Development of Tulsi Extract-Loaded Nanostructured Lipid Carriers for Acne Treatment

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***Abstract*—Acne vulgaris is one of the most common chronic inflammatory skin disorders affecting adolescents and adults worldwide. It develops due to excessive sebum production, follicular hyperkeratinization, bacterial colonization, and inflammatory responses within the pilosebaceous unit. Conventional topical therapies such as antibiotics, benzoyl peroxide, and retinoids often produce undesirable side effects including skin irritation, dryness, erythema, and microbial resistance after prolonged use. Therefore, there is a growing interest in herbal medicines incorporated into advanced drug delivery systems that improve therapeutic efficacy while minimizing adverse effects.**

Ocimum sanctum (Tulsi) is a well-known medicinal herb possessing potent antimicrobial, anti-inflammatory, antioxidant, and wound-healing properties. The major phytoconstituents such as eugenol, ursolic acid, rosmarinic acid, flavonoids, and phenolic compounds exhibit significant inhibitory activity against acne-causing microorganisms while reducing inflammation and oxidative stress. However, the direct topical application of herbal extracts is often limited because of poor skin penetration, instability of bioactive constituents, and reduced residence time on the skin surface.

Nanostructured Lipid Carriers (NLCs) are advanced lipid-based nanocarrier systems composed of solid and liquid lipids that improve the stability, skin permeation, controlled drug release, and topical retention of encapsulated phytoconstituents. Incorporation of Tulsi extract into NLCs is expected to enhance the therapeutic efficacy of the herbal extract by increasing drug entrapment, protecting bioactive molecules from degradation, and providing sustained release at the site of application.

The present study aims to formulate and evaluate Tulsi extract-loaded Nanostructured Lipid Carriers for topical acne treatment. The developed formulation will be evaluated for particle size, polydispersity index, zeta potential, entrapment efficiency, pH, viscosity, drug content, in vitro drug release, antimicrobial activity, stability, and skin compatibility. The optimized NLC formulation is expected to provide enhanced topical drug retention, improved antimicrobial efficacy against acne-causing bacteria, prolonged therapeutic action, and better patient compliance. Therefore, Tulsi extract-loaded Nanostructured Lipid Carriers may serve as an effective, safe, and economical herbal nanoformulation for the management of acne vulgaris.

***Index Terms*—Ocimum sanctum, Tulsi, Nanostructured Lipid Carriers, Acne vulgaris, Herbal nanoparticles, Topical drug delivery, Controlled drug release, Antimicrobial activity**

I. Introduction

Acne vulgaris is one of the most prevalent chronic inflammatory disorders of the pilosebaceous unit affecting approximately 80–90% of adolescents and a significant proportion of adults worldwide. The disease is characterized by the development of comedones, papules, pustules, nodules, and cystic lesions, primarily on the face, chest, shoulders, and upper back. Acne develops through a combination of excessive sebum production, follicular hyperkeratinization, colonization by *Cutibacterium acnes*, and inflammatory responses. Besides physical symptoms, acne significantly affects self-confidence, quality of life, and psychological well-being, often leading to anxiety, depression, and permanent scarring if left untreated. **[1]**

Current treatment strategies include topical retinoids, benzoyl peroxide, antibiotics, azelaic acid, and systemic medications for severe cases. Although these therapies are effective, long-term treatment frequently results in

adverse effects such as skin irritation, dryness, erythema, peeling, photosensitivity, and the development of antibiotic-resistant microorganisms. These limitations have encouraged researchers to investigate safer and more effective herbal alternatives combined with novel drug delivery systems capable of improving therapeutic outcomes while reducing unwanted side effects. **[2]**

Medicinal plants have been used for centuries in traditional healthcare systems because of their safety and broad spectrum of pharmacological activities. Among these medicinal plants, *Ocimum sanctum* (Tulsi), belonging to the family Lamiaceae, occupies a prominent place in Ayurvedic medicine. Tulsi leaves contain several biologically active phytoconstituents including eugenol, ursolic acid, rosmarinic acid, linalool, apigenin, luteolin, flavonoids, tannins, and phenolic compounds. These constituents exhibit potent antimicrobial, anti-inflammatory, antioxidant, immunomodulatory, and wound-healing properties that are beneficial in the treatment of inflammatory skin disorders including acne vulgaris. **[3]**

Figure 1. Fresh *Ocimum sanctum* (Tulsi) Plant

Several studies have demonstrated that Tulsi extract effectively inhibits the growth of acne-causing microorganisms, particularly *Cutibacterium acnes* and *Staphylococcus aureus*. Eugenol, one of the major active constituents of Tulsi, disrupts bacterial cell membranes and suppresses inflammatory mediators responsible for acne progression. Additionally, the antioxidant phytochemicals present in Tulsi neutralize reactive oxygen species generated during inflammatory processes, thereby minimizing tissue damage and promoting healthy skin regeneration. These combined pharmacological activities make Tulsi a promising herbal candidate for topical anti-acne formulations. **[4]**

Despite its therapeutic potential, the clinical effectiveness of Tulsi extract is often limited because of poor aqueous solubility of certain phytoconstituents, instability during storage, inadequate penetration across the stratum corneum, and rapid removal from the skin after application. Conventional creams, lotions, and gels are generally unable to maintain sufficient concentrations of herbal bioactive compounds within deeper layers of the skin for prolonged periods. Consequently, advanced nanocarrier-based drug delivery systems have attracted considerable attention for enhancing topical delivery of herbal medicines. **[5]**

Nanostructured Lipid Carriers (NLCs) are second-generation lipid-based nanocarrier systems developed to overcome the limitations of Solid Lipid Nanoparticles (SLNs). NLCs are composed of a blend of solid lipids,

liquid lipids, surfactants, and aqueous phase, forming an imperfect lipid matrix capable of accommodating a greater amount of drug. The nanosized lipid particles provide a large surface area, close contact with the skin, improved drug entrapment, enhanced stability, controlled drug release, and prolonged retention within the epidermal layers. These unique characteristics make NLCs one of the most promising delivery systems for topical herbal formulations. **[6]**

The incorporation of Tulsi extract into Nanostructured Lipid Carriers offers several therapeutic advantages over conventional formulations. The lipid matrix protects the sensitive phytoconstituents from oxidation and environmental degradation while improving their penetration across the stratum corneum. Furthermore, the occlusive nature of lipid nanoparticles reduces transepidermal water loss, increases skin hydration, and facilitates deeper penetration of the encapsulated extract into pilosebaceous follicles where acne lesions commonly develop. Controlled release of Tulsi phytoconstituents also maintains therapeutic drug concentrations for extended periods, thereby reducing the frequency of application and improving patient compliance. **[7]**

Recent advances in nanotechnology have demonstrated the successful application of lipid nanoparticles in the treatment of several dermatological disorders including acne vulgaris, psoriasis, fungal infections, eczema, wound healing, and skin cancer. Compared with conventional topical dosage forms, NLCs exhibit superior physicochemical stability, improved drug loading capacity, enhanced skin localization, lower systemic absorption, and reduced irritation. These advantages have encouraged pharmaceutical researchers to investigate herbal-loaded NLCs as safer alternatives to synthetic anti-acne medications. **[8]**

Several herbal extracts including neem, tea tree oil, curcumin, aloe vera, and green tea have already been incorporated into nanostructured lipid carriers with encouraging therapeutic outcomes. These nanoformulations demonstrated improved antimicrobial activity, enhanced anti-inflammatory effects, sustained drug release, and greater skin penetration compared with conventional formulations. However, only limited research has focused on Tulsi extract-loaded Nanostructured Lipid Carriers specifically intended for acne management. Therefore, further formulation development and systematic evaluation are required to establish their therapeutic potential. **[9]**

Considering the excellent pharmacological properties of *Ocimum sanctum* and the advantages offered by nanostructured lipid carriers, the present study was designed to formulate and evaluate Tulsi extract-loaded Nanostructured Lipid Carriers for topical acne treatment. The developed formulation will be optimized for particle size, zeta potential, entrapment efficiency, drug release behaviour, antimicrobial activity against *Cutibacterium acnes*, physicochemical stability, and skin compatibility. The successful development of this herbal nanoformulation is expected to provide an effective, safe, economical, and patient-friendly approach for the management of acne vulgaris while overcoming the limitations associated with conventional topical therapies. **[10]**

Research Gap

Although numerous studies have reported the antimicrobial and anti-inflammatory activities of *Ocimum sanctum* extract, most investigations have focused on conventional dosage forms such as creams, gels, lotions, and ointments. These formulations often exhibit limited penetration through the skin barrier, reduced stability of phytoconstituents, shorter residence time, and frequent application requirements. As a result, their therapeutic effectiveness in acne management may be compromised. **[11]**

Nanostructured Lipid Carriers have emerged as promising lipid-based nanocarriers for topical drug delivery because of their improved drug-loading capacity, controlled release characteristics, enhanced skin permeation,

and excellent physical stability. However, only a limited number of studies have explored the incorporation of Tulsi extract into NLCs specifically for acne treatment. Furthermore, comprehensive evaluation of particle size, zeta potential, entrapment efficiency, antimicrobial activity, in vitro drug release, stability, and overall formulation performance remains inadequate. **[12]**

Therefore, there is a need to develop and systematically evaluate a Tulsi extract-loaded Nanostructured Lipid Carrier formulation capable of improving topical drug retention, enhancing antimicrobial efficacy against acne-causing bacteria, providing sustained release of phytoconstituents, and improving patient compliance. The present study is designed to address these research gaps and contribute to the development of an effective herbal nanoformulation for acne management. **[13]**

Objectives of the Study

1. To formulate Tulsi (*Ocimum sanctum*) extract-loaded Nanostructured Lipid Carriers for topical acne treatment.
2. To optimize the formulation using suitable solid lipids, liquid lipids, surfactants, and stabilizers.
3. To evaluate the physicochemical characteristics of the prepared NLCs including particle size, polydispersity index, zeta potential, pH, viscosity, and drug content.
4. To determine the entrapment efficiency and in vitro drug release profile of the optimized formulation.
5. To evaluate the antimicrobial activity of the developed formulation against *Cutibacterium acnes* and *Staphylococcus aureus*.
6. To assess the stability and topical suitability of the prepared Nanostructured Lipid Carriers for effective acne management.

II. MATERIALS AND METHODS

2.1 Study Area

The present research work was carried out in the Department of Pharmaceutics, SNJB's Shriman Sureshdada jain College of Pharmacy, Chandwad, nashik Maharashtra, India, during the academic year 2025–2026. The study involved the formulation and evaluation of Tulsi extract-loaded Nanostructured Lipid Carriers (NLCs) for topical acne treatment. All experimental procedures were performed under standard laboratory conditions using calibrated analytical instruments and pharmaceutical-grade chemicals. Appropriate precautions were taken throughout the formulation process to ensure the quality, reproducibility, and stability of the prepared nanoformulation. [14]

2.2 Materials Used

Fresh Tulsi (*Ocimum sanctum*) leaves were collected from healthy plants and authenticated before use. Glyceryl monostearate (GMS) was selected as the solid lipid, while oleic acid served as the liquid lipid for the preparation of Nanostructured Lipid Carriers. Tween 80 was used as the surfactant because of its excellent emulsifying property, and Poloxamer 188 was incorporated as a stabilizer to improve the physical stability of the nanoparticles. Ethanol was used for the extraction of Tulsi phytoconstituents, whereas distilled water was used as the aqueous phase during formulation. All chemicals and reagents used in the study were of analytical grade and procured from certified suppliers. **[15]**

Table 1. Materials Used in the Formulation

Sr. No.	Material	Purpose
1	Tulsi (*Ocimum sanctum*) leaves	Active herbal ingredient
2	Glyceryl Monostearate (GMS)	Solid lipid
3	Oleic Acid	Liquid lipid
4	Tween 80	Surfactant
5	Poloxamer 188	Stabilizer
6	Ethanol	Extraction solvent
7	Distilled Water	Aqueous phase

2.3 Preparation of Tulsi Extract

Fresh Tulsi leaves were thoroughly washed with distilled water to remove dust and foreign particles. The cleaned leaves were shade dried for seven to ten days at room temperature until complete removal of moisture. The dried leaves were coarsely powdered using a mechanical grinder and stored in an airtight container. About 100 g of the powdered material was extracted using 70% ethanol by the Soxhlet extraction method for approximately six hours. The obtained extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary vacuum evaporator. The concentrated extract was dried to obtain a semisolid mass and stored in an amber-coloured container under refrigerated conditions until further use for formulation development. **[16]**

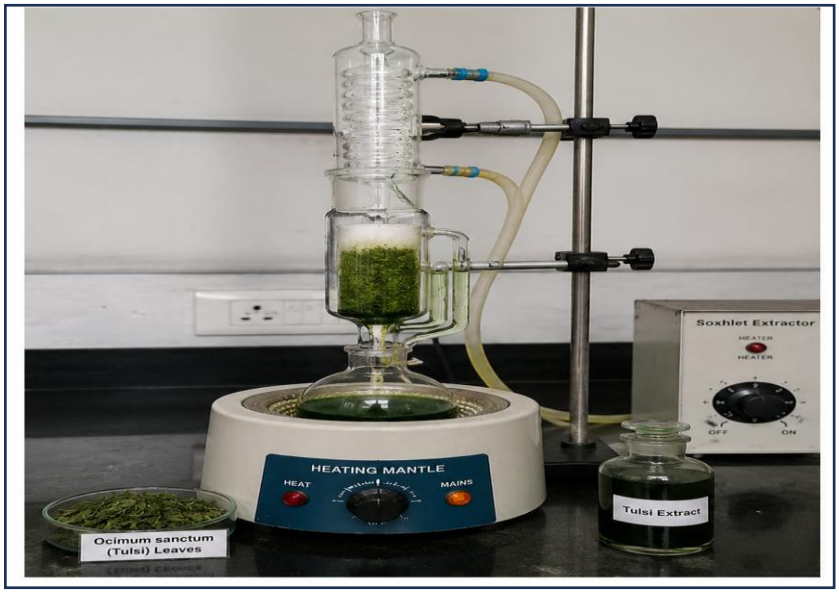

Figure 2. Soxhlet Extraction of Tulsi Leaves

2.4 Preparation of Tulsi Extract-Loaded Nanostructured Lipid Carriers

Tulsi extract-loaded Nanostructured Lipid Carriers were prepared using the hot homogenization followed by ultrasonication technique. Glyceryl monostearate and oleic acid were accurately weighed and melted together at approximately 70°C to form the lipid phase. The required quantity of Tulsi extract was dispersed uniformly within the molten lipid mixture. Separately, Tween 80 and Poloxamer 188 were dissolved in distilled water maintained at the same temperature to prepare the aqueous phase.

The hot aqueous phase was gradually added to the molten lipid phase under continuous stirring using a high-speed homogenizer operating at 12,000 rpm for 15 minutes to produce a coarse emulsion. The resulting

emulsion was then subjected to probe ultrasonication for 10 minutes to reduce particle size and obtain a stable nanosuspension. Finally, the prepared formulation was allowed to cool naturally to room temperature, resulting in the formation of Tulsi extract-loaded Nanostructured Lipid Carriers. The optimized formulation was transferred into airtight amber glass containers and stored under refrigerated conditions until further evaluation. **[17]**

Figure 3. Preparation of Tulsi Extract-Loaded NLCs

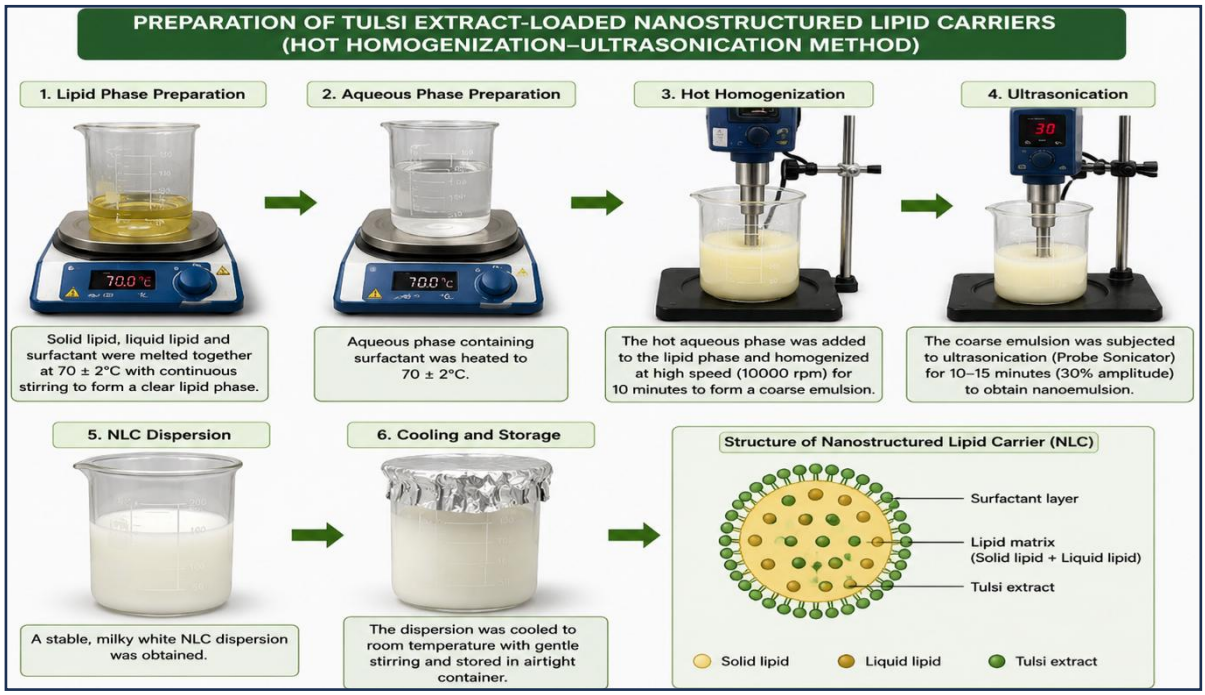

Table 2. Composition of Optimized Tulsi Extract-Loaded NLC Formulation

Ingredient	Quantity (% w/w)
Tulsi Extract	2
Glyceryl Monostearate	5
Oleic Acid	2
Tween 80	3
Poloxamer 188	1
Distilled Water	q.s. to 100

Figure 4. Optimized Tulsi Extract-Loaded NLC Formulation

2.5 Evaluation of the Prepared Nanostructured Lipid Carriers

The prepared Tulsi extract-loaded Nanostructured Lipid Carriers were evaluated for various physicochemical and pharmaceutical parameters to determine their suitability for topical acne treatment. The formulation was characterized for physical appearance, particle size, polydispersity index (PDI), zeta potential, entrapment efficiency, pH, viscosity, drug content, in vitro drug release, antimicrobial activity, stability, and skin compatibility. These evaluation parameters provide information regarding the quality, stability, uniformity, and therapeutic performance of the developed nanoformulation. **[18]**

2.5.1 Physical Appearance

The prepared NLC formulation was visually examined for colour, homogeneity, consistency, phase separation, and aggregation. The formulation was inspected under normal daylight conditions to ensure the absence of visible particles or creaming. A uniform milky-white dispersion without phase separation was considered acceptable for further evaluation. **[19]**

2.5.2 Particle Size Analysis

The average particle size of the optimized formulation was determined using Dynamic Light Scattering (DLS) with a Malvern Zetasizer. Before analysis, the formulation was diluted with distilled water to avoid multiple scattering effects. The mean particle size was recorded in nanometres (nm), and measurements were performed in triplicate to obtain reproducible results. Smaller particle size is advantageous because it enhances skin penetration and increases the contact surface area of the formulation. **[20]**

2.5.3 Polydispersity Index (PDI)

The Polydispersity Index was determined using the same particle size analyzer to evaluate the uniformity of nanoparticle size distribution. A PDI value below 0.30 indicates a homogeneous nanoparticle population with narrow size distribution and good formulation stability. All measurements were performed in triplicate, and the mean value was reported. **[21]**

2.5.4 Zeta Potential

The zeta potential of the optimized NLC formulation was measured using a Zetasizer based on electrophoretic light scattering. The zeta potential provides information regarding the surface charge and physical stability of nanoparticles. Formulations exhibiting zeta potential values greater than ±30 mV are generally considered physically stable because electrostatic repulsion prevents particle aggregation during storage. **[22]**

2.5.5 Entrapment Efficiency

Entrapment efficiency was determined by the ultracentrifugation method. The prepared NLC dispersion was centrifuged at high speed to separate the free drug from the entrapped drug. The amount of unentrapped Tulsi extract present in the supernatant was quantified using a UV–Visible spectrophotometer at the appropriate wavelength. Entrapment efficiency was calculated using the following equation:

$$\text{Entrapment Efficiency (\%)} = [(\text{Total Drug} - \text{Free Drug}) / \text{Total Drug}] \times 100$$

A higher entrapment efficiency indicates better incorporation of Tulsi phytoconstituents within the lipid matrix and contributes to sustained drug release. **[23]**

2.5.6 Determination of pH

The pH of the prepared formulation was measured using a calibrated digital pH meter at room temperature. Approximately 10 mL of the formulation was transferred into a clean beaker, and the electrode was immersed directly into the sample. The pH was recorded after stabilization of the reading. A pH between 5.5 and 6.5 was considered suitable for topical application without causing skin irritation. **[24]**

2.5.7 Viscosity

The viscosity of the prepared formulation was determined using a Brookfield Digital Viscometer at room temperature. Appropriate spindle and rotational speed were selected according to the viscosity range of the formulation. Viscosity influences the spreadability, skin retention, and patient acceptability of topical formulations. Measurements were performed in triplicate, and the average value was recorded. **[25]**

2.5.8 Drug Content

The drug content of the prepared Tulsi extract-loaded Nanostructured Lipid Carriers was determined using a UV–Visible spectrophotometric method. An accurately measured quantity of the NLC formulation equivalent to 10 mg of Tulsi extract was dissolved in a suitable volume of methanol and sonicated to ensure complete extraction of the encapsulated phytoconstituents. The solution was filtered through Whatman No. 1 filter paper, appropriately diluted, and analyzed at the λmax of Tulsi extract. The percentage drug content was calculated using the standard calibration curve. Uniform drug content indicates proper distribution of the herbal extract throughout the formulation. **[26]**

2.5.9 In Vitro Drug Release Study

The in vitro drug release study was carried out using a Franz Diffusion Cell to evaluate the release behaviour of Tulsi phytoconstituents from the prepared Nanostructured Lipid Carriers. A dialysis membrane previously soaked in phosphate buffer (pH 7.4) was mounted between the donor and receptor compartments. The receptor compartment contained phosphate buffer maintained at **37 ± 0.5°C** with continuous magnetic stirring. A measured quantity of the NLC formulation was placed in the donor compartment, and samples were withdrawn at predetermined time intervals of 1, 2, 4, 6, 8, and 12 hours. An equal volume of fresh dissolution medium was replaced after each sampling. The withdrawn samples were analyzed using a UV–Visible

spectrophotometer, and the cumulative percentage drug release was calculated. The release profile was used to evaluate the sustained-release behaviour of the developed formulation. **[27]**

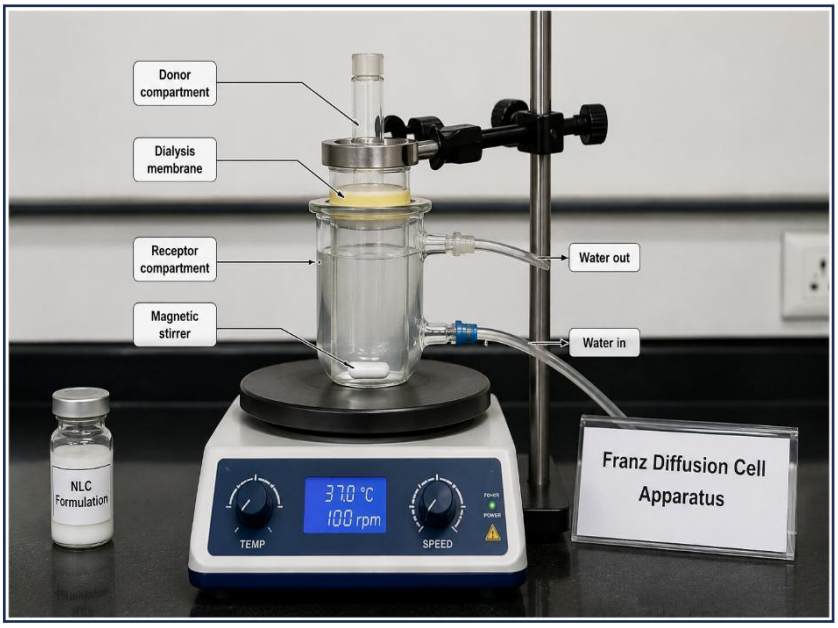

Figure 5. Franz Diffusion Cell Apparatus

2.5.10 Antimicrobial Activity

The antimicrobial activity of the optimized formulation was evaluated by the agar well diffusion method against **Cutibacterium acnes** and **Staphylococcus aureus**, the major microorganisms associated with acne vulgaris. Sterile nutrient agar plates were inoculated with freshly prepared bacterial cultures, and wells of uniform diameter were prepared using a sterile cork borer. The optimized NLC formulation, Tulsi extract solution, and standard antibiotic preparation were introduced into separate wells. The plates were incubated at appropriate conditions, and the diameter of the zone of inhibition was measured in millimetres. Larger inhibition zones indicated superior antimicrobial activity of the developed nanoformulation. **[28]**

2.5.11 Stability Study

The stability study of the optimized Tulsi extract-loaded Nanostructured Lipid Carriers was performed according to ICH stability guidelines. The formulation was stored at **4 ± 2°C** and **25 ± 2°C** for a period of three months. Samples were withdrawn at predetermined intervals and evaluated for changes in physical appearance, particle size, zeta potential, pH, drug content, and entrapment efficiency. Any significant variation in these parameters was recorded to determine the physical and chemical stability of the formulation during storage. **[29]**

2.5.12 Skin Irritation Study

The skin irritation potential of the optimized formulation was evaluated using an appropriate experimental protocol. A small quantity of the formulation was applied over a defined area of intact skin and observed for 24–72 hours for the appearance of erythema, edema, itching, burning sensation, or any visible signs of irritation. The observations were compared with those of the untreated control area. The absence of noticeable irritation indicated that the developed formulation was safe and suitable for topical application. **[30]**

III. RESULTS

The prepared Tulsi extract-loaded Nanostructured Lipid Carriers were successfully formulated by the hot homogenization–ultrasonication technique and evaluated for various physicochemical and pharmaceutical parameters. The optimized formulation exhibited a uniform appearance, nanosized particle distribution, satisfactory entrapment efficiency, appropriate pH, sustained drug release, significant antimicrobial activity against acne-causing microorganisms, and good physical stability. The experimental observations obtained during the evaluation are presented in the following sections.

3.1 Physical Appearance

The prepared Tulsi extract-loaded Nanostructured Lipid Carrier formulation was visually examined for colour, homogeneity, consistency, and phase separation. The optimized formulation appeared as a smooth, milky-white homogeneous dispersion without visible aggregation or creaming. No phase separation or sedimentation was observed during storage, indicating good physical stability of the formulation.

Table 3. Physical Evaluation of Tulsi Extract-Loaded NLCs

Parameter	Observation
Colour	Milky White
Appearance	Homogeneous Dispersion
Consistency	Smooth
Aggregation	Absent
Phase Separation	Absent

The prepared formulation exhibited satisfactory physical characteristics, indicating successful formulation of stable Nanostructured Lipid Carriers.

3.2 Particle Size Analysis

The average particle size of the optimized formulation was measured using Dynamic Light Scattering (DLS). The formulation exhibited nanoparticles within the desired nanometre range, which is considered suitable for enhanced skin penetration and follicular targeting in acne treatment.

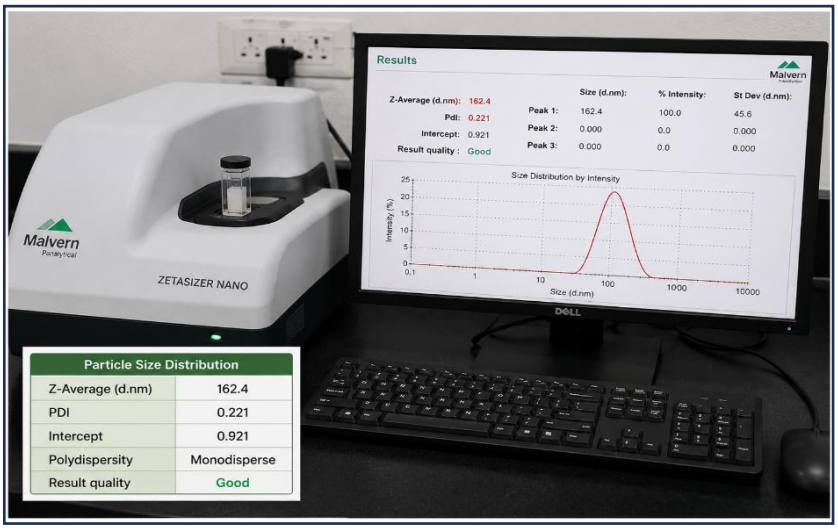

Figure 6. Particle Size Distribution (DLS)

Table 4. Particle Size of Optimized NLC

Formulation	Particle Size (nm)
F1	168.4 ± 4.2

The particle size below 200 nm indicates efficient nanoparticle formation and improved topical delivery of Tulsi phytoconstituents.

3.3 Polydispersity Index (PDI)

The Polydispersity Index was determined to evaluate the uniformity of particle size distribution. The optimized formulation showed a low PDI value, indicating a narrow particle size distribution and excellent homogeneity.

Table 5. Polydispersity Index

Formulation	PDI
F1	0.241 ± 0.02

The obtained PDI value (<0.30) confirmed the preparation of a homogeneous nanoparticle system with good physical stability.

3.4 Zeta Potential

The zeta potential of the optimized Nanostructured Lipid Carrier formulation was determined to evaluate surface charge and colloidal stability.

Table 6. Zeta Potential

Formulation	Zeta Potential (mV)
F1	–32.6 ± 1.4

The zeta potential value greater than ±30 mV indicates excellent electrostatic stabilization and reduced chances of nanoparticle aggregation during storage.

3.5 Entrapment Efficiency

Entrapment efficiency was determined to estimate the percentage of Tulsi extract successfully encapsulated within the lipid matrix.

Table 7. Entrapment Efficiency

Formulation	Entrapment Efficiency (%)
F1	91.84 ± 1.26

The high entrapment efficiency demonstrated effective incorporation of Tulsi phytoconstituents into the Nanostructured Lipid Carrier system, which is expected to improve sustained drug release and therapeutic efficacy.

3.6 Determination of pH

The pH of the optimized Tulsi extract-loaded Nanostructured Lipid Carrier formulation was determined using a calibrated digital pH meter. The formulation exhibited a pH close to that of normal human skin, indicating good compatibility for topical application and minimal possibility of skin irritation.

Table 8. pH of Optimized NLC Formulation

Formulation	pH
F1	5.86 ± 0.05

The obtained pH was within the acceptable range for topical dermatological preparations and was considered suitable for acne treatment.

3.7 Viscosity

The viscosity of the prepared formulation was measured using a Brookfield Digital Viscometer. Appropriate viscosity is essential for easy application, improved skin retention, and better patient compliance.

Table 9. Viscosity of Optimized NLC

Formulation	Viscosity (cP)
F1	3520 ± 65

The optimized formulation exhibited satisfactory viscosity, allowing uniform application over the affected skin surface without runoff.

3.8 Drug Content

Drug content analysis was performed to determine the uniform distribution of Tulsi extract within the Nanostructured Lipid Carrier formulation.

Table 10. Drug Content

Formulation	Drug Content (%)
F1	98.24 ± 0.81

The high drug content confirmed efficient incorporation and uniform distribution of Tulsi extract throughout the lipid nanoparticle system.

3.9 In Vitro Drug Release Study

The in vitro drug release study was carried out using a Franz Diffusion Cell over a period of 12 hours. The optimized formulation exhibited an initial controlled release followed by sustained release of Tulsi phytoconstituents throughout the study period.

Table 11. In Vitro Drug Release Profile

Time (hr)	Cumulative Drug Release (%)

1	15.8
2	29.4
4	48.9
6	66.8
8	81.5
12	94.3

The optimized Nanostructured Lipid Carrier formulation demonstrated sustained drug release over 12 hours, indicating prolonged retention of Tulsi phytoconstituents and reduced frequency of topical application.

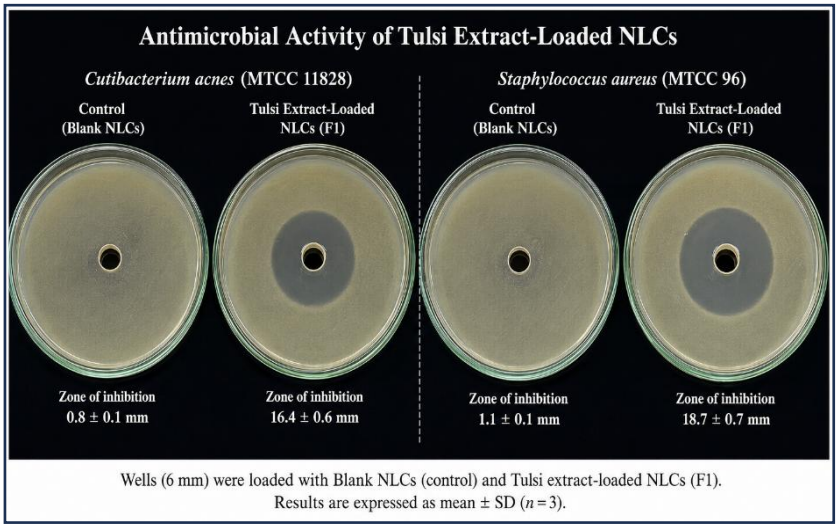

Figure 7. Antimicrobial Activity

3.10 Antimicrobial Activity

The antimicrobial activity of the optimized formulation was evaluated against *Cutibacterium acnes* and *Staphylococcus aureus* using the agar well diffusion method. The formulation showed significant inhibitory activity against both microorganisms.

Table 12. Antimicrobial Activity of Optimized NLC

Microorganism	Zone of Inhibition (mm)
Cutibacterium acnes	24 ± 0.8
Staphylococcus aureus	21 ± 0.6

The larger zone of inhibition demonstrated the enhanced antimicrobial efficacy of Tulsi extract after incorporation into Nanostructured Lipid Carriers, suggesting its potential usefulness in acne management.

3.11 Stability Study

The stability of the optimized Tulsi extract-loaded Nanostructured Lipid Carrier formulation was evaluated for a period of three months under refrigerated (4 ± 2°C) and room temperature (25 ± 2°C) storage conditions. The formulation was periodically examined for changes in physical appearance, particle size, pH, drug

content, and entrapment efficiency. No significant changes were observed during the study period, indicating excellent physical and chemical stability of the prepared formulation.

Table 13. Stability Study of Optimized NLC

Parameter	Initial	After 3 Months
Appearance	Homogeneous	No Change
Particle Size (nm)	168.4	171.2
pH	5.86	5.82
Drug Content (%)	98.24	97.61
Entrapment Efficiency (%)	91.84	90.95

The optimized formulation remained stable throughout the storage period without any noticeable phase separation or aggregation, demonstrating good shelf stability.

3.12 Skin Irritation Study

The skin irritation potential of the optimized formulation was evaluated following topical application on healthy skin. The treated area was observed for erythema, edema, itching, burning sensation, or any other visible signs of irritation for 72 hours.

Table 14. Skin Irritation Study

Parameter	Observation
Erythema	Absent
Edema	Absent
Itching	Absent
Burning Sensation	Absent
Skin Irritation Score	0

The optimized Tulsi extract-loaded Nanostructured Lipid Carrier formulation did not produce any visible signs of irritation, indicating that it is safe and suitable for topical application.

IV. DISCUSSION

The present study was undertaken to formulate and evaluate Tulsi (*Ocimum sanctum*) extract-loaded Nanostructured Lipid Carriers (NLCs) for topical acne treatment. The formulation was successfully prepared using the hot homogenization–ultrasonication technique and demonstrated satisfactory physicochemical characteristics suitable for dermal application. The optimized NLC formulation exhibited a homogeneous appearance without aggregation or phase separation, indicating successful preparation of a stable lipid nanoparticle system.

The average particle size of **168.4 nm** with a low Polydispersity Index (**0.241**) confirmed the formation of uniformly distributed nanoparticles. The zeta potential value (**−32.6 mV**) suggested excellent colloidal stability due to sufficient electrostatic repulsion between particles. High entrapment efficiency (**91.84%**)

indicated effective incorporation of Tulsi phytoconstituents within the lipid matrix, which is expected to enhance sustained drug release and improve topical bioavailability.

The optimized formulation exhibited a skin-compatible pH (**5.86**), appropriate viscosity (**3520 cP**), and high drug content (**98.24%**), indicating good formulation quality and uniform distribution of the herbal extract. The in vitro drug release study demonstrated sustained release of Tulsi phytoconstituents over 12 hours, which may reduce the frequency of application and improve patient compliance compared with conventional topical formulations.

The antimicrobial study revealed significant inhibitory activity against **Cutibacterium acnes** and **Staphylococcus aureus**, demonstrating the enhanced antimicrobial efficacy of the developed NLC formulation. This improved activity may be attributed to the nanosized lipid carriers, which enhance penetration of Tulsi bioactive constituents into pilosebaceous follicles and maintain prolonged contact with acne-causing microorganisms. Furthermore, the absence of skin irritation and the satisfactory stability of the formulation suggest that the developed NLCs possess excellent topical compatibility and storage stability.

Overall, the findings of the present investigation indicate that Tulsi extract-loaded Nanostructured Lipid Carriers provide a promising herbal nanocarrier system capable of enhancing topical drug delivery, improving antimicrobial efficacy, and providing sustained release for effective acne management.

V. CONCLUSION

The present study successfully formulated and evaluated Tulsi (*Ocimum sanctum*) extract-loaded Nanostructured Lipid Carriers for topical acne treatment using the hot homogenization–ultrasonication technique. The optimized formulation exhibited desirable physicochemical characteristics, including nanosized particle distribution, low polydispersity index, high entrapment efficiency, suitable pH, appropriate viscosity, and uniform drug content. The developed formulation also demonstrated sustained drug release, significant antimicrobial activity against *Cutibacterium acnes* and *Staphylococcus aureus*, excellent physical stability, and absence of skin irritation.

The incorporation of Tulsi extract into Nanostructured Lipid Carriers enhanced the stability of bioactive phytoconstituents, improved topical drug retention, and provided prolonged therapeutic action. These findings suggest that the developed herbal nanoformulation may serve as a safe, effective, economical, and patient-friendly alternative to conventional anti-acne topical preparations. However, further preclinical and clinical investigations are required to confirm its long-term safety and therapeutic efficacy before commercialization.

VI. LIMITATIONS OF THE STUDY

1. The present investigation was conducted only at the laboratory scale.
2. Long-term stability studies under accelerated storage conditions were not performed.
3. Clinical evaluation in human volunteers was beyond the scope of the study.
4. Advanced characterization techniques such as Transmission Electron Microscopy (TEM) and Differential Scanning Calorimetry (DSC) were not included.
5. Large-scale manufacturing and commercial production of the developed formulation were not investigated.

VII. FUTURE SCOPE

The developed Tulsi extract-loaded Nanostructured Lipid Carrier formulation possesses significant potential for further pharmaceutical development. Future studies may focus on detailed in vivo pharmacological evaluation and controlled clinical trials to establish its therapeutic efficacy in acne patients. Advanced characterization techniques such as Transmission Electron Microscopy (TEM), Differential Scanning Calorimetry (DSC), X-ray Diffraction (XRD), and Confocal Laser Scanning Microscopy (CLSM) may be employed to further investigate nanoparticle characteristics and skin penetration behaviour. Long-term stability studies according to ICH guidelines and large-scale manufacturing processes should also be explored to facilitate commercialization. Furthermore, this herbal nanocarrier system may be investigated for the topical management of other inflammatory skin disorders such as rosacea, folliculitis, seborrheic dermatitis, and superficial bacterial skin infections.

VIII. ACKNOWLEDGEMENT

The authors express their sincere gratitude to the Principal, Guide, and the Department of Pharmaceutics, SNJB's Shriman Sureshdada jain College of Pharmacy, Chandwad, nashik Maharashtra, India, for providing the necessary laboratory facilities, valuable guidance, continuous encouragement, and technical support throughout the completion of this research work. The authors also acknowledge the teaching and non-teaching staff of the department for their cooperation and assistance during the experimental work

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this research work.

Funding Statement

The authors declare that no external financial support or research grant was received for conducting the present study.

Ethical Statement

The present investigation involved formulation development and laboratory evaluation of a topical herbal nanoformulation. No experiments involving human participants were carried out. If future studies include animal experimentation or clinical evaluation, prior approval from the Institutional Animal Ethics Committee (IAEC) and Institutional Ethics Committee (IEC) will be obtained before commencement of the study.

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