

## DEVELOPMENT AND EVALUATION OF POLYHERBAL ANTI-ACNE NANO GEL INCORPORATING *AZADIRACHTA INDICA*, *ALOE BARBADENSIS*, AND *RUBIA CORDIFOLIA*

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DOI: <https://doi.org/10.5281/zenodo.20641404>

**How to cite this Article:** Pushpak Sanjay Kaule\*, Aparna Sanjay Jadhav, Avinash Dhondiba Shelake, Shital Shamrao Amare, Manisha M. Murgude. (2026). Development and Evaluation of Polyherbal Anti-Acne Nanogel Incorporating Azadirachta Indica, Aloe Barbadensis, And Rubia Cordifolia. European Journal of Pharmaceutical and Medical Research, 13(6), 677-687. This work is licensed under Creative Commons Attribution 4.0 International license.



Article Received on 15/05/2026

Article Revised on 04/06/2026

Article Published on 10/06/2026

### ABSTRACT

Acne vulgaris is a common skin disorder caused by excessive sebum production, bacterial growth, and inflammation of the pilosebaceous unit. The present study aimed to develop and evaluate a polyherbal anti-acne nanogel incorporating extracts of Neem (*Azadirachta indica*), Aloe Vera (*Aloe barbadensis*), and Manjistha (*Rubia cordifolia*). These medicinal plants possess antimicrobial, anti-inflammatory, antioxidant, and wound-healing properties that are beneficial in acne management. Herbal nanoparticles were synthesized using a green synthesis method and incorporated into a Carbopol-based gel formulation to enhance skin penetration and therapeutic efficacy. Three formulations (B1, B2, and B3) were prepared and evaluated for particle size, zeta potential, appearance, pH, viscosity, spreadability, extrudability, in-vitro drug release, and antimicrobial activity. The prepared nanogels exhibited acceptable physicochemical properties with pH values suitable for topical application and good stability. Among the formulations, batch B2 showed the most desirable characteristics, including optimum viscosity (2843 cP), spreadability (27.33 g·cm/s), and extrudability (100 g/cm<sup>2</sup>). In-vitro diffusion studies demonstrated sustained drug release, reaching 57.9% within 120 minutes. Antimicrobial evaluation revealed significant antibacterial activity, with formulation B2 producing the highest zone of inhibition (22 mm) against acne-associated microorganisms. The results indicate that the developed polyherbal nanogel is a promising, safe, and effective alternative to conventional anti-acne treatments, offering enhanced therapeutic efficacy, controlled drug release, and reduced risk of side effects.

**KEYWORDS:** Acne vulgaris, Polyherbal Nanogel, *Azadirachta indica*, *Rubia cordifolia*, *Aloe barbadensis*, Antimicrobial activity.

## 1. INTRODUCTION

### 1.1 Gel

The term "gel" is derived from "gelatin," which comes from Latin words meaning "ice" and "freeze." Gels are semi-solid, jelly-like substances characterized by a three-dimensional network of molecules dispersed in a liquid medium. This unique structure allows gels to retain significant amounts of liquid while maintaining a solid-like consistency. The texture of gels can vary from soft to firm, depending on the concentration of gelling agents and the properties of the liquid phase. Common gelling agents used in face gel formulations include Hydroxypropyl Methylcellulose (HPMC), Carbopol, and

Sodium Carboxymethyl Cellulose (CMC). These agents are often combined with stabilizers, antibacterial additives, and other beneficial compounds to enhance the gel's properties. Typically, the concentration of gelling agents in these formulations ranges from 0.5% to 2.5%.<sup>[1]</sup>

### Classification

Gels can be categorized according to their physical characteristics, content, and application.

1. Hydrogels: Perfect for hydration, they are primarily made of water and a network of hydrophilic polymers.

2. Emulsion Gels: Emulsifiers stabilize blends of water and oil phases.
3. Transparent Gels: Eye-catching and transparent.
4. Thickened Gels: When thickeners are applied, the consistency becomes denser.

#### Advantages of Gel Formulations

1. Easy to produce.
2. Sophisticated and non-greasy.
3. Outstanding adherence to application domains.
4. Biocompatible and environmentally friendly.
5. Stress-resistant.

**Disadvantages of Gel Formulations:** Despite their advantages, gel formulations also have certain limitations:

1. They may produce a slower onset of action and prolonged therapeutic effect.
2. Some ingredients, such as gelling agents and additives, can cause skin irritation in sensitive individuals.
3. Their high water content makes them more susceptible to microbial and fungal contamination.
4. Loss of solvent during storage can result in drying and shrinkage of the gel.
5. Certain gel systems may exhibit instability due to particle aggregation or flocculation.

#### Ideal Properties of Facial Gel

An ideal gel formulation should possess the following characteristics:

1. It should exhibit a uniform appearance and good transparency.
2. It should readily liquefy or break down when subjected to shear stress or mechanical agitation, such as shaking.
3. The components of the gel should be chemically inert.
4. It should have a non-sticky texture to ensure patient comfort and ease of application.
5. The gel should be compatible with all other ingredients present in the formulation and should not undergo undesirable interactions.
6. It should maintain its physical and chemical stability throughout storage and use.
7. It should be non-irritating and safe for application to the skin or other intended sites of administration.<sup>[2]</sup>

#### 1.2 Nanoparticle

Nanoparticles possess distinctive and often enhanced physicochemical properties compared to their bulk counterparts. Unlike bulk materials, whose properties generally remain unchanged regardless of size, the characteristics of nanoparticles are strongly influenced by their dimensions. Due to their extremely small size and large surface area-to-volume ratio, nanoparticles exhibit increased reactivity and remarkable chemical, optical, electronic, magnetic, and mechanical properties. These unique features have generated significant interest in various scientific and technological fields.

Nanotechnology provides a versatile platform for the design, modification, and optimization of nanoparticle characteristics, including particle size, shape, surface charge, and hydrophilicity, enabling their application in a wide range of biomedical, pharmaceutical, and industrial areas.<sup>[3]</sup>

#### 1.3 Acne

Acne is a long-term inflammatory disorder of the skin that primarily involves the pilosebaceous units, including hair follicles and sebaceous glands. Its development is influenced by multiple factors such as genetic predisposition, hormonal fluctuations during puberty or pregnancy, stress, dietary habits, and the use of certain medications. Acne is considered a multifactorial condition characterized by excessive sebum secretion, colonization of the pilosebaceous unit by microorganisms, and inflammatory responses within the skin. In recent years, polyherbal formulations have attracted considerable attention as a promising approach for acne management due to their potential therapeutic effectiveness and natural origin.<sup>[4]</sup>

#### TYPES OF ACNE

1. **Inflammatory-** Inflammatory acne is characterized by redness, swelling, and inflammation of the skin and is typically more severe than non-inflammatory acne. Common forms of inflammatory acne include papules, pustules, nodules, and cysts, which result from the body's inflammatory response to blocked and infected hair follicles.
2. **Non-inflammatory-** The most common forms of acne include non-inflammatory acne, such as whiteheads and blackheads. Although these lesions are generally milder than inflammatory acne, persistent or improperly treated non-inflammatory acne may contribute to skin discoloration and, in some cases, scarring.<sup>[5]</sup>

#### Acne vulgaris is caused by

1. Follicular hyperkeratosis
2. Perifollicular inflammation and
3. Excessive sebum production and secretion.<sup>[6,7]</sup>

#### 1.4 Microbes Included

##### 1. Staphylococcus aureus (S. aureus)

This bacterium is a natural inhabitant of the skin microbiota; however, it can become pathogenic and cause infections, particularly in individuals with compromised immune function. In cases of acne vulgaris, Staphylococcus aureus may play a role in promoting inflammation and worsening the severity of the condition.



Fig. No. 1: S.Aureus.

## 2. Escherichia coli

Escherichia coli is a Gram-negative, rod-shaped bacterium that naturally inhabits the gastrointestinal tract of humans and animals. Owing to its rapid growth rate, well-established genetic profile, and ease of laboratory cultivation, it is frequently used as a model microorganism in antimicrobial research. Although E. coli is not directly associated with the pathogenesis of acne, it is commonly utilized to assess the broad-spectrum antibacterial efficacy of pharmaceutical, herbal, and polyherbal formulations.



Fig. No. 2: E Coli.

## 3. Bacillus subtilis

Bacillus subtilis is a Gram-positive, rod-shaped bacterium capable of forming endospores and is commonly present in soil and various environmental habitats. It is generally regarded as a non-pathogenic microorganism and is extensively employed in microbiological studies as well as pharmaceutical and biotechnological research due to its well-characterized biological properties.<sup>[4]</sup>



Fig. No. 3: Bacillus Subtilis.

## 1.5 PLANT PROFILE

### a. Manjistha

- **Botanical Name:** *Rubia cordifolia*
- **Family:** Rubiaceae

Manjistha (*Rubia cordifolia*) is a prominent medicinal herb in Ayurvedic medicine and has been traditionally used for the treatment of various skin ailments. It is rich in bioactive constituents, including anthraquinones, glycosides, flavonoids, and tannins, which are believed to play a significant role in its anti-acne and skin-protective properties.



Fig. No. 4: Manjistha.

### Pharmacological Roles of Manjeeshta in Anti-Acne Gel

Mechanism of Action of Manjistha (*Rubia cordifolia*) in Acne Management

#### 1. Antibacterial Activity

Manjistha possesses significant antibacterial properties against acne-associated microorganisms, including *Cutibacterium acnes* and *Staphylococcus epidermidis*. This activity helps limit bacterial growth within the pilosebaceous units and reduces the risk of infection.

#### 2. Anti-inflammatory Activity

The herb suppresses the production of inflammatory mediators such as cytokines and prostaglandins, thereby alleviating redness, swelling, and discomfort associated with acne lesions.

#### 3. Antioxidant Activity

Rich in flavonoids and other phenolic compounds, Manjistha exhibits potent antioxidant effects by neutralizing free radicals and protecting skin cells from oxidative damage, which may contribute to acne development.

#### 4. Regulation of Sebum Production

Manjistha may assist in controlling excessive sebum secretion, a key factor involved in the formation and progression of acne.

#### 5. Blood-Purifying Effect

In Ayurvedic medicine, Manjistha is recognized as a *Rakta-shodhak* (blood purifier). It is believed to help eliminate toxins from the body, supporting the

prevention and long-term management of skin disorders, including acne.

### 6. Promotion of Wound Healing and Scar Reduction

The herb aids in the repair of damaged skin tissue, accelerates the healing of acne lesions, and helps minimize post-acne scarring and hyperpigmentation.<sup>[8-12]</sup>

#### b. Aloe Vera

- **Botanical Name:** *Aloe barbadensis*
- **Family:** Asphodelaceae

Aloe vera contains several bioactive constituents that contribute to its effectiveness in acne management. One of these is salicylic acid, which possesses keratolytic and astringent properties that help unclog pores and reduce the development of new acne lesions. Aloe vera is also rich in polysaccharides, which enhance skin hydration, soothe irritation, and support the repair of damaged skin tissue. Additionally, it contains phytosterols that exhibit anti-inflammatory and antioxidant activities, helping to calm inflammation and protect the skin from oxidative stress. The presence of essential amino acids further promotes skin health by supporting tissue regeneration and facilitating the healing process.<sup>[13-14]</sup>



**Fig. No. 5: Aloe vera gel.**

Aloe vera gel, obtained from the inner parenchymatous tissue of *Aloe barbadensis* leaves, is widely recognized as a versatile ingredient in cosmetic and pharmaceutical formulations. It contains several bioactive compounds, including acemannan, an immunomodulatory polysaccharide; aloe-emodin, known for its antimicrobial activity; chromones, which possess anti-inflammatory properties; and enzymes such as bradykinase. These constituents contribute to the therapeutic effects of Aloe vera by regulating inflammatory cytokines, stimulating fibroblast growth, enhancing wound healing, and exhibiting antibacterial activity against acne-associated microorganisms such as *Cutibacterium acnes* (*Propionibacterium acnes*).

#### c. Neem

- **Botanical Name:** *Azadirachta indica*
- **Family:** Meliaceae

Neem (*Azadirachta indica*), a member of the Meliaceae family, is a medicinal plant known for its rich antioxidant content and diverse therapeutic properties. It has been extensively utilized in traditional systems of medicine, including Ayurvedic, Unani, and Chinese medicine, particularly in India, for the treatment and prevention of various health conditions. For centuries, neem has been valued as a natural remedy for numerous skin disorders and has also been widely employed to protect against agricultural pests and microbial skin infections.<sup>[15]</sup>



**Fig. No. 6: Neem.**

The medicinal benefits of neem are attributed to its diverse range of bioactive constituents, including azadirachtin, nimbinolin, nimbin, nimbidin, nimbidol, sodium nimbin, gedunin, salannin, and quercetin. Neem leaves are also rich in several phytochemicals such as nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol, various amino acids, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, and nimbiol. These compounds collectively contribute to the plant's wide range of pharmacological activities.<sup>[16]</sup>

## 2. METHOD OF EXTRACTION

Maceration method is used for extraction.

### Maceration process for herbal extraction

Maceration is a conventional extraction technique commonly employed to obtain bioactive constituents from medicinal plants using an appropriate solvent. The method is straightforward, economical, and adaptable for both laboratory-scale and large-scale industrial extraction processes.<sup>[17]</sup>

#### Maceration Process

##### 1. Preparation of Plant Material

- **Drying:** The plant material (leaves, roots, flowers, etc.) is usually dried to reduce moisture content.
- **Grinding/ Shredding:** The material is crushed or ground to increase surface area for better extraction.

##### 2. Selection of Solvent

- **Common solvents:** Water, ethanol, methanol, glycerin, or mixtures (e.g., hydroalcoholic solutions).

- Solvent choice depends on the type of compounds targeted (e.g., polar or non-polar).

### 3. Soaking (Maceration)

- The plant material is soaked in the solvent in a closed container.
- Duration: Typically 3 to 7 days at room temperature.
- Agitation: Occasional shaking or stirring helps improve extraction efficiency.

### 4. Filtration

- After soaking, the mixture is filtered to separate the liquid extract (menstruum) from the solid plant residues (marc).

#### 2.1 Advantages of Maceration

- Simple and inexpensive.
- No specialized equipment needed.
- Suitable for heat-sensitive compounds.

#### 2.2 Disadvantages Of Maceration

- Time-consuming.
- Less efficient than other methods (e.g., percolation or Soxhlet).
- Risk of microbial growth if not handled properly.<sup>[17]</sup>



Fig. No. 7: Extracts.

### 3. Procedure

#### 1. Preparation of Zinc Sulphate Solution

- Dissolve 0.288gm, 1.44gm & 2.88gm of Zinc sulphate powder in 100ml of distilled water to make 0.01, 0.05 & 0.1 M  $ZnSO_4 \cdot 7H_2O$  solution.
- Stir well to ensure complete dissolution.

#### 2. Green Synthesis reaction

- Mix the  $ZnSO_4$  solution and herbal extraction in a 1:9 ratio.
- Stir the mixture continuously at room temperature by using Magnetic Stirrer (around 60-70°C) for 1-4 hours.
- A colour change indicates nanoparticle formation due to reduction of  $Zn^{2+}$ .

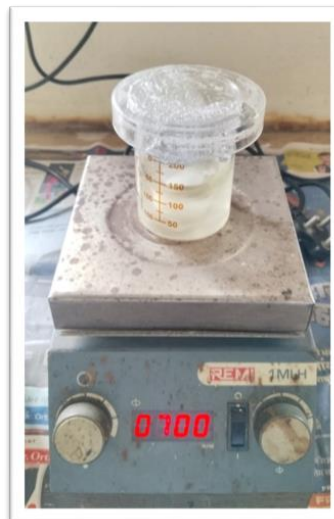


Fig. No. 8: Magnetic Stirrer.

### 3. Procedure for Preparation of Gel

1. Dissolve Carbopol in 20 ml of water.
2. Add 1.2 ml of triethanolamine dropwise with continuous stirring.
3. Dissolve the extract in 1 ml of water and add it to the prepared gel. Continue stirring for 30 minutes.
4. Dissolve 7 ml of glycerine and 0.5 g of methyl paraben in water, then add it to the gel.
5. Add 5 ml of polyethylene glycol and continue stirring.
6. Add sufficient quantity (q.s.) of water to adjust the consistency (total 70 ml of water).
7. For uniform smell, rose oil was added after dissolving it in water.
8. A uniform gel was prepared.



Fig. No. 9: Formulation.

#### 4. FORMULATION TABLE

Table No. 1: Formulation Table.

Sr No.	Ingredients	B1	B2	B3	Role
1.	Carbopol 940	1 gm	1.5 gm	2 gm	Gelling Agent
2.	Nanoparticle of herbal extract	2 ml	2 ml	2 ml	API
3.	Triethanolamine	1.2 ml	1.2 ml	1.2 ml	Neutralizing Agent
4.	Glycerine	7 ml	7 ml	7 ml	Humectant
5.	Methyl Paraben	0.5 gm	0.5 gm	0.5 gm	Preservative
6.	Polyethylene Glycol	5 ml	5 ml	5 ml	Humectant
7.	Rose Oil	q.s	q.s	q.s	Fragrance
8.	Distilled Water	q.s	q.s	q.s	Solvent

#### 5. EVALUATION PARAMETERS

##### 5.1 Characterization of Nanoparticles

###### 1. Particle Size and Zeta Potential

The particle size and size distribution of the formulation were analyzed using photon correlation spectroscopy (PCS) with a Horiba Particle Size Analyzer equipped with PCS software. Prior to analysis, each sample was appropriately diluted with distilled water. The surface charge of the nanoparticles, expressed as zeta potential, was determined by measuring their electrophoretic mobility using a Horiba Zeta Sizer. For zeta potential measurements, the samples were similarly diluted with distilled water before analysis.<sup>[18]</sup>

##### 5.2 Characterization of Gel

###### 1. Physical examination

The prepared formulation was inspected visually for its colour, odour and appearance.

###### 2. Determination of Viscosity

- Take 50–100 g of gel in a clean beaker.
- Place spindle (e.g., #64) of Brookfield Viscometer into the center of the gel.
- Set temperature to 25°C and speed to 10 RPM.
- Turn on the viscometer and allow it to stabilize (about 1–2 minutes).
- Record viscosity in centipoise (cP).
- Repeat 3 times and take the average.<sup>[17]</sup>



Fig. No. 10: Brook Field Viscometer.

##### 3. pH Evaluation

The pH of the nanogel formulation was determined using a digital pH meter. Prior to measurement, the instrument was calibrated with a phosphate buffer solution of pH 7.4. A small quantity of the optimized nanogel formulation was then transferred into a clean glass beaker, and the electrode of the pH meter was immersed in the sample for approximately one minute to obtain the reading. The pH of each formulation was measured in triplicate, and the average value was calculated and recorded.<sup>[19]</sup>



Fig. No. 11: Digital pH Meter.

##### 4. Spreadability

The spreadability of the nanoemulgel was evaluated by placing 0.5 g of the formulation on a glass plate marked with a 1 cm diameter circle. A second glass plate was carefully placed over the sample, and a weight of 500 g was applied for 5 minutes. The increase in the diameter of the spread gel was measured using a linear scale. The study was performed in triplicate, and the average spreadability value was calculated.<sup>[20]</sup> Spreadability was calculated using the following formula:

$$S = M \times L/T$$

S = spreadability

M = mass of upper slide

L = length of moved glass slide

T = time in seconds.<sup>[17]</sup>

##### 5. Extrudability (Tube Test)

The extrudability of the gel formulation was evaluated using a simple method based on the amount of force required to expel the gel from a collapsible tube. A sealed tube containing the gel was pressed firmly at the

crimped end, and after removing the cap, the gel was allowed to extrude until the applied pressure was released. Extrudability was expressed as the weight (in grams) required to extrude a 0.5 cm ribbon of gel within 10 seconds. A greater quantity of gel extruded under the applied force indicated better extrudability of the formulation.

The extrudability is then calculated by using the following formula:

Extrudability = Applied weight to extrude gel from tube (gm) / Area (cm<sup>2</sup>)

The measurement of extrudability of nano-emulgel formulation was in triplicate.<sup>[20]</sup>

### 6. In vitro Diffusion studies

The in vitro drug release study of the topical gel formulation was performed using a Franz diffusion cell.

Approximately 1.0 g of gel was placed on an egg membrane mounted between the donor and receptor compartments. The receptor compartment contained 250 mL of phosphate buffer (pH 7.4), which served as the dissolution medium and was maintained at  $37 \pm 1^\circ\text{C}$  throughout the study. Aliquots of 5 mL were withdrawn from the receptor compartment at predetermined time intervals of 1, 2, 3, and 4 hours. After each sampling, an equal volume of fresh phosphate buffer was added to maintain a constant volume. The collected samples were analyzed using a UV-visible spectrophotometer, with phosphate buffer (pH 7.4) used as the blank solution.<sup>[21]</sup>

$$\% \text{ Drug Release} = (\text{Amt. of Drug release} / \text{Dose}) \times 100$$



Fig No. 11: Egg Membrane.



Fig No.12: Franz Diffusion Cell.

### 7. Antimicrobial screening

The antibacterial activity of the gel formulation was evaluated using the disc diffusion method. Microbial cultures were first grown in nutrient broth and incubated at  $37^\circ\text{C}$  for 24 hours. The inoculum was then standardized and aseptically introduced into sterile Petri dishes. Approximately 20 mL of Mueller–Hinton agar medium was poured into each plate and allowed to solidify. The agar surface was inoculated with 0.1 mL of

microbial suspension containing approximately  $5 \times 10^5$  CFU/mL. Sterile discs (6 mm in diameter) impregnated with the gel formulation were placed on the inoculated agar surface. The plates were then allowed to stand for 30 minutes to facilitate diffusion of the test samples into the medium. Subsequently, the plates were incubated at  $37^\circ\text{C}$  for 24 hours. After incubation, the antibacterial activity was assessed by measuring the diameter of the zone of inhibition surrounding each disc.<sup>[22]</sup>

## 6. RESULTS AND DISCUSSION

### 6.1. Particle Size and Zeta Potential

Table No.2.

Sample No.	Particle Size(nm)	Zeta Potential(mV)
Sample 1	391.4	-13.5
Sample 2	329.2	-14.0
Sample 3	707.4	-14.8

• Particle Size

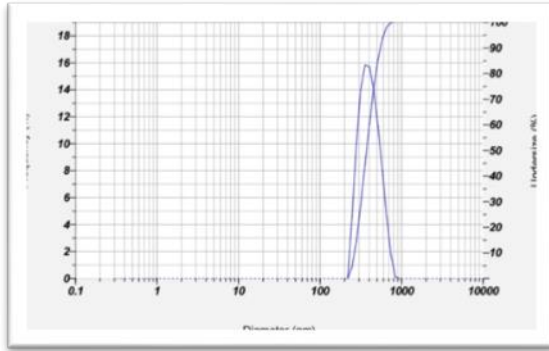


Fig. No. 13: Sample 1

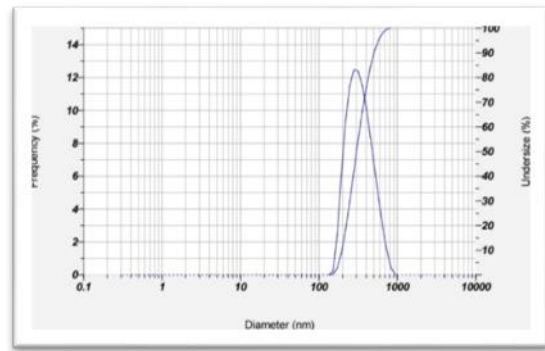


Fig.No.14: Sample 2

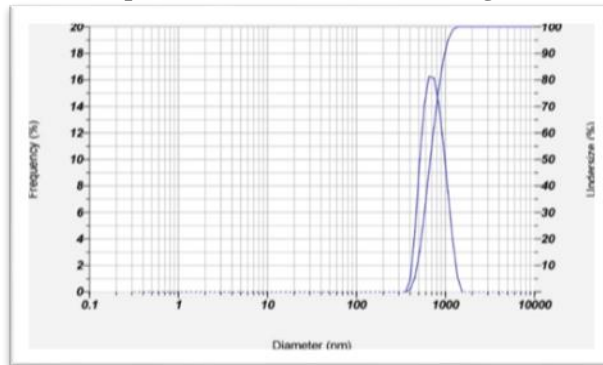


Fig. No. 15: Sample 3.

• Zeta Potential

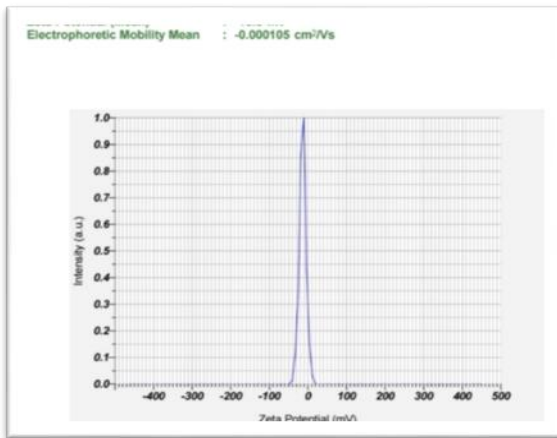


Fig. No. 16: Sample 1

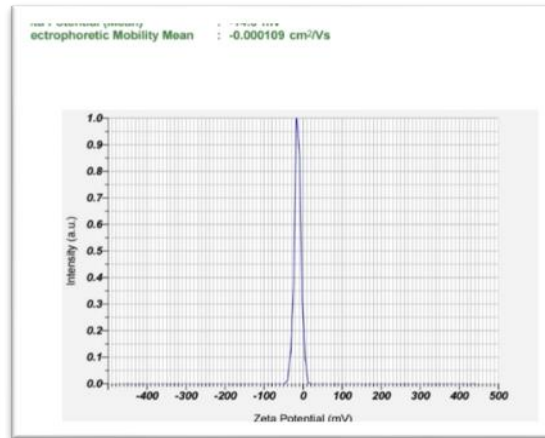


Fig. No. 17: Sample 2

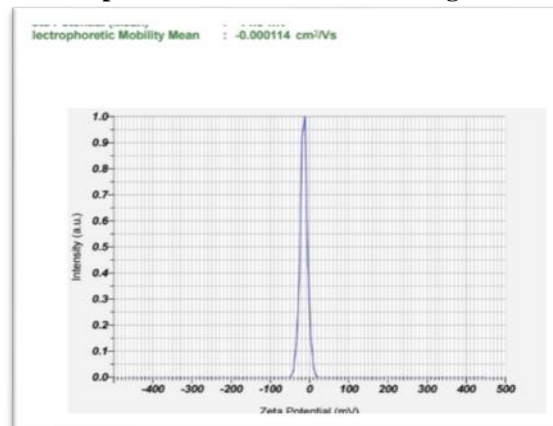


Fig. No. 18: Sample 3.

## 6.2 Physical Examination

Table No. 3.

Batch	Colour	Odour	Appearance
B1	Transparent	Pleasant	Smooth
B2	Transparent	Pleasant	Smooth
B3	Transparent	Pleasant	Smooth

## 6.3. Viscosity, pH, Spreadability, Extrudability

Table No. 4.

Batch	Viscosity (in cP)	pH	Spreadability (gm.cm/sec)	Extrudability ((g/cm <sup>2</sup> ))
B1	2244	5.58	26.04	80
B2	2843	5.77	27.33	100
B3	2112	5.40	20.07	70

## 6.4. Franz Diffusion Cell

Table No.5.

Sr No.	Time (Min)	Absorbance	% DR
1.	30	0.344	34.4
2.	60	0.414	41.4
3.	90	0.493	49.3
4.	120	0.579	57.9

## 6.5. Antimicrobial Assay: ( Zone of Inhibition Diameter in mm)

Table No.6.

Batch	Standard(S)	Formulation	Control
B1	18mm	16mm	0mm
B2	18mm	22mm	0mm
B3	15mm	14mm	0mm

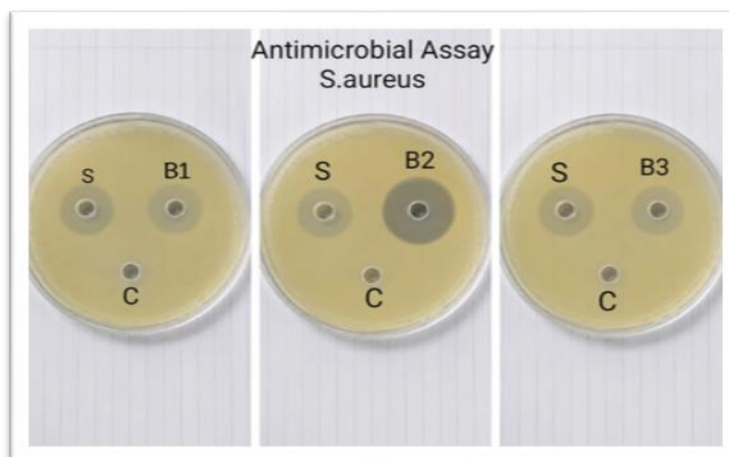


Fig. No. 19: Antimicrobial Assay.

## 7. CONCLUSION

The present study successfully developed and evaluated a polyherbal nanoparticle-based anti-acne gel containing extracts of *Azadirachta indica*, *Aloe barbadensis*, and *Rubia cordifolia*. The incorporation of herbal nanoparticles into the gel formulation enhanced the therapeutic effectiveness of the active phytoconstituents by improving skin penetration and providing controlled drug release.

The prepared nano gel exhibited satisfactory physicochemical characteristics such as appropriate pH,

good viscosity, smooth appearance, excellent spreadability, and acceptable extrudability, indicating its suitability for topical application. Among all formulations, batch B2 showed the best overall performance with optimum viscosity, spreadability, and significant antimicrobial activity.

The antimicrobial studies demonstrated effective inhibition against acne-associated microorganisms including *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis*, confirming the synergistic antibacterial potential of the selected herbal extracts. In vitro diffusion

studies further indicated sustained and controlled drug release from the nano gel formulation.

Overall, the developed polyherbal anti-acne nano gel can be considered a promising, safe, cost-effective, and natural alternative to conventional anti-acne therapies. The formulation may help reduce side effects commonly associated with synthetic treatments while improving patient compliance and therapeutic efficacy. Further clinical and stability studies are recommended to establish its long-term safety, efficacy, and commercial applicability.

## 8. ACKNOWLEDGEMENT

It's our great pleasure to acknowledge the help that we have received during project work.

Though it is sure that the debt which we owe to our guide, cannot be expressed within this line, still it is most pleasant duty to acknowledge our deepest sense of gratitude to my Project guide, **Smt. Manisha M. Murgude** for the invaluable guidance and support throughout this project. Their expertise and insights greatly contributed to the success of this endeavor.

We express our sincere gratitude to **Dr. S.K. Mohite, Principal, Rajarambapu College of Pharmacy, Kasegaon** for his constant encouragement and making the requisite arrangement to able us to complete our Project work.

We are thankful to all teaching and non-teaching staff for their cooperation and moral support during the course of our Project work we must place on record special thanks to our friends for their time to time help and encouragement.

We would like to express our deepest gratitude to my family and friends for their unwavering support and encouragement throughout the duration of this project.

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