meter was calibrated using standard buffer solutions at pH 4 and 7. Approximately 0.5 g of the gel was weighed, dissolved in 50 mL of distilled water, and the pH was then measured[20-23].

Extrudability -

The herbal gel formulation was filled into standard collapsible tubes, sealed by crimping at the ends, and the tube weight was recorded. The tube was then placed between two glass slides and compressed. A 100 g weight was applied to the slides, and after removing the cap, the expelled gel was weighed to determine its extrusion capability[20-23].

Homogeneity -

The formulations were assessed for homogeneity through visual inspection[19].

Spreadability -

Two glass slides measuring 20 cm × 20 cm were selected, and a small amount of the sample was placed between them. A 100 g weight was applied to the top slide to evenly press the sample into a thin layer. After removing the weight, the slides were fixed to a stand without disturbance, allowing the upper slide to slide freely due to the force of a tied weight. The time taken for the upper slide to completely separate from the lower one was recorded using a stopwatch. This parallel plate method is widely used to determine and quantify the spreadability of semisolid formulations due to its simplicity and low cost[19]. The spreadability was calculated using the following equation:

$S = m \times L/T$

Where

S - Spreadability

m - Weight tied to the upper slide l - Length of the glass

t - Time taken in seconds.

Viscosity -

The viscosity of the phytosomal gel formulations was measured using a Brookfield viscometer (Brookfield Engineering Labs, USA) with an LV2 spindle. The spindle was immersed in the gel formulation, and the viscosity was recorded at a temperature of 25 ± 1°C and a shear rate of 10 rpm[20-23].

***In-Vitro* Skin Permeation Study -**

Confocal laser scanning microscopy was utilized to evaluate skin permeability. Rhodamine B-loaded phytosomal gel and a hydroalcoholic solution of rhodamine B were applied

uniformly to excised rat skin mounted on Franz diffusion cells and allowed to incubate for 8 hours at 37 ± 1°C. After treatment, the skin samples were rinsed with distilled water to remove excess gel and solution. The cutaneous specimens were then dissected into small fragments and placed on a glass slide with the stratum corneum facing up for examination. Using a confocal laser scanning microscope, the rat dermal tissue was scanned optically at 5 µm intervals along the z-axis. Fluorescence emission was stimulated and captured with an argon laser at 488 nm and 532 nm, respectively[24].

Skin Irritation Study -

Cells were incubated at a concentration of 1×10^4 cells/mL in culture medium for 24 hours at 37°C with 5% CO2. They were then seeded in 96-well tissue culture plates at a concentration of 10^4 cells/well with 100 µL of culture medium and varying sample concentrations (20, 40, 60, 80, and 100 µg/mL). Control wells were maintained to assess cell survival and the percentage of live cells after culture. Following incubation for another 24 hours under the same conditions, the medium was removed, and 20 µL of MTT reagent (5 mg/mL in PBS) was added. The cells were incubated for an additional 4 hours at 37°C in the CO2 incubator. Absorbance of each sample was measured at 550 nm using a microplate reader, with triplicate samples analyzed for accuracy[25,26].

***In-Vitro* Antimicrobial Study**

Determination of Zone of Inhibition -

The phytosomal gel was evaluated for its antibacterial activity against *Propionibacterium acnes* (ATCC 11827). The inoculum was adjusted to approximately 5×10^5 CFU/mL using a sterile saline solution. Different volumes of the gel (20, 40, 60, and 80 µL) were added to separate wells, and Muller Hinton agar was used as the culture medium. After incubation at 37°C, the diameter of the zone of inhibition (in mm) was measured to assess the gel's effectiveness against the bacterial strain[27,28].

Determination Minimum Inhibitory Concentrations (MIC) and Minimum bactericidal concentrations (MBC) -

Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) were assessed using the broth dilution method against the bacterial strain *P. acnes* (ATCC 11827). Samples were serially diluted from 0 to 120 µL/mL (v/v) and loaded into different wells. An equal volume of 10 µL of the inoculum, adjusted to 5×10^5 CFU/mL, was added to the wells and mixed with the growth medium. The bacterial plates were incubated at 37°C for 48 hours. The MIC was determined as the lowest concentration that inhibited the growth of the bacteria. For the MBC, 50 µL of culture from the wells showing no growth was

transferred to agar plates, and the concentration at which no colonies formed was recorded as the MBC[27,28].

Stability study -

The stability of the refined *Nigella sativa* phytosomal gel formulation was evaluated over approximately 45 days at varying temperatures (specifically, 4 ± 2 °C and 25 ± 2 °C). The formulations were stored in amber-coloured containers to protect against light-induced degradation. After the storage period, the formulation was tested for characteristics such as physical appearance, pH, and spreadability[29].

RESULT AND DISCUSSION:

Characterization of *N. sativa* -loaded Phytosomes

Particle Size -

The particle size of the *N. sativa*-loaded phytosomes was measured to be 202.5 nm.

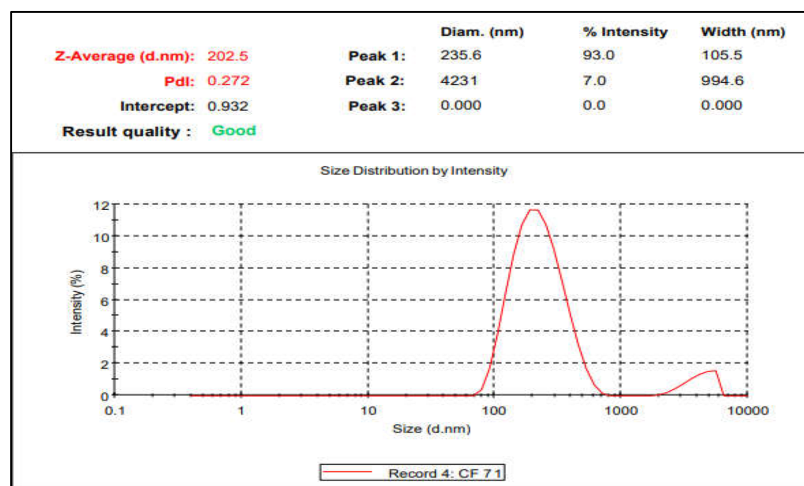

Figure 1. Particle size of *N. sativa*-loaded phytosomes

Zeta Potential

The magnitude of the zeta potential for the similarly charged *N. sativa* phytosomes indicates strong integrity within the complex, with a zeta potential value of -2.43, suggesting good stability of the formulation.

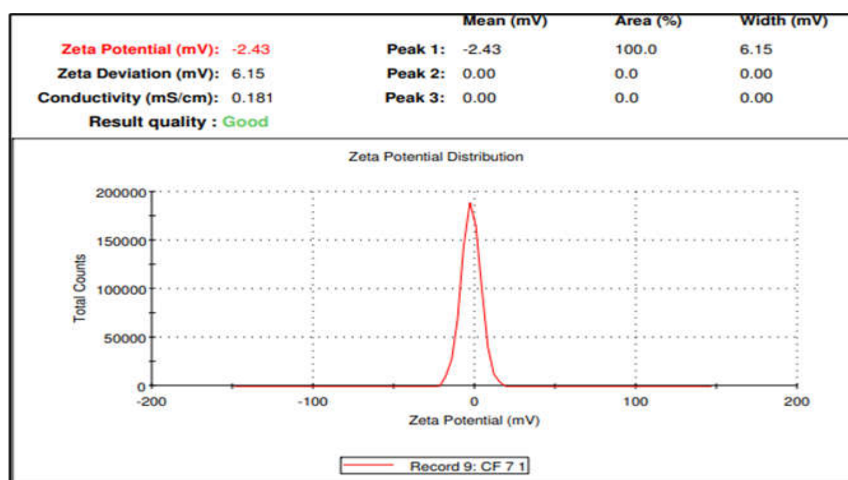

Figure 2. Zeta potential of *N. sativa*-loaded phytosomes

Scanning Electron Microscopy (SEM)

Scanning electron microscopy revealed that the vesicles are spherical in shape and have a smooth surface, as illustrated in Figure 3.

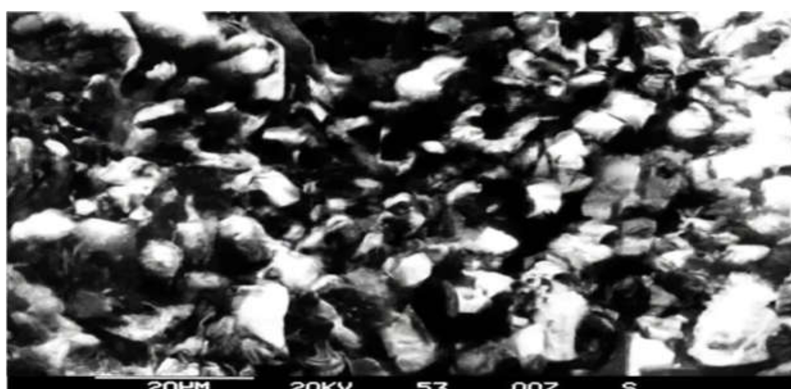

Figure 3. SEM of *N. sativa*-loaded phytosomes

Optimization by Design of Experiment (DoE) and Evaluation of Prepared Gel Formulation

The gels were optimized using a 3^2-level factorial randomized central composite design in Design Expert. Nine trial batches were prepared and evaluated for spreadability and viscosity, with responses obtained from the Design of Experiments (DoE). The spreadability ranged from 5.4 ± 0.4 to 13.6 ± 0.3 gm.cm/sec, while the viscosity varied between 9865 to 19768 cps. The optimal drug content was achieved with a ratio of Carbopol 934P to PEG 400 (1.5:1.5).

Effect of Different Factors on the Selected Responses

Effect of Factors on Spreadability -

The model developed for spreadability yielded a p-value of 0.0005 and an F-value of 211.15, indicating that the model is significant. Analysis of the contour plot and response surface plot

revealed that as the concentration ratio of Carbopol 934P to PEG 400 increased from -1 to +1, there was a notable enhancement in spreadability. The ratio of Carbopol 934P to PEG 400 had a significant impact on the gel's spreadability, as represented in the following equation:

$$Spreadability = +8.5958 + 1.2375A - 3.0167B$$

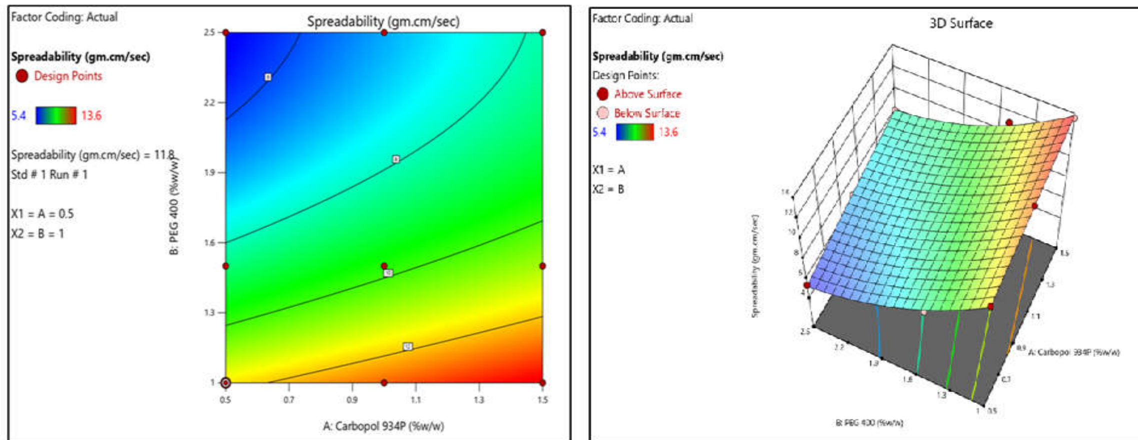

Figure 4: Contour plot and 3D surface plot for the effect of factors on Spreadability.

Effect of Factors on Viscosity -

The model developed for the Viscosity had a p-value of 0.0394 and an F-value of 10.73, indicating the model to be significant. The amount of Carbopol 934P to PEG 400 ratio significantly affected the Viscosity study of gel, as shown in the following equation.

$$Viscosity = +11441.8611 + 647.25A - 1890.8333B$$

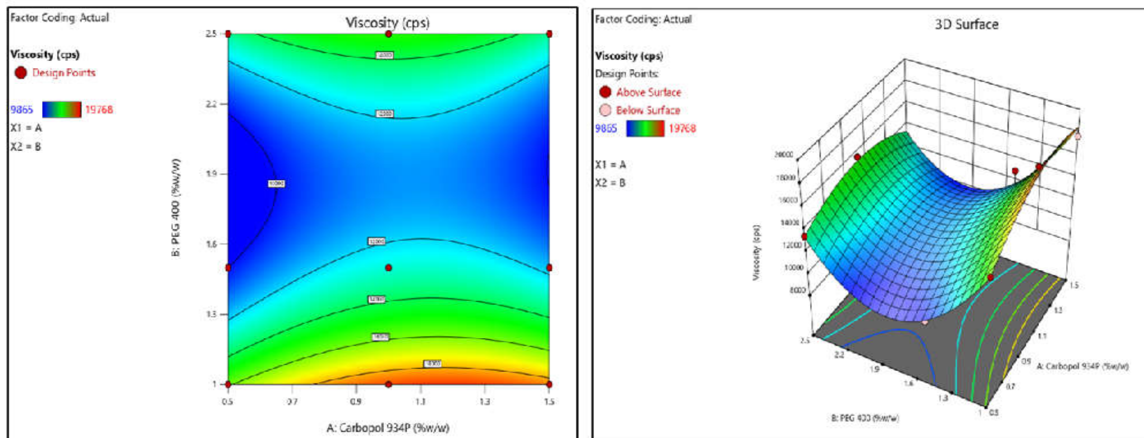

Figure 5: Contour plot and 3D surface plot for the effect of factors on Viscosity

Table 3. Observed responses for gels obtained from DoE.

Formulation Code	Factor 1	Factor 2	Response 1	Response 2
	Carbopol 934P	PEG 400	Spreadability (gm.cm/sec)	Viscosity (cps)
HF1	-1	-1	11.8	15925

HF2	0	-1	12.9	19768
HF3	+1	-1	13.6	17692
HF4	-1	0	8.3	9865
HF5	0	0	9.7	11632
HF6	+1	0	11.1	12923
HF7	-1	+1	5.4	13478
HF8	0	+1	6.7	15576
HF9	+1	+1	8.1	12986

Evaluation of *N. sativa* Loaded Phytosomal Gel

pH – Table 4 indicates that the pH of the various gel formulations ranged from 5.87 ± 0.1 to 7.01 ± 0.2, falling within the normal physiological range and suggesting that they are unlikely to cause skin irritation.

Extrudability -Table 4 presents the extrudability values for all prepared gel formulations, which range from 88.8 ± 2.9 to 92.8 ± 1.9.

Homogeneity -Table 4 indicates that the visual inspection of the prepared gel formulations revealed a good appearance and homogeneity.

Spreadability -Table 4 displays the spreadability values for all prepared gel formulations, which ranged from 5.4 ± 0.4 to 13.6 ± 0.3 gm.cm/sec.

Viscosity -Table 8 indicates that the viscosity of the various gel formulations ranged from 9865 to 19768.

Table 4. Results for pH, spreadability, viscosity, homogeneity and Extrudability -

Batch	pH	Spreadability (gm.cm/sec)	Viscosity (cps)	Homogeneity	Extrudability
HF1	6.69±0.3	11.8±0.2	15925	Good	88.8±2.9
HF2	6.63±0.2	12.9±0.1	19768	Good	91.8±1.0
HF3	6.54±0.4	13.6±0.3	17692	Good	92.7±1.5
HF4	6.87±0.3	8.3±0.2	9865	Good	90.2±1.1
HF5	6.78±0.2	9.7±0.2	11632	Good	90.5±2.3
HF6	6.69±0.3	11.1±0.1	12923	Good	92.5±0.6
HF7	7.01±0.2	5.4±0.4	13478	Good	90.4±1.9
HF8	6.92±0.3	6.7±0.3	15576	Good	92.8±1.9
HF9	5.87±0.1	8.1±0.3	12986	Good	91.6±2.5

*Each value represents mean ± S.D.of three observations.

***In-Vitro* Skin Permeation Study -**

During acne outbreaks, there is an increased production of sebum by hair follicles in the skin's epidermis. To enhance drug penetration into the deeper skin layers, a trial using confocal laser microscopy was conducted with a rhodamine-infused phytosomal gel derived from *Nigella sativa* L. seed extract and a hydro-alcoholic solution containing Rhodamine B.

Figure 6A presents confocal laser microscopy images of rat skin after 8 hours of treatment with both the hydro-alcoholic solution and the rhodamine B-loaded phytosomal gel. The results showed that the phytosomal gel exhibited uniform red fluorescence throughout the stratum corneum, epidermis, and dermis, indicating deeper penetration compared to the hydro-alcoholic solution. The increased fluorescence observed in the deeper skin layers signifies an accumulation of the phytosomal gel within the skin.

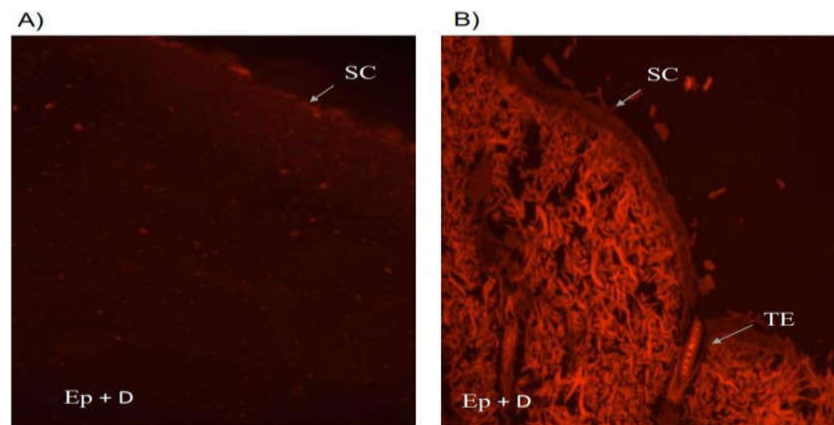

Figure 6: Confocal laser micrographs; 6A: Hydro-alcoholic solution of Rhodamine B showing 10 µm depth of penetration; 6B: Rhodamine B-loaded *Nigella sativa* Phytosomal gel showing 33.6 µm depth of penetration.

Skin Irritation Study -The MTT assay for skin irritation was conducted at various concentrations (Figure 7). The Sample Code H Gel (i.e., *Nigella sativa* L. seed extract-loaded phytosomal gel) demonstrated a moderate percentage of viability in comparison to the standard drug 5FU. Based on the percentage of viability (Figure 8) and percentage of inhibition (Figure 9), it can be concluded that the sample is moderately non-irritating to skin cells.

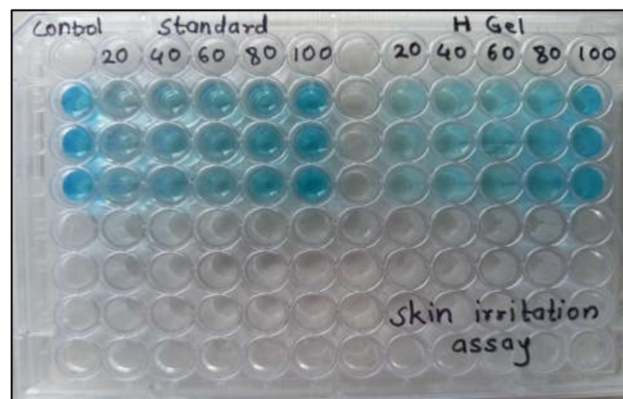

Figure 7: Image of Skin Irritation Assay

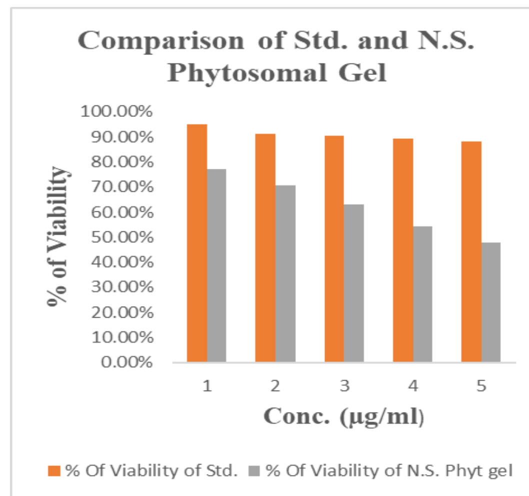

Figure 8. % of Viability Graph

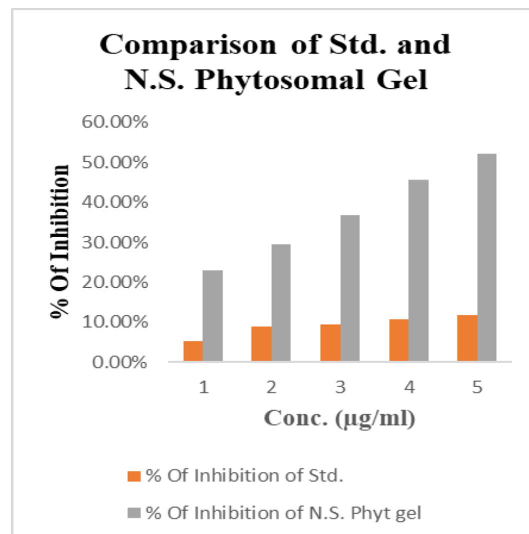

Figure 9. % of Inhibition Graph

In-Vitro Antimicrobial Study

Determination of Zone of Inhibition -

The results from the agar well diffusion method demonstrated the antimicrobial effect of *Nigella sativa* L. loaded phytosomal gel at various concentrations (20 µL, 40 µL, 60 µL, and 80 µL) against *Propionibacterium acnes* (*P. acnes*) ATCC 11827, as shown in Figure 10. Clear zones of inhibition were observed around the phytosomal gel disk, indicating significant antimicrobial activity capable of inhibiting pathogen growth. The measured zones of inhibition for different bacterial strains are presented in Table 5. Comparing the different zones of inhibition reveals that the phytosomal gel exhibited stronger antimicrobial activity specifically against the *Propionibacterium acnes* pathogen, demonstrating its effectiveness against acne-causing bacteria.

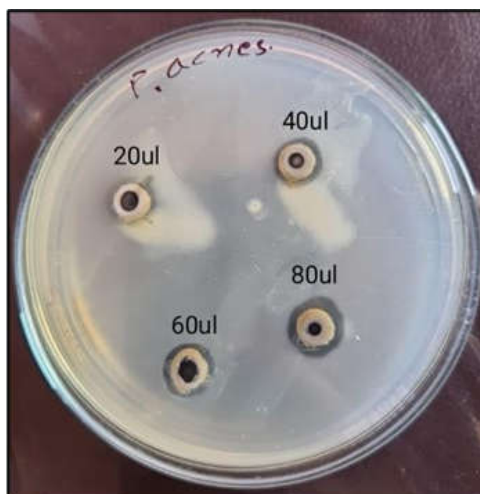

Figure 10.Zone of inhibition of*Nigella sativa* loaded Phytosomal gel, against *Propionibacterium acne*bacterial strain

Table 5.Antimicrobialactivity'sdiameter ofzone of inhibition(mm)

Sr. No.	SampleName	Conc.(µl)	*Propionibacterium acnes (mm)*
1.	*Nigella sativa* L. loaded Phytosomal gel	20	6.23 ± 0.35
		40	7.51 ± 0.39
		60	8.89 ± 0.57
		80	9.35 ± 0.59

*Each value represents mean ± S.D.of three observations.

Determination Minimum Inhibitory Concentrations (MIC) and Minimum bactericidal concentrations (MBC)

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the phytosomal gel were evaluated against the bacterial strain *Propionibacterium acnes* (*P. acnes*) ATCC 11827. The MIC, defined as the lowest concentration at which no visible growth of the pathogen occurred, was found to be 80 µL for the *Nigella sativa* L. loaded phytosomal gel, indicating that this concentration effectively inhibits bacterial growth. The MBC, which represents the minimum concentration required to completely eliminate the bacterial population, was determined to be 120 µL for the phytosomal gel, as illustrated in Figure 11 and recorded in Table 6. This investigation demonstrates that the *Nigella sativa*-loaded phytosomal gel not only hinders the proliferation of *P. acnes* but is also effective in eliminating it, suggesting its potential as an antimicrobial agent against acne-causing bacteria.

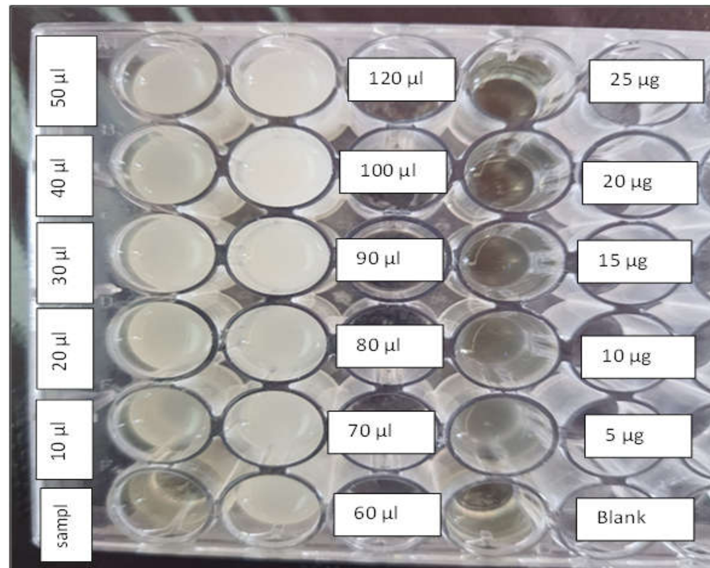

Figure 11. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration(MBC)activity of *Nigella sativa* loaded Phytosomal gel.

Table 6.Antibacterial property of sample in MIC and MBC

Sr. No.	Sample Name	*P.acnes*	
		MIC(µl)	MBC(µl)
1	*Nigella sativa* loaded Phytosomal gel	80	120
2	Std.penicillin	10	20

Stability study -

The optimized formulation of *Nigella sativa* L. seed extract phytosomal gel underwent a stability study over 45 days. The assessment indicated no significant changes in the results before and after the stability trials, as shown in Table 7. Therefore, the refined formulation, designated as HF6, comprising the phytosomal gel derived from *Nigella sativa* L. seed extract, was considered stable.

Table 7. Stability of the Optimized Phytosomal Gel from *Nigella sativa* L. Seed Extract under Various Storage Environments

Storage Condition						
	4± 2°C			25± 2°C		
Days	**Physical appearance**	**pH**	**Spreadability**	**Physical appearance**	**pH**	**Spreadability**
15	Good	6.69±0.2	8.82 ± 0.1	Good	6.67±0.2	9.2±0.2
30	Good	6.74±0.2	11.88 ±0.2	Good	6.65±0.1	8.76±0.3
45	Good	6.72±0.3	12 ±0.2	Good	6.71±0.1	10.8±0.3

*Each value represents mean ± S.D. of three observations.

CONCLUSION:

The aim of this study was to develop a transdermal gel formulation of *Nigella sativa* L. seed extract using phytosomes for the treatment of acne vulgaris. *Nigella sativa* is known for its use in treating various conditions like asthma, allergies, autoimmune diseases, hypertension, diabetes, and cancer. The study successfully utilized a 3^2 factorial design to optimize the *Nigella sativa* phytosome-loaded gel. The optimized gel demonstrated favorable characteristics, including spreadability (11.1 ± 0.1 gm.cm/sec), viscosity (12923 cps), extrudability (92.5 ± 0.6), and good homogeneity. The optimized formulation contained 1.5% w/w Carbopol 934P and 1.5% w/w PEG 400. SEM analysis revealed that the *Nigella sativa* phytosomes had a spherical shape with a smooth surface. The pH of the prepared phytosomal gel was found to be 6.69 ± 0.3, which is within the normal range for topical applications. Overall, the optimized formulation showed promise for managing acne vulgaris.

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