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# Design and Evaluation of Nanoparticle Loaded Topical Gel Containing Andrographis Echioides Linn Extract For Anti Inflammatory Activity

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**ABSTRACT**

The present study focuses on the design, formulation, and evaluation of a nanoparticle-loaded topical gel containing Andrographis echioides Linn extract for enhanced anti-inflammatory and wound-healing activity. Plant-mediated copper nanoparticles (CuNPs) were successfully synthesized using aqueous leaf extract of Andrographis echioides, which acted as a reducing and capping agent. The synthesized nanoparticles were characterized using UV–Visible spectroscopy, FTIR, SEM, and particle size analysis, confirming their formation, morphology, and nanoscale size. The CuNPs were incorporated into a topical gel formulation using suitable excipients, and the prepared nano-gel was evaluated for physicochemical properties such as appearance, pH, viscosity, spreadability, homogeneity, gel fraction, and stability.In vitro drug release studies demonstrated a sustained release pattern of the nanoparticle-loaded gel compared to the extract alone. Anti-inflammatory activity was assessed using the egg albumin denaturation assay, where the nanoparticle-loaded topical gel exhibited significantly higher percentage inhibition compared to the standard extract and nanoparticles alone. Pharmacological evaluation using an incision wound model in Swiss albino mice showed enhanced wound-healing activity in the nanoparticle-loaded topical gel. Stability studies conducted as per ICH guidelines indicated good formulation stability over the test period. Overall, the results suggest that the Andrographis echioides Linn copper nanoparticle-loaded topical gel is a promising and effective novel drug delivery system for topical anti-inflammatory and wound-healing applications.

**KEYWORDS:** Andrographis echioides Linn, Copper nanoparticles, Topical nano-gel,Anti-inflammatory activity, Wound healing, Plant-mediated nanoparticles.

## I. INTRODUCTION

A Novel Drug Delivery System (NDDS) refers to an advanced and innovative approach designed to deliver therapeutic agents to the body in a way that improves the drug's therapeutic effectiveness, reduces side effects, or enhances patient compliance. NDDS are distinct from traditional drug delivery systems by their ability to offer controlled, targeted, and sustained release of drugs, allowing for better treatment outcomes and more personalized therapy. These systems are often developed using new technologies and materials to overcome the limitations of conventional drug delivery, such as poor bioavailability, rapid metabolism, or inadequate targeting of the disease site.

### 1.1 Types of Novel Drug Delivery Systems:

1. **Liposomes**: Liposomes are spherical vesicles made of lipid bilayers that can encapsulate both hydrophilic and hydrophobic drugs. They are used to improve the solubility, stability, and controlled release of drugs, as well as to enable targeted drug delivery.
2. **Nanoparticles**: Nanotechnology is used to design nanoparticles that can carry drugs and target specific cells or tissues. These nanoparticles can be designed to release drugs in response to external stimuli like pH, temperature, or magnetic fields.
3. **Microspheres and Nano-sponges**: Microspheres (larger particles) and nano-sponges (smaller particles) are used to encapsulate drugs and provide controlled, sustained release, as well as to enhance solubility or target specific tissues.

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4. **Polymeric Drug Delivery Systems**: Polymers can be engineered to release drugs in a controlled manner over time. These systems include biodegradable polymeric nanoparticles, hydrogels, and microspheres, which are widely used for sustained-release formulations.
5. **Transdermal Drug Delivery Systems**: These systems, including patches, are designed to deliver drugs through the skin. They provide controlled and sustained release over extended periods, offering a non-invasive alternative to oral or injectable administration.
6. **Protein and Peptide Delivery Systems**: Delivery systems for biologics like proteins and peptides often include nanoparticles, liposomes, or other encapsulation technologies that protect the drug from degradation and enhance its delivery to specific tissues or cells.
7. **Implants and Depot Systems**: These systems are designed to release drugs over a prolonged period, sometimes for weeks or months, after being implanted into the body. They are used for chronic conditions or hormone delivery.
8. **Gene Delivery Systems**: Gene therapies often use novel delivery systems to transfer genetic material into cells for the treatment of genetic diseases. These systems may include viral vectors or non-viral nanoparticles.
9. **Targeted Drug Delivery Using Antibodies**: Monoclonal antibodies or antibody-drug conjugates (ADCs) are designed to selectively target specific cells (e.g., cancer cells) and deliver cytotoxic drugs directly to the site of disease, reducing systemic toxicity.
10. **Smart or Stimuli-Responsive Drug Delivery**: These systems respond to environmental stimuli such as pH, temperature, light, or magnetic fields. For example, a drug might be released only in the acidic environment of a tumor or after exposure to heat.

## II. PLANT PROFILE:ANDROGRAPHIS ECHIOIDES NEES

### 2.1 Scientific Classification:

Scientific classification of plant can be defined as "The arrangement of entities ofthat plant" in a hierarchical series of nested classes, in which similar or related classes at one hierarchical level are combined comprehensively into more inclusive classes at the next higher level. The scientific classification of Andrographis echioidesis as follows.

**KINGDOM : Plantae**
**DIVISION : Magnoliphyta**
**CLASS : Magnoliopesida-dicotyledons**

**ORDER : Scrophulariales**
**FAMILY : Acanthaceae - Acanthus Family**
**GENUS : Andrographis**

**SPIECES : Echioides**
**SUB FAMILY : Acanthoideae**
**SUB CLASS : Lamiidae**

**Fig: 1 Plant Profile Of Andrographis Echioides**

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**2.2 Vernacular Names:**

A vernacular name of a species can be defined as name that is used generally within a community. It is differentiated with the scientific name for the same species. Thesynonyms for vernacular names are common name, colloquial name, and popular name. The various vernacular names of the plant Andrographis echioidesare as follows.

- **English :** False Water willow
- **Tamil :** Gopuram tangi
- **Hindi :** Charayetah
- **Malayalam :** Pitumba, Mala kulukki
- **Telugu :** Chalavala puri kada
- **Marathi :** Ranchimani
- **Oriya :** Lavalata
- **Gujarati :** Kalukariyatun

**Fig: 2Andrographis Echioides**

**2.3 Chemical Constituents Of Andrographis Echioides:**

Phytochemical investigations have identified several bioactive compounds in Andrographis echioides, including:

1. **Flavonoids** – Known for their antioxidant and anti-inflammatory activities.
2. **Tannins** – Have astringent and antimicrobial properties.
3. **Saponins** – Support immune modulation and have antimicrobial effects.
4. **Alkaloids** – Often possess analgesic and anti-inflammatory actions.
5. **Terpenoids** – Contribute to anti-inflammatory and anticancer effects.
6. **Phenolic compounds** – Act as antioxidants and support metabolic health.

**2.4 Pharmacological Uses:**

| | |
|---|---|
| **Antidiabetic** | Helps reduce blood glucose levels; used in managing diabetes. |
| **Anti-inflammatory** | Used to treat swellings, joint pain, and related conditions. |
| **Hepatoprotective** | Believed to protect the liver from toxins and infections. |
| **Antimicrobial** | Effective against bacterial and fungal infections. |
| **Antioxidant** | Helps combat oxidative stress and supports cellular health. |
| **Antipyretic** | Traditionally used to reduce fever. |
| **Wound healing** | Crushed leaves or extracts are applied to wounds for faster healing. |
| **Digestive Aid** | Used in the treatment of stomach disorders and indigestion. |

**Table: 1 Pharmacological Uses**

## III. MATERIALS AND METHODS

### 3.1 Plant Collection:

Fresh leaf of andrographis echioides linn were collected from the local area of Thiruvannamalai district. It was washed gently with distilled water in order to remove the dust particles may present on the surface of the leaf.

### 3.2 Preparation Of Plant Extract:

Fresh andrographis echioides linnwere collected. It was washed gently with distilled water in order to remove the dust particles. The leaf are crushed into smaller pieces and stored in an air-tight container for further use. Set up the magnetic stirrer. Take 25g of leaf in 100ml De ionized water & set temperature to 80˚-90˚C. Filter the leaf extract using filter paper to use as a reducing agent and capping agent.

### 3.3 Synthesis Of Copper Nanoparticles With Extract:

Take 10gm of Copper sulphate and 3gm of Ascorbic acid in 100ml DI-water & set temperature to 80˚-90˚. The andrographis echioides linnextract has been mixed dropwise until the color develops to light brownish orange. The plant andrographis echioides linnextract has been used as a reducing agent and capping agent. The mechanism involved in the synthesis of CuNPs is reduction. The product has been centrifuged using centrifugal apparatus. CuNPs are stored in an air tight container.

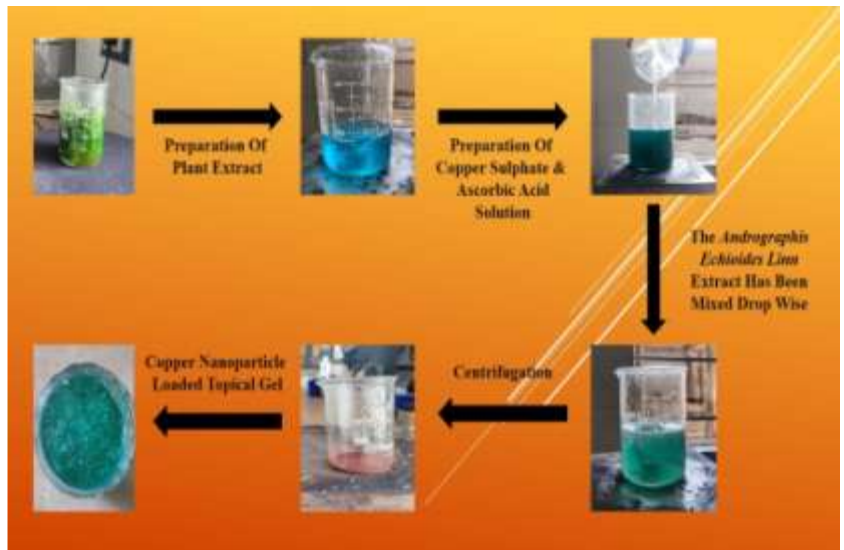


**Fig: 3 Preparation Of Copper Nanoparticle**

### 3.4 Preparation Of Nanoparticle Loaded Topical Gel:

| S.NO | INGREDIENTS | AETG |
|---|---|---|
| 1 | Andrographis Echioides Linn Copper Nanoparticle | 1 gm |
| 2 | Xanthan Gum | 0.50 gm |
| 3 | Tea Tree Oil | 1 ml |
| 4 | Formaldehyde | 1 ml |
| 5 | Vitamin E | 0.5 ml |
| 6 | Polyethylene Glycol | 0.85ml |
| 7 | Polysorbate 80 | 2 ml |
| 8 | Purified water | 37 ml |

**Table: 2 Composition Of Andrographis Echioides Linn Extract Containing Nano-Gel**

**Fig: 4 Copper Nanoparticle Loaded Topical gel**

**Procedure:**
**Preparing the Aqueous Phase:**Start with the PEG (Polyethylene Glycol) and polysorbate (Polysorbate 80) in a beaker. These act as emulsifiers and stabilizers for the formulation. Add 5 mL of water (distilled or deionized) to the beaker if required, as a solvent for dispersing the components. Heat the mixture to around 40°C–50°C (use a water bath) while stirring. This will help dissolve the PEG and polysorbate and improve their emulsification properties.
**Mixing Oil Phase:**In a separate beaker, combine the tea tree oil (1 mL) and Andrographis Echioides Linnnanoparticle(1 gm). The tea tree oil is lipophilic (oil-based), while the Andrographis Echioides Linnnanoparticle, but it needs to be incorporated efficiently into the gel matrix. Add vitamin E(0.5 mL) to this oil phase. Vitamin E will help stabilize the formulation and provide antioxidant properties. Heat this oil phase to around 40°C (if necessary) while stirring to ensure all oil-based components mix together properly.

**Emulsification Step:**Slowly add the oil phase (tea tree oil + Andrographis Echioides Linnnanoparticle + vitamin E) to the aqueous phase (PEG + polysorbate + water). Use a magnetic stirrer or a high-speed mixer to emulsify the oil and water phases, forming a stable oil-in-water emulsion**.** Polysorbate will help emulsify the oil (tea tree oil) in the aqueous phase.
**Incorporating Formaldehyde (1 mL)**:Formaldehyde is usually used as a preservative in some formulations. It is important to add it after the emulsion has formed. Add 1 mL of formaldehyde slowly into the emulsion while stirring, making sure it's fully integrated into the formulation. It will help preserve the topical gel for extended shelf life.
**Size Reduction (Topical-gel Formation)**:To achieve the topical gel consistency, you can use an ultrasonicator or high-speed homogenizer for size reduction. This step is optional but recommended if you want to achieve the nano-scale particle size (typically 50-200 nm). Sonicate the mixture for around 10–15 minutes to break down the particle size and ensure uniform distribution of the components. This will ensure the consistency is uniform and the active ingredients are evenly distributed.
**Adjusting the pH and Viscosity**:Check the pH of the formulation using a pH meter. The pH should

ideally be between 5 and 7 to ensure stability and compatibility with the skin or other intended uses. If the pH is too high or low, adjust it using citric acid. If necessary, adjust the **viscosity** of the nano-gel by adding xanthan gum (a thickening agent), (if more viscosity is needed for gel-like consistency).

**Final Mixing**:Once the emulsion has been created and particle sizes reduced, you can allow the mixture to cool down to room temperature while stirring gently to avoid air bubbles. Check the viscosity and consistency again after cooling. If it's too thin, you can add a small amount of xanthan gum to thicken it. If it's too thick, you can add a small amount of water to achieve the desired consistency.

**Purification (Optional)**:If needed, purify the formulation by dialysis or centrifugation to remove excess solvents, unreacted particles, or larger aggregates.

**Packaging**:Once the nano-gel is prepared**,** store it in an airtight container to preserve its stability. Ensure it is kept in a cool, dry place, away from direct sunlight to prevent degradation.

### 3.5 Evaluation Of Nanoparticle:

Specific methods must first confirm the formation of CuNPs before they can be used for their intended application. The most basic method to monitor CuNPs production by visually observing the change in the color of the solution from yellow to brown. A spectrophotometer can further confirm the tracking process and detect nanoparticle peaks in the visible area of the UV–vis spectrum at a wavelength between 400 and 450 nm. Other techniques, including SEM can be used to investigate the size, morphology, dispersion, and composition of nanoparticles. Moreover, FTIR spectroscopy can help track biomolecules that influence nanoparticle formation and stability.

### 3.5.1 UV–Vis Spectrophotometry Of CuNPs:

UV–Visible spectroscopy is an effective technique that can help characterize synthesized CuNPs. The absorbance spectra can confirm the formation of synthesized CuNPs in a solution, The analysis measures the intensity of light transmitted through the sample and compares it with a reference measurement of the incident light source**.** Wavelengths ranging from 400 to 800 nm are commonly used to indicate the presence of nanoparticles.

CuNPs are established to induce surface plasmon resonance (SPR) at a certain range of wavelengths. Lower and higher maximum wavelength (λmax) values are associated with a smaller average size and higher concentration of CuNPs, respectively. Moreover, broad and narrow peaks at higher and shorter wavelengths, respectively, indicate an increase and decrease in CuNPs size, respectively. The quality of the synthesized nanoparticles can be illustrated by the intensity and position of the SPR peak, which occurs at wavelengths between 380 and 450 nm. A narrow and low wavelength absorption peak implies a small size of the nanoparticles, while a broad peak at a high wavelength implies a large size or aggregated CuNPs, as, Additionally, SPR peaks of the same wavelength, obtained using UV–Visible spectroscopy, indicate that the stability of green synthesized CuNPs can be maintained for several months. Thus, UV–vis spectroscopy is a valuable technique for characterizing synthesized CuNPs. Sharma et al. (2020) presented spectroscopic results of CuNPs synthesized using Andrographis Echioides Linnnanoparticle.

### 3.5.2 SEM Imaging Of Nanoparticles:

Among other electron microscopy techniques, SEM can determine the surface morphology of nanoparticles, such as their shape, size, and size distribution. Field emission SEM (FESEM) involves the emission of electrons that are accelerated using a powerful electric field. In SEM imaging, an electric current passed through electromagnetic coils and lenses to generate a focused beam of electrons that collide with a sample surface to create secondary electrons. The information on the resulting electrons is utilized to reconstruct a very detailed representation of the sample surface morphology. SEM scans the surface of a test sample and records the backscattered rays.

Metal nanoparticles, such as silver and gold nanoparticles, are very electrically conductive, making them easy to scan employing SEM. This microscopy tool offers a significant advantage because it can be performed by placing the samples directly on a black surface to avoid undesirable incident beam scattering. Although SEM cannot observe the internal structure of samples, it can provide helpful information regarding the purity and aggregation of particles. CuNPs are typically spherical, cubical, triangular, oval, pebble-like, and circular in shape, and appear as single or aggregated particles. The variations in shape may be caused by changes in the synthesis parameters such as pH, temperature, and plant concentrations.

Most particles have sizes ranging from 20 to 30 nm.

**3.5.3 Drug Excipients Compatibility:**

The IR spectrum of substance was compared with that obtained concurrentlyfor the corresponding USP reference standard provides perhaps the most conclusive evidence of the identity of the substance. Compatibility studies were carried out to study the possible interactions between drug and other inactive ingredients. After the storage of drug-excipients for a period of 30days at 50°C, the compatibility studies should be performed using FTIR. Potassium bromide (KBr) pellet method was carried out. All the ingredients were individually mixed with KBr(1:10) and compressed under 10 tones pressure in a hydraulic pressure to form a transparent KBr pellets. The pellets was scanned from 4000 to 400cm$^{-1}$ in FTIR spectrometer. From the spectrum, the spectral studies between active and other inactive ingredients were analyzed.

**3.5.4In- Vitro Drug Release:**

Dialysis bags (cut off 12-14kDa) were filled with a fixed amount of formulation put into 100ml of buffer solution (pH 7.4) and stirred at 50 rpm was used as receptor phase. The dissolution medium was phosphate buffer having pH 7.4. The dialysis sacs were equilibrated with dissolution medium for few hours prior to experiment. The experiment was conducted at 37°C±0.5°C. sampling was performed with interval of every half an hour once for upto 24 hours, 1ml of sample was withdrawn and 1ml of phosphate buffer pH 7.4 was replaced into the same to maintain sink condition. After appropriate dilutions the samples were analyzed by UV spectrophotometer at 250 nm. The sample procedure was repeated for studying the in-vitro drug release.

**3.6 Evaluation Of Topical Gel:**

Evaluating Topical gel involves assessing their properties, performance, and suitability for specific applications. This evaluation typically includes various physical, chemical, and mechanical tests. Below is an overview of the key aspects considered when evaluating Topical gel.

**3.6.1 Appearance:** The formulations physical appearance was analyzed visually.The Topical gel was Soft,flexible with a smooth surface. Moderately firm, as the borax concentration provides some cross-linking but not excessive stiffness. Elasticand slightlysticky, due to unreacted starch molecules retaining water.

**3.6.2 Color:** The color of the formulation was checked out against white & black backgrounds. The Topical gel was white or translucent in color.

**3.6.3 Odor:** The odor of the Topical gel was checked by taking smell of little amount of Topical gel. The Topical gel was mild or odorless.

**3.6.4Determination of PH:**To determine the PH of the product, dilute the product in distilled water (1:10) then measure the PH of the dilution. The PH of the Hydro gel was 7.0-7.4. Citric acid is used to adjust the PH of the product.

**3.6.5 Rheology**: Viscosity of Topical gel is evaluated by using Cone plate type viscometer under constant temperature at 4ºC. This viscometer is highly specific for the evaluation of viscosity.

**3.6.6 Spreadibility study:** The apparatus was made of wooden block with scale and two glass slides having a pan mounted on a pulley. Excess formulation was placed between two glass slides and 100 gm weight was placed on upper glass slide for 5 minutes to compare the formulation to achieve uniform thickness. Weight can be added and the time to separate the two slides was taken as spreadibilty time.

$$S = (m \times l) / t$$

Where,

- S is spreadibilty,
- m is weight tied on upper slide,
- l is length of glass slide and
- t is time taken in seconds

**3.6.7 Viscoelastic Properties:** Rheological tests are conducted to measure the Topical gel viscoelastic behavior, which includes both its elastic (solid-like) and viscous (liquid-like) responses.

**3.6.8 Gel Fraction:** The gel fraction represents the proportion of the Topical gel that is cross-linked and does not dissolve in water. It is measured by extracting the soluble components from the Topical gel and weighing the remaining material.

$$GF (\%) = Wg / Wo \times 100$$

- Wg = weight of the dried gel after extraction (i.e., after removing soluble fractions)
- W0 = initial dry weight of the polymer before extraction.

**3.6.9 Scanning Electron Microscopy (SEM)**: SEM can be used to provide information about the sample's composition, surface topography, and other properties such as electrical conductivity.

**3.6.10 Homogeneity:**All developed Topical gelwere tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates.

**3.6.11 Irritancy Test:** Mark an area (1sq.cm) on the left hand dorsal surface. The gel was applied to the specified area and time was noted. Irritancy, erythema, edema, was checked if any for regular intervals up to 24 hrs and reported.

**3.6.12In-VitroDiffusionStudy:**

**Cellophane Membrane Treatment for Permeation study:** Cellophane membrane was boiled in the distilled water for 1 hour and washed with fresh distilled water for three times and kept in ethanol for 24 hours. It was treated with 0.3% sodium sulphite and soaked in distilled water for 2 min at $60^0$C followed byacidifiedwith0.2%sulphuric.Finallythemembrane wasdippedinboricacidbufferpH (9) till it is used for permeation study.

**In-vitro diffusion study:** The in- vitro permeation rate of selected formulations of gel were evaluated by open ended tube through using pH 7.4 as diffusion medium upto 10 hours studies. The cellophane membrane was tied in one end of the tube and then immersed in the receptor compartment containing 200ml of 7.4 buffer solution which was stirred at 100±10rpm and maintained at $37^0$C ±$2^0$C. A quantity of 5ml samples were withdrawn from the receptor fluid at the time intervals of 0, 1, 2, 4,6, 7, 8, 10 hr. and 5ml of phosphate buffer of pH 7.4 was replaced immediately each time.

**3.6.13 Stability Studies:**

Stability testing of drug products begins as a part of drug discovery and ends with the demise of the compound or commercial product. To assess the drug and formulation stability, stability studies were done according to ICH guidelines. The stability studies were carried out as per ICH guidelines. The gel filled in bottle and kept in humidity chamber maintained at 40 ± 2°C / 75 ± 5 % RH for TWO months. At the end of studies, samples were analyzed for the physical properties, pH and viscosity.

**3.7Anti Inflammatory Study:**

**Egg Albumin Assay**: The anti-inflammatory activity of unknown crude extracts can be determined in vitro for inhibition of the denaturation of egg albumin (protein).

- 0.2 mL of 1-2% egg albumin solution (from fresh hen's egg/ or commercially available egg albumin powder), 2 mL of sample extract or standard (Diclofenac sodium) at varying concentrations, and 2.8 mL of phosphate buffered saline (pH 7.4) were mixed to form a reaction mixture of a total volume of 5 mL.
- A total volume of 5 mL of the control was created by combining 2 mL of triple-distilled water, 0.2 mL of 1-2% egg albumin solution, and 2.8 mL of phosphate-buffered saline.
- The reaction mixtures were then incubated at 37±2°C for 30 min and will be heated in a water bath at 70±2°C for 15 min.
- After cooling, the absorbance was measured at 280 nm by a suitable UV/Vis spectrophotometer using triple distilled water as the blank [1].
- The following equation was used to determine the % inhibition of protein denaturation.

**Percentage Of Inhibition:**

**Absorbance of control -Absorbance of test sample /Absorbance of control ×100**

**3.8 Pharmacological Activity:**

**Animals:** Male Swiss albino mice weighing between 20 – 25 gm was used for the study. The animals were obtained from animal house, Aadhibhgawan College of Pharmacy, rantham. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals werehouse data temperature of 24±2°C and relative humidity of 30– 70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethics Committee of Aadhibhagawan College of Pharmacy.

**Preparing test animals and making incisions:** The test animals used were 15 male mice. Before treatment, mice were acclimatized to the research environment for 7 days. The day before the incision was made, the fur on the back of the test animals were shaved. Then anesthetized with lidocaine.

Next, an incision was made with a length of 2 cm and a wound depth of 1.5 mm with a scalpel. The incision will be treated twice a day.

**Testing the effectiveness of ethanol extract gel on incision wounds:** Observation was carried out for 14 days to see the wound healing process by measuring the diameter of the wound

# IV. RESULTS AND DISCUSSION

## 4.1 Preliminary Phytochemical Screening:

Result of the preliminary phytochemical constituents present in Extract:

| S.NO | CONSTITUENTS | EXTRACT |
|---|---|---|
| 1. | Phenolic Compounds | Present |
| 2. | Essential Oils | Present |
| 3. | Flavonoids | Present |
| 4. | Polysaccharides | Present |
| 5. | Saponins | Present |

**Table: 3 Preliminary Phytochemical Constituents**

## 4.2 Evaluation Of Nanoparticle:

### 4.2.1 UV Analysis:

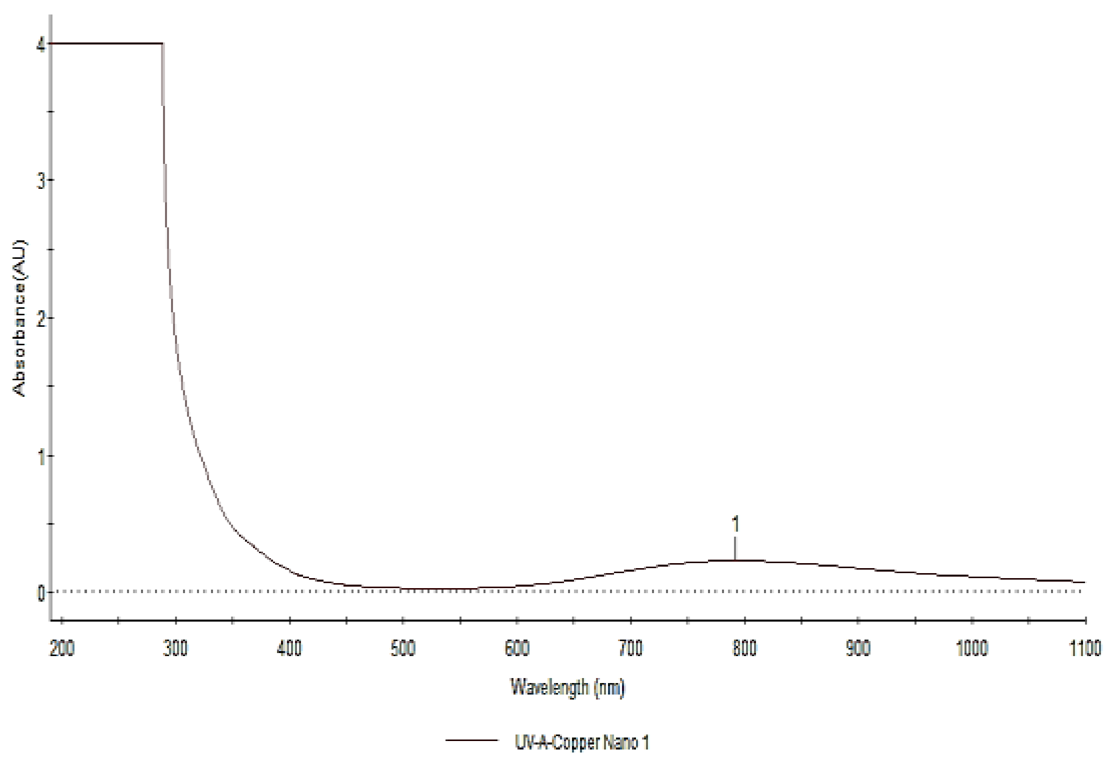


**Fig: 5 UV Analysis Of Copper Nanoparticle**

Name No. Peak(nm) Peak(AU) No. Valley(nm) Valley(AU)
UV-A-Copper Nano 1 791.5 0.23

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**4.2.2 IR Analysis:**

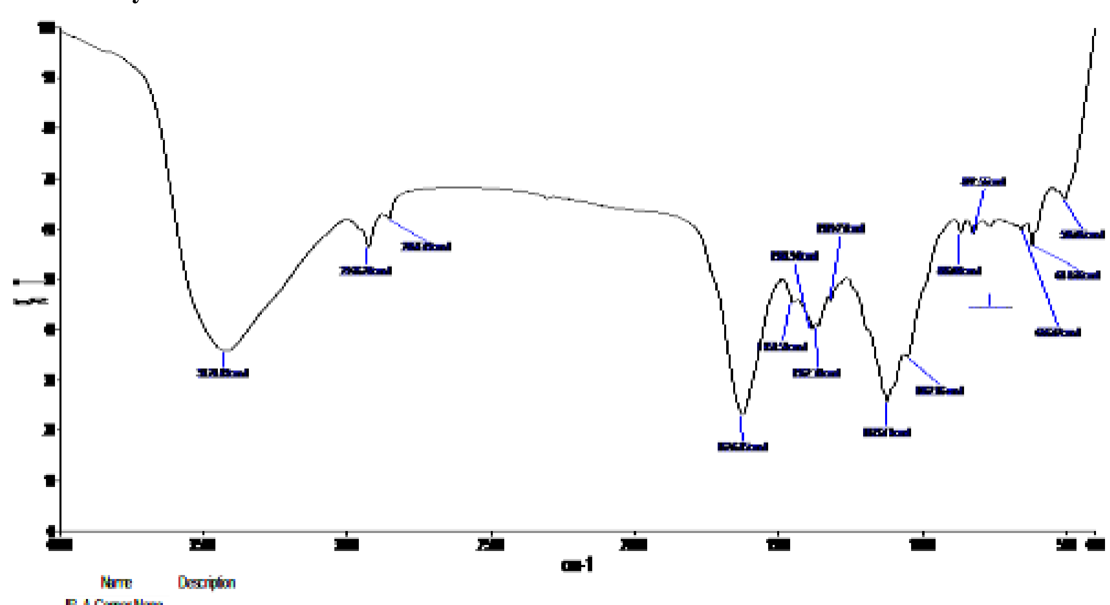


**Fig: 6 IR Analysis Of Copper Nanoparticle**

**4.2.3 SEM Analysis:**

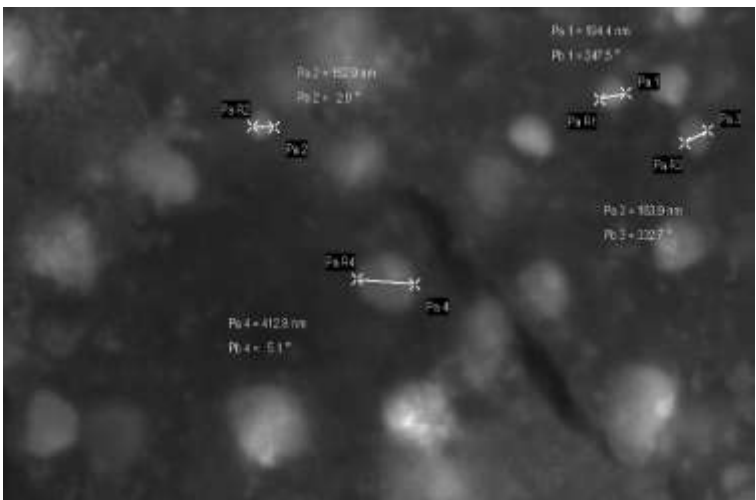

**Fig: 7 SEM Analysis Of Copper Nanoparticle**

| S.NO | PARTICLE | SIZE |
|---|---|---|
| 1 | Particle 1 | 194.4nm |
| 2 | Particle 2 | 183.9nm |
| 3 | Particle 3 | 152.9nm |
| 4 | Particle 4 | 412.8nm |

**Table: 4 SEM Analysis Of Copper Nanoparticle**

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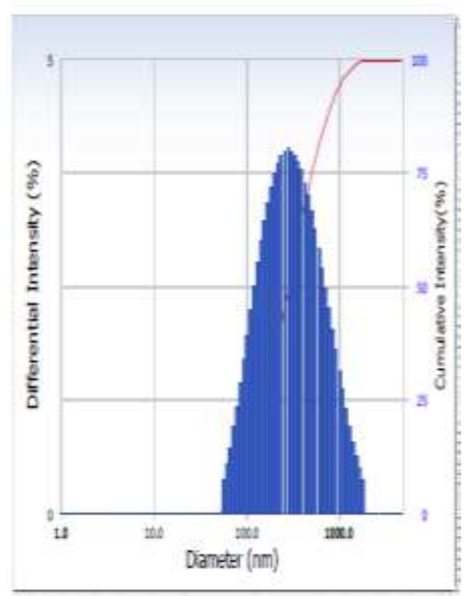
#### 4.2.4 Particle Size Analysis:



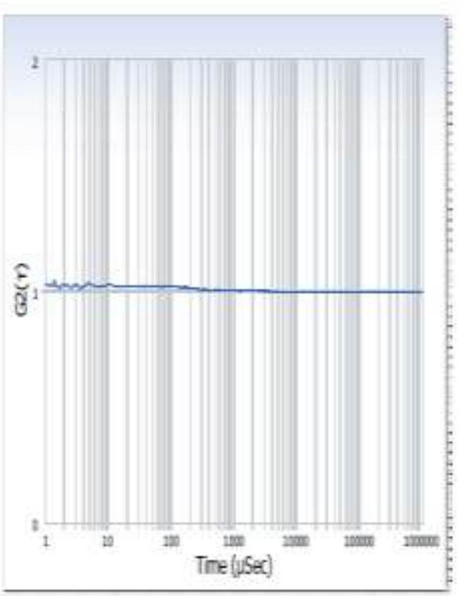




**Fig: 8 Particle size Analysis Of Copper Nanoparticle**

Distribution Results (Contin)

| Peak | Diameter (nm) | Std. Dev. |
|---|---|---|
| 1 | 410.5 | 334.0 |
| 2 | 0.0 | 0.0 |
| 3 | 0.0 | 0.0 |
| 4 | 0.0 | 0.0 |
| 5 | 0.0 | 0.0 |
| Average | 410.5 | 334.0 |
| Residual : | 2.816e-003 | (O.K) |

Cumulants Results

| | | | |
|---|---|---|---|
| Diameter | (d) | : 227.4 | (nm) |
| Polydispersity Index | (P.I.) | : 0.339 | |
| Diffusion Const. | (D) | : 2.163e-008 | (cm²/sec) |

Measurement Condition

| | | |
|---|---|---|
| Temperature | : 25.0 | (°C) |
| Diluent Name | : WATER | |
| Refractive Index | : 1.3328 | |
| Viscosity | : 0.8878 | (cP) |
| Scattering Intensity | : 30927 | (cps) |
| Attenuator 1 | : 7.28 | (%) |

Intensity Distribution Table

| d (nm) | f(%) | f(cum.%) | d (nm) | f(%) | f(cum.%) | d (nm) | f(%) | f(cum.%) | d (nm) | f(%) | f(cum.%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 7.8 | 0.0 | 0.0 | 66.4 | 0.7 | 1.6 | 564.5 | 3.1 | 78.4 | 4800.0 | 0.0 | 100.0 |
| D (10%) : | 106.30 | (nm) | D (50%) : | 289.40 | (nm) | D (90%) : | 835.20 | (nm) | | | |

Distribution Results: 410.5
Standard Deviation: 334.0
Cumulant Result: 227.4

**4.2.5 Dissolution Study:**

| S.NO. | TIME (IN HOURS) | % OFRELEASEOF AECUNP FORMULATION |
|---|---|---|
| 1 | 0 | 0.000 |
| 2 | 1 | 6.62 ± 0.133 |
| 3 | 2 | 16.14 ± 0.253 |
| 4 | 4 | 36.09 ± 0.343 |
| 5 | 8 | 61.35 ± 0.39 |
| 6 | 10 | 92.33 ± 1.35 |

**Table: 5InVitroReleaseProfileOf AECUNP Formulation**

| Time (Hr) | Cumulative % Drug Released | % Drug Remaining | Square Root Time | Log Cumu % Drug Remaining | Log Time | Log Cumu % Drug Released | % Drug Released | Cube Root Of % Drug Remaining (Wt) | Wo-Wt |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 100 | 0.000 | 2.000 | 0.000 | 0.000 | 100 | 4.642 | 0.000 |
| 2 | 6.62 | 93.38 | 1.414 | 1.970 | 0.301 | 0.821 | 6.62 | 4.537 | 0.105 |
| 4 | 16.14 | 83.86 | 2.000 | 1.924 | 0.602 | 1.208 | 9.52 | 4.377 | 0.265 |
| 6 | 36.09 | 63.91 | 2.449 | 1.806 | 0.778 | 1.557 | 19.95 | 3.998 | 0.644 |
| 8 | 61.35 | 38.65 | 2.828 | 1.587 | 0.903 | 1.788 | 25.26 | 3.381 | 1.261 |
| 10 | 92.33 | 7.67 | 3.162 | 0.885 | 1.000 | 1.965 | 30.98 | 1.972 | 2.670 |

**Table: 6 Cumulative % Drug Release Of AECUNP Formulation**

**4.3 Evaluation Of Nanoparticle Loaded Topical Gel:**
**4.3.1 Evaluation Of Gel:**

| S.NO | PARAMETERS | AECUTG |
|---|---|---|
| 1. | State | Semi Solid |
| 2. | Color | Greenish |
| 3. | Odor | Herbaceous Aroma |
| 4. | pH | 8.31 |
| 5. | Grittiness | Smooth |
| 6. | Viscosity | 8719 |
| 7. | Sensitivity Test | No Irritation |
| 8. | Irritation Test | No Irritation |
| 9. | Spreadability | 7.5 cm |
| 10. | Homogeneity | Homogenous |
| 11. | Gel Fraction | 66% |
| 12. | Viscoelastic | Higher Elasticity Gel-Like Behavior |

**Table: 7Evaluation Of Nanoparticle Loaded Topical Gel**

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### 4.3.2 UV Analysis:

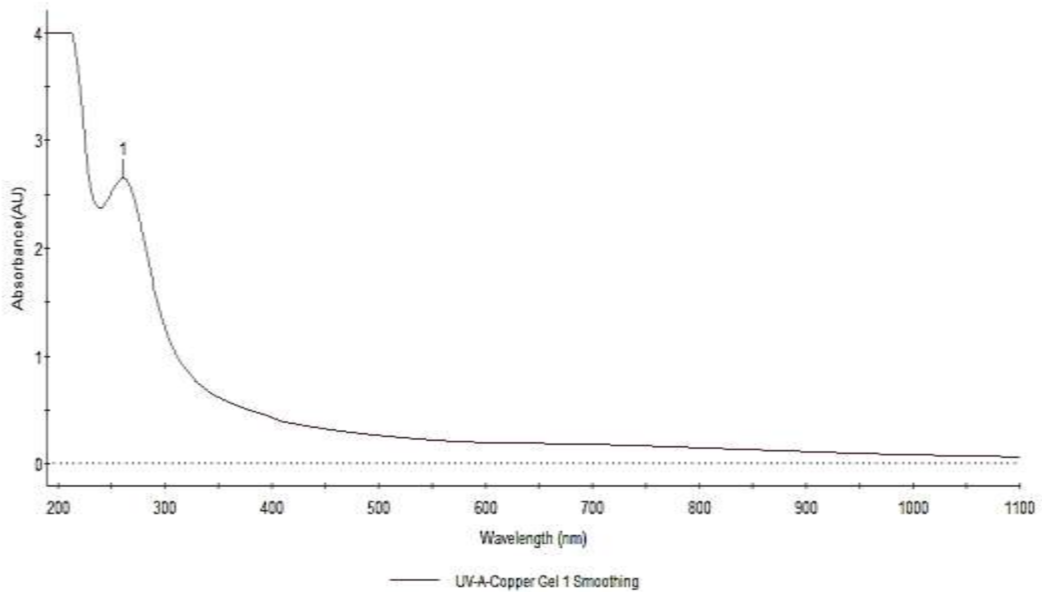



**Fig: 9 UV Analysis Of Copper Nanoparticle Loaded Topical Gel**

### 4.3.3 IR Spectrum:

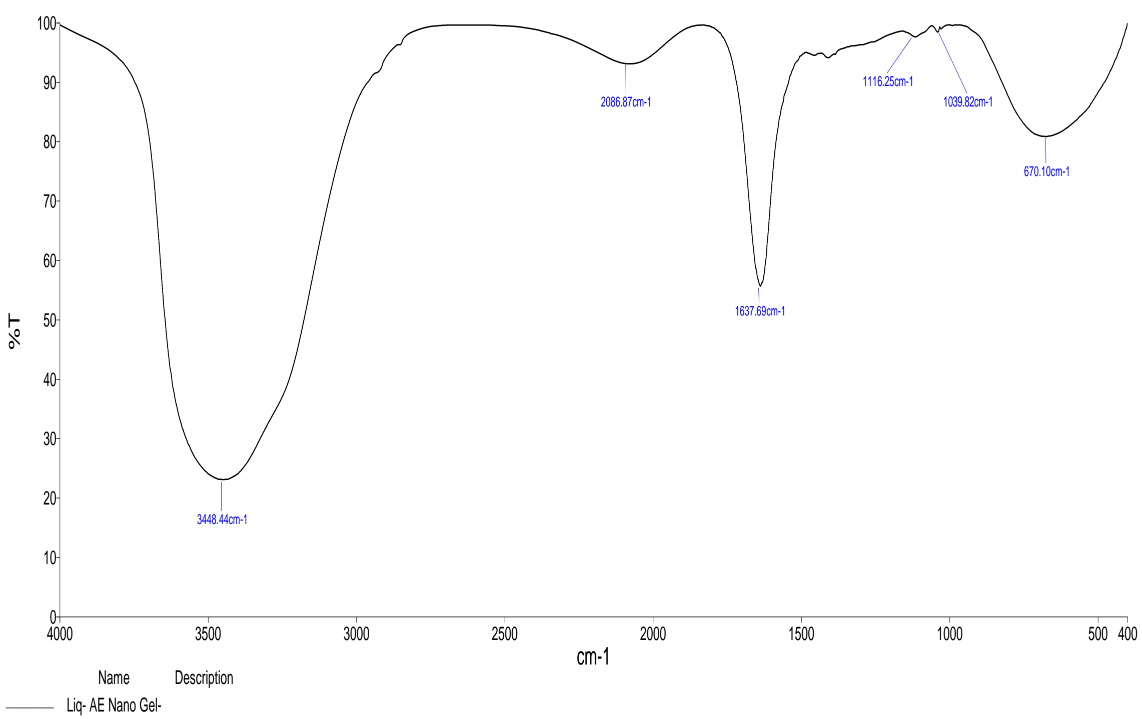



**Fig: 10 IR Spectrum Of Copper Nanoparticle Loaded Topical Gel**

**4.3.5 SEM Analysis:**

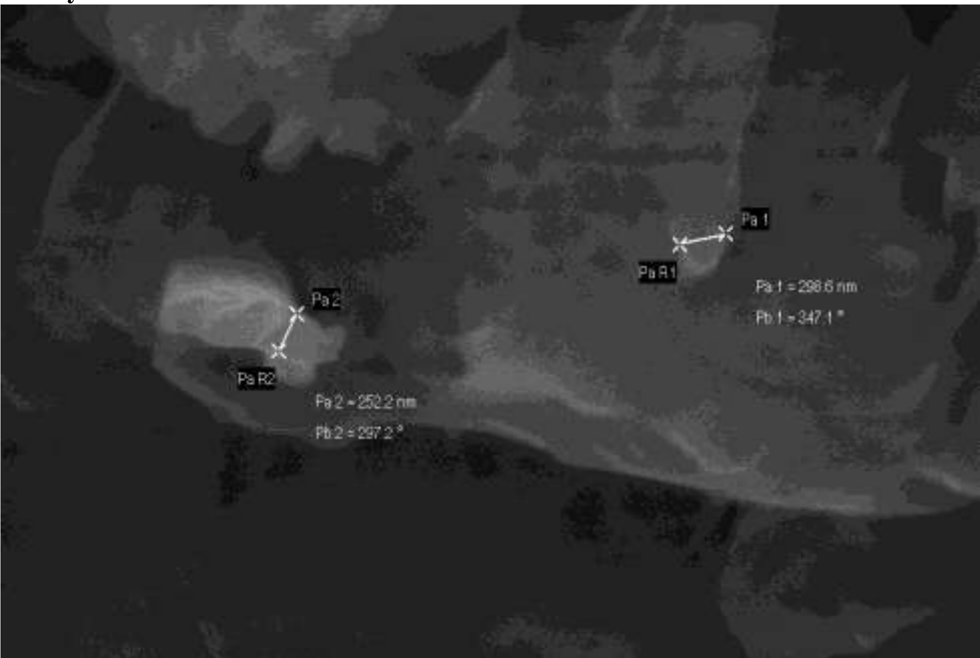
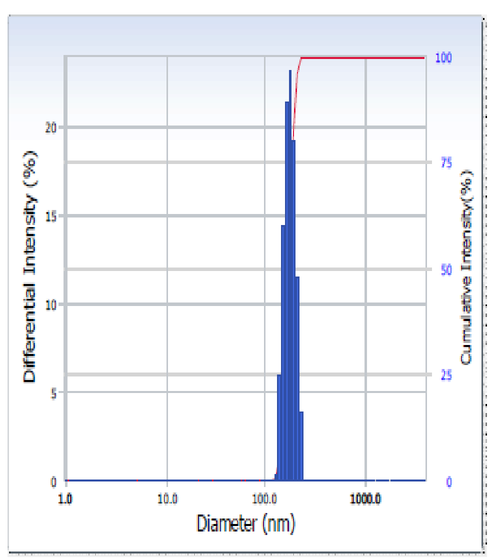



**Fig: 11 SEM Analysis Copper Nanoparticle Loaded Topical Gel**

| S.NO | PARTICLES | SIZE |
|---|---|---|
| 1 | Particle 1 | 296.6nm |
| 2 | Particle 2 | 252.2nm |

**Table: 8SEM Analysis Copper Nanoparticle Loaded Topical Gel**

**4.3.6 Particle Size Analysis:**



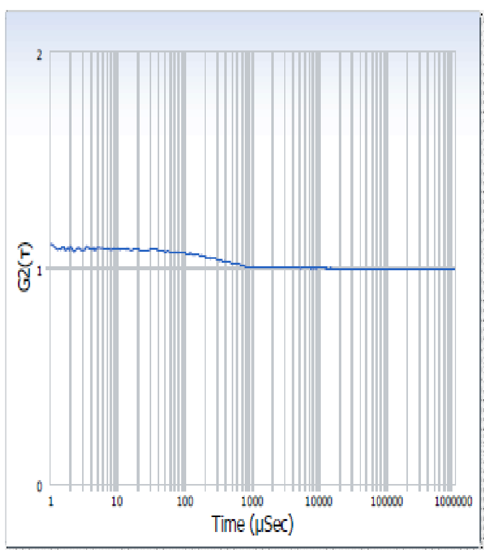




**Fig: 12 Particle Analysis Copper Nanoparticle Loaded Topical Gel**

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Distribution Results (Contin)

| Peak | Diameter (nm) | Std. Dev. |
|---|---|---|
| 1 | 182.2 | 23.5 |
| 2 | 0.0 | 0.0 |
| 3 | 0.0 | 0.0 |
| 4 | 0.0 | 0.0 |
| 5 | 0.0 | 0.0 |
| Average | 182.2 | 23.5 |

Residual : 5.955e-003 (O.K)

Cumulants Results

| | | | |
|---|---|---|---|
| Diameter | (d) | : 219.3 | (nm) |
| Polydispersity Index | (P.I.) | : 0.269 | |
| Diffusion Const. | (D) | : 2.243e-008 | (cm²/sec) |

Measurement Condition

| | | |
|---|---|---|
| Temperature | : 25.0 | (°C) |
| Diluent Name | : WATER | |
| Refractive Index | : 1.3328 | |
| Viscosity | : 0.8878 | (cP) |
| Scattering Intensity | : 29628 | (cps) |
| Attenuator 1 | : 15.71 | (%) |

Intensity Distribution Table

| d (nm) | f(%) | f(cum.%) | d (nm) | f(%) | f(cum.%) | d (nm) | f(%) | f(cum.%) | d (nm) | f(%) | f(cum.%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 7.5 | 0.0 | 0.0 | 61.4 | 0.0 | 0.0 | 501.7 | 0.0 | 100.0 | 4100.0 | 0.0 | 100.0 |
| D (10%) : | 145.40 | (nm) | D (50%) : | 173.20 | (nm) | D (90%) : | 207.10 | (nm) | | | |

Distribution Results: 182.2
Standard Deviation: 23.5
Cumulant Result: 219.3

**4.3.7InVitroReleaseStudies:**

| S.NO. | TIME (IN HOURS) | % OFRELEASEOF AECUTG FORMULATION |
|---|---|---|
| 1 | 0 | 0.000 |
| 2 | 1 | 5.531 ± 0.135 |
| 3 | 2 | 18.143 ± 0.255 |
| 4 | 4 | 35.083 ± 0.353 |
| 5 | 8 | 60.035 ± 0.311 |
| 6 | 10 | 96.335 ± 1.331 |

**Table: 9 In Vitro Release Profile Of AECUTG Formulation**

| Time (Hr) | Cumulative % Drug Released | % Drug Remaining | Square Root Time | Log Cumu % Drug Remaining | Log Time | Log Cumu % Drug Released | % Drug Released | Cube Root Of % Drug Remaining( Wt) | Wo-Wt |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 100 | 0.000 | 2.000 | 0.000 | 0.000 | 100 | 4.642 | 0.000 |
| 2 | 5.531 | 94.469 | 1.414 | 1.975 | 0.301 | 0.743 | 5.531 | 4.554 | 0.088 |
| 4 | 18.143 | 81.857 | 2.000 | 1.913 | 0.602 | 1.259 | 12.612 | 4.342 | 0.300 |
| 6 | 35.083 | 64.917 | 2.449 | 1.812 | 0.778 | 1.545 | 16.94 | 4.019 | 0.623 |
| 8 | 60.035 | 39.965 | 2.828 | 1.602 | 0.903 | 1.778 | 24.952 | 3.419 | 1.223 |
| 10 | 96.335 | 3.665 | 3.162 | 0.564 | 1.000 | 1.984 | 36.3 | 1.542 | 3.100 |

**Table: 10 Cumulative % Drug Release Of Copper Nanoparticle Loaded Topical Gel**

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**4.4 Anti – Inflammatory Activity:**

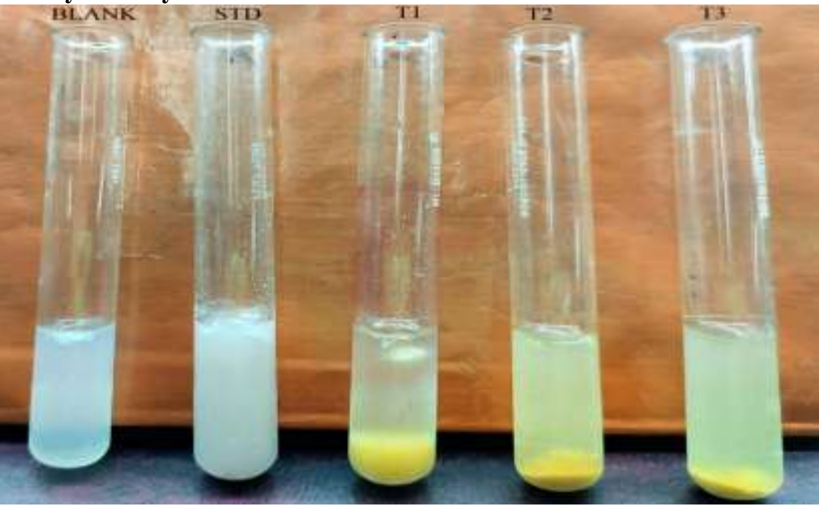


**Fig: 13Anti-Inflammatory Activity of Copper Nanoparticle**

| S.NO | COPPER NANOPARTICLE | OD VALUE | % OF INHIBITION |
|---|---|---|---|
| 1 | DISTILLED WATER | 0.00 | - |
| 2 | CONTROL | 0.449 | - |
| 3 | STANDARD | 0.146 | 67.48 |
| 4 | 100 mg | 0.185 | 58.79 |
| 5 | 200 mg | 0.179 | 60.13 |
| 6 | 300mg | 0.173 | 61.46 |

**Table: 11 Anti-Inflammatory Activity of Copper Nanoparticle**

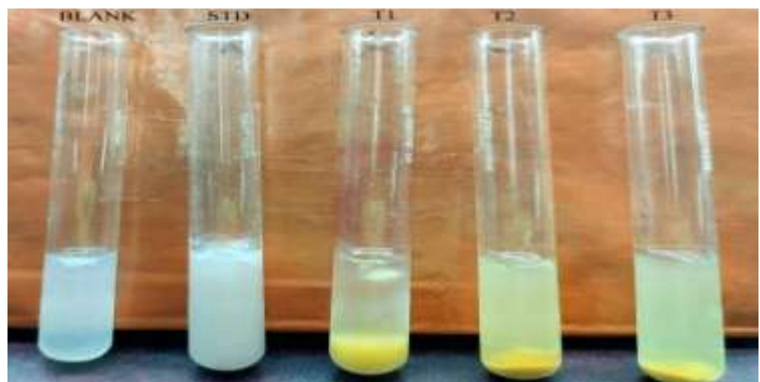


**Fig: 14Anti-Inflammatory Activity Copper Nanoparticle Loaded Topical Gel**

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| S.NO | COPPER NANOPARTICLE LOADED TOPICAL GEL | OD VALUE | % OF INHIBITION |
|---|---|---|---|
| 1 | DISTILLED WATER | 0.00 | - |
| 2 | CONTROL | 0.449 | - |
| 3 | STANDARD | 0.146 | 67.48 |
| 4 | 100 mg | 0.129 | 71.26 |
| 5 | 200 mg | 0.123 | 72.60 |
| 6 | 300mg | 0.117 | 73.94 |

**Table: 12 Anti-Inflammatory Activity Copper Nanoparticle Loaded Topical Gel**

**4.5 Pharmacological Activity:**

Wound Length Measurement Data The data of the observation for 14 days on wound healing and measuring it diameter shown on Table 18.

| OBSERVATION DAYS | TREATMENT | WOUND LENGTH cm |
|---|---|---|
| 3 | Negative Control | 2 |
| | Positive Control | 1.8 |
| | AE Extract | 1.7 |
| | Copper Nanoparticle Loaded Topical Gel | 1.8 |
| 5 | Negative Control | 2 |
| | Positive Control | 1.7 |
| | AE Extract | 1.7 |
| | Copper Nanoparticle Loaded Topical Gel | 1.6 |
| 7 | Negative Control | 2 |
| | Positive Control | 1.5 |
| | AE Extract | 1.6 |
| | Copper Nanoparticle Loaded Topical Gel | 1.8 |
| 14 | Negative Control | 1.9 |
| | Positive Control | 0.4 |
| | AE Extract | 1 |
| | Copper Nanoparticle Loaded Topical Gel | 1.2 |

**Table: 13 Wound Length Measurement**

| OBSERVATION DAYS | COPPER NANOPARTICLE LOADED TOPICAL GEL |
|---|---|
| **3** | |

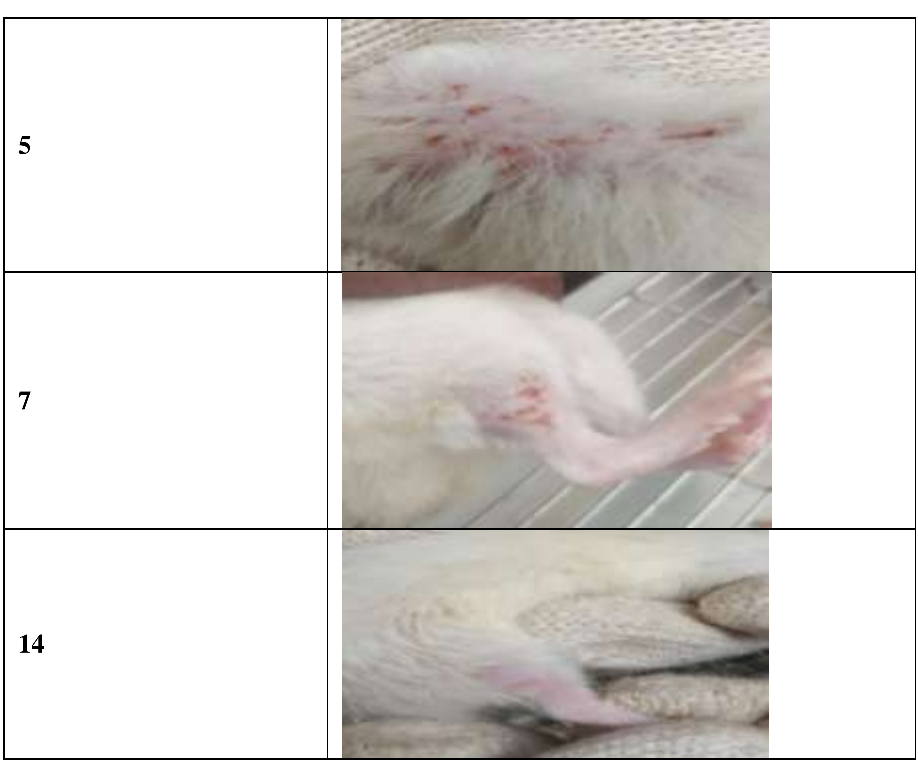


**Table: 14 Wound Healing Copper Nanoparticle Loaded Topical Gel**

**4.6 Stability Studies:**

| S.No | Parameter | Observation | | | | |
|---|---|---|---|---|---|---|
| | | Initial | At the end of 1st month | | At the end of 2nd month | |
| | | | RT | 40 ± 2oC & RH 70 ± 5% | RT | 40 ± 2oC & RH 70 ± 5% |
| 1. | Appearance | Smooth | Smooth | Smooth | Smooth | Smooth |
| 2. | pH | 8.23 | 8.23 | 8.23 | 8.23 | 8.22 |
| 3. | Spreadability | 8.2 cm | 8.2 cm | 8.2 cm | 8.1 cm | 8.1 cm |
| 4. | Extrudability | Excellent | Excellent | Excellent | Excellent | Excellent |
| 5. | % drug content | 96.33 | 99.16 | 99.14 | 99.16 | 99.16 |

**Table: 15 Stability Study Of Copper Nanoparticle Loaded Topical Gel**

## V. CONCLUSION

The findings of this study conclude that plant-mediated copper nanoparticles can be effectively synthesized and incorporated into a topical gel formulation. The nanoparticle-loaded topical gel exhibited superior anti-inflammatory and wound healing activities compared to the extract and nanoparticles alone. The formulation demonstrated sustained drug release, good stability, and excellent skin compatibility, making it a

promising candidate for topical therapeutic applications. Further in vivo and clinical studies are recommended to establish its safety and efficacy for pharmaceutical use.

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