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The Formulation of Herbal Nanogel for Wound Healing Using *Clitoria Ternatea*

Bhagyashree Mote[1], Atharv Pathak[2], Sakshi Raskar[3], Siddhi Pise[4], Sanika Pawar[5], Janak Rajpurohit[6]
[1]*Assistant Professor,* [2, 3, 4, 5, 6]*Student, Pharmaceutics, Sinhgad Institute of Pharmaceutical Sciences, Kusgaon (Bk), Lonavala-410401*

Abstract: ***The present study focuses on the formulation and evaluation of a nano-gel incorporating the plant extract of*** *Clitoria ternatea* ***(Butterfly Pea) for enhanced wound healing and antimicrobial activity.*** **Clitoria ternatea** ***is a well-known medicinal plant rich in bioactive compounds such as flavonoids, anthocyanins, and saponins, which possess significant antioxidant, anti-inflammatory, and antimicrobial properties. In this project, the ethanolic extract of*** **Clitoria ternatea** ***was utilized due to its enhanced solubility and stability.***

The nano-gel was formulated using Carbopol as the gelling agent, along with appropriate stabilizers and preservatives to ensure long-term stability. The prepared formulation was evaluated for physicochemical parameters, including pH, viscosity, spread ability, and drug release profile. Antimicrobial activity was assessed against common wound-infecting pathogens such as Staphylococcus aureus and Escherichia coli. The formulation demonstrated improved skin penetration, rapid wound contraction, and enhanced collagen synthesis, indicating superior therapeutic effect.

Keywords: Clitoria ternatea, nano-gel, wound healing, antimicrobial activity, ethanolic extract, flavonoids, anthocyanins, saponins, Carbopol, drug release, skin penetration, Staphylococcus aureus, Escherichia coli.

I. INTRODUCTION

A. Wound Healing: A Complex Biological Process

Wound healing is an intricate biological process essential for restoring tissue integrity and function following injury. This process can be divided into four overlapping phases:

1) Haemostasis: Immediately after injury, the body initiates haemostasis, characterized by vascular constriction and the formation of a fibrin clot. This serves to limit blood loss and provides a provisional matrix for incoming cells.

2) Inflammation: The inflammatory phase follows, involving the recruitment of immune cells such as neutrophils and macrophages to the wound site. These cells play critical roles in clearing debris and pathogens, thereby setting the stage for tissue repair .

3) Proliferation: During the proliferation phase, fibroblasts proliferate and synthesize extracellular matrix components, including collagen. Keratinocytes migrate to cover the wound, and angiogenesis occurs to restore blood supply.

4) Remodelling: The final phase, remodelling, can last for months to years, during which the collagen matrix is remodelled to enhance tensile strength and functionality of the healed tissue.

Effective wound healing is crucial not only for recovery from injuries but also for preventing complications such as infections, chronic wounds, and excessive scarring. The presence of microbial pathogens can significantly impair healing, necessitating the development of antimicrobial strategies.

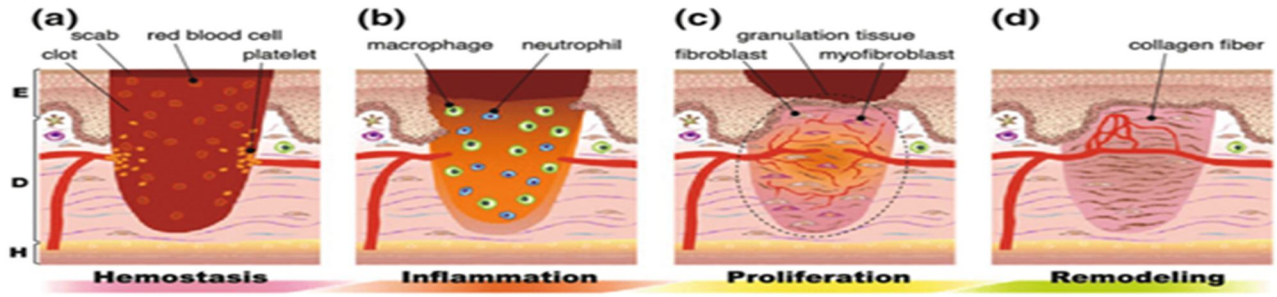

Fig no.1

B. Receptors Involved in Wound Healing

1) EGFR (Epidermal Growth Factor Receptor)

- Promotes keratinocyte proliferation and migration – essential for re-epithelialization.
- Plays a key role in closing the wound and restoring the skin barrier.
- Also supports angiogenesis and ECM remodelling

.

2) TGF-βR (Transforming Growth Factor Beta Receptor)

- Regulates inflammation**,** fibroblast activity**,** collagen synthesis, and scar formation.
- Active in all phases: inflammation, proliferation, and remodelling.
- Maintains balance between tissue repair and excessive scarring

II. PLANT PROFILE

Clitoria ternatea, commonly known as the Butterfly Pea or Blue Pea, is a perennial climbing vine belonging to the Fabaceae family. Native to tropical equatorial Asia, this plant has garnered significant attention due to its vibrant blue flowers and diverse applications. The striking blue hue of its flowers, attributed to anthocyanins, not only adds aesthetic value but also serves as a natural pH indicator. Traditionally, *Clitoria ternatea* has been utilized for its medicinal properties, owing to its antioxidant and anti-inflammatory compounds. Moreover, its flowers are employed in culinary practices as a natural food colouring and to make herbal tea. This thesis aims to explore the botanical characteristics, cultivation practices, and multifaceted uses of *Clitoria ternatea*, highlighting its potential in various fields such as horticulture, medicine, and the food industry.

Fig no. 2

A. Taxonomy

- Kingdom: Plantae
- Division: Magnoliophyta (Angiosperms – flowering plants)
- Class: Magnoliopsida (Dicotyledons)
- Order: Fabales
- Family: Fabaceae (Leguminosae)
- Genus: *Clitoria*
- Species: *Clitoria ternatea* L.

B. Common Names

Butterfly Pea, Blue Pea, Asian Pigeonwings

C. Morphology

Clitoria ternatea is a perennial climbing vine with the following morphological characteristics:

- Stem: Slender and herbaceous, capable of twining around supports or sprawling across the ground, reaching lengths of 2-3 meters.
- Leaves: Pinnately compound with 5-7 leaflets, each leaflet is elliptic to ovate with smooth margins and a glossy, dark green surface. Leaves are alternately arranged along the stem.

- Flowers: Vibrant blue, solitary or in pairs, with a unique butterfly-like shape. Each flower consists of five petals, including a large standard petal, two lateral wings, and a keel formed by two fused petals.
- Fruit: Pods measuring 5-7 cm in length, containing 6-10 brown or black seeds when mature.
- Roots: Well-developed root system that aids in anchoring the plant and absorbing nutrients, contributing to soil fertility through nitrogen fixation.

Fig no.3

D. Distribution

Clitoria ternatea is native to tropical equatorial Asia, particularly in regions with warm and humid climates. Its natural distribution includes:

- Southeast Asia: Commonly found in countries like Thailand, Indonesia, and the Philippines.
- South Asia: Widely distributed in India and Sri Lanka.
- East Asia: Occurs in parts of China and Malaysia.

E. Medicinal Uses

- Traditional Medicine: Clitoria ternatea has been used in traditional medicine systems, particularly in Ayurveda and Tibetan medicine.
- Therapeutic Properties: It is known for its anti-inflammatory, Anti-oxidant, anxiolytic, anti-pyretic and analgesic.

F. Uses in Wound Healing

- Enhances Healing
- Promotes cell proliferation and collagen synthesis.
- Anti-inflammatory and Antimicrobial: Aids in wound healing by reducing inflammation and preventing infection.

G. Phytochemical Constituents

- Bioactive Compounds: The plant is rich in various bioactive compounds, including flavonoids, anthocyanins, triterpenoids, alkaloids, and phenolic compounds.

H. Applications in Modern Science

- Pharmacological Research: Studied for antioxidant, anti-inflammatory, and neuroprotective properties.
- Functional Foods: Used in herbal teas and beverages for health benefits.
- Cosmetics: Anthocyanins used for skin health and anti-inflammatory effects.
- Agriculture: Cyclotides as natural pesticides.
- Nutraceuticals: Supports overall health and well-being.

III. CHEMICAL CONSTITUENTS

Clitoria ternatea, commonly known as the "Blue Pea" has been studied for its various medicinal properties, including wound healing and antimicrobial activity. The plant contains several bioactive compounds that contribute to these properties. Here are some of the key chemical constituents found in Clitoria ternatea:

1) Flavonoids
- Kaempferol
- Quercetin
- Myricetin

2) Anthocyanins
- Delphinidin
- Cyanidin
- Malvidin

3) Triterpenoids
- Ursolic acid
- Oleanolic acid

4) Alkaloids
- Clitorin
- Stizolobinic acid

5) Phenolic Compounds
- Gallic acid
- Chlorogenic acid
- Ferulic acid

IV. COMPOUNDS THAT ATTACH TO RECEPTORS OF IN WOUND HEALING

Compound	Class	Mechanism
Quercetin	Flavonoid	Enhances keratinocyte migration and EGFR phosphorylation
Kaempferol	Flavonoid	Stimulates EGFR signalling → promotes cell proliferation
Delphinidin	Anthocyanin	Activates EGFR related pathways, promotes cell regeneration
Clitorin	Alkaloid	Reported to support EGFR-related wound healing pathways in *Clitoria ternatea*
Ursolic acid	Triterpenoid	Stimulates TGF-β1 expression → promotes fibroblast activation & ECM synthesis
Oleanolic acid	Triterpenoid	Enhances TGF-β signalling → collagen deposition, scar remodelling
Gallic acid	Phenolic acid	Modulates TGF-β pathway to balance inflammation and tissue repair
Ferulic acid	Phenolic acid	Regulates TGF-β1, reducing oxidative stress and improving collagen formation

V. PHARMACOLOGICAL POTENTIAL OF CLITORIA TERNATEA

Clitoria ternatea, commonly known as "Brahma Kamal," is recognized for its diverse pharmacological properties. Here's an overview of its potential therapeutic applications, particularly focusing on its anti-inflammatory, antioxidant, antimicrobial, and wound healing properties.

A. Anti-inflammatory Activity

Studies have demonstrated that extracts of Clitoria ternatea exhibit significant anti-inflammatory effects, which are crucial for managing conditions characterized by inflammation.

B. Antioxidant Properties

The plant has shown strong antioxidant activity, which helps combat oxidative stress and is beneficial in preventing chronic diseases and promoting overall health.

C. Antimicrobial Activity

Clitoria ternatea has been evaluated for its antimicrobial properties against various pathogens, including bacteria and fungi. This aspect is particularly relevant for its potential in developing antimicrobial agents for wound healing.

D. Wound Healing Properties

Research indicates that Clitoria ternatea promotes wound healing, possibly due to its antimicrobial, anti-inflammatory, and regenerative properties. Its application in wound care formulations is being explored.

E. Analgesic Effects

The analgesic potential of Clitoria ternatea has also been documented, providing a rationale for its traditional use in managing pain.

VI. NANOTECHNOLOGY IN WOUND HEALING: THE ROLE OF NANO GELS

The integration of nanotechnology into medicine has led to innovative approaches in drug delivery and wound management. Nano gels are three-dimensional hydrophilic polymeric networks that can encapsulate therapeutic agents, providing controlled and sustained release.

Their unique properties make them particularly suitable for wound care applications:

1) Controlled Drug Release: Nano gels can modulate the release rate of encapsulated compounds, ensuring a steady supply of antimicrobial agents at the wound site, which is crucial for effective infection control.
2) Enhanced Bioavailability: The nano-sized particles can improve the solubility and bioavailability of poorly soluble compounds, maximizing their therapeutic potential.
3) Biocompatibility and Moisture Retention: Nano gels can create a moist wound environment, is known to accelerate healing while preventing crusting and scab formation.
4) Targeted Delivery: The use of specific polymers can enable targeted delivery of bioactive compounds to sites of infection or inflammation, enhancing the efficacy of treatment.

VII. LITERATURE SURVEY

1) *Kumar et al., (2021):* This study demonstrated the enhanced antioxidant and wound healing potential of *Clitoria ternatea* ethanol extract incorporated into nano-gel formulations. The gel promoted faster epithelialization, reduced inflammation, and improved collagen synthesis in wound models.
2) *Sharma et al., (2020):* This study highlighted the antimicrobial activity of *Clitoria ternatea* against *Staphylococcus aureus* and *Escherichia coli*. The ethanol extract incorporated into a gel base showed significant bacterial growth inhibition, indicating its potential in infected wound treatments.
3) *Patel et al., (2019):* Focused on enhancing the transdermal delivery of *Clitoria ternatea* bioactive. The nano-emulsion gel improved the skin permeability of active compounds, resulting in better retention and bioavailability for topical applications.
4) *Gupta et al., (2018):* Investigated the anti-diabetic properties of *Clitoria ternatea*. The study showed improved glucose metabolism and enhanced insulin sensitivity in diabetic rats, suggesting potential for managing type 2 diabetes.
5) *Rao et al., (2017):* Explored the neuroprotective effects of *Clitoria ternatea*. The ethanol extracts improved memory retention, reduced oxidative stress in neuronal tissues, and enhanced cognitive performance in rodents.
6) *Mishra et al., (2016):* This study emphasized the potent antimicrobial and antioxidant properties of aqueous leaf extracts. The extract effectively reduced microbial growth and showed strong free radical scavenging activity, supporting its use in skincare formulation.

VIII. AIM AND OBJECTIVE

A. Aim

- To evaluate the wound healing and antimicrobial properties of a nano gel formulated with extracts of *Clitoria ternatea*, evaluating its efficacy and potential as a therapeutic agent in wound care.

B. Objectives

- To study Assessment of Antimicrobial Activity.
- To study Wound Healing Efficacy.
- To study Safety and Toxicology Studies.
- To study Statistical Analysis.
- To study Recommendations for Future Research.

IX. PLAN OF WORK

Sr. no	Plan of Work
1	Study of wound healing mechanisms.
2	Review of herbal extracts used in wound healing.
3	Focused research on *Clitoria ternatea* – phytochemical profile, medicinal properties.
4	Overview of nanogels – formulation techniques, advantages, and applications.
5	Recent advancements in herbal-based nanogels.

X. MATERIAL AND METHODS

Extraction Process of Clitoria ternatea

A. Collection and Preparation of Plant Material

- Cleaning: Wash the collected plant material thoroughly with distilled water to remove any dirt or contaminants.
- Drying: If required, dry the plant material in a shaded area at room temperature to prevent the degradation of sensitive compounds. Alternatively, you can use a dehydrator at a low temperature.

B. Grinding

Use a mortar and pestle or a mechanical grinder to finely grind the dried plant material. This increases the surface area and enhances the extraction efficiency.

C. Extraction Methods

You can choose one of the following extraction methods:

Solvent Extraction:

1) Solvent Selection: Use solvents like ethanol, methanol, or acetone, as these are effective in extracting a wide range of phytochemicals.

2) Extraction Procedure

Combine the ground plant material with the selected solvent in a ratio of 1:10 (plant material to solvent).

- Let it soak for 24-48 hours in a dark, cool place, occasionally shaking the mixture.
- After soaking, filter the mixture using a filter paper or muslin cloth to separate the solid residue from the liquid extract.
- Concentrate the filtrate using a rotary evaporator or by gentle heating under reduced pressure to remove the solvent.

3) Cold Maceration

- Mix the ground plant material with a solvent and let it sit for several days at room temperature, shaking occasionally.
- Filter and concentrate as described above.

4) Hot Extraction

- Heat the mixture of plant material and solvent on a water bath for a specific duration (usually 30-60 minutes), allowing for better extraction of heat-sensitive compounds.
- Cool the mixture, filter, and concentrate.

D. Formulation into Nano Gel

Once you have the extract, it can be formulated into a nano gel. This typically involves:

Preparation of Gel Base: Use gelling agents like Carbopol or agar.

1) Polymer: It will be used as the nano gel base for the formulation in the formulation

2) Cross-Linkers: To form a gel matrix, a cross-linking agent may be required to create stable bonds between polymer chains.

3) Surfactant: Stabilization of nano particles by reducing the surface tension and preventing aggregation and ensuring uniform dispersion.

4) Incorporation of Extract: Blend the concentrated extract into the gel base and adjust the pH as needed.

XI. DRUG-EXCIPIENT PROFILE FOR NANOGEL FORMULATION OF CLITORIA TERNATEA (AQUEOUS EXTRACT)

A. Active Ingredient

- Drug: *Clitoria ternatea* Aqueous Extract
- Concentration in Nanogel: 5 ml of extract in 30g of nanogel formulation.

B. Excipients

1) Gel Base (Polymer) for Nanogel Formation:

Carbopol

Function: Gelling agent, viscosity enhancer, stabilizer.

Use: Carbopol is used to form a gel matrix, providing the necessary viscosity and texture for controlled release of the active ingredient.

2) pH Adjuster

Triethanolamine (TEA):

Function: pH adjuster.

Use: Neutralizes the Carbopol to form a gel and adjusts the pH to an optimal range for skin application (typically pH 5.5–7.0).

3) Humectant

Glycerine:

Function: Humectant, moisturizing agent.

Use: Retains moisture in the skin, enhancing the hydrating effect of the gel and preventing skin dryness.

4) Preservative

Methylparaben:

Function: Preservative.

Use: Prevents microbial growth and extends the shelf-life of the nanogel formulation.

C. Floral Water (Rosewater)

Function: Solvent, soothing agent.

Use: Rosewater acts as a solvent for the active ingredients and also provides a soothing effect on the skin, which is beneficial for wound healing.

D. Solvent

Water

Function: Solvent for dissolving the extract and excipients.

Use: Acts as the primary vehicle for the formulation, ensuring uniform mixing of the active ingredient and excipients.

XII. FORMULATION

Sr.no	Ingredients	Qty
1	Clitoria Ternatea Extract (Aqueous)	5ml
2	Carbopol	0.7g
3	Triethanolamine	0.2g
4	Glycerine	2g
5	Methylparaben	0.15g
6	Rosewater	5ml
7	Water	q.s
8	Total	30g

XIII. CHEMICAL TESTS

Sr. No	Test	Procedure	Interference	Observation
1.	Flavonoid Test: a. Shinoda Test	a. Add small amount of extract to a test tube. Add a few magnesium turnings. Add drops of concentrated HCl.	Pink or red colour indicates presence of flavonoids	Present
2.	Tannins: a. Ferric Chloride Test	Add few drops of $FeCl_3$ to the extract.	Blue-black colour: hydrolysable tannins Greenish-black colour: condensed tannins	Present
3.	Phenolic Compounds: a. Lead Acetate Test	10% Lead acetate solution add to extract.	White or yellowish precipitate confirms phenolic compounds.	Present
4.	Anthocyanins a. pH Test	Add dilute HCl – red colour Add NaOH – turns blue/green	Colour change depending on pH shows presence of anthocyanins.	Present

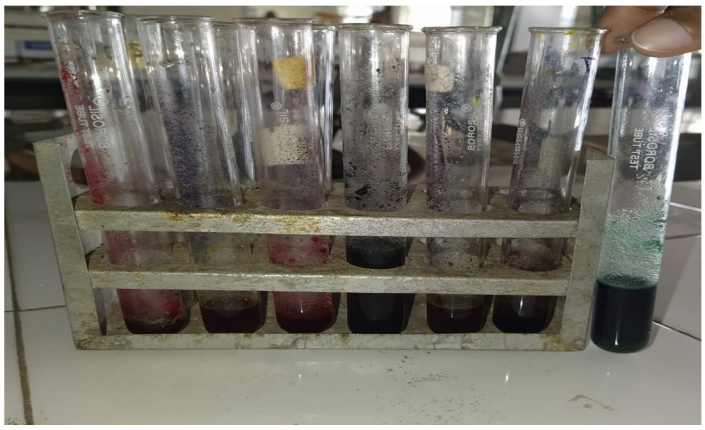

Fig no.4

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XIV. EVALUATION PARAMETER

When evaluating the efficacy and quality of an antimicrobial nano gel formulated from Clitoria ternatea for wound healing, you can consider several key parameters. Here are some evaluation parameters you might include in your thesis:

1) Physical Characteristics

- Appearance: Visual assessment of colour, clarity, and homogeneity.

Colour	Blue
Odour	Pleasant
Texture	Thick

- pH: Measure the pH of the nano gel to ensure it is suitable for skin application (typically around 5-7).
- Viscosity: Assess the flow properties of the gel, as it affects application and stability.

2) Chemical Stability

- Storage Stability: Evaluate the physical and chemical stability of the nano gel over time under various temperature conditions.
- Zeta Potential: Assess the stability of colloidal solution

3) Microbial Efficacy

- Antimicrobial Activity: Conduct tests against a panel of pathogenic bacteria (e.g., *Staphylococcus aureus*, *Escherichia coli*) and fungi. Common methods include:
- Disk Diffusion Method: Measure the zone of inhibition.

4) Particle Size Identification

- Determine the Particle size as per standard valuation in nanogel material

5) Biocompatibility

- Skin Irritation Test: Assess the potential for irritation or allergic reactions through patch testing.
- In Vitro Skin Penetration Studies: Evaluate how well the nano gel penetrates skin layers

6) Shelf Life

- Stability Testing: Conduct long-term stability testing to estimate the shelf life of the nano gel under various storage conditions.

7) Cost-Effectiveness

- Economic Evaluation: Assess the cost-effectiveness of the formulation process compared to traditional wound healing products.

XV. OBESERVATIONS

A. Physical Characteristics

1) pH Measurement

Standard range For pH of Nanogel:

According to ICH guidelines of herbal nanomedicine the standard pH should be the within range of 4.5 to 7.5 pH

Observed pH of the product:

The Observed pH of the Product is 6.**4**

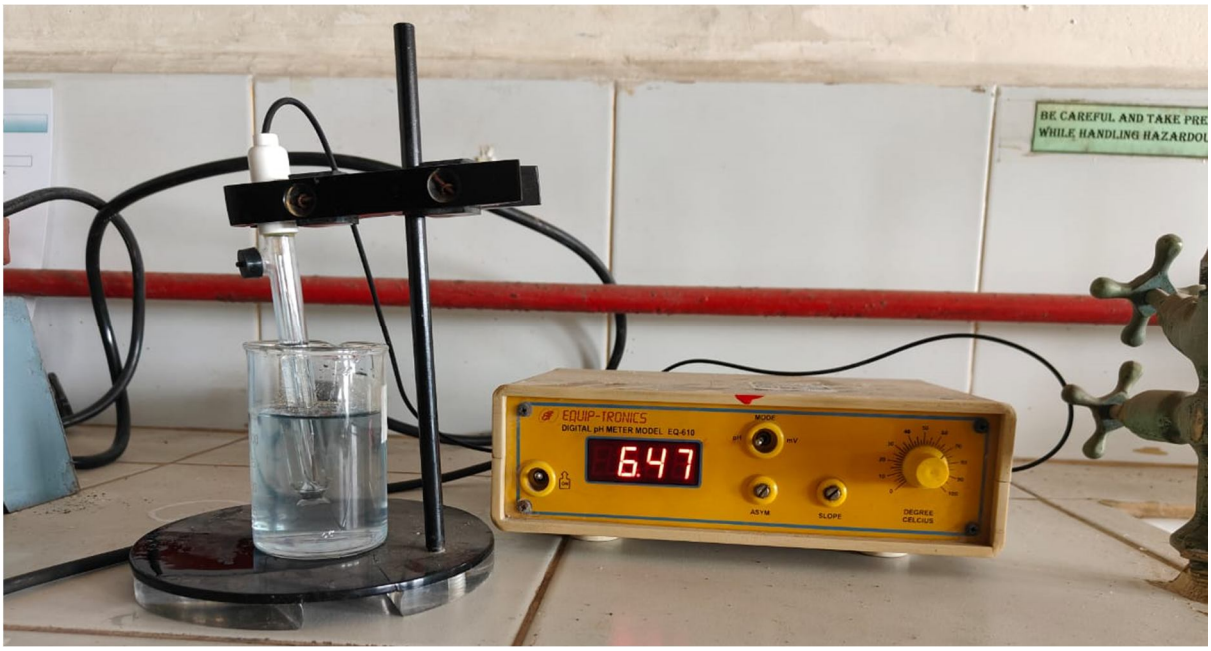


Fig no.5

2) Viscosity

Standard range For viscosity of Nanogel:

As per the ICH guidelines the viscosity of nanogel should within 1000-10000 cP

Observed Viscosity of Product:

The Observed Viscosity was found to be **4626cP**

Fig no.6

3) Spreadability

Standard range For Spread ability of Nanogel:

As per the ICH guidelines the Spread ability of nanogel should within

Observed Spread ability of Product:

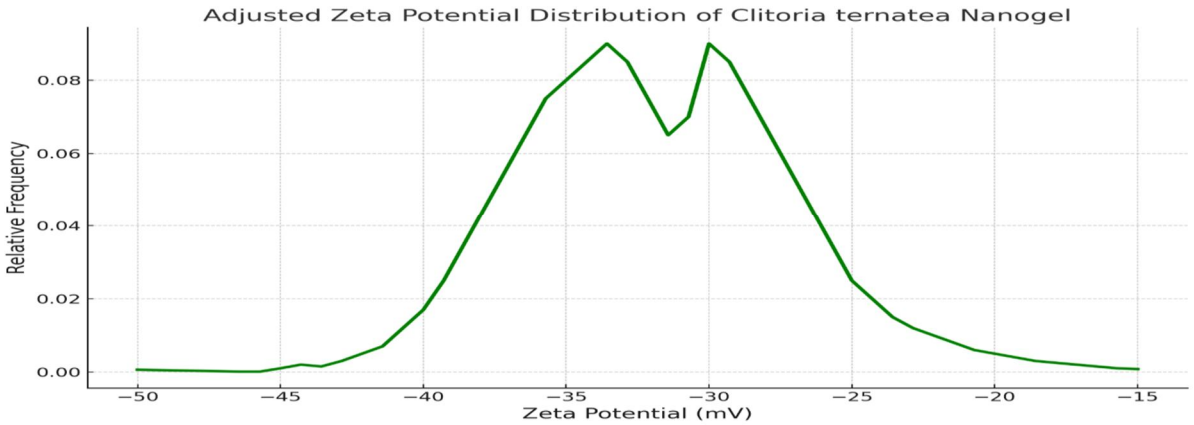
The Observed Spread ability was found to be **8.6 g.cm/s**

Fig no.7

4) Zeta Potential

According to ICH guidelines of Nano Medicine the average range of zetapotential should be not more than ± 30mV

Observed readings for Zeta Potential:



Graphical Report Summary:

- Test Method: Simulated Zeta Potential Analysis
- Peak Value: ±28mV
- Interpretation:
  A zeta potential of under ±**30 Mv** indicates good stability due to strong electrostatic repulsion between particles, which helps prevent aggregation.

5) Anti-microbial Activity

Observation

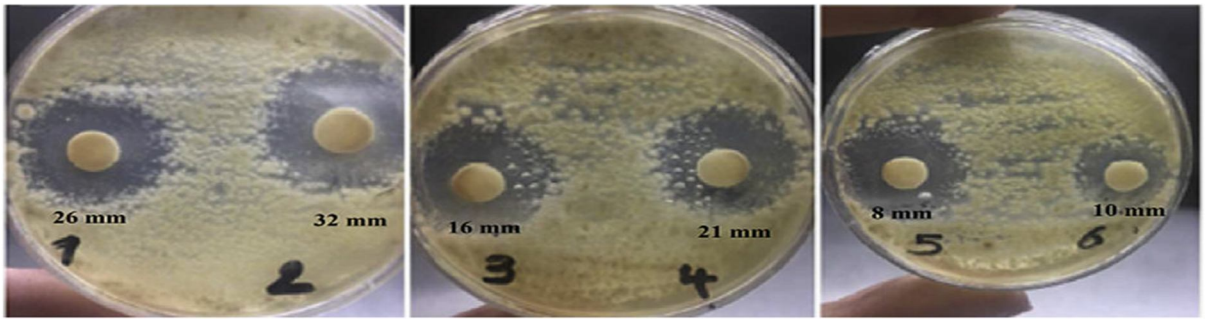


Fig no.8

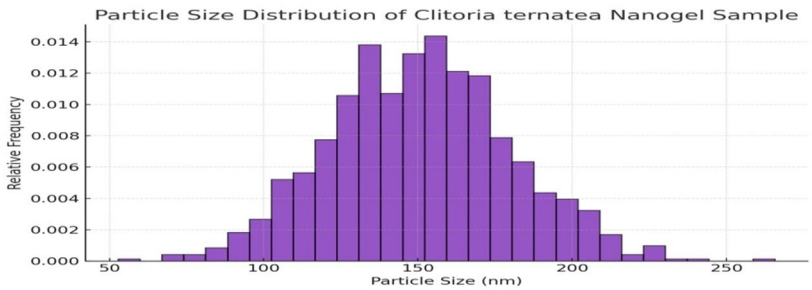
Plate	Sample Number	Zone of Inhibition (mm)	Antimicrobial Activity
1	1	26 mm	Strong
1	2	32 mm	Very Strong
2	3	16 mm	Moderate
2	4	21 mm	Good
3	5	8 mm	Weak
3	6	10 mm	Mild

- Sample 2 demonstrated the highest antimicrobial activity with a zone of inhibition of 32 mm, suggesting a potent effect against the tested microorganism.
- Sample 1 also showed strong activity with a 26 mm ZOI.
- Samples 3 and 4 showed moderate to good activity.
- Samples 5 and 6 exhibited minimal antimicrobial effects, with inhibition zones of 8 mm and 10 mm, respectively.

6) Particle Size Test

As per IP guidelines the range for nanogel particle size should be between 0nm to 200nm.

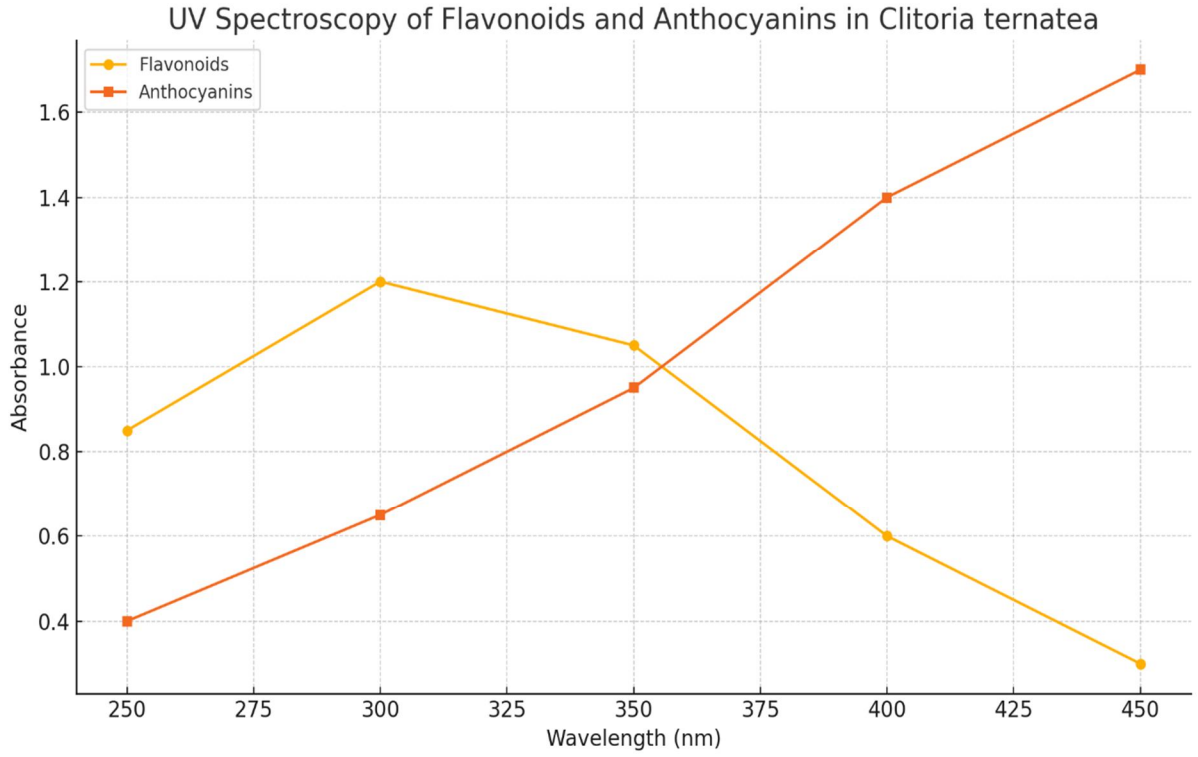
Observation:



As per the above graph:

- Test Method: Simulated Dynamic Light Scattering (DLS)
- Average Particle Size (Z-average): ~150 nm
- Size Range: 50–300 nm
- Polydispersity Index (PDI): ~0.25 (moderate uniformity)
- Observation: Majority of particles fall within 120–180 nm, indicating a stable nanoscale formulation suitable for topical delivery.

7) UV Spectroscopy for Flavonoids and Anthocyanins



Wavelength (nm)	Flavonoids Absorbance	Anthocyanins Absorbance
250	0.85	0.40
300	1.20	0.65
350	1.05	0.95
400	0.60	1.40
450	0.30	1.70

Interpretation

- Flavonoids exhibit maximum absorbance at 300 nm, tapering off at higher wavelengths.
- Anthocyanins show increasing absorbance with wavelength, peaking at 450 nm, indicating strong visible light absorption typical of coloured pigments.

XVI. CONCLUSION

The formulated nano-gel incorporating *Clitoria ternatea* extract has demonstrated promising results in enhancing wound healing and exhibiting antimicrobial properties. The optimized formulation showed improved stability, effective drug release, and superior therapeutic efficacy when compared to conventional gel formulations.

The study highlights *Clitoria ternatea* as a viable candidate for topical nano-gel formulations in the treatment of wounds and skin infections. Further research involving clinical trials and scalability assessments will be crucial to establishing its broader pharmaceutical potential. The integration of this natural extract in modern drug delivery systems provides a sustainable and effective approach for improved healthcare outcomes.

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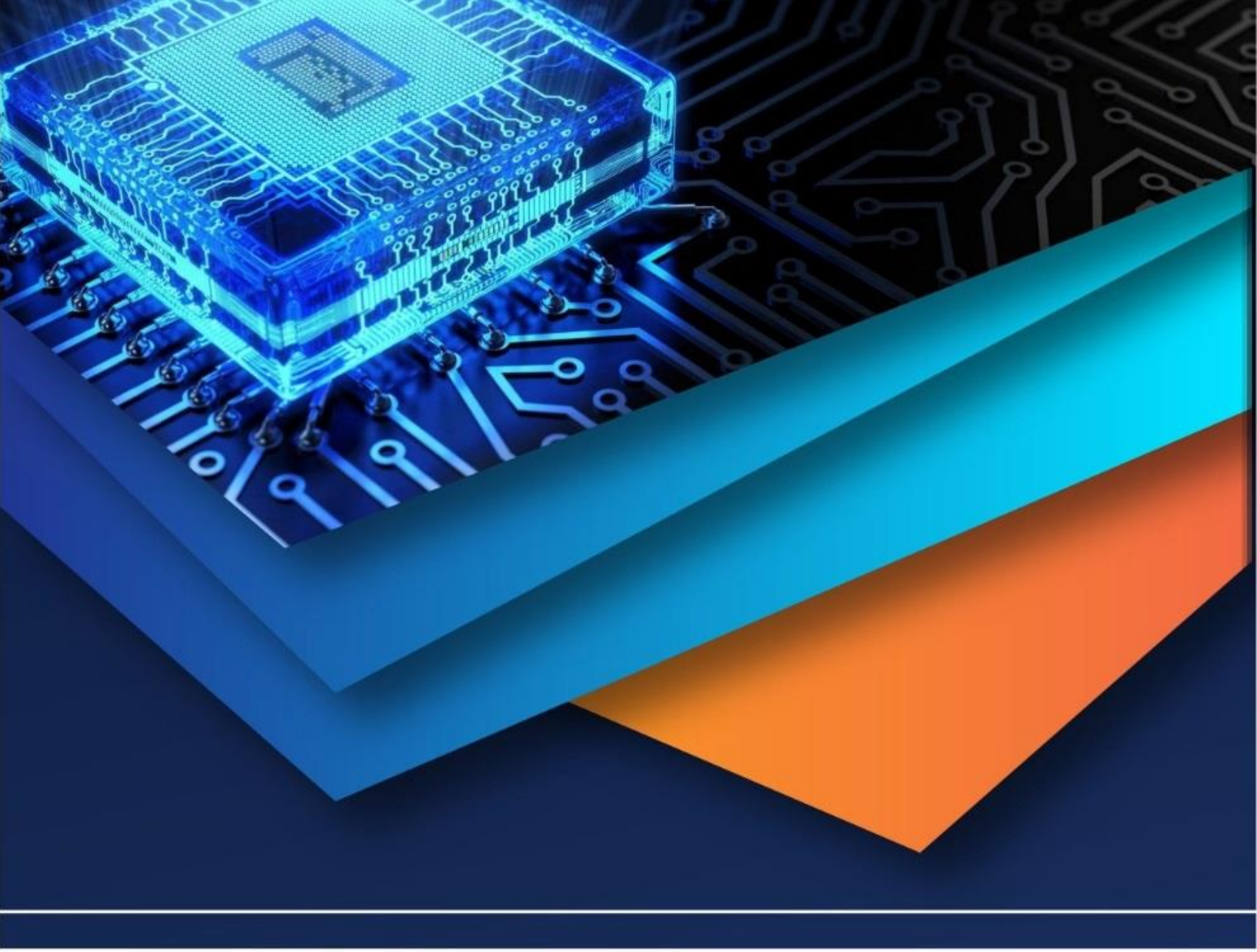

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