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DEVELOPMENT AND EVALUATION OF ANTI-INFLAMMATORY GEL FORMULATION FROM MIMOSA PUDICA LINN WITH CYTOTOXICITY SCREENING VIA BSLA

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Abstract:

The present study focused on the development and evaluation of an anti-inflammatory gel formulated from Mimosa pudica Linn. extract and perform Brine Shrimp Lethality Assay (BSLA) for cytotoxicity screening. The plant extract was prepared by maceration extraction method using methanol as solvent and perform preliminary phytochemical screening, which confirmed the presence of alkaloids, carbohydrates, flavonoids, tannins, steroids. To perform protein denaturation assay at different concentrations (100–500 µg/mL) for evaluation of anti-inflammatory activity of the extract. The extract showed significant inhibition of protein denaturation with an IC_{50} value of around 220 µg/mL and represent good anti-inflammatory potential. Cytotoxicity screening using Brine Shrimp Lethality Assay (BSLA) demonstrated moderate biological activity, with 100% lethality at 1000 µg/mL and LC_{50} value of 100 µg/mL. Herbal gel was formulated using the different conc. of Carbopol and evaluated for pH, viscosity, Spreadability, washability, transparency and extrudability. MPG F4 batch showed optimum physicochemical properties and was selected for optimized formulation. The study recommends that Mimosa pudica Linn. gel is useful and effective topical formulation for inflammation management.

Keywords: *Mimosa pudica L., Anti-inflammatory gel, Brine Shrimp Lethality Assay (BSLA), Cytotoxicity, protein denaturation assay.*

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INTRODUCTION:

Anti- Inflammatory activity [1]

Inflammation – Inflammation is the body's natural response to injury, infection, or harmful stimuli.

Inflammation is a natural defence and healing process of the body. It helps protect us when we face injuries, infections, autoimmune reactions, toxins, or reduced blood flow. While inflammation is essential for healing, chronic inflammation can be harmful and is linked to conditions like diabetes, heart disease, autoimmune disorders, and even cancer.

Even in acute Inflammation, controlling swelling can be important, not just to support healing but also to reduce discomfort, such as pain and fever. Many current medications are available to manage inflammation, and new formulations are frequently being developed.

At the same time, several Ayurvedic medicines are recognized for their strong anti-inflammatory effects, often with fewer side effects. One of the most noticeable benefits of controlling inflammation is pain relief, especially for conditions like arthritis or muscle injuries.

Gel Formulation [2]

Gels are the semisolid formulation in which small or large molecules dispersed in a suitable liquid and rendered jelly like with the help of gelling agent or gelator (proteins, starch, gelatine, cellulose derivatives, carbomers etc.)

Gels are semi-rigid mixtures where the liquid is held in place by a three-dimensional network of tiny particles or dissolved macromolecules. This network limits the movement of the liquid, giving the gel its structure.

Types of Gels:

Hydrogels:

- Contain water as the dispersion medium.
- Example: Carbopol-based anti-inflammatory gel containing diclofenac or herbal extracts (like turmeric or aloe vera).

Organogels:

- Contain an organic solvent such as propylene glycol or isopropyl myristate.
- Often used for lipophilic (oil-soluble) drugs.

Emulgels:

- Combination of gel and emulsion systems.
- Provide enhanced penetration and stability for poorly water-soluble drugs.

Cytotoxic Screening:

Cytotoxic screening refers to in vitro methods used to evaluate whether a substance (such as a plant extract, synthetic compound, nanoparticle, or formulation) is toxic to cells. In pharmacology, toxicology and natural-product research, cytotoxicity assays are often used as preliminary screening tools to identify candidates with either harmful effects (toxicity) or useful effects (e.g., anticancer activity) before moving to further studies.

- **Methods of cytotoxic screening:**
  1. BSLA.
  2. MTT Assay.
  3. Trypan Blue Exclusion Assay.
  4. Lactate Dehydrogenase (LDH) Release Assay.
  5. Neutral Red Uptake (NRU) Assay.

Brine Shrimp Lethality Assay (BSLA) :[3]

The Brine Shrimp Lethality Assay is useful method for the evaluation of toxicity. It is also broadly recommended for screening the pharmacological activities of plant extracts. Additionally, BSLA is for determining the suitability and bioactivity of natural products.

This assay is suitable method for the primary assessment of toxicity. It is generally used to detect fungal toxins, assessment of plant extract toxicity, for cytotoxicity testing of dental materials, heavy metals, bacterial toxins, pesticides.

Plant Information: [4]

Mimosa pudica L. is a low-growing annual herb, generally known in Ayurveda as *Lajjalu*. Traditionally, it has been valued for a wide range of healing properties, with antiasthmatic, aphrodisiac, pain-relieving, and antidepressant effects. The plant is also known for its calming, emetic, and restorative qualities. Over the years, it has been used in traditional medicine to help treat conditions such as hair loss, diarrhoea, dysentery, insomnia, tumours, and various urogenital infections.

Plant profile

Plant Name: *Mimosa pudica Linn.*

Common Name: It is Commonly known as Sensitive Plant or Touch-Me-Not. In Ayurveda, it is known as Lajjalu. Family: It belongs to the Fabaceae family, commonly known as the legume, pea, or bean family.

Origin and global distribution: This plant originally comes from tropical regions of the Americas. Over time, it has spread and become naturalized throughout many tropical areas, including most parts of India's tropical belt.

Habitat: Generally dispersed in open- spaces, specifically rural areas, cultivated land, and waste area. Propagation: With the help of seeds and vegetative styles.

Explanation: This plant grows low to the ground and can have a slightly spreading habit. It is a small shrub that typically reaches up to about 0.5 meters in height. [5]

Fig.no.01. *Mimosa pudica* linn…

Botanical description

Table no.01 Scientific classification [6]

Kingdom	Plantae
Division	Magnoliophyte
Class	Magnoliopsida
Order	Magnoliopsida
Family	Fabaceae
Subfamily	Mimosoideae
Genus	Mimosa M.
species	pudica

Morphology:[7]

Mimosa pudica is a little, diffuse, prickly, perennial herb that produces as an annual plant.

- **Stem:** Trailing, branched, slender and covered with scattered prickles.
- **Fruits:** Bristly margins with Flat pods.
- **Leaves:** Alternate and bipinnate, having 1–2 pairs of pinnae, with each pinna containing 10–26 small leaflets.
- **Flowers:** Pink-coloured, globose flower heads that are axillary, solitary, or arranged in clusters.

Phytochemistry:[7]

Different type of phytoconstituents with a range of pharmacological potential are present in the plant.

• Alkaloids: derivatives of mimosine and tryptamine
• Flavonoids: isorhamnetin, kaempferol, and quercetin
• Tannins: catechol tannins
• Saponins: Derivatives of oleanolic acid
• Terpenoids: luteol, β-sitosterol;
• Phenolic compounds: gallic acid, ellagic acid

MATERIAL AND METHODOLOGY

Material: *Mimosa pudica* Linn., Methanol, Carbopol 940, Triethanolamine, HCL, Mayer's reagent, Dragendorff's reagent, Magnesium ribbon, Fecl3, Chloroform, Nacl.

Collection of plant: The selected plants, *Mimosa pudica* L…were collected from the rural zone of Sindhudurg district (Maharashtra) on 30 October 2025.

Authentication of plant: The plant was authenticated by the head of PG department and research Centre of botany of SPK Mahavidyalaya, Sawant-wadi, Maharashtra via Ref. NO 12-B/556/2025 date – 06/11/2025 by comparing morphology features of crude drug sample.

Drying and size Reduction of plant material: The leave of mimosa pudica were dried under shade then dried plant material were crush to coarse powder. The coarse powder was passed through the sieve no. 40 to maintain uniformity.

Extraction of mimosa pudica:

Method:[8]

- **Maceration:** In this extraction procedure, powdered drug material is placed in a container (beaker) and completely soaked powdered drug material with suitable solvent. The container is then covered with foil

paper and kept aside for at least three days. During this period, the mixture contents are stirred occasionally with glass rod and the bottle is shaken at specific time intervals to ensure whole extraction of the active constituents. After the extraction process is completed, the micelle is separated from marc by filtration. The solvent (menstruum) is then removed from micelle by evaporation using a water bath. This method is especially suitable for the extraction of thermolabile plant materials.

- **Preparation of Extract:**[9]

Methanol was used as the solvent, and 100 g of *Mimosa pudica* Linn. powder was weighed.300 ml of solvent was added to the powder and mixture was left at room temperature for 48 hours, with stirring every morning. After 48 hours, the mixture was filtered by using Whatman No. 1 filter paper to separate the residue.

Phytochemical Screening:[10-11]

1. Test for Alkaloids:

To detect the presence of alkaloids, about 0.5 g of sample is mixed with 8 ml of 1% HCL. The mixture was heated and filtered. From the clear filtrate, 2 ml was taken and treated with following reagents. With the help of observation, we will consider alkaloid present or absent.

- Mayer's Test: To 2–3 mL of the filtrate, a few drops of Mayer's reagent are added. The formation of a cream-colored ppt indicates the presence of alkaloids.
- Dragendorff's Test: To 2–3 mL of the filtrate, some drops of Dragendorff's reagent are added. The formation of a reddish-brown ppt shows the alkaloids are present.

2. Test for Flavonoids:

- Shinoda Test: Some amount of the dried sample is mixed with 5% ethanol. Few drops of conc. HCL are added, followed by approximately 0.5 g of magnesium ribbon. The appearance of red, pink, orange or purple color indicates the presence of flavonoids.
- Ferric chloride ($FeCl_3$) test: A few drops of neutral $FeCl_3$ solution are added to a small quantity of the methanolic extract. If blackish-green color appears then shows presence of flavonoids.

3. Test for Tannins:

- Ferric chloride test: A few drops of neutral ferric chloride solution is added to a small amount of the methanolic extract. If a blackish-green color appears, it indicates the presence the phenolic nucleus.
- Lead Acetate Test: Add 2 ml extract with 1 mL of 10% lead acetate solution. Observe White ppt indicates the presence of tannins.

2. Test for Steroids:

- Salkowski test: A sample of 0.5 g is taken and dissolved in 3 ml of conc. sulfuric acid and 2 ml of chloroform. A yellow to brown layer is observe showing that presence of steroid.

3. Test for Carbohydrates:

- Molisch's Test: Mix 2 ml extract with 2 drops of Molisch's reagent (1% α-naphthol in ethanol). Then, carefully add concentrated sulfuric acid along the side of the test tube. The formation of a violet ring at the junction of the two layers indicates the presence of carbohydrates.
- Benedict's Test: Add 2 ml extract with 2 ml Benedict's reagent, boil 2 min. Brick-red precipitate will be observe which indicate the presence of reducing sugar.

Screening of Anti-inflammatory activity of mimosa pudica:[12]

To perform protein denaturation assay, start by preparing a 5 mL reaction mixture for each concentration of your plant extract (100,200,300,400 and 500µg/mL). In separate test tubes, combine 0.2 mL of egg albumin with 2.8 mL of PBS buffer (pH 6.4), then add 2 mL of the plant extract at the required concentration. For the control tube, follow the same steps but use 2 mL of double-distilled water instead of the extract. You should also prepare the standard samples in the same way, replacing the extract with 2 mL of paracetamol solution at the matching concentrations. Once all your tubes are prepared, place them in an incubator at 37 °C for 15 minutes to allow the reaction to settle, and then transfer them to a water bath at 70 °C for 5 minutes to induce protein denaturation.

After heating, let all tubes cool naturally to room temperature. Finally, measure the absorbance of each mixture at 660 nm using a spectrophotometer, ensuring the instrument is zeroed with a PBS-only blank before reading the samples.

Calculation:

The percentage inhibition of protein denaturation was calculated by using the following formula:

% Inhibition = [(Vc – Vt)/Vc] x 100

Cytotoxic screening of mimosa pudica:

- **Brine Shrimp Bioassay:** [13]: The cytotoxicity of the plant extracts was evaluated using the Brine Shrimp Lethality Assay (BSLA). Brine shrimp eggs were obtained and placed in a hatching chamber filled with filtered seawater. About 50 mg of eggs is added, and the setup was left undisturbed for 48 hours to allow the eggs to hatch into nauplii (brine shrimp larvae). Since the nauplii are attracted toward light, they naturally migrated through the holes in the divider into the smaller

illuminated compartment. For testing, the samples were prepared in vials to achieve final concentrations of 1, 10, 100, and 1000 µg/ml.

- **Preparation of Stock Solution and hatching brine shrimp:**[14]

1. About 10 g of brine shrimp eggs were added to a clean transparent jar (separating funnel) containing 3 litres of water with approximately 27 g of table salt.
2. Then dissolves salt completely stir the mixture well using a spatula.
3. To provide proper aeration, place the air tube connected to an air pump at the bottom of the separating funnel, then gently sprinkle the eggs onto the surface of the water and mix carefully.
4. Switch on a light source (60-to-100-watt bulb) placed at a slight distance below the jar to provide warmth.
5. After 20–24 hours of incubation, the nauplii were hatch from the eggs, and the newly hatched nauplii are observed and collected after 24 hours.
6. To separate the nauplii from the empty egg shells, switch off the light and air pump before collecting the nauplii.

- **Testing**

1. Prepare the final concentrations of the gel for cytotoxicity testing: 1, 10, 100, and 1000 µg/ml.
2. Using a Dropping pipette, transfer 10 nauplii into each test tube for the toxicity test.

After 24 hours, count how many nauplii are still alive and calculate the percentage of mortality.

Fig. No. 02 Brine shrimp hatching

Fig. No. 03 Brine shrimp growth

Formulation of Mimosa pudica Gel: [15]

Table no.02: Formulation of mimosa pudica gel containing

Sr.no	Ingredient name	Quantity
1.	Lajjalu extract	1gm
2.	Demineralised water	100 ml
3.	Carbapol	0.25-1.50 gm
4.	TEA	1-10 drops
5.	Phenoxyethanol	0.2 ml

- **Procedure:**

Take demineralized water 100 ml and extract of Lajjalu 1 gram. Mix it well and add Carbopol 934 about 1 gram mix it until it dissolves completely wait for 3 hours filter it well. Then add TEA (Triethanolamine) drop by drop up to 20 drops. Finally, gel like consistency will be obtained. Then add 0.2 ml of Phenoxyethanol as preservative.

Evaluation of Herbal Gel:

- **Homogeneity and Appearance:** By visual observation formulated gels were evaluate for physical appearance and homogeneity.
- **pH:** The pH of the gel was measured by using the digital pH meter by gently inserting the glass electrode into gel. The measurement was taken three times, and the average of those three readings was noted.
- **Viscosity:** The viscosity of gel is measure by using Brookfield viscometer at 25°C having spindle speed of 100 rpm.
- **Determination of Spreadability:** Take a clean dry petri plate. Place about 0.5–1 g of gel at the centre. Cover it with another petri plate. Place a known weight (100 g) on the upper plate. Allow it to stand for 1–5 minutes. Measure the diameter (cm) of the spread gel.
- **Extrudability Test:** Fill the gel into a collapsible tube (like ointment tube) and seal it properly. Press the tube by applying uniform pressure (using fingers or a weight). Note the amount of gel extruded from the tube. Measure the length or weight of gel

extruded in a fixed time. Evaluate extrudability.

- **Washability:** Apply a small amount of gel on the skin (hand/forearm). Leave it for a few minutes to form a thin film. Wash the area gently with water. Observe the ease of removal and any residue left. Record as good, moderate, or poor washability based on removal.
- **Transparency:** Around 5 ml of prepared gel was taken in to 10 ml test tube and its transparency was checked visually.

RESULT

Morphological description: Morphological description of plant material is the study of external characters like size, shape, color, texture, and structure of different plant parts.

Fig. No.4 *mimosa pudica* linn

Table no.03 Characteristics

Sr. No	Characteristics	Observation
1	Parameter	Characteristics
2	Appearance	Small, sensitive herb with compound leaves
3	Color (Leaves)	Bright green to pale green
4	Color (Flowers)	Pink or light purple, fluffy, globular inflorescence
5	Odor	Characteristic mild, slightly earthy odour
6	Taste	Slightly bitter and astringent
7	Parameter	Characteristics
8	Appearance	Small, sensitive herb with compound leaves

Extraction

Extraction was performed on *Mimosa pudica* with the help of maceration process with 95% methanol around 3 days. 200 g of dried mimosa pudica powder in 600 ml of methanol was used for extraction. A total of 15 g of extract was obtained and used for the further studies.

Fig. No. 05 Maceration of dried powder

Fig. No. 06 Filtration through Whatman filter paper no.1

Phytochemical screening

TABLE NO.04 PRELIMINARY PHYTOCHEMICAL SCREENING:

PHYTOCHEM CAL TEST	TEST	OBSERVATION	INFERANCE
1. Alkaloids	Mayer's Test	Cream ppt	
	Dragendorff's Test	Reddish brown ppt	++
2. Flavonoids	Shinoda Test	Orange to purple	
	Ferric chloride test (FeCl3)	Dark greenish black color	++
3.Tannins	Ferric chloride test (FeCl3)	Dark greenish black color	
	Lead Acetate Test	White ppt	++
4.Steroids	Salkowski test	Red brown color	++
5.Carbohydrates	Molisch's Test	Violet color ring	
	Benedict's Test	Brick red color	++

Screening of anti-inflammatory activity of mimosa pudica

The methanolic extract of mimosa pudica was evaluated for anti-inflammatory activity with the help of protein denaturation assay method.

Table no.05 Absorbance readings of standard, sample and control

CONCENTRATION (µg/ml.)	STANDARD		SAMPLE	
	ABSORBANCE	% INHIBITION	ABSORBANCE	% INHIBITION
100	1.440	48.99	1.569	44.42
200	1.401	50.37	1.434	49.20
300	1.239	56.11	1.321	53.20
400	1.050	62.80	1.123	60.21
500	0.905	67.94	0.970	65.63
CONTROL READING – 2.823				

Calculation: For IC50 1**. Standard:**

$$IC50 = 100 + \frac{(50.37-48.99)}{(50-48.99)} \times (200 - 100)$$

IC_{50} (Standard) ≈ 173 µg/mL

2.Sample:

$$IC50 = 200 + \frac{(53.20 - 49.20)}{(50 - 49.20)} \times (300 - 200)$$

IC_{50} (Sample) ≈ 220 µg/mL

Sample (Mimosa pudica extract) shows good activity but is slightly less potent than standard (IC_{50} = 220 µg/mL).

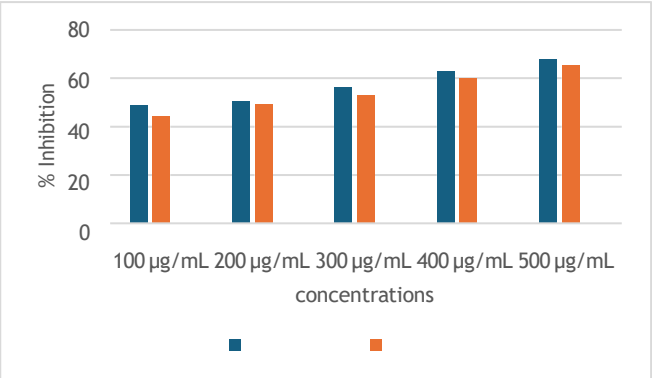

Fig. No. 07 Graph of comparative study of protein denaturation assay.

Cytotoxicity screening of mimosa pudica

Cytotoxic effect of the methanolic extract of Mimosa pudica via BSLA: Cytotoxicity activity of methanolic extract of mimosa pudica was evaluated using BSLA. The extract shows moderate cytotoxic activity; At the conc. 1000µg/ml. the extract was found to be 100% lethal to brine shrimp.

Table no.06 Cytotoxicity effect of the mimosa pudica extract and fraction on BSLA

PLANT MATERIAL	PLANT EXTRACT	% OF DEATH OF NAUPLII AFTER 24 HRS (Conc. µg/mL) LC50 (PPM)				
		1 µg/ml	10 µg/ml	100 µg/ml	1000 µg/ml	LC_{50}
Mimosa pudica	methanolic extract of *Mimosa pudica*	20	30	50	100	100

The test sample shows moderate biological activity/toxicity, as the LC_{50} value is 100 µg/ml. This indicates that the active ingredient possesses significant bioactivity, which may contribute to the therapeutic effect of the gel formulation (such as anti-inflammatory)

FIG NO.08 DEATH OF NAUPLII

FIG NO.09 ALIVE NAUPLII

FORMULATION TABLE

Table no.07 Formulation table (batches)

Ingredients	F1	F2	F3	F4	F5	F6
Lajjalu extract (gm)	1	1	1	1	1	1
Demineralised water(ml)	100	100	100	100	100	100
Carbapol(gm)	0.25	0.50	0.75	1.00	1.25	1.50
TEA (drops)	q. s	q. s	q. s	q. s	q. s	q. s
Phenoxyethanol(ml)	0.2	0.2	0.2	0.2	0.2	0.2

EVALUATION TESTS FOR GEL

1. **Homogeneity and Appearance:** By utilizing sensory organs like eyes or nose, the examination of the formulation is performed. The texture, colour, odour and appearance are checked.
2. **pH Test:** The gel we developed exhibited a pH range of 5 to 7, which aligns closely with the recommended optimal pH values for gel, typically between 6 and 7.

Table no.08:PH test

Batches	F1	F2	F3	F4	F5	F6
Reading (1)	5.83	5.93	5.97	6.54	5.88	5.94
Reading (2)	5.80	5.87	6.01	6.53	5.85	5.91
Reading (3)	5.63	5.80	5.99	6.54	5.87	5.93
Mean	5.75	5.86	5.99	6.53	5.85	5.92

3. **Viscosity:** Viscosity plays a crucial role in determining the stability, performance and patient acceptability of gel formulations. Proper optimization of viscosity ensures ease of application, efficient drug delivery and improved therapeutic effectiveness.

Table no.09 Viscosity

Batches	F1	F2	F3	F4	F5	F6
Viscosity(cp)	957.1	1058	1148.1	2064.1	920.1	2568.1

4. **Determination of Spreadability:**

Spreadability is an important parameter for topical gels, indicating how easily the gel spreads on the skin surface. Good Spreadability ensures easy application of gel, uniform drug distribution and better patient compliance.

Table no.10 Spreadability

Batches	F1	F2	F3	F4	F5	F6
Spreadability (cm)	8.4	7.2	6.2	5.2	4.4	4.00

The Spreadability test confirms that the gel possesses adequate uniformity for easy application and uniform distribution onto the skin surface.

5. **Extrudability Test:** The test is based on applying a constant force on the tube and measuring the amount of gel extruded from tube. A good gel should come out from tube easily without excessive force.

Table no.11 Extrudability test

Batches	F1	F2	F3	F4	F5	F6
Extrudability (%)	90.4	93.00	86.83	96.70	87.34	83.66

Optimization: The formulation batch MPG F4 was considered as the optimized formulation of *Mimosa pudica* gel. In the present study on the Anti-inflammatory activity of *mimosa pudica*, batch optimization was carried out to achieve maximum efficacy and consistency in the formulation. Various parameters such as the extraction method (maceration Method), solvent type (methanol), plant material ratio were systematically varied to determine their impact on the anti-inflammatory potential of the final product. The optimized batch was selected based on basis of physical parameters such as Homogeneity and Appearance**,** pH, viscosity, Spreadability, Extrudability etc. Repeated trials ensured reproducibility, and the optimized batch showed a good stability than individual extracts, confirming the improved efficacy of the combined formulation. This optimization step was crucial in standardizing the preparation process and ensuring consistent therapeutic outcomes.

6. **Washability:** The prepared gel formulation showed good washability, as it was easily removed with water without leaving any residue on the skin.

7. **Transparency:** Transparency is a parameter that reflects the homogeneity and aesthetic acceptability of gel formulations. The prepared gel was found to be translucent due to the presence of plant extract.

CONCLUSION:

The present study was carried out to evaluate morphological characteristics, preliminary phytoconstituent screening, anti-inflammatory activity, cytotoxicity of *Mimosa pudica*. Macroscopic and microscopic evaluation of the plant serves as the primary step to establish its identity and characteristics.

The study revealed that *Mimosa pudica* Linn. contains alkaloids, flavonoids, tannins, steroids, and carbohydrates.

Extract of *Mimosa pudica* Linn was found the anti-inflammatory and cytotoxic properties. Extracts demonstrate strong inhibitory action against heat-induced protein denaturation and albumin denaturation, crucial in treating inflammatory skin diseases.

The results of the BSLA shows that the extract was 100% lethal to brine shrimp at conc. of 1000 µg/ml. The extract showed moderate biological activity with an LC_{50} value of 100 µg/ml, indicating its potential suitability for the formulation of herbal gel.

Thus, it can be concluded that the prepared gel formulation of Mimosa pudica exhibits significant anti-inflammatory and cytotoxic activities, indicating its potential therapeutic applications.

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