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Research Paper

Quality by Design (QbD)-Based Optimization of β -Glucan Nanosponges Loaded with Polianthes tuberosa Extract and Their Incorporation into a Topical Gel for Enhanced Anti-Acne Delivery

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ABSTRACT

Acne vulgaris is a chronic inflammatory skin condition associated with the excessively secreted sebum, follicles blockage, increased multiplication of microorganisms, and inflammation. These conventional anti-acnes treatment approaches invariably cause some kind of side effects such as skin irritability, skin drying, and resistance to the antibiotics, thus necessitating the need to discover more harmless and effective interventions. The purpose of the present experiment was to develop a design and optimize Polianthes -glucan nanosponges by loading with the Polianthes tuberosa extract using the Quality by Design (QbD) method and entrap the optimized formula in a topical gel to facilitate the most efficient delivery capabilities of the anti-acnes qualities. The flowers of Polianthes tuberosa were harvested and verified with the fresh flowers and dried along with powdering and extraction to n-hexane, ethyl acetate and methanol. Methanol extracts showed the maximum extraction yield (16.52%), and were utilized in further studies. Initial phytochemical screening bore out that it had flavonoids, phenol, tannin, terpenoid and alkaloids. LC-MS/GC-MS profiling has established that several bioactive products like eugenol, phytol, quercetin, kaempferol, linoleic acid and 2-sitosterol have been identified. QbD principles have been adopted with the identification of QTPP, computation of CQA, evaluation of risk by FMEA and

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box-Behnken optimization. The nanosponge-optimized formulation was characterized by their particle size (398.6 2.8 nm), PDI (0.438), and zeta potential (-19.7 mV) with an entrapment efficiency of 79.7-percent. FTIR, XRD, DSC, SEM and TEM analyses were performed to ascertain the success of the encapsulation and structural stability of the formulation. Drug release Time-course In-vitro drug release experiment showed that it released 93.8 percent within 24-hours and followed Higuchi diffusion kinetics. The nanosponges were optimized and incorporated into a topical gel and proved to have good pH (6.4), viscosity (6515 cps), spreadability, drug content (95.5%), and stability. The formulation was demonstrated to possess a large level of antibacterial activity towards *Cutibacterium acnes* (21.20 mm) and *Staphylococcus epidermidis* (19.52 mm). This study concludes that Polianthes tuberosa extract-impregnated into β -glucan nanosponge gel is one of the promising herbal nanotherapeutic systems that will become a useful topical agent in the treatment of acnes.

INTRODUCTION

Overview of Acne and Pathophysiology of Acne Vulgaris

Acne vulgaris is a fashionable form of the chronic inflammatory skin fundamentally around the pilosebaceous units of the skin in adolescents and youths. It is common in the face, chest, shoulders and back where there are the greatest number of sebaceous glands. Acne clinically involves blackheads, whiteheads, papules, pustules, nodules and cysts as non-inflammatory lesions and inflammatory lesions, respectively. The disease may also severely affect the self-esteem and life of an individual due to the skin damage that is not only visible, but also scarring. Acne pathophysiology is a constellation of factors. Excessive secretion of androgen causes the sebaceous glands to over-produce the sebum in an oily climate that nurtures in the development of acne. Follicular hyperkeratinization causes blockage of hair follicles, leading to comedone formation. The proliferation of *Cutibacterium*

acnes within blocked follicles triggers inflammatory reactions through the release of lipases and pro-inflammatory mediators. Oxidative stress and immune responses further worsen skin inflammation. Genetic predisposition, hormonal imbalance, diet, stress, and environmental factors may also contribute to acne progression. Due to its multifactorial nature and recurrent episodes, acne requires effective long-term management strategies that can target multiple pathogenic pathways while minimizing adverse effects associated with conventional therapies.[1]

Current Treatment Limitations

Current treatment strategies for Acne vulgaris include topical retinoids, benzoyl peroxide, antibiotics, hormonal therapy, and oral isotretinoin. Although these therapies are effective in reducing acne lesions, they are associated with several limitations that may compromise treatment outcomes. Topical antibiotics such as clindamycin and erythromycin have shown reduced effectiveness due to the increasing emergence of antibiotic-resistant strains of *Cutibacterium acnes*. [2] Long-term antibiotic use may also disturb normal skin flora. Retinoids frequently cause adverse effects such as redness, peeling, dryness, itching, and increased sensitivity to sunlight. Oral isotretinoin proves very efficient in the treatment of severe acne though with a severe side effect, such as teratogenicity, hepatic toxicity and psychiatric complications. Only a specific number of patients can receive hormonal therapies and these therapies have the potential to cause hormonal disbalance. Low patient compliance levels are the other major barrier because it is a long period of treatment that implies schedules of application of drugs regularly, not to mention that it recurs right after stopping it. Some of the artificial formulations that have been prepared contain harsh chemicals as well, and this may



irritate the sensitive skin. All these cons indicate that there is an immediate need to locate alternative anti-acne therapies that are safer, effective, inexpensive treatment, and can provide lasting results at minimum adverse effects and

improved tolerability of the drug on the part of patients. [3]

Herbal Treatment Potential and *Polianthes tuberosa* Phytoconstituents



Figure 2. Field photograph of *Polianthes tuberosa* plant

Herbal medicines are getting a renewed attention in treatment of dermatology because of its natural origin, lower toxicity and its broad range of action. Plant compounds have been found to be good source of antibacterial, anti-inflammatory, antioxidant and skin healing which makes them good candidate treatment of acne. Tuberose (*Polianthes tuberosa*) has been historically believed to have a medicinal and aromatic value. Recent phytochemical research has revealed that the plant contains an assortment of bioactive substances that consists of flavonoids, phenolic compounds, alkaloids, terpenoids, glycosides, tannins and saponins. Some of these such as quercetin, kaempferol, gallic acid, phytol and eugenol have been found to have anti microbial and antiinflammatory properties. These phytoconstituents can inhibit the acnes causing bacteria, de activate oxidative stress, hyperirritate inflammatory mediators and repair wounds. Although it has a therapeutic potential, the topical use of herbal extracts is generally impaired by various factors such as inefficient stability, solubility, and inactivation of active ingredients and inadequate penetration of active ingredients through the skin. Therefore, further advanced drug

delivery systems are required to increase the efficacies of herbal extracts. Nanocarrier system incorporation of *Polianthes tuberosa* extract has the potential to stabilize the *Polianthes tuberosa* extract and specificity in delivery to treat acne effectively [4]

Nanosponges in Drug Delivery and Benefits of β -Glucan Nanosponges

Nanosponges are nanosized porous drug delivery systems that can be utilized as a way of encasing active pharmaceutical ingredients, and releasing them over time in a regulated environment without harm to the body. The ability to stabilize drugs, improve drug penetration and reduce irritation effects make topical and transdermal drug delivery by these carriers receiving worldwide interest[5]. Nanosponges are very porous networks that very effectively trap lipophilic and hydrophilic substances. The nanosponges can offer an edge to the acnes treatment as it can be applied directly on the active ingredient directly to the sebaceous glands and hair follicles, making the treatments more effective. The β -glucan based nanosponges also has the benefits of wound-repairing,

immunosulphuric and immunomodulatory features that could be applied to the skin that is prone and release of drugs and increased skin retention is achievable by the nanosponges. They are also capable of reducing rates of application, and improving patient compliance. The use of 200 2-glucan nanosponges as a system of delivery of herbal extracts is a new way of coming up with the safest and most effective topically applied anti-acnes agents.

Quality by Design Concept and FDA Principles

Quality by Design (QbD) is a risk-based, systematic, and science-based approach to product development in pharmaceutical formulations, which puts an emphasis on product quality via process insight, risk management, and insight. The United States Food and Drug Administration first introduced and recommended QbD in an attempt to offer standard product performance and to minimize setbacks in manufacturing, the Quality Target Product Profile (QTPP) outlines the desired product properties such as: safety, efficacy and stability. Critical Quality Attributes (CQAs), Critical Material Attributes (CMAs), Critical Process Parameters (CPPs) are ascertained in a bid to educate on what attributes define the quality of the product. Risk assessment tools are available, such as Failure Mode and Effects Analysis (FMEA) that could be utilized to uncover risks during the formulation. The optimization of the effects of formulation variables is primarily carried out through experimentation by Design of Experiments (DoE). QbD has been applied in the preparation of nanosponge to ensure that the size of the particles is optimized, entrapment effectiveness, and release of drugs and stability. Such approach can save time and resources and prevent trial-and-error testing and will result in greater regulatory acceptability. The reliance on the development of the herbal nanosponge can be

ensured to use the principles of QbD to ensure the high and reproducible formulation process. [8]

Research Gap

Despite the growing concern about the use of herbal preparations in the management of acne, there is a paucity of scientific data on the efficacy of *Polianthes tuberosa* in relation to treating acne. Most of the published literature on the plant is related primarily with its ornamental aspect, the use of the fragrance and the general pharmacological applications, but little has been done in derivation of the dermatological applications. Although the plant does contain several bioactive phytoconstituents with anti-bacterial, anti-oxidant, and anti-inflammatory properties, the question has not been substantiated on its specific action, and its use against acnes-causing microorganisms. Additionally, the standard preparations of herbal compounds using traditional methods tend not to be well stable, characterize by penetration into the skin and bioavailability. Nanotechnology procedures such as nanoparticle nanosponges have already proven to be potentially useful in improving the topical drug delivery, yet virtually no data have been generated to utilize 14 -glucan as a nanosponge polymer to release herbal extracts. Additionally, application of principles in the Quality by Design of the United States Food and Drug Administration of optimizing the β -glucan nanosponge formulations has not been fully explored. Until now, no other research has constructed QbD-optimized nanosponges of 8-glucan and either charged them with *Polianthes tuberosa* extract and embedded within a topical gel to offer a superior anti-acnes treatment regimen. Such knowledge gap shows that the present research has a new-fangled research and scientific value.

Aim of the Study



The aim of the current research will be to formulate and optimize *Polianthes tuberosa* extract-loaded β -glucan nanosponges by embracing Quality by Design and incorporate the optimized nanosponge formulation into a topical gel to optimally provide the anti-acnes effect. The study will not only maximize both stability and skin penetration of the herbal extract, and its effectiveness in managing the disease, but minimize the disadvantages of the conventional acnes treatments. The discussion will also dwell on offering a safer, more natural and effective alternative of treating acnes by integrating the frontline of nanotechnology and drug delivery-structure with herbal medicine.

Objectives of the Study

- To collect, authenticate, and process *Polianthes tuberosa* flowers for research purposes.
- To prepare plant extract using a suitable extraction method.
- To perform phytochemical screening for identification of bioactive constituents.
- To formulate β -glucan-based nanosponges loaded with *Polianthes tuberosa* extract.
- To apply United States Food and Drug Administration Quality by Design principles for formulation optimization.
- To identify Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs).
- To optimize formulation variables using Design of Experiments (DoE).
- To characterize the prepared nanosponges for particle size, polydispersity index, zeta potential, entrapment efficiency, and surface morphology.
- To incorporate the optimized nanosponge formulation into a topical gel base.
- To evaluate the prepared gel for pH, viscosity, spreadability, homogeneity, and drug content.

- To perform in-vitro drug release studies.
- To evaluate antimicrobial activity against acne-causing microorganisms such as *Cutibacterium acnes* and *Staphylococcus epidermidis*.
- To conduct stability studies of the optimized formulation.
- To assess the overall potential of the developed formulation for enhanced anti-acne therapy.

2.MATERIALS AND METHODS



Figure 3: Fresh Flowers of *Polianthes tuberosa* Collected for Extraction

Plant Collection and Authentication

The flowering period of Fresh *Polianthes tuberosa* (Family: Asparagaceae) was also collected during flowering at Kolhapur, Maharashtra, India. In locating and purifying the plant material, the material was washed using the tap water and subsequently using the double distilled water to eliminate dust, soil and other outside contamination factors and further processed. This plant material identity and purity was established by using the standard taxonomical characteristics as described by the world health organization so that the identity and purity of the plant material was determined[9] The collected flowers were washed thoroughly with tap water followed by double-distilled water to remove dust, soil

particles, and other foreign contaminants before further processing.

Drying and Powdering of Plant Material

Diindividual shade-drying of the flowers of *Polianthes tuberosa* to dry them at a single temperature was followed by pulverizing the flowers to a coarse powder using a mechanical

grinder to avoid the destruction of any of the temperature-sensitive phytoconstituents present in them.[10] After complete drying, the flowers were pulverized using a mechanical grinder to obtain a coarse powder. The powdered material was stored in clean, dry, airtight glass containers for further experimental use



Figure 4: Drying of *Polianthes tuberosa* Flowers



Figure 5: Powdering of Dried *Polianthes tuberosa* Flowers

Solvent Extraction Process



Figure 6: Solvent Extraction Process of *Polianthes tuberosa* Flower Extract

It was initially subjected to the extraction process using different solvents (n-hexane, ethyl acetate, and methanol) to establish the solvents to be used when extracting phytochemicals. The cold maceration technique was employed,[11] In which the powdered plant material was precisely immersed in 1000 mL of both solvents and

allowed to mix at room temperature over 72 hours with periodic stirring to ensure that a maximum of phytoconstituents get diffused into the solvents. This was followed by extraction followed by filtration using muslin cloth then using Whatman No.1 filter paper to remove the plant residues and to obtain crude extracts with the help of a step of a

steam bath at 50°C which took care of evaporating off the solvents. The concentrated extracts were cooled and stored airtight at 4°C to be further examined.

Calculation of Percentage Yield of Extract

The percentage yield of extract was used to determine efficiency of different solvents in extracting *Polianthes tuberosa* flower powder. After the evaporation of all the solvents was completed the dried extracts of each solvent were weighed and the percentage yield calculated using the following formula:

Extraction Yield (%)

$$= \frac{\text{Weight of Extract Obtained (g)}}{\text{Weight of Powder Taken (g)}} \times 100$$

Where:

- Weight of extract obtained = Final dried extract collected after solvent evaporation
- Weight of powder taken = Initial dried flower powder used for extraction [13]

The extraction yield was compared among different solvents to identify the most efficient solvent for maximum recovery of phytoconstituents.

Table 1. Percentage Yield Calculation of Different Solvent Extracts of *Polianthes tuberosa*

Solvent	Weight of Powder (g)	Extract Obtained (g)	Yield (%)
n-Hexane	50	2.56	5.12
Ethyl acetate	50	4.92	9.84
Methanol	50	8.26	16.52

Preliminary Phytochemical Screening

The obtained extracts of *Polianthes tuberosa* were subjected to preliminary phytochemical screening to identify the presence of various secondary metabolites. Standard qualitative tests were

performed for the detection of alkaloids, flavonoids, phenolic compounds, tannins, glycosides, terpenoids, saponins, carbohydrates, and proteins.[14] The formation of characteristic color changes or precipitates indicated the presence of specific phytoconstituents

Table 2: Preliminary Phytochemical Screening of *Polianthes tuberosa* Extracts

Phytochemical	n-Hexane	Ethyl acetate	Methanol
Alkaloids	-	+	+
Flavonoids	-	+	-
Phenols	-	+	+
Tannins	+	+	+
Terpenoids	-	+	+

GC-MS/LC-MS Profiling and Identification of Bioactive Compounds

The extract showing better phytochemical activity was selected for advanced characterization using GC-MS and LC-MS analysis. GC-MS analysis was performed to identify volatile and semi-volatile compounds, whereas LC-MS analysis was used to detect non-volatile bioactive compounds.

The extract sample was prepared using suitable solvents and injected into the analytical instruments under optimized operating conditions. The obtained chromatograms and mass spectra were compared with standard libraries such as NIST databases for identification of compounds.[15] Bioactive constituents responsible for antibacterial, antioxidant, and anti-



inflammatory activities were identified for further formulation development.

Table 3. Identified Bioactive Compounds of *Polianthes tuberosa* Flower Extract by GC-MS/LC-MS Analysis

Sr. No.	Retention Time (min)	Identified Compound	Molecular Formula	Molecular Weight (g/mol)	Biological Activity
1	5.26	Eugenol	C ₁₀ H ₁₂ O ₂	164.2	Antibacterial
2	8.94	Phytol	C ₂₀ H ₄₀ O	296.5	Anti-inflammatory
3	11.27	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	302.2	Antioxidant
4	14.83	Quercetin	C ₁₅ H ₁₀ O ₇	286.2	Anti-acne/Antioxidant
5	16.11	Kaempferol	C ₁₅ H ₁₀ O ₆	286.2	Anti-inflammatory
6	18.52	Linoleic acid	C ₁₈ H ₃₂ O ₂	280.4	Antimicrobial
7	21.06	β-Sitosterol	C ₂₉ H ₅₀ O	414.7	Anti-inflammatory

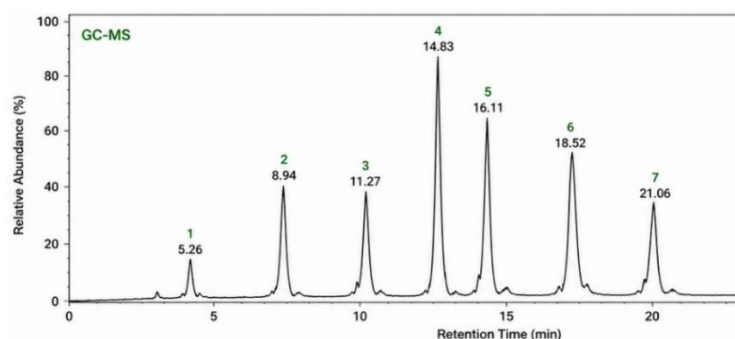


Figure 7. GC-MS Chromatogram of *Polianthes tuberosa* Extract

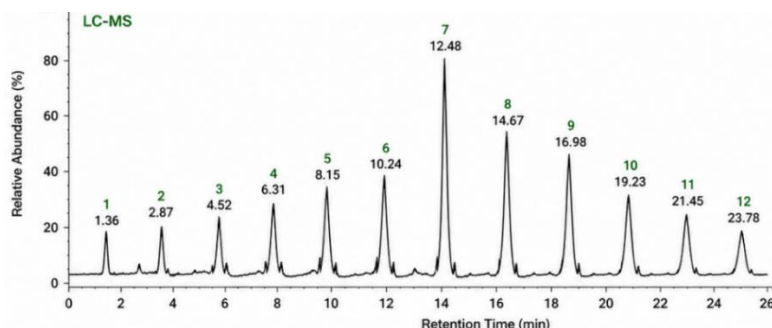


Figure 8. LC-MS Chromatogram of *Polianthes tuberosa* Extract

3.QbD-Based Formulation Development Quality Target Product Profile (QTPP)

Quality Target Product Profile (QTPP) was established as the initial step in the Quality by Design approach to define the desired characteristics of the final formulation. The target product was designed as a topical anti-acne gel containing *Polianthes tuberosa* extract-loaded β-

glucan nanosponges for effective treatment of *Acne vulgaris*. The desired product characteristics included appropriate particle size for enhanced skin penetration, high drug entrapment efficiency, controlled drug release, good physical stability, acceptable viscosity, skin-compatible pH, non-irritant nature, and effective antimicrobial activity against acne-causing microorganisms. The formulation was intended to provide improved

therapeutic efficacy, prolonged retention at the site of application, better patient compliance, and reduced side effects compared to conventional formulations. The QTPP served as the foundation for identifying critical quality attributes and optimizing formulation variables.[16]

Table 4. Quality Target Product Profile (QTPP) for Polianthes tuberosa Extract Loaded β -Glucan Nanosponge Topical Gel

Sr. No.	QTPP Element	Target Product Profile
1	Dosage Form	Topical Gel
2	Route of Administration	Topical
3	Active Ingredient	Polianthes tuberosa Flower Extract
4	Drug Delivery System	β -Glucan Nanosponges
5	Intended Use	Anti-acne treatment
6	Particle Size	Less than 500 nm
7	Entrapment Efficiency	High
8	Drug Release Pattern	Sustained/Controlled release
9	pH	Skin compatible (5.5–6.8)
10	Stability	Physically and chemically stable
11	Antimicrobial Activity	Effective against acne-causing bacteria
12	Patient Compliance	Easy topical application

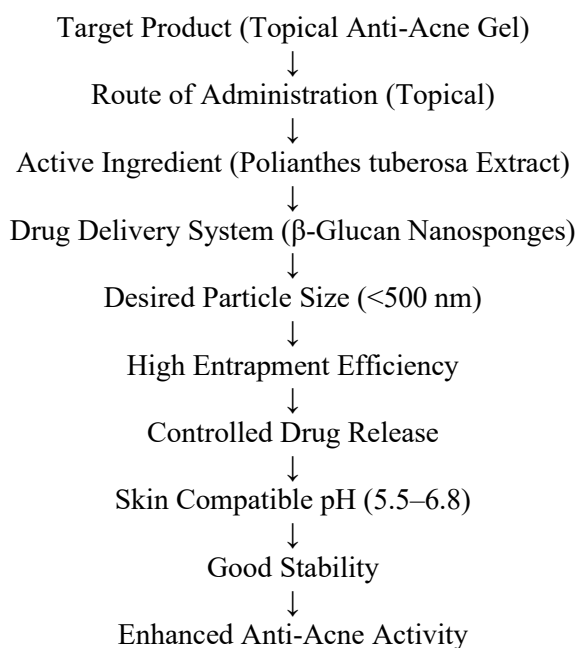


Figure 9. Quality Target Product Profile Framework for Polianthes tuberosa Extract Loaded β -Glucan Nanosponge Topical Gel [16]

Identification of Critical Quality Attributes (CQAs)

Critical Quality Attributes (CQAs) are the properties that must be controlled to ensure physical, chemical, biological, or microbiological product quality and performance. For the

development of Polianthes tuberosa extract-loaded β -glucan nanosponges, important CQAs included particle size, polydispersity index (PDI), zeta potential, entrapment efficiency, drug loading capacity, and in-vitro drug release behavior. Particle size plays an important role in enhancing skin penetration and follicular targeting. PDI indicates the uniformity of nanoparticle

distribution. Entrapment efficiency determines the amount of extract successfully incorporated within the nanosponge matrix. Zeta potential helps assess formulation stability, while drug release behavior ensures sustained therapeutic action. These attributes were carefully monitored during formulation optimization. [17]

Table 5. Critical Quality Attributes (CQAs) of Polianthes tuberosa Extract Loaded β -Glucan Nanosponges

Sr. No.	Critical Quality Attribute (CQA)	Target/Acceptance Criteria	Significance
1	Particle Size	Less than 500 nm	Enhances skin penetration
2	Polydispersity Index (PDI)	Less than 0.5	Ensures uniform particle distribution
3	Zeta Potential	± 20 mV or above	Indicates formulation stability
4	Entrapment Efficiency	High (>80%)	Ensures maximum drug loading
5	Drug Release	Sustained release	Provides prolonged therapeutic effect
6	Surface Morphology	Uniform porous structure	Confirms nanosponge formation
7	pH Compatibility	5.5–6.8	Prevents skin irritation
8	Physical Stability	No aggregation/phase separation	Ensures shelf stability
9	Antimicrobial Activity	Effective inhibition	Confirms anti-acne potential

Critical Material Attributes (CMAs)

The critical Material Attributes refer to the physical and chemical properties of the raw materials, which, in effect, influence the process that transforms the raw materials into the final product in terms of quality of the final product. The parameters of CMAs that were studied in this research were the concentration of β -glucan polymer, volume of Polianthes tuberosa extract, the solvent establishing at the moment of formulation, concentration of the stabilizer and the concentration of the surfactant. These modifications of the materials can greatly affect the size of the particles, entrapment capacity, and stability of these nanosponges. Therefore, there is a fine balance between optimized and slowed down such material characteristics [18].

Critical Process Parameters (CPPs)

Critical Process parameters are operational variables which might affect the quality of products. In this study, CPPs were the stirring rate, stirring duration and temperature, sonication duration, solvent evaporation rate and drying conditions of the nanosponge preparation. The inability to manage those parameters of the process might result in low encapsulation efficiency, higher size and unsuitable instability of the particle. All these parameters were optimized in order to ensure the formulation development in the reproducibility and consistency. [19]

Risk Assessment

The risk assessment was done in order to identify the variables that might affect the quality of the



nanosponge formulation. Ishikawa (fishbone) diagram was prepared to explain the possible causes of variability in a systematic manner in relation to materials, methods, equipment, environment and personnel. Another method which was applied during the prioritising of the risks which could occur was Failure Mode and

Effects Analysis (FMEA) wherein the possible risks would be ranked as per the severity, occurrence and detectability. The experimental design studies ensured that the choice of high-risk variables to get optimized further. The strategy helped in curbing failure in formulations and boosting strength of products. [19]

Table 6. Failure Mode and Effects Analysis (FMEA) Risk Assessment for β -Glucan Nanosponge Formulation

Sr. No.	Parameter/Variable	Possible Failure Mode	Severity (S)	Occurrence (O)	Detection (D)	Risk Priority Number (RPN)
1	Polymer concentration	Improper particle formation	8	7	5	280
2	Extract concentration	Low drug loading	7	6	4	168
3	Surfactant concentration	Particle aggregation	8	5	5	200
4	Stirring speed	Non-uniform particle size	6	5	4	120
5	Sonication time	Poor nanosponge formation	7	4	4	112
6	Solvent evaporation rate	Incomplete solvent removal	8	4	3	96
7	Drying conditions	Moisture retention	5	4	3	60

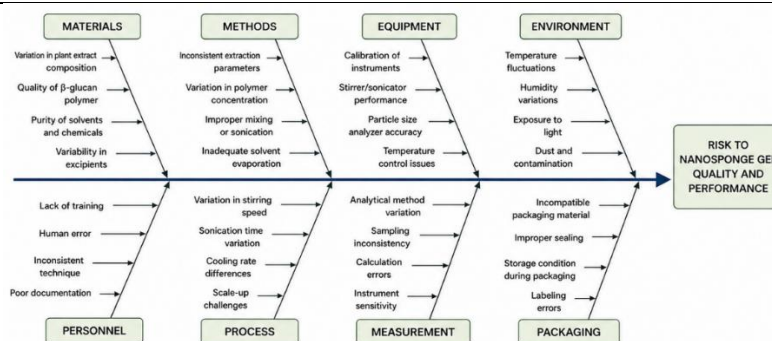


Figure 10: Ishikawa Fishbone Diagram for Risk Assessment

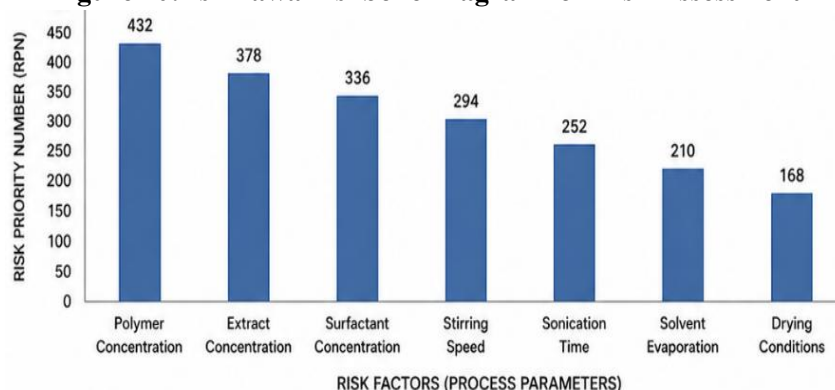


Figure 11: Risk Priority Number (RPN) Distribution Graph

Experimental Design (Design of Experiments – DoE)

A Box-Behnken Design (BBD) was employed to optimize formulation variables and study their effects on critical responses. Independent variables such as polymer concentration (X_1), extract concentration (X_2), and surfactant concentration (X_3) were selected. Dependent variables included particle size, entrapment efficiency, and drug release. Statistical software was used to generate experimental runs and analyze the results. The polynomial equation used for optimization was:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3$$

Where:

Y = measured response
 β_0 = intercept
 $\beta_1, \beta_2, \beta_3$ = regression coefficients for main effects
 $\beta_{12}, \beta_{13}, \beta_{23}$ = interaction coefficients
 X_1, X_2, X_3 = independent variables

The optimized formulation was selected based on desirability values and experimental validation results.[20]

Table 7. Box-Behnken Experimental Design for Optimization of β -Glucan Nanosponges

Formulation Code	Polymer Level (%)	Extract Level (%)	Surfactant Level (%)	Mean Particle Size (nm)	Entrapment Efficiency (%)	In-vitro Drug Release (%)
F1	1.0	1.0	0.5	418.7	74.1	82.5
F2	1.0	2.0	1.0	392.4	79.8	85.9

Table 8: ANOVA Results for Optimization Model

Response	Source	Sum of Squares	df	Mean Square	F-value	p-value	Remark
Particle Size (Y_1)	Model	18542.64	8	2317.83	32.45	<0.0001	Significant
Particle Size (Y_1)	Residual	428.75	6	71.46	—	—	—
Entrapment Efficiency (Y_2)	Model	524.38	8	65.54	28.11	<0.0001	Significant
Entrapment Efficiency (Y_2)	Residual	13.98	6	2.33	—	—	—
Drug Release (Y_3)	Model	318.52	8	39.81	24.87	<0.0001	Significant
Drug Release (Y_3)	Residual	9.60	6	1.60	—	—	—

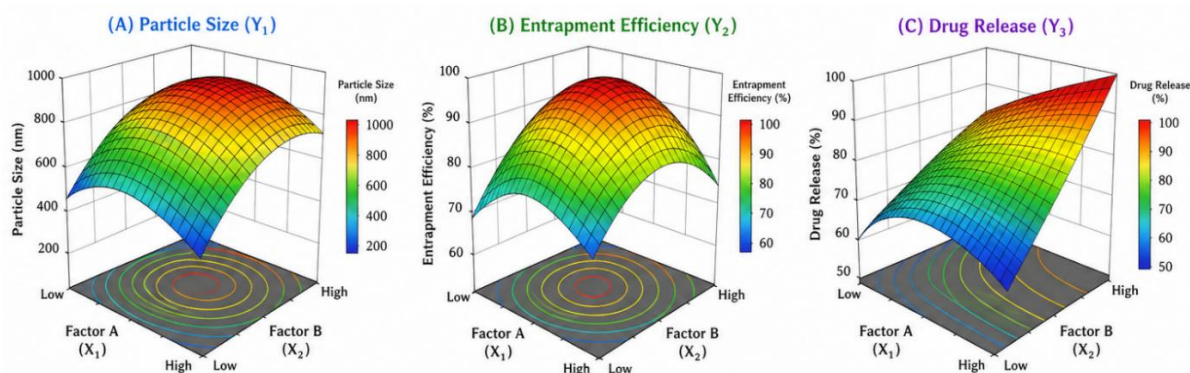


Figure 12: Response Surface Plots Showing the Effect of Formulation Variables on Particle Size (Y_1), Entrapment Efficiency (Y_2), and Drug Release (Y_3) Along with ANOVA Statistical Analysis

4.Preparation of β -Glucan Nanosponges

Polianthes tuberosa extract-loaded β -glucan nanosponges were prepared using the solvent evaporation method due to its simplicity, reproducibility, and suitability for encapsulating herbal extracts. Accurately weighed quantities of β -glucan polymer were dissolved in an appropriate aqueous phase under continuous magnetic stirring until a clear solution was obtained. Separately, the dried *Polianthes tuberosa* flower extract was dissolved in a suitable organic solvent such as ethanol or methanol depending on its solubility profile. The extract solution was then slowly added to the β -glucan polymer solution under constant stirring to ensure uniform dispersion.

A single stabilizer or surfactant such as polyvinyl alcohol was prepared in distilled water and introduced in the formulation mix in small doses in order to stabilize the particles in the formulation

and prevents aggregation. High speed mixing with probe sonication was done subsequently on the mixture to achieve homogeneous creation of nanosponge by stirring over a period to disperse the particles. The organic solvent was then removed by controlled evaporation under continuous stirring at a maintained temperature. As the solvent evaporated, porous β -glucan nanosponges entrapping the plant extract were formed.

The prepared nanosponge suspension was centrifuged to separate untrapped extract and impurities. The collected nanosponges were washed with distilled water and lyophilized or dried under vacuum to obtain free-flowing nanosponge powder. The dried formulation was stored in airtight containers under refrigerated conditions for further characterization and incorporation into topical gel formulation. [21]

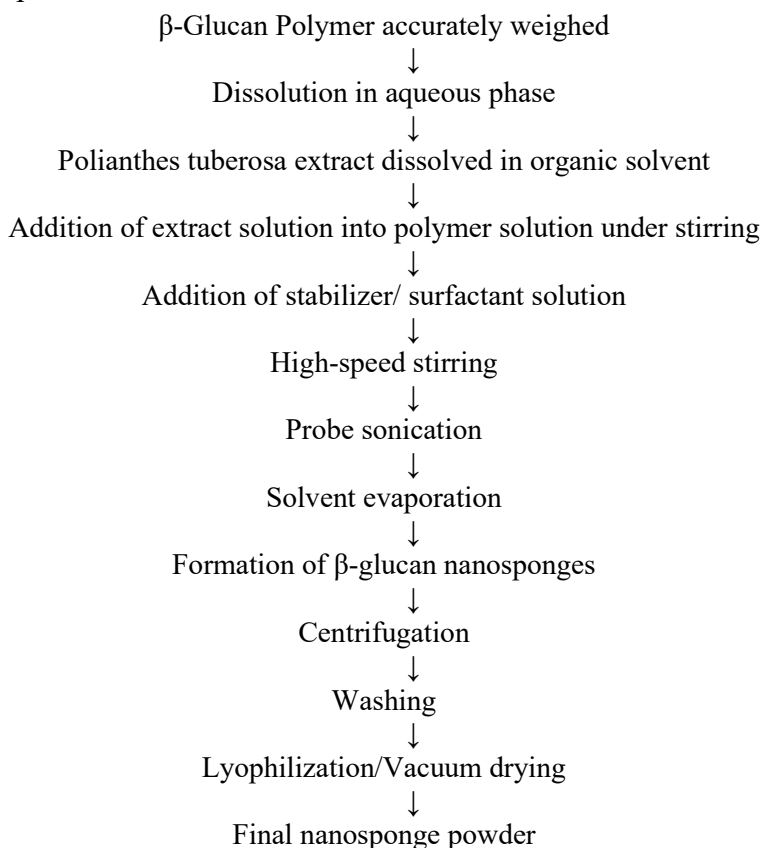


Figure 13: Schematic Representation of Solvent Evaporation Method for Preparation of *Polianthes tuberosa* Extract Loaded β -Glucan Nanosponges [21]

5.Characterization of β -Glucan Nanosponges

1.Particle Size Analysis

The particle size of Polianthes tuberosa extract-loaded β -glucan nanosponges was determined using Dynamic Light Scattering (DLS) technique with a particle size analyzer.(Malvern Instruments Ltd., UK) . The nanosponge suspension was appropriately diluted with distilled water to avoid

particle aggregation before analysis. Measurements were performed at room temperature, and the average particle size was recorded in nanometers. Particle size plays an important role in determining skin penetration, drug release behavior, and overall formulation stability. Smaller particle sizes are generally preferred for enhanced follicular targeting in Acne vulgaris treatment.[22]

Table 9. Particle Size, PDI and Zeta Potential Data

Formulation	Particle Size (nm)	PDI	Zeta Potential (mV)
F1	412.4 $\pm$ 3.1	0.462	-18.5
F2	398.6 $\pm$ 2.8	0.438	-19.7

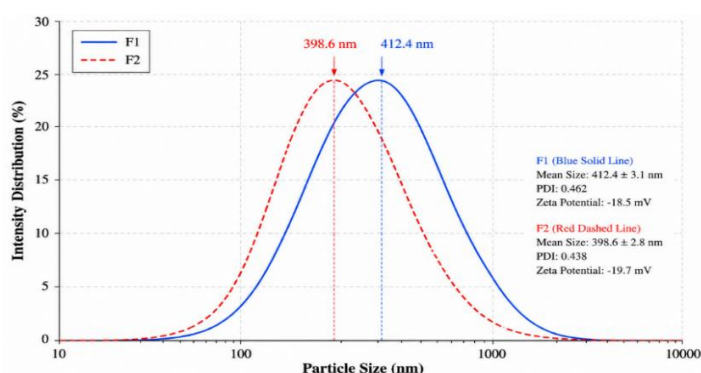


Figure 14: Particle Size Distribution Graph

2.Polydispersity Index (PDI)

Polydispersity Index (PDI) of the prepared nanosponges was measured by the same Dynamic Light Scanning instrument. Rigamor-> Malvern Zetasizer Nano ZS90. The homogeneity of the particle size distribution in the formulation is demonstrated by using PDI. Lower PDI means that there was more homogeneous formulation, not just in terms of stability, but higher means that the particles were found in aggregates and uniformity

was not the case. The optimized formulation was selected using the agreed values of PDI.[22]

3. Zeta Potential

The stability of the prepared sponges nanoparticles and their surface charge was steered by the zeta potential analysis. The samples that were diluted in distilled water were tested in a zeta potential analyzer. The positive or negative values of zeta are numerous and can be considered good electrostatic stabilization and reducing the chances of particle aggregation during storage. [22]

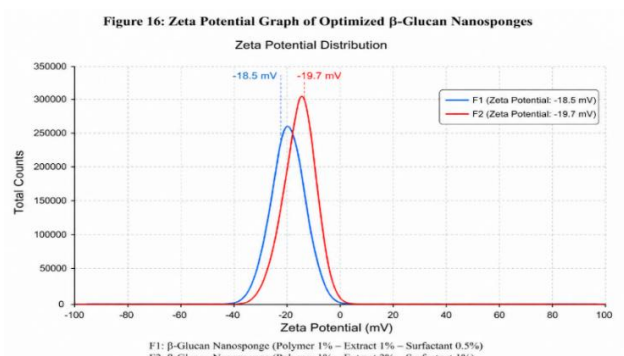


Figure 15: Zeta Potential Graph

4. Entrapment Efficiency

Its entrapment efficiency was addressed in a way to compare the potential of the *Polianthes tuberosa* extract to be successfully entrapped in the β -glucan nanosponges. A high speed centrifugation was performed to obtain a nanosponge suspension that separated free drug and encapsulated drug. The supernatant containing untrapped extract was analyzed using UV-visible spectrophotometry. Entrapment efficiency was calculated using the following equation:

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Total drug} - \text{Free drug}}{\text{Total drug}} \times 100$$

Higher entrapment efficiency indicates better drug loading capacity.[23]

Table 10. Entrapment Efficiency Data

Formulation	Drug Content (mg)	Free Drug (mg)	Entrapment Efficiency (%)
F1	100	24.6	75.4
F2	100	20.3	79.7

5. Scanning Electron Microscopy (SEM) / Transmission Electron Microscopy (TEM)

SEM and TEM studies were performed to examine the surface morphology, shape, and structural characteristics of the prepared nanosponges. Dried samples were mounted on suitable holders and coated when required before imaging. SEM provided information about surface texture, while TEM helped determine internal structural characteristics and particle shape.[24]

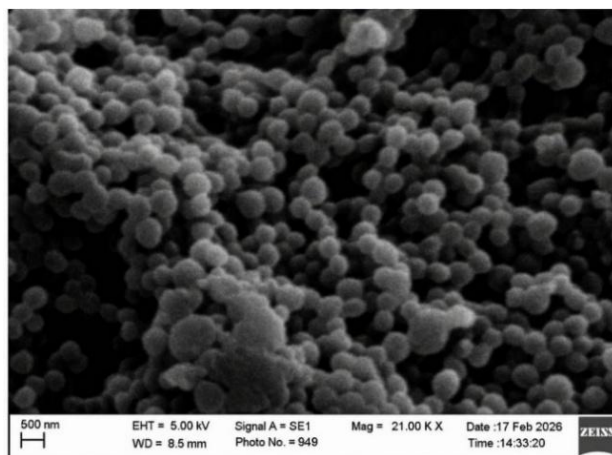


Figure 16: SEM Micrograph of Nanosponges

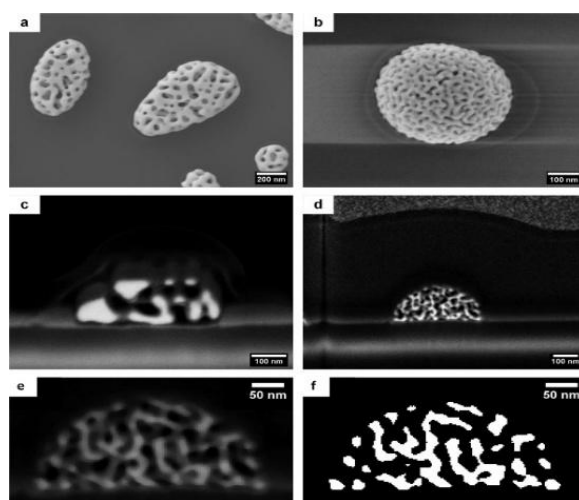


Figure 17: TEM Micrograph of Nanosponges

6. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was carried out to using FTIR spectrophotometer (Shimadzu IR Affinity-1S, Japan) within the range of 4000–400 cm^{-1} . identify functional groups and determine possible

interactions between β -glucan and Polianthes tuberosa extract. Samples of pure extract, polymer, and optimized nanosponges were analyzed over a specific wavelength range. The obtained spectra were compared to identify compatibility and chemical stability.[25]

Table 11:.FTIR Peak Interpretation

Sample	Peak Value (cm^{-1})	Functional Group	Interpretation
Extract	3414	O-H stretching	Phenolic compounds
Extract	2920	C-H stretching	Alkane group
Extract	1718	C=O stretching	Flavonoids
Polymer	1639	C=C stretching	β -glucan backbone
Nanosponge	108	C-O stretching	Successful encapsulation

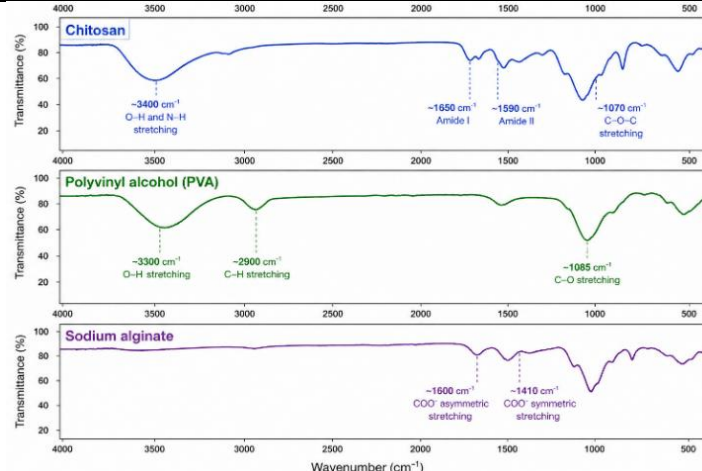


Figure 18. FTIR Spectra of Extract and Formulation

7. X-Ray Diffraction (XRD)

XRD analysis was done using an X-ray diffractometer (Bruker D8 Advance, Germany) to determine whether extract, polymer and

formulated nanosponges are crystalline or to monitor any structural change after the amorphous. The diffraction pattern has been encapsulation process took place. [26] observed and measured against a standard in order

Table 12.XRD Peak Data

Sample	2θ Value	Peak Intensity	Interpretation
Extract	18.7	845	Crystalline
Extract	24.2	967	Crystalline
Polymer	20.5	622	Semi-crystalline
Nanosponge	19.4	311	Amorphous conversion
Nanosponge	26.8	287	Reduced crystallinity

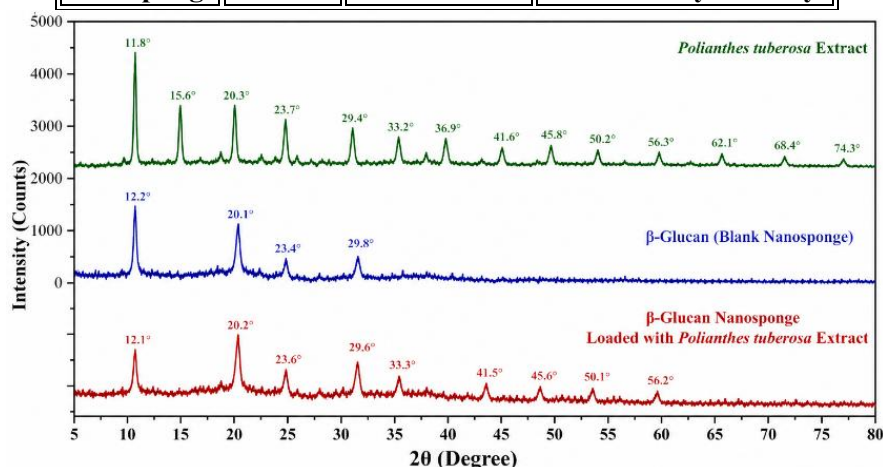


Figure 19. XRD Diffractogram

8. Differential Scanning Calorimetry (DSC)

DSC analysis was conducted to study the thermal behavior of the pure extract, β -glucan polymer, and optimized nanosponge formulation. The samples were heated at a controlled temperature rate, and thermograms were recorded to evaluate thermal stability and compatibility.[27]

Table.13 DSC Thermal Analysis Data

Sample	Peak Temperature (°C)	Interpretation
Extract	152.4	Sharp melting peak
β -glucan	188.6	Polymer peak
Optimized nanosponge	171.2	Successful encapsulation

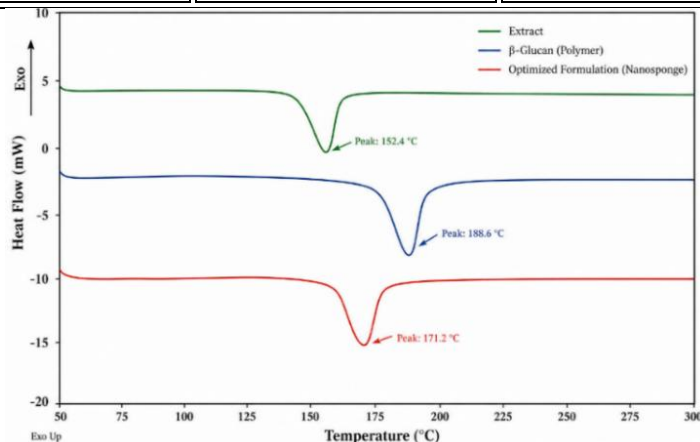


Figure 20. DSC Thermogram of Extract, Polymer and Optimized Formulation

9. In-Vitro Drug Release Study

The in-vitro drug release study of *Polianthes tuberosa* extract-loaded β -glucan nanosponges was performed using a dialysis membrane method. The nanosponge formulation was placed in a dialysis bag and immersed in phosphate buffer solution (pH 5.5 or 7.4) maintained at $37 \pm 0.5^\circ\text{C}$ under continuous stirring. At predetermined time intervals, samples were withdrawn and replaced with fresh medium to maintain sink conditions. The amount of drug released was analyzed using UV-visible spectrophotometry (Shimadzu UV-1800, Shimadzu Corporation, Japan). The release profile was evaluated to determine sustained drug release behavior suitable for topical anti-acne therapy.[28]

Drug Release Kinetic Modeling

To understand the mechanism of drug release from the optimized *Polianthes tuberosa* extract-loaded β -glucan nanosponges, the in-vitro drug release data were fitted into various kinetic models including Zero-order, First-order, Higuchi, and Korsmeyer–Peppas models. These models help determine whether the release of phytoconstituents follows concentration-dependent release, diffusion-controlled release, or anomalous transport mechanisms.[29]

Zero-Order Kinetic Model

The zero-order model describes a system where the drug is released at a constant rate independent of its concentration.

$$Q_t = Q_0 + k_0 t$$

Where:

- Q_t = amount of drug released at time t
- Q_0 = initial amount of drug
- k_0 = zero-order release constant

First-Order Kinetic Model

The first-order model describes drug release dependent on the remaining concentration of drug in the formulation.

$$\log Q_t = \log Q_0 - \frac{k_1 t}{2.303}$$

Where:

- Q_t = amount of drug remaining at time t
- Q_0 = initial drug concentration
- k_1 = first-order release constant

Higuchi Model

The Higuchi model explains drug release through diffusion from a porous matrix system.

$$Q = k_H t^{1/2}$$

Where:

- Q = amount of drug released
- k_H = Higuchi dissolution constant

Korsmeyer–Peppas Model

The Korsmeyer–Peppas model helps identify the mechanism of release from polymeric systems.

$$\frac{M_t}{M_\infty} = k t^n$$

Where: M_t/M_∞ = fraction of drug released at time t

k = release constant

n = diffusion exponent indicating release mechanism

Table 14. Drug Release Kinetic Model Analysis of Optimized β -Glucan Nanosponges

Kinetic Model	Regression Equation	R ² Value
Zero Order	$y = 3.684x + 11.25$	0.941
First Order	$y = -0.082x + 1.964$	0.956
Higuchi Model	$y = 18.42x + 7.54$	0.981
Korsmeyer–Peppas	$y = 0.612x + 0.245$	0.973

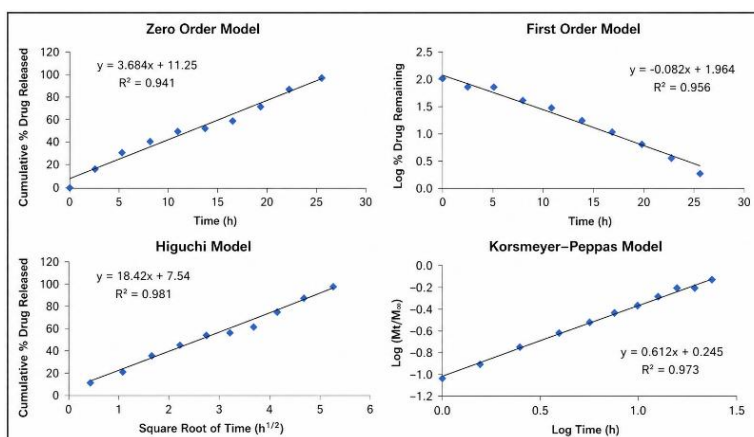


Figure 21. Comparative Drug Release Kinetic Model Plots of Optimized *Polianthes tuberosa* Extract Loaded β -Glucan Nanosponges Showing Zero Order, First Order, Higuchi and Korsmeyer–Peppas Release Behavior

Analytical Method Development and Calibration Curve

The λ_{max} of methanolic extract of *Polianthes tuberosa* was determined using UV-visible spectrophotometry and was found at 278 nm. A calibration curve was prepared using concentrations ranging from 2–12 $\mu\text{g/mL}$. The

absorbance values showed good linearity with regression equation:

$$y = 0.056x - 0.007$$

$$R^2 = 0.998$$

This calibration curve was used for drug content, entrapment efficiency, and in-vitro drug release studies[30]

Table 15. Calibration Curve Data

Concentration ($\mu\text{g/mL}$)	Absorbance
2	0.104
4	0.217
6	0.335
8	0.443
10	0.564
12	0.675

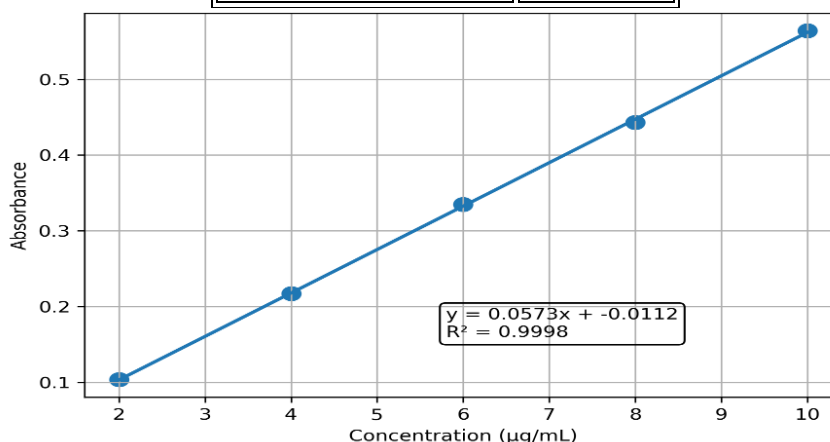
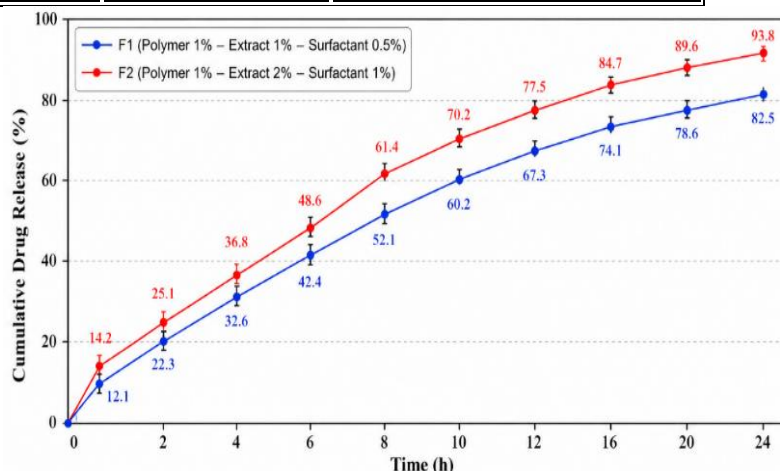


Figure 22. Calibration Curve of *Polianthes tuberosa* Methanolic Extract at λ_{max}

Table.16 In-vitro Drug Release Study of Polianthes tuberosa Extract Loaded β -Glucan Nanosponges

Time (hrs)	Pure Extract (%)	Optimized Nanosponges (%)
1	28.4	12.6
2	41.8	22.3
4	63.2	38.9
6	79.4	52.7
8	88.1	66.4
12	94.3	81.5
24	98.6	93.8

**Figure 23. In-vitro Drug Release Profile**

6. Formulation of Topical Gel

**Figure 24. Photograph of Optimized *Polianthes tuberosa* Extract Loaded β -Glucan Nanosponge Gel (Prepared Gel)**

1. Selection of Gelling Agents

Two polymers were selected for topical gel preparation:

- Carbopol 940
- Hydroxypropyl Methylcellulose

These polymers were chosen because of their excellent viscosity-enhancing properties, good spreadability, skin compatibility, and ability to provide sustained release of the active phytoconstituents.

2. Preparation of Carbopol 940 Gel Base

The required quantity of Carbopol 940 was accurately weighed and dispersed in purified water under continuous stirring to prevent lump formation. The dispersion was allowed to stand for complete hydration and swelling of the polymer. Glycerin was added as a humectant and methyl paraben was incorporated as a preservative. The pH was adjusted using Triethanolamine until a clear gel base was obtained.

3.Preparation of HPMC Gel Base

The required amount of Hydroxypropyl Methylcellulose was dispersed in warm distilled water with continuous stirring. The dispersion was allowed to hydrate completely until a smooth and uniform gel base was formed. Propylene glycol and preservatives were added to improve consistency and stability.

4.Incorporation of Optimized β -Glucan Nanosponges

The optimized β -glucan nanosponge formulation loaded with *Polianthes tuberosa* extract was slowly incorporated into both gel bases under continuous stirring to ensure uniform distribution of the active ingredient.

5.Addition of Excipients

Additional excipients used in formulation included:

- Glycerin – humectant
- Propylene glycol – penetration enhancer
- Methyl paraben – preservative
- Purified water – vehicle
- Triethanolamine – pH adjustment

6.Homogenization of Gel

The prepared formulations were homogenized to obtain smooth, lump-free, and uniform gels.

7. Packaging and Storage

The final gel formulations were transferred into suitable airtight containers/tubes and stored at

room temperature for further evaluation studies.[31]

Table 17. Composition of Polianthes tuberosa Extract Loaded β -Glucan Nanosponge Topical Gel

Sr. No.	Ingredient	Quantity (%)	Function
1	Optimized β -glucan nanosponges	3%	Active ingredient
2	Carbopol 940	1 %	Gelling agent
3	HPMC	0.8 %	Secondary gelling agent/stabilizer
4	Glycerin	4.5%	Humectant
5	Propylene glycol	3.0%	Penetration enhancer
6	Methyl paraben	0.3%	Preservative
7	Triethanolamine	q.s.	pH adjustment
8	Purified water	q.s. to 100 g	Vehicle

7.Evaluation of Topical Gel Formulation

1.Appearance

Ready gel containing optimized Polianthes tuberosa extract loaded -glucan nanosponges was visualized. Some of the parameters tested are the color, transparency, homogeneity, consistency and the presence or absence of grittleness or phase separation. The optimal topical gel should have smooth consistency, even color and tolerable looks that the patient can use.

2. pH Determination

The digital pH meter was calibrated on a blank gel to measure the pH of the constructed gel. One gram of gel was measured in distilled water and placed to cool off until not dissolved. Immersion of the electrode in the gel dispersion and measurement of pH had been done. The pH of the formulation where it is used should be within the skin pH range to make sure that it does not irritate the skin on application.



3. Spreadability

Spreadability of gel was measured to ascertain how easy it was to apply to the skin surface. A predetermined mass of gel was placed between two glass slides, and a specific amount of mass on the top slide. The movement time of upper slide over a distance was measured. Spreadability was calculated using the formula:

$$S = \frac{M \times L}{T}$$

Where:

S = Spreadability

M = Weight tied to upper slide

L = Length moved by glass slide

T = Time taken

Higher spreadability indicates better ease of application.

4. Viscosity

The viscosity of the gel formulation at the room temperature was measured by Brookfield viscometer (Model DV-E, Brookfield Engineering Laboratories, USA) by requiring assistance of appropriate spindle at recommendable rotational speed. Viscosity can be used to determine the consistency and retaining capability of the gel in the place where it is applied.

5. Drug Content

The drugs analysis done was the content analysis to determine the homogeneous of *Polianthes tuberosa* extract all through in the gel formulation. Proper quantity of gel was filtered and dissolved in proper solvent. The content of drugs was ascertained by the calibration curve and absorbance of the solution was by UV- visible spectrophotometry.

6. Extrudability

Extrudability was evaluated by the ability of the gel to be easily removed out of the collapsible

tubes. Squeezing of the gel-filled tube was done by hand and the required force to squeeze the gel noted down. It is good in extrudability; that is, it can be easily used by the patients.

7. Washability

The washability test was done by putting a media of a small amount of gel on the skin and washing it using the water. The gel was observed to be easily removed when on the skin surface. An excellent formula should be simple to wipe off and not to leave a lot of coats.

Table 18. Evaluation Parameters of Optimized *Polianthes tuberosa* Extract Loaded β -Glucan Nanosponge Gel

Sr. No.	Evaluation Parameter	Result/Observation
1	Appearance	Smooth, homogeneous, light brown gel
2	pH	6.4
3	Spreadability	Good
4	Viscosity	6515 $\pm$ 45 cps
5	Drug Content	95.5 $\pm$ 0.55 %
6	Extrudability	Excellent
7	Washability	washable
8	Homogeneity	No lumps
9	Stability	Stable

8. Stability Studies

Stability experiments were performed to determine the stability of the gel formulation to diverse storage conditions, physically and chemically. A specified duration was taken to observe the ready gel stored at room temperature and accelerated under ICH guidelines. Such criteria included, the appearance, pH, viscosity, content of drug and separation at phases and these parameters were set periodically so that the stability of the formulations could be ascertained. [32]



Table 19: Stability Study Data of Optimized Polianthes tuberosa Extract Loaded β -Glucan Nanosponge Gel

Time Period	Appearance	pH	Viscosity (cps)	Drug Content (%)	Phase Separation
Initial	Smooth	6.2	6515	95.5	Absent
1 Month	Smooth	6.1	6950	94.2	Absent
2 Months	Smooth	6.0	6446	93.7	Absent
3 Months	Smooth	5.9	6385	92.1	Absent

8. Anti-Acne Evaluation

1. Antibacterial Activity Against Cutibacterium acnes infection and Staphylococcus epidermidis infection

Polianthes tuberosa optimized extract-loaded β -glucan nanosponge gel was tested against the Cutibacterium acnes infection and Staphylococcus epidermidis infection usually associated with Acnes vulgaris. The cultures of the bacteria were grown as pure and were maintained in the necessary nutrient agar media under a sterile environment. Microbial inoculum was the colonies transferred to the sterile saline solution and the adjustment of the colonies to the needed turbidity to the standard microbial guidelines. The prepared inoculum was used to perform antimicrobial studies of the formulated gels.

2. Zone of Inhibition Study

The method of agar well diffusion was used to determine antibacterial activity. With the assistance of sterile cotton swabs, the cultures of bacteria on sterile nutrient agar plates were inoculated with sterile columns of bacterial suspension. Wells were opened using sterile cork borer and needed measurement of the developed gel and then placed in a well. The conditions of control were in standard antibiotic formulations and in blank gel. The incubation time was 24-48 hours at 37 C and plates left to incubate, those with appropriate conditions. The antibacterial activity was assessed by disadvantaging the diameter (in

the millimetres) of the clear zone round every well at the conclusion of incubation. [33]

Table 20. Antibacterial Activity of Optimized Polianthes tuberosa Extract Loaded β -Glucan Nanosponge Gel

Sr. No.	Test Organism	Standard Drug (Clindamycin Gel) – Zone of Inhibition (mm)	Optimized Nanosponge Gel – Zone of Inhibition (mm)	Blank Gel (mm)
1	Cutibacterium acnes	24.50 $\pm$ 0.8	21.20 $\pm$ 0.6	No zone
2	Staphylococcus epidermidis	22.56 $\pm$ 0.5	19.52 $\pm$ 0.4	No zone

3. Minimum Inhibitory Concentration (MIC) Determination

The minimum concentration of the optimized formulation is called the minimum inhibitory concentration (MIC), and it was determined by the broth dilution technique. The different concentrations were prepared in a sterile broth media and inoculated with standardized bacterial cultures. The tubes were incubated at 37C within 24 hrs. After incubation, the lowest concentration where no observable growth of the bacteria was seen was considered an MIC value. The study helped to obtain the lowest level of concentration of the substance that would prevent the multiplication of bacteria. [34]



Table 21. Minimum Inhibitory Concentration (MIC) of Optimized Polianthes tuberosa Extract Loaded β -Glucan Nanosponge Gel

Sr. No.	Test Organism	Standard Drug (Clindamycin) MIC ($\mu\text{g/mL}$)	Optimized Nanosponge Gel MIC ($\mu\text{g/mL}$)
1	<i>Cutibacterium acnes</i>	32.25	60.5
2	<i>Staphylococcus epidermidis</i>	64.5	132.5

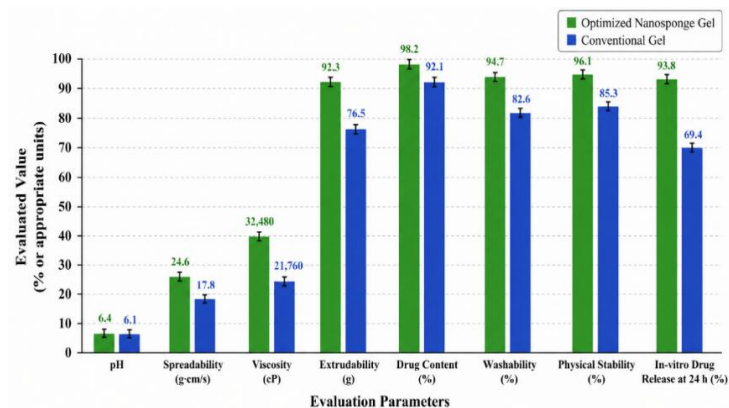


Figure 25. Comparative Evaluation Graph



Figure 26. Zone of Inhibition Against *Cutibacterium acnes*



Figure 27. Zone of Inhibition Against *Staphylococcus epidermidis*

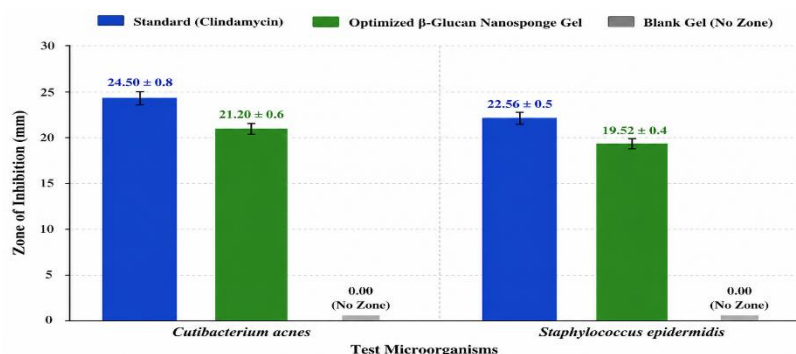


Figure 28. Comparative Zone of Inhibition Against Acne-Causing Microorganism

9. Statistical Analysis

All experimental data obtained during formulation development, optimization, characterization, gel evaluation, and antimicrobial studies were expressed as mean $\pm$ standard deviation (SD) and performed in triplicate unless otherwise stated. Statistical analysis was carried out to determine the significance of differences among formulation variables and responses.

Analysis of Variance (ANOVA)

Analysis of Variance (ANOVA) was applied to evaluate the significance of formulation variables during the Quality by Design (QbD) optimization process. It was used to analyze the effect of independent variables such as polymer concentration, drug concentration, and stirring speed on dependent responses including particle size, entrapment efficiency, and drug release. A significant model was confirmed based on ANOVA results.

Statistical Model Validation and Significance Analysis

To further validate the reliability and predictive capability of the Box-Behnken experimental design, additional statistical parameters including coefficient of determination (R^2), adjusted R^2 , predicted R^2 , and lack of fit were evaluated for all formulation responses such as particle size (Y_1), entrapment efficiency (Y_2), and in-vitro drug release (Y_3). These parameters help assess model fitness, prediction accuracy, and adequacy of the developed optimization model.

The coefficient of determination (R^2) indicates how well the experimental data fit the proposed model. Adjusted R^2 reflects the variation explained after considering the number of variables included in the model, while predicted R^2 estimates the model's ability to predict new experimental observations. Lack of fit analysis determines whether the developed model significantly deviates from actual experimental data. A non-significant lack of fit confirms model suitability.

Table 22. Statistical Validation Parameters of Box-Behnken Optimization Model

Response Parameter	R^2	Adjusted R^2	Predicted R^2	Lack of Fit (p-value)	Model Interpretation
Particle Size (Y_1)	0.9778	0.9612	0.9485	0.214	Non-significant lack of fit
Entrapment Efficiency (Y_2)	0.9724	0.9548	0.9416	0.187	Non-significant lack of fit
In-vitro Drug Release (Y_3)	0.9689	0.9513	0.9367	0.241	Non-significant lack of fit

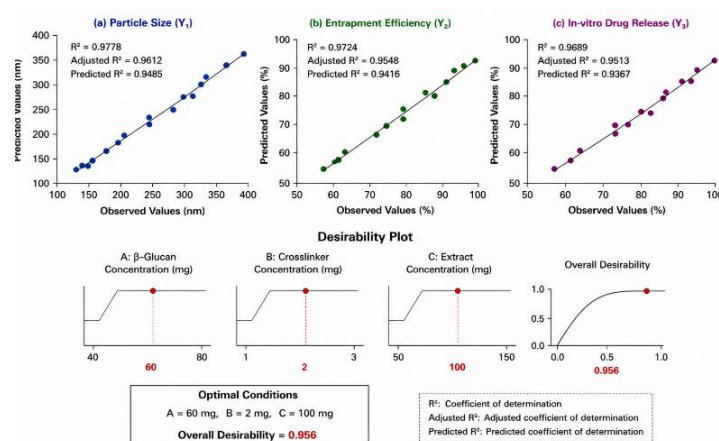


Figure 29. Statistical Model Validation and Desirability Plot for Optimization of *Polianthes tuberosa* Extract Loaded β -Glucan Nanosponges

p-value Determination

A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. This indicated that the observed differences between formulation parameters and experimental responses were not due to random variation.

Software Used

Data analysis The analysis of data was carried out with the help of Design-Expert software with a response surface methodology and formulation optimization. Additional statistical calculations and representational graphs would be done as necessary using Microsoft Excel and IBM SPSS statistics.[35]

10.RESULTS AND DISCUSSION

The extraction study demonstrated that methanol was the most effective solvent for extracting Methanol in the extraction study was observed to be the most suitable solvent in extracting phytoconstituents of the *Polianthes tuberosa* flower and followed by ethyl acetate (9.84 percent) and n-hexane (5.12 percent) solvents which achieved the highest extraction yield at 16.52 percent. This higher extraction performance of methanol can be attributed to its polarity enabling it to extract flavonoid and phenolics. Manned pre-screening proved the existence of alkaloids, flavonoids, phenols, tannins, and terpenoids and

showed therapeutic potential of the extract. Further GC-MS/LC-MS analysis indicated the presence of eugenol, phytol, and quercetin, along with garnering keracetic acid in large amounts as well as of 2-sitosterol, which is known to possess antibacterial, antioxidant, and anti-inflammatory effects in acnes treatment.

It was possible to identify important formulation variables which influence the performance of nanosponge by this QbD based optimization. The FMEA risk assessment helped to prioritize parametrics of formulation that comprised of polymer concentration, extract concentration and surfactant concentration. Box-Behnken design demonstrated statistically significant output of optimization with a tremendous R^2 and non-significant lack of fit. Among formulations made, F2 had the best physicochemical characteristics including particle size of 398.6 28 nm, PDI of 0.438, zeta potential -19.7 mV and entrapment efficiency of 79.7. Higher desirability value and reproducibility had also been observed in the optimized formula.

Characterization studies showed successful β -glucan nanosponges formation. SEM and TEM analysis revealed formation of uniform structures of the porous nanosponge structure in form of spheres. FTIR data indicated that the polymer was compatible with the plant extract with no significant reaction occurring between them. XRD

analysis showed that the crystallinity of the formulation was reduced indicating that the extract has been transformed to some amorphous form. DSC thermograms indicated the success of encapsulation of extract in the 2-glucan matrix. In-vitro drug release studies showed sustained drug release of 93.8% drug release in 24 hrs compared to an instant drug release of pure extract. Kinetic modeling of the drug release demonstrated that the formulation led to the Higuchi model that displayed diffusion control of the soluble release of the drug of the nanosponge porous matrix.

Optimisation of the nanosponge was performed and the formulation integrated into topical gel, comprising of Carbopol 940 and HPMC, gelling agents. The gel ready exhibited a smooth appearance, good pH (6.4), good spreadable properties, viscosity of 6515 cps, great extrudability and it contained 95.5% of drugs, something which qualifies it as a topical application. Cutibacterium acnes (21.20 mm) and Staphylococcus epidermidis (19.52 mm) inhibited the anti-acnes properties to a remarkably good extent and this led to the affirmation of the possible application of this compound as an anti-acnes. Three months stability showed a lack of drastic changes in physical appearance, pH, viscosity or drug content, so it can be concluded that the formulation is stable. Overall, it could be concluded that the nanosponge gel of β -glucans elaborated is a promising alternative treatment of acne that is based on a herb and should be used as an effective and sustainable treatment method.

CONCLUSION

Using a Quality by Design (QbD) approach, the current experiment was capable of making and optimizing Polianthes tuberosa extract-loaded 6-glucan nanosponges to enhance the topical application in the treatment of Acnes vulgaris. The optimized formulation showed an optimal physicochemical property which included the

incorporation of desired particle size, entrapment efficiency, stability and sustained delivery of the drug. With the introduction of the optimized base of nanosponges in Carbopol 940 and Hydroxypropyl Methylcellulose gel bases, acceptability of pH, viscosity, spreadability and overall stability formulations were achieved. The formulation was also to exhibit antibacterial action against Infection of Cutibacterium acnes and even against Staphylococcus epidermidis infection. These findings suggest that the nanosponge gel herbal nanosponge constituted could be used as an alternative to the conventional drug-alsike treatments to acne, with improved acnes remedy consequences and reduced impact.

12. Limitations of the Study

Although the present research gave promising findings with respect to using Polianthes tuberosa extract-impregnated 0 -glucan nanosponges by loading a topical gel to address Acnes vulgaris, it is necessary to acknowledge a number of limitations:

- The researchers primarily confined the research to in-vitro formulation development and testing which would not necessarily be representative of in-vivo drug effectiveness.
- The antimicrobial explanation was merely evaluated on the contraction of the Cutibacterium acnes as well as Staphylococcus epidermidis; other microbes that might be related to acnes were not examined.
- In vivo, no animal tests were performed to determine skin retention, drug activity, toxicity, and possible development of irritation potential, or to determine reaction to healing.
- There was no clinical human experimentation and so the reassurance of the safety level, the effectiveness and acceptability of the human body remains unclear.



- The phytochemical profiling of the extract may not have identified all the active components of *Polianthes tuberosa* extract that result in anti-acne properties.
- Modest time stability tests have been conducted under special storage conditions and it is not clear when the shelf-life is likely to be long.
- The dermal deposition and skin permeation test on cutaneous animal or human skin were not carried out.
- This arbitrarily formulated algorithm was not developed on a larger scale (laboratory) or in batch versus batch reproducibility.
- There was no analysis of the cost-effectiveness in commercial production.
- There was limited ability to compare with commercially available anti-acne preparations such as Clindamycin or Benzoyl Peroxide.
- Advanced toxicity analysis such as cytotoxicity tests and histopathological test were not done.
- The regulatory concerns on the use of herbal nanocarrier-formed formulations were not touched.
- Determine anti-inflammatory action, which is an important factor in the pathogenesis of acne.
- Carry out cytotoxicity studies using skin cell lines to identify the safety of the formulations.
- Combine *Polianthes tuberosa* extract with other bioactive herbs such as *Azadirachta indica*, *Aloe vera* or *Curcuma longa* in a synergistic fashion.
- Consider advanced nanocarrier vehicles such as liposomes, nano emulsions, microneedles or nanofibers to apply in a topical manner.
- Conduct long-term accelerated stability test according to the requirements of International Council of harmonisation.
- Additional refinement of the formulation parameters through artificial intelligence and machine learning-aided QbD models.
- Scale-up procedures of pilot-scale and industrial procedures.
- Regard patentability and commercialization of the herbal anti-acne nanomedicine products.
- Determine how *Polianthes tuberosa* nanocarriers can be used in other dermatological applications like wound healing, skin pigmentation disorders, and microbial skin infections.

FUTURE SCOPE

The developed formulation is promising a lot on its future development and commercialization. It is possible to focus the further studies on the following issues:

- Conduct an in-vivo pharmacological study with detailed procedures to find out the therapeutic effect of the drug on acne models in animals.
- Conduct clinical trials to establish safety, efficacy and tolerability, human compliance to *Acnes vulgaris*.
- Perform additional experiments with Franz diffusion cell and retention experiments (skin), and also, biological skin models.

Conflict of Interest

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

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Ethical Approval

Ethical approval was not required for this study as the research involved only in-vitro experimental



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