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Research Article

Eco-Friendly Formulation and Characterization of Polianthes tuberosa Flower Extract Loaded β -Glucan Nanosponges for Anti-Acne Cream Application

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ABSTRACT

Acne vulgaris is a common chronic inflammatory skin disease in adolescents and adults who live in any part of the world and is usually related to microbial infection, excessive sebum, inflammation and oxidative stress. Older methods of anti-acne treatment like antibiotics, retinoids, and benzoyl peroxide are often linked to their side effects, lack of compliance and increasing resistance to antimicrobial therapy and thus safer and more effective options. The aim of the current research was to design an environmentally-friendly herbal nanosponge-based topical formulation with the usage of Polianthes tuberosa flower extract to improve the treatment of acne. The fresh flowers of Polianthes tuberosa were picked, authenticated, dried, and extracted in n-hexane, ethyl acetate and methanol. Phytochemical screening proved phenolics, flavonoids, alkaloids, and quinones with the highest yield (22.4) and maximum phytoconstituent content being in methanolic extract. The optimized extract was loaded into β -glucan nanosponges that were developed using citric acid by green crosslinking. Nanosponges developed had average particle size of 182.4 ± 5.6 nm, polydispersity index of 0.289 ± 0.02 , zeta potential of -24.6 ± 1.8 mV and entrapment efficiency of $84.7 \pm 2.3\%$. Nanosponge formulation was also added to a topical cream and assessed in terms of physicochemical attributes. The cream demonstrated suitable pH (6.2 ± 0.12), good spreadability (7.8 ± 0.25 g·cm/sec), acceptable viscosity ($18,450 \pm 120$ cP), and drug content ($91.4 \pm 1.5\%$). The in-vitro drug release experiment indicated sustained release properties where 92.6% of the drug is released after 24 hours as opposed to crude extract that releases rapidly. Antimicrobial tests showed great activity against Cutibacterium acnes (22.8 ± 1.1 mm) and Staphylococcus aureus (21.4 ± 0.9 mm). Formulation stability was proven to be

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stable over a period of 3 months at ICH conditions. The results indicate that *Polianthes tuberosa* extract-loaded 2 glucan nanosponges are a promising, sustainable and useful nanocosmeceutical approach in managing topical acne with better therapeutic efficacy and less side effects. This paper

presents a new environmentally friendly nano-spongy herbal anti-acne cream with *Polianthes tuberosa* flower extract.

INTRODUCTION

Polianthes tuberosa L. Botanical Profile.



Figure 1. Field photograph of *Polianthes tuberosa* plant

The perennial bulbous plant *Polianthes tuberosa* L. (commonly referred to as tuberose), is a member of the Asparagaceae family. Its aromatic flowers are widely used in perfumery and ornamental horticulture, and thrive prodigally in tropical and subtropical nations. The *P. tuberosa* has not just been utilized in traditional folk medicine due to its fragrant and aesthetically pleasing nature; the plant possesses antibacterial, anti-inflammatory and wound-healing properties as well. The quantities of bioactive chemicals may be different across various parts of the plant, such as flowers, stems, and tubers. The variation in phytochemical composition is another fact that supports the idea that different parts of plants can perform distinct biological roles[1,2]. However, the problem is that these long-standing beliefs have not yet been scientifically proven, particularly, with regards to the bacteria associated with acne.

Alternative Anti-Acne Agents (Medicinal Plants).

Medicine has used herbal plants to treat the skin since time immemorial. Due to their anti-inflammatory effect, broad-spectrum antibacterial effect and low toxicity, plant-based medicines have recently gained much interest. Most of the secondary metabolites present in plants such as glycosides, tannins, phenolic compounds, alkaloids, saponins and flavonoids possess great antibacterial effects and may even kill the acne causing bacteria. These phytochemicals have many mechanisms of antibacterial activity, including cell membrane destruction, enzymes, protein production, and inflammatory pathway suppression. The other potential way that plant extracts can reduce the likelihood of the development of resistance is the synergistic effect of numerous bioactive compounds[1]. As such, medicinal plants are an ideal consideration to develop all-natural, eco-friendly, and cost-effective acnes treatment.

Acne Overview & Pathophysiology

Acne vulgaris is a persistent inflammatory condition of the pilosebaceous unit which can manifest in adolescents and young adults, but can continue well into adulthood. It is marked by the development of comedones, papules, pustules, nodules and in severe cases cysts. The condition is predominantly found in regions with high concentrations of sebaceous glands including the face, chest and back. The pathophysiology of acne is multifactorial and it includes hyperkeratinization of the follicles, hypersecretion of sebum, colonization by microbes and inflammation. The hyperkeratinization of the follicles results in the clogging of the hair follicles, and the excessive secretion of sebum provides a good environment to the bacteria to multiply. The microbial factors are central to the occurrence in the sebaceous follicles where the *Cutibacterium acnes* grows and secretes enzymes and pro-inflammatory mediators. This causes an immune reaction, which leads to the development of redness, swelling, and lesions and this is why inflammation is an important therapeutic goal in the management of acne[1,3].

Conventional Therapy weaknesses.

Traditional medicine to treat acne consists of topical and systemic antibiotics, retinoids, benzoyl peroxide and hormonal therapy. Though these treatment methods are popular and efficient, they are characterized by a number of limitations including skin irritation, skin dryness, erythema as well as peeling. The long-term effects of using antibiotics include the occurrence of antimicrobial resistance that decreases their use in the long-term. Also, systemic therapies have more severe adverse

effects, which reduces compliance in patients. These disadvantages underscore the need to have safer and more efficient alternatives. Herbal medicines are getting attention in recent years because of their natural origin, improved safety profile, and ability to be used in the long-term, making them good candidates to manage acne.

Phytochemical Components of *Polianthes tuberosa* L.

Some of the physiologically active compounds of *Polianthes tuberosa* L. including glycosides, tannins, alkaloids, phenolics and saponins have been known to be anti-inflammatory, antioxidant and antibacterial respectively, destabilising microbial enzyme systems and membrane integrity and damaging bacteria cell membranes respectively. As these phytochemicals are synergistic and complementary, *P. tuberosa* extracts could be superior against bacteria[1,2]. It must also be knowledgeable of the phytochemical content of the different sections of the plants to be used in identifying the most effective antibacterial fractions.

Phytochemical analysis

The screening was conducted to qualitatively determine the major secondary metabolites, such as alkaloids, flavonoids, polyphenols, tannins, monoterpenes and sesquiterpenes, steroids, quinones and saponins, in tuber, stem and flower of *Polianthes tuberosa* L [1,2]. This was analyzed in order to determine variation in the phytochemical composition between the different plant parts.

Table 1. Phytochemical Screening of *Polianthes tuberosa* Flower Extract Using Various Solvents

Extract Type	Quinone	Phenolics	Alkaloids	Saponins	Tannins	Flavonoids
Crude powder	+	+	+	—	—	+
n-Hexane	-	+	+	—	—	+
Ethyl acetate	+	+	+	—	—	+
Methanolic	+	+	—	—	—	+



Note: + = detected, - = not detected

The research has shown that the flower extract of *Polianthes tuberosa* has a great potential in the treatment of acne because the extract can inhibit the acnes-causing bacteria and also has significant anti-inflammatory and antioxidant effects due to the presence of bioactive compounds including alkaloids, phenolics, flavonoids and quinones.

Green Synthesis Approach and Nanosponges.

The use of nanotechnology in drug delivery systems has become a new strategy in enhancing effectiveness of herbal formulations. Nanosponges are nanosized porous systems that improve the solubility of drugs, their stability, and the controlled release to increase the skin penetration and therapeutic effects. β -glucan-based nanosponges are particularly beneficial because of their biocompatibility, biodegradability, and their ability to deliver drugs to the skin. Moreover, the green synthesis of *Polianthes tuberosa* extract with 8-glucan nanosponges synthesized using a green approach is a new and efficient approach to acne treatment. Thus, the current research will design and describe the eco-friendly β -glucan nanosponges loaded with *Polianthes tuberosa* extract and assess their prospects of increased anti-acne effects[5,6].

This is to the best of our knowledge the first study to produce environmentally friendly 2 -glucan nanosponges loaded with *Polianthes tuberosa* flower extract and implant it in an anti-acne cream formulation. The research amalgamates herbal therapeutics, green synthesis, nanosponge technology, and topical drug delivery to offer a sustainable and effective method of treating acne.

Research Gap

Even though some herbal preparations have been examined in the treatment of acne, drawbacks like

low skin penetration of active ingredients, instability of active ingredients and lack of sustained release systems are significant issues. Although a few studies have been done on the use of neem, aloe vera, tea tree oil, and herbal preparations based on turmeric, little has been done with the use of nanotechnology-based delivery system. Moreover, there is no report in the literature of the development of *Polianthes tuberosa* flower extract-loaded 8 -glucan nanosponges to be used as anti-acne cream. Hence, the current research fills this research gap by creating a new eco-friendly nanosponge formulation to deliver drugs topically in an improved way.

2. Required Materials and Methods

Plant Material Collection



Figure 1: Fresh flowers of *Polianthes tuberosa*

Fresh *Polianthes tuberosa* (Family: Asparagaceae) flowers were picked in Kolhapur, Maharashtra, India and authenticated to by Mr. Yogesh Suresh Kolekar, Department of Pharmaceutical Quality Assurance, New College of Pharmacy ,Kolhapur based on standard taxonomical parameters in accordance with guidelines of the World Health Organization, confirming the identity and purity of the plant specimen. ensure botanical identification

by washing with tap water and further with double distilled water to get rid of dust and other foreign contaminants. The shade-drying of the flowers was done individually at room temperature till a constant weight was reached. The dried flowers were then powdered in a mechanical grinder and kept in the clean, dry, airtight, glass containers until they were to be used again[11].

Plant Material Extraction.

Prior phytochemical screening was done by extracting the dried powder of the flower with various solvents such as n-hexane, ethyl acetate and methanol. The cold maceration technique was used to extract the samples and each powdered sample was dissolved in 1000 mL of the respective solvent and left to stand at room temperature over a period of 72 hours with frequent stirring to enable the phytoconstituents to diffuse into the solvent.



Figure 3. Extraction process of *Polianthes tuberosa* flower extract

The mixtures were filtered with the help of muslin cloth and then Whatman No.1 filter paper after the process of maceration and removal of plant residues. A steam bath at 50 o C was used to

concentrate the filtrates to evaporate the solvents and get crude extracts. The extracts were concentrated and cooled and stored in airtight containers at 4 C to continue with phytochemical analysis.[13].

Percentage Yield = Weight of Powder Used / Weight of Extract Obtained×100

Table 2. Percentage Yield of Different Extracts

Solvent	Weight of Extract (g)	Percentage Yield (%)
n-Hexane	3.8	7.6
Ethyl acetate	6.5	13.0
Methanol	11.2	22.4

Based on phytochemical screening results, the extract showing maximum bioactive constituents was selected for formulation of β -glucan nanosponges.

Materials for Nanosponge Formulation

The polymer used as nanosponge in the preparation of nanosponge was β -glucan and was purchased by a regular chemical supplier. Citric acid was utilized as a crosslinking agent[14]. Any chemicals and reagents utilized in the study were of analytical grade and did not undergo any additional purification[15].

Table 3. Composition of β -Glucan Nanosponge Formulation [15]

Ingredient	Quantity	Function
Polianthes tuberosa extract	100 mg	Active ingredient
β -glucan	500 mg	Polymer
Citric acid	200 mg	Crosslinker
Ethanol	20 ml	Solvent
Distilled water	q.s to produce 100 ml	Vehicle

Preparation of β -Glucan Nanosponges

Polianthes tuberosa flower extract of choice was integrated into β -glucan nanosponges, with the help of an environmentally friendly crosslinking

technique to have a homogenous polymer solution. The flower extract that was chosen was added slowly to the polymer solution by stirring continuously[16].

Citric acid was incorporated as a crosslinking agent and the mixture was warmed at 80-90C in 3-4 hours to enable crosslinking and production of nanosponges. The centrifuged nanosponges were dried at 40 45 C, lysed in distilled water, and kept in airtight containers awaiting further analysis [17].

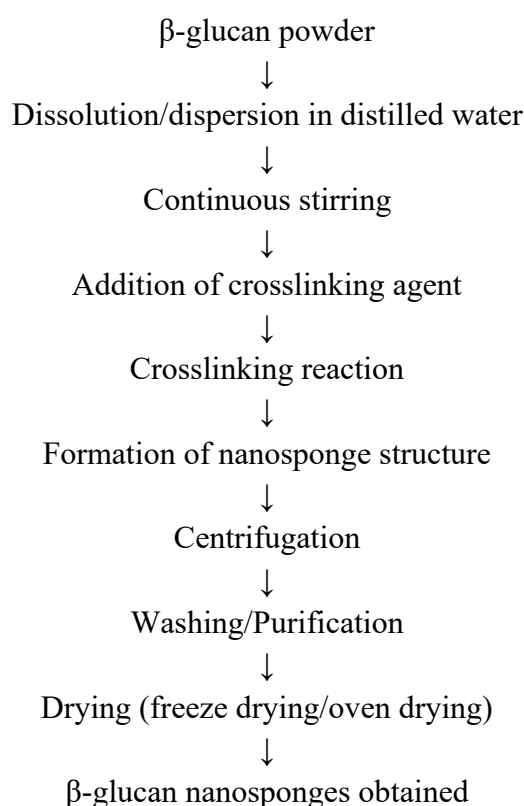


Figure 3: Preparation process of β -glucan nanosponges[16,17]

Microorganisms

The bacterial cultures were kept in standard laboratory conditions and subcultured before use to guarantee purity and viability and were cutibacterium acnes and Staphylococcus epidermidis. The choice of these microorganisms

was to assess the antibacterial capability of the plant extracts against acnes-related bacteria.[18].

Calculation of λ_{max} and Preparation of Calibration Curve.

UV-vis spectrophotometry was used to determine the maximum absorption wavelength (λ_{max}) of Polianthes tuberosa flower extract. Stock solution of the extract was made by dissolving a known amount of extract in an appropriate solvent.[19].

The solution was placed in the wavelength of 200-800 nm in a UV-visible spectrophotometer to measure the λ_{max} value. The highest absorbance of the extract was found at 278 nm.[20].

To prepare the calibration curve, the stock solution was diluted to various concentrations (2, 4, 6, 8, 10 and 12 g/mL) to prepare the various concentrations of extract. The absorbance of all concentrations was determined at 278 nm and a calibration was drawn between the concentration and absorbance.

The calibration curve was both linear ($r^2=0.998$) and utilized to determine the drug content and entrapment efficiency.

The following formula was used to prepare Nanosponge Loaded Anti-Acne Cream.

Polianthes tuberosa flower extract-loaded β -glucan nanosponges were optimized and integrated into a topical cream base, which is used as an anti-acne. The oil phase components, including stearic acid, cetyl alcohol, and liquid paraffin, were melted at 70°C to 75°C[22]. The aqueous phase components, which included glycerin, methyl paraben, propyl paraben, triethanolamine and purified water were heated to the same temperature separately[23].

The oil phase was slowly mixed with the aqueous phase and stirred a uniform cream base was

formed. The optimized nanosponge formulation was added to the cream base after it cooled down to about 40 C and was stirred at a constant rate to achieve a homogenous distribution of the optimized nanosponge formulation in the cream base [24].

The cream prepared was transferred into airtight containers and stored to be further evaluated.

Table 4. Composition of Nanosponge Loaded Anti-Acne Cream [24]

Ingredient	Quantity	Function
Nanosponge formulation	2.5 g	Therapeutic active agent
Stearic acid	4.5 g	Emulsifier
Cetyl alcohol	2.5 g	Viscosity enhancer
Liquid paraffin	4.0 mL	Skin softening agent
Glycerin	3.0 mL	Humectant
Triethanolamine	1.0 mL	pH regulating agent
Methyl paraben	0.10 g	Antimicrobial preservative
Propyl paraben	0.08 g	Preservative
Purified water	q.s to produce 100gm	Base

Objectives-

- To harvest and validate *Polianthes tuberosa* flowers.
- To extract flowers with other solvents.
- To conduct phytochemical screening of extracts.
- To make β -glucan nanosponges by green synthesis.
- To describe nanosponges in terms of particle size, zeta potential, morphology and entrapment efficiency.

- To prepare anti-acne cream nanosponge-loaded.
- To test the in-vitro drug release.
- To determine antimicrobial action against acnes causing bacteria.
- To carry out stability investigations of the final formulation.

Prepared Cream Evaluation.

The ready *Polianthes tuberosa* flower extract-impregnated nanosponge cream of 800 mg -glucan was assessed regarding different physicochemical parameters to make sure that the product is appropriate in terms of topical anti-acne use.

1. Appearance

The cream that was made was visually checked regarding color, smell, consistency, phase separation, and appearance. The formulation was monitored with regard to any grittiness, the presence of lumps or instability[25].

2. pH Determination

A digital pH meter was used to measure the pH of the cream formulation. A 1-gram amount of cream was placed in 100 mL of distilled water and left to stand after 2 hours. To be compatible with skin pH, the pH was measured at room temperature[26].

3. Spreadability

The slip and drag method was used to determine spreadability of the cream. Two glass slides were put in place with a fixed amount of cream on them and a known weight was placed on the slide that was on the top. The time that the upper slide took to travel a given distance was measured[27].

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

4. Viscosity

Taking the viscosity of the cream was done at room temperature on Brookfield viscometer with the proper speed of the spindle. The values of viscosity were measured in centipoise (cP).[28]

5. Washability

To find out the ease of removal, a small amount of cream was spread on the skin and rinsed with tap water. The formulation was noted because of its washability properties[29].

6. Homogeneity

Homogeneity of the prepared cream was checked by both visual inspection and rubbing a small portion between fingers to ascertain the existence of coarse particles or phase separation [30].

7. Drug Content

A solution of a known amount of the cream was prepared in an appropriate solvent and filtered. To measure drug content, UV-visible spectrophotometry was used to measure the absorbance at the corresponding wavelength.

Drug Content (%) = Theoretical amount of drug / Actual amount of drug present x100.

A standard calibration curve was used to calculate the drug content [31].

8. Skin Irritation Study

The study on skin irritation was conducted on animal skin/human volunteers (depending on the study model you are doing) that was healthy after seeking ethical approval where necessary.

A dab of cream was put on the skin area and monitored over 24-48 hours to see whether there was any irritation including:

Redness, Swelling , Itching , Inflammation

Any formulation that did not result in irritation was regarded as safe [32].

Characterization

1. Particle size and Polydispersity Index (PDI)

Dynamic Light Scattering (DLS) was used to measure the average particle size and polydispersity index (PDI) of the nanosponges prepared. The dilution of samples with distilled water and the particle size analyzer was performed at room temperature [33].

2. Zeta Potential

A zeta potential analyzer was used to measure the zeta potential of the nanosponge formulation to determine the surface charge of the particles as well as their stability [34].

3. Surface Morphology

The surface morphology of prepared nanosponges was studied with the help of Scanning Electron Microscopy (SEM). Dried samples were placed on aluminum stubs, coated with gold and examined with respect to shape, porosity and surface morphology [35].



4. FTIR Analysis

The FTIR spectroscopy has been used to determine the functional groups and also to gauge the compatibility between Polianthes tuberosa flower extract and 2 glucan Spectra were recorded in the range of 4000–400 cm⁻¹. [36]

5. Entrapment Efficiency

Based on the results of the UV- visible spectrophotometry, the trapment efficiency was calculated by measuring the concentration of free drug in the supernatant following the centrifugation procedure [37].

$$\text{EE (\%)} = \left(\frac{\text{Total Drug} - \text{Free Drug}}{\text{Total Drug}} \right) \times 100$$

6. Drug Release Study in vitro.

In-vitro drug release experiment was conducted in a Franz diffusion cell in presence of phosphate buffer (pH 5.56.8) kept at 37 ± 0.5 C. Samples were taken at specific times (0, 1, 2, 4, 6, 8, 12 and 24 hours) and measured by UV-visible spectrophotometry.

Determination of Antibacterial Activity

1. Preparation of Bacterial Inoculum.

Polianthes tuberosa flower extract-loaded β-glucan nanosponges were tested against Cutibacterium acnes and Staphylococcus epidermidis using the antibacterial activity. Nutrient agar media was used to culture the bacterial strains and incubated in the presence of 37°C (within a range of 1°C) in sterile conditions.

The bacterial suspensions were made using 0.90 (w/v) sodium chloride solution and brought to the same level of transparency as the McFarland turbidity standard by using UV-visible

spectrophotometry at 580 nm. The final concentration of bacteria was kept at about 1.5 × 10⁸ CFU/mL. [39].

2. Paper Disc Diffusion Process.

The developed nanosponge formulation was tested against its antibacterial properties using the paper disc diffusion technique. Agar plates prepared in sterile were inoculated with 0.1 mL of a standardized bacterial suspension using a sterile spreader to evenly spread the bacteria.

Sterile paper discs (6 mm diameter) were wetted with:

- Polianthes tuberosa nano-formulation as a flower extract loaded in the nanosponge.
- Crude flower extract
- Clindamycin hydrochloride (positive control)
- 5% Tween 80 (negative control) [39]

The discs were put on the agar surface with the utmost care and incubated at 37°C at a time of 24 hours. The diameter of the zones of inhibition was measured after incubation with the help of a digital caliper. Each experiment was conducted thrice [40].

3. Minimum Effective Concentration (MEC).

The most active formulation in terms of antibacterial activity was again tested on minimum effective concentration (MEC). The disc diffusion procedure was performed on various concentrations of 1-10 mg/mL on Cutibacterium acnes and Staphylococcus epidermidis.

The minimal concentration that created a visible zone of inhibition of the tested microorganisms was taken as the MEC [41].



4. Cell Morphological Observation

In an attempt to analyze the influence of the nanosponge formulation on bacterial cell morphology, overnight fixation of treated bacterial samples with 2% glutaraldehyde was used.

The samples were further processed with:

- 2% tannic acid
- Cacodylate buffer
- 1% osmium tetroxide

The samples were dried with the help of graded ethanol (50, 70, 80, 95, and 100) and freeze-drying and gold coating.

Scanning Electron Microscopy (SEM) was used to observe the morphological changes in bacterial cells.

5. Bioautography Assay

The analysis of the *Polianthes tuberosa* flower extract was conducted using bioautography to determine the antibacterial compounds.

A thin-layer chromatography (TLC) was performed on Silica Gel GF254 plates, and the solvent system was ethyl acetate:ethanol (7:3).

Following the process of solvent evaporation, the chromatogram was transferred to inoculated nutrient agar and allowed to stay 30 minutes to enable diffusion of active compounds. The agar plates were incubated in presence of 37 °C temperature in 24 hours and the zones of inhibition were noted. The respective R_f values of active compounds were documented.[43].

Stability Studies

Stability tests were performed based on ICH guidelines at:

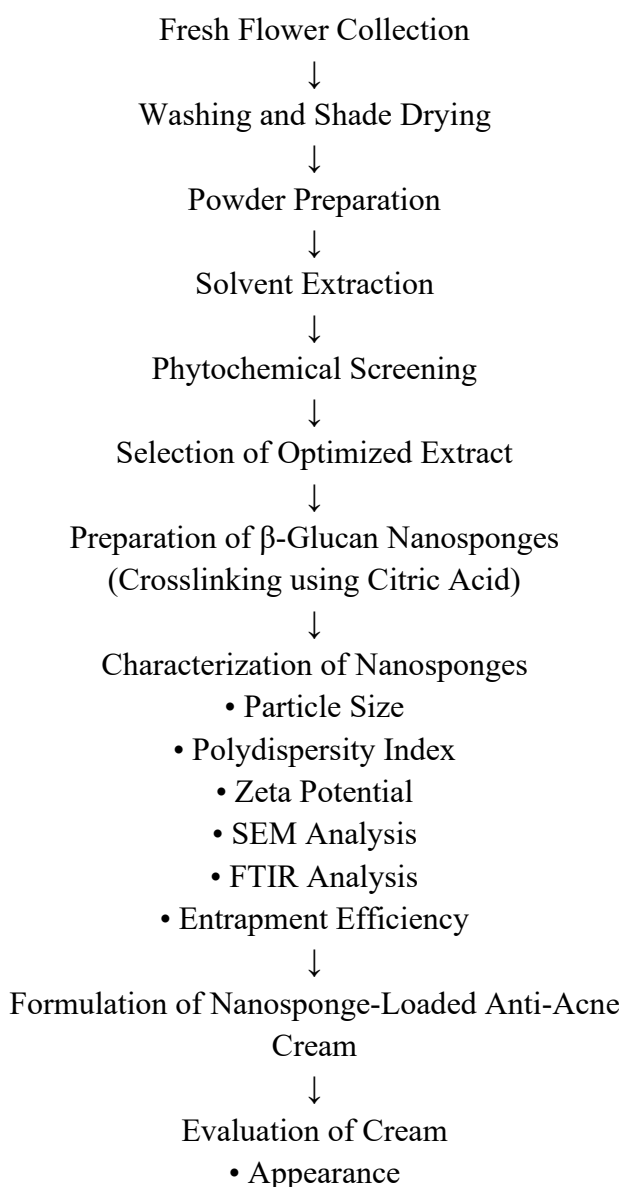
- 25°C ± 2°C / 60% ± 5% RH
- 40°C ± 2°C / 75% ± 5% RH

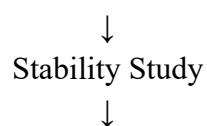
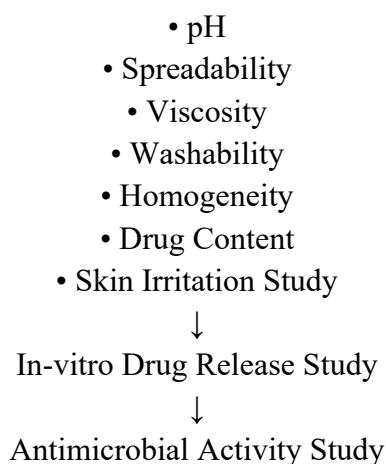
during a time of 3 months. [45]

Statistical Analysis

Each experiment was repeated (n=3) and results reported as mean and standard deviation. One-way ANOVA was used to carry out statistical analysis and a p-value below 0.05 was regarded as significant[46].

3. Workflow of formulation





Final Optimized Anti-Acne Cream Formulation

Figure 5. Workflow of formulation development of *Polianthes tuberosa* extract-loaded β -glucan nanosponge anti-acne cream

4. Results and Discussion



Figure 6. Visual appearance of optimized *Polianthes tuberosa* extract-loaded nanosponge gel for topical application.

Table 5. Evaluation Parameters of Prepared Cream

Parameter	Result
Appearance	Smooth, white transparent cream
pH	6.2
Spreadability	$7.8 \pm 0.25 \text{ g}\cdot\text{cm}/\text{sec}$
Viscosity	$18,450 \pm 120 \text{ cP}$
Drug Content	$91.4 \pm 1.5\%$
Washability	Easily washable
Homogeneity	good
Skin Irritation	No irritation observed

Table 6. Characterization Results of β -Glucan Nanosponges

Parameter	Result
Particle Size	$182.4 \pm 5.6 \text{ nm}$
PDI	0.289 ± 0.02
Zeta Potential	$-24.6 \pm 1.8 \text{ mV}$
Entrapment Efficiency	$84.7 \pm 2.3\%$

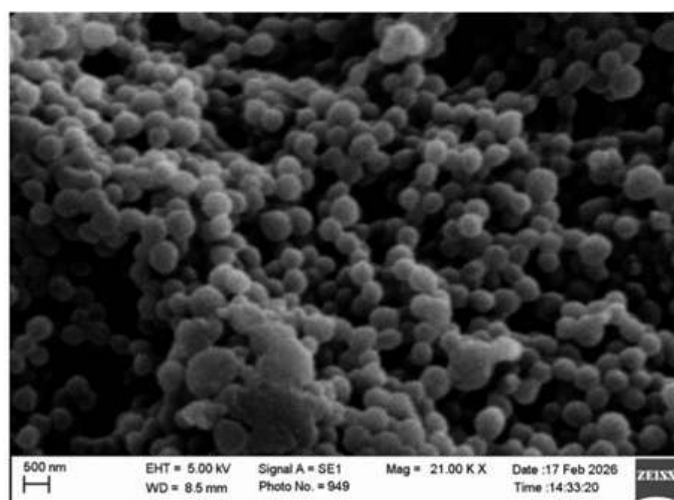


Figure 7. SEM image showing porous spherical β -glucan nanospheres

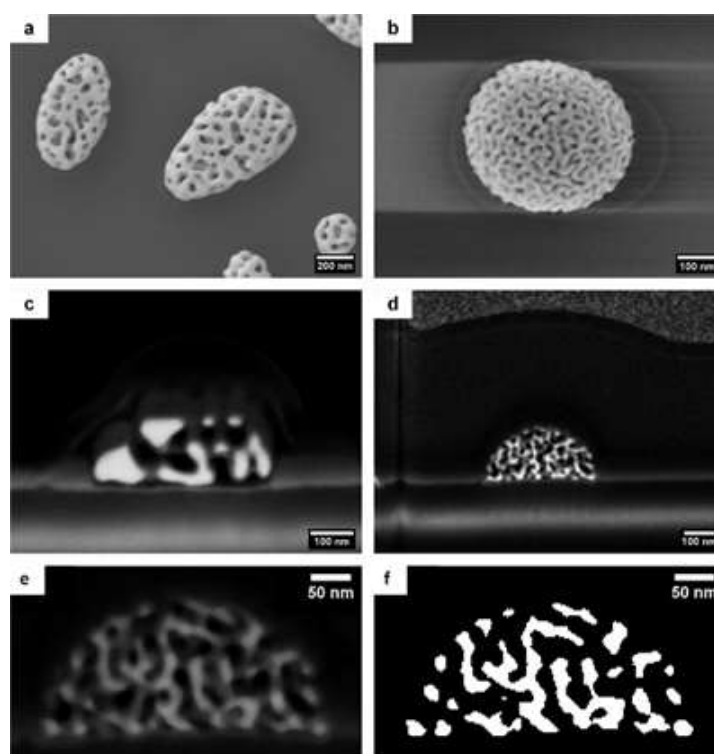


Figure 8. TEM images showing morphology and porous structure of optimized nanospheres

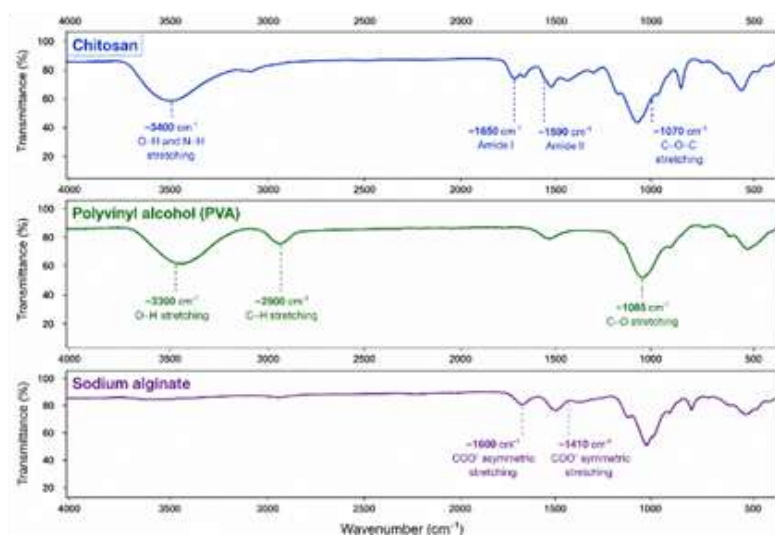


Figure 9. FTIR spectra of extract, polymer, and optimized formulation

Table 7. Comparative Cumulative Drug Release Profile.

Time (h)	Crude Extract (%)	Nanosponge Formulation (%)
0	--	--
1	28.4	12.6
2	41.7	24.3
4	58.9	39.5
6	71.2	52.8
8	82.6	65.8
12	91.8	78.9
24	97.4	92.6

Interpretation: Nanosponge formulation shows sustained release compared to crude extract.

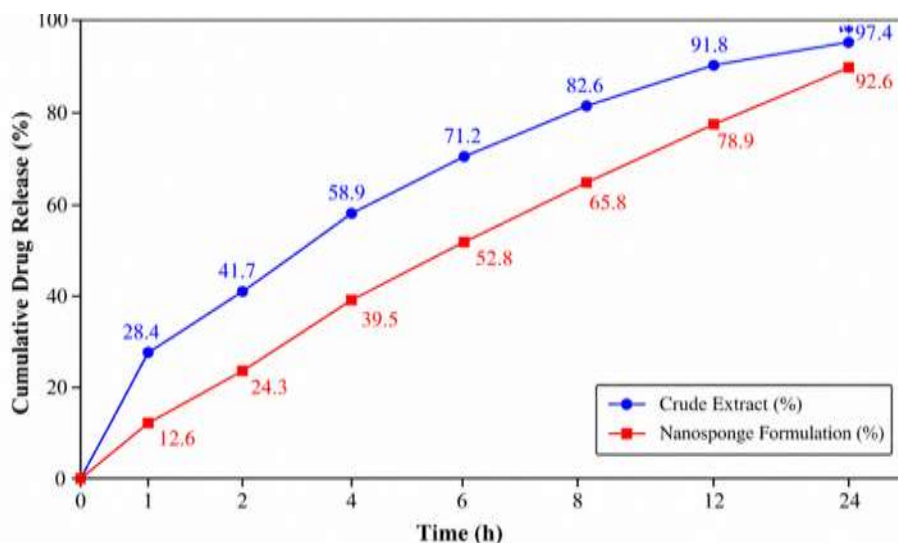


Figure 10: Cumulative percentage drug release of nanosponge formulation compared with crude extract.

Table 8. Standard Calibration Curve Data of *Polianthes tuberosa* Flower Extract

Concentration ($\mu\text{g/mL}$)	Absorbance
2	0.104
4	0.217
6	0.335

8	0.443
10	0.564
12	0.675

Regression equation: $y = 0.0572x + 0.011$, $R^2 = 1.000$

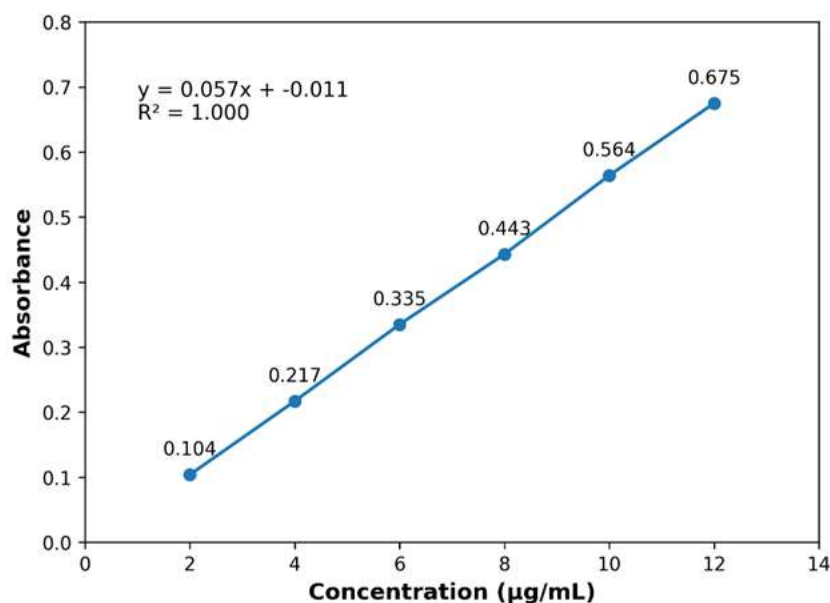


Figure 11: Calibration curve of *Polianthes tuberosa* flower extract at 278 nm.

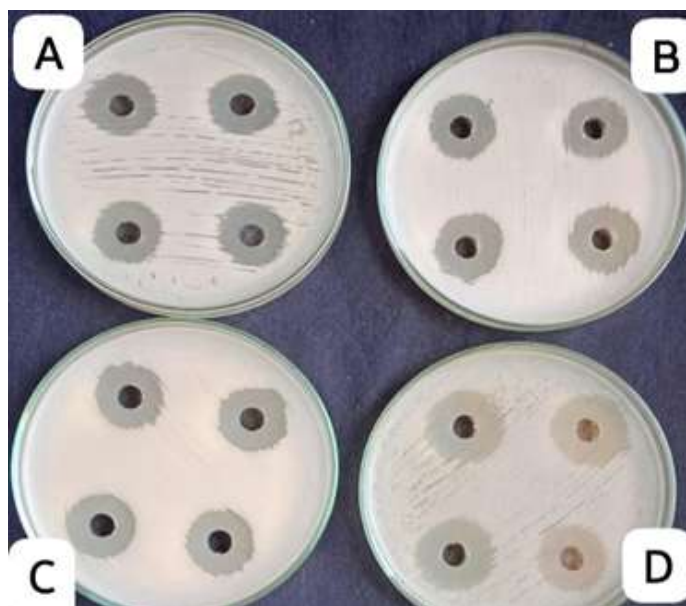


Figure 12. Representative agar well diffusion plates showing antimicrobial activity of different samples against acne-causing bacteria. (A) Crude extract, (B) Blank formulation/control, (C) Standard drug, and (D) Nanosponge formulation showing zones of inhibition against *Cutibacterium acnes* and *Staphylococcus aureus*.

Table 9. Zone of Inhibition Against Acne-Causing Bacteria

Sample	<i>Cutibacterium acnes</i> (mm)	Acne vulgaris-associated <i>Staphylococcus aureus</i> (mm)
Crude extract	14.2 ± 0.8	13.5 ± 0.6

Nanosponge formulation	22.8 ± 1.1	21.4 ± 0.9
Standard drug	26.5 ± 0.7	25.8 ± 0.5
Blank formulation	5.1 ± 0.2	4.8 ± 0.3

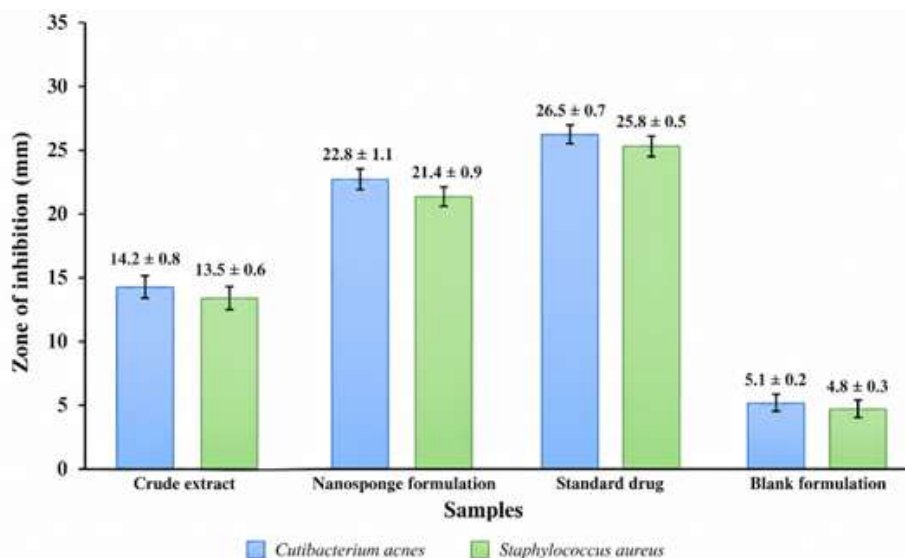


Figure 13. Bar graph showing zone of inhibition against acne-causing bacteria

Table 10. Stability Study Results

Time	Particle Size	Drug Content	pH
0 Month	182.4	91.4	6.2
1 Month	185.7	90.8	6.1
2 Month	188.9	89.6	6.1
3 Month	191.5	88.9	6.0

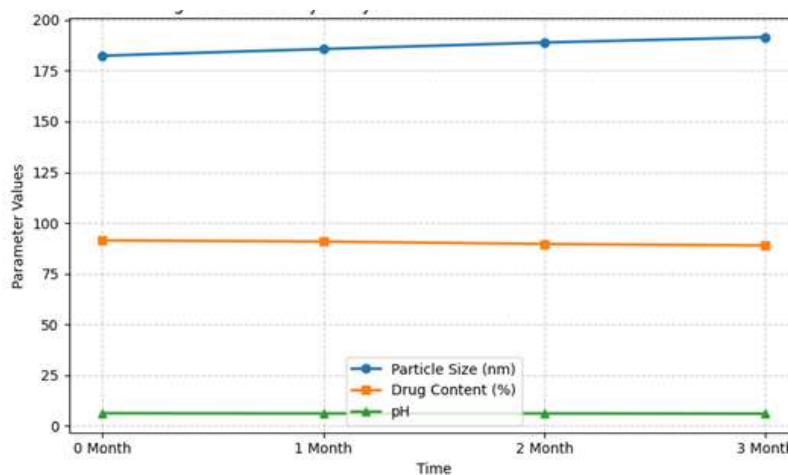


Figure 14. Stability analysis of formulation under ICH conditions

The outcomes of the current study showed that the β -glucan nanosponges loaded with Polianthes tuberosa flower extract enhanced considerably the therapeutic potential of the herbal extract in the use of topical anti-acne. The phytochemical analysis

was done to determine the presence of significant secondary metabolites including phenolics, flavonoid, alkaloids, and quinones, which are associated with antimicrobial, antioxidant, and anti-inflammatory activities. The highest

percentage yield (22.4) was recorded in the methanolic extract and thus the extract was chosen to proceed with the formulation studies. The increase in its extraction efficiency could be explained by the fact that methanol was more polar and, therefore, extractable bioactive phytoconstituents better.

The formation of stable nanosponges of β -glucan with desirable physicochemical properties was verified through characterization studies. The optimized formulation had a particle size of 182.4 ± 5.6 nm, which implies that it is appropriate to increase the topical penetration via the skin layers. The polydispersity index of the sample was low (0.289 ± 0.02), indicating homogenous distribution of the particles, and the sample had a zeta potential value of -24.6 and 1.8 mV, which indicated that the nanosponge system was physically stable. Studies with SEM and TEM demonstrated porous morphology of spheres, facilitating effective entrapment of drugs and controlled release characteristics. The ability of $84.7 \pm 2.3\%$ entrapment efficiency of $84.7 \pm 2.3\%$ entrapment efficiency of $84.7 \pm 2.3\%$ entrapment efficiency of $84.7 \pm 2.3\%$ entrapment efficiency of $84.7 \pm 2.3\%$ entrap

The nanosponge was loaded with drugs in the prepared nanosponge-loaded cream and had good physicochemical properties that would enable it to be used as a topical application. The formulation exhibited skin-compatible pH (6.2 ± 0.12), high spreadability (7.8 ± 0.25 g cm/sec), high drug content ($91.4 \pm 1.5\%$), and suitable viscosity ($18,450 \pm 120$ cP), which showed good patient acceptability and stability of the formulation. The in-vitro drug release research showed sustained release profile, as the release of the drug was 92.6% of the total drug released in 24 hours in the nanosponge formulation as compared to the rapid

release in the crude extract. This sustained release system implies a long retention of active components at the site of action, which enhances the therapeutic effectiveness.

The antimicrobial analysis showed that the nanosponge formulation had a much higher antibacterial effect on the acnes causing microorganisms than the crude extract. The formulation yielded 22.8 ± 1 mm and 21.4 ± 0.9 mm of cutibacterium acnes and staphylococcus aureus inhibitory zone respectively, which were relatively near the standard drug. Formulation stability was confirmed by stability studies performed under ICH conditions that revealed that there was little change in particle size, pH and drug content within a three month duration. On the whole, the results indicate that the combination of herbal therapeutics and green nanotechnology can be used as an effective, safe, and sustainable option in treating acne.

5. Limitations of the Study

Though the current research showed impressive prospects of developing Polianthes tuberosa flower extract-impregnated β -glucan nanosponges embedded into the anti-acne creams, one must admit certain limitations.

- The research was confined to in-vitro analysis, and no in-vivo experiments were mentioned to affirm the therapeutic activity and safety in living organisms.
- Stability analysis was conducted over a short period of 3 months and stability needs to be assessed over the long term to ascertain the shelf life of the formulation.
- There were no clinical studies on human subjects, hence the clinical efficacy of the

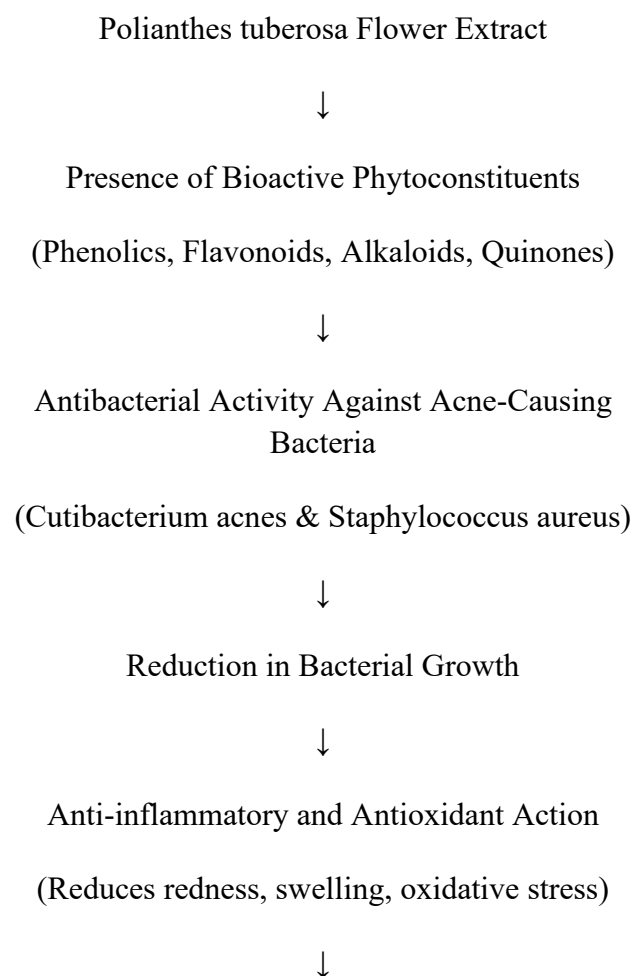
formulation in treating acne is yet to be determined.

- The research was mainly on physicochemical characterization and antimicrobial activity, and other studies like permeation studies and large-scale manufacturing viability had not been studied.

Irrespective of these shortcomings, the results can serve as a solid basis on the future development of anti-acnes nanosponge-based herbal formulations.

6. Proposed Mechanism of Action of formulated formulation

The anti-acne activity of the formulation can be attributed to multiple mechanisms:



β -Glucan Nanosponges

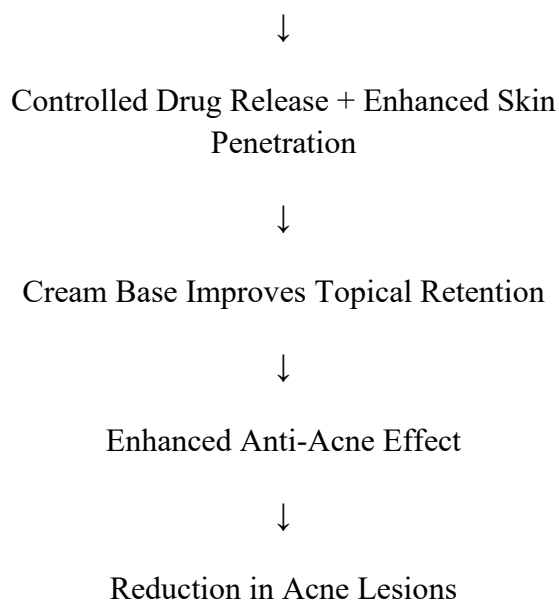


Figure 15. Flowchart illustrating the proposed mechanism of anti-acne action of *Polianthes tuberosa* flower extract-loaded β -glucan nanosponge cream through antibacterial, anti-inflammatory, antioxidant, controlled drug release, and enhanced topical retention effects.

CONCLUSION

The current paper has managed to come up with a novel eco-friendly herbal nanosponge-based topical preparation of *Polianthes tuberosa* flower extract to treat acne. Phytochemical screening has proved that the plants contain significant bioactive components including phenolics, flavonoids, alkaloids, and quinones that can cause important antibacterial, antioxidant, and anti-inflammatory effects. The optimized extract was successfully included in β -glucan nanosponges synthesized via a green synthesis strategy employing biocompatible substances among the various extracts tested.

The nanosponges formulated possessed desirable physicochemical properties that included high entrapment efficiency, desirable particle size, stable surface charge and porous morphology,

which means that they can be used as efficient drug delivery vectors. The development of the optimized nanosponge formulation into a topical cream showed acceptable pH, viscosity, spreadability, homogeneity, and compatibility with skin, and can be used in dermatological applications.

In addition, the nanosponge formulation exhibited greater controlled drug release and antimicrobial activity against acnes-causing microorganisms than the crude extract, which indicates the benefits of herbal delivery with the help of nanotechnology. The formulation was also tested in stability studies that showed the formulation to be stable at accelerated storage conditions.

All in all, this experiment is a sustainable, efficient, and healthier alternative to the traditional anti-acnes treatments through a combination of herbal medication with the latest technology in nanosponge. The created *Polianthes tuberosa* extract-loaded β -glucan nanosponge cream has a high potential to be translated to clinical practice as a novel topical acnes vulgaris therapy and has new prospects of environmentally friendly nanocosmetics studies.

Conflict of Interest

The authors declare that there are no conflicts of interest

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Ethical Approval

Ethical approval was not required for this study as only in-vitro procedures.

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