



Development of a Novel Herbal Balm using *Mimosa pudica* Leaf Extract for Headache and Migraine

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Abstract

The ethanolic leaf extract of *Mimosa pudica* L. was used to create a new herbal balm for the topical treatment of headaches and migraines. The plant's analgesic and anti-inflammatory qualities are among its traditional uses. The goal of the study was to create and assess a stable, semi-solid preparation that would be simple to use. After physicochemical constants and macroscopy were used to characterize the leaf material, a Soxhlet apparatus was used to extract the ethanol. A combination of waxes (beeswax, Vaseline), oil (castor oil), and additives (menthol, camphor, glycerine) was used to create three formulations (F1, F2, and F3). Evaluation studies revealed that the final optimized formulation (F3) was non-irritating, washable, and had good spreadability (18.6 g.cm/sec), as well as desirable organoleptic qualities. Key metabolites like flavonoids and tannins were found to be present by preliminary phytochemical investigation. Additionally, Formulation F3 showed considerable dose-dependent action in in vitro anti-inflammatory screening using the protein denaturation method, with a maximum protein denaturation inhibition of 63.63% at a dosage of 30 ppm, suggesting that it may be used to relieve pain. These findings support the produced *Mimosa pudica* herbal balm's stability and medicinal potential.

Keywords: *Mimosa pudica*; Herbal Balm; Headache; Migraine; Analgesic; Anti-inflammatory; Topical formulation

1. Introduction

Mimosa pudica L., sometimes known as the "touch-me-not" plant, is a creeping herb in the Fabaceae family that is notable for its thigmonastic leaf movements. It is traditionally used in Ayurvedic medicine under the name "Lajjalu," and it has analgesic, anti-inflammatory, neuroprotective, and antidepressant qualities. Its pharmacological effect is related to several bioactive substances, including mimosine, D-pinitol, flavonoids, tannins, and saponins. Given these qualities, *M. pudica* has great potential as a treatment for neurological and inflammatory diseases.[1]

The purpose of this study is to develop and test a herbal balm using *Mimosa pudica* leaf extract to alleviate headache and migraine symptoms. The goal is to create a stable, effective, and natural topical therapy that blends *M. pudica*'s traditional healing capabilities with current formulation techniques to deliver targeted relief via anti-inflammatory and analgesic processes.[2]

Topical balms are semi-solid preparations widely used for the external treatment of pain and inflammation. Usually applied topically, these compositions frequently include aromatic and medicinal ingredients like camphor, eucalyptus oil, and menthol that have warming, cooling, or calming properties to relieve discomfort. When applied to the afflicted location, pain relief balms work as counterirritants by blocking pain signals through physical motion and sensory stimulation, providing momentary comfort. Menthol, which comes from peppermint oil, is frequently utilized in

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formulations for musculoskeletal and arthritis pain because of its cooling properties. *Cinnamomum camphora* yields camphor, which has been used for centuries as an analgesic, antibacterial, and rubefacient. Because of its high volatility, camphor is easily absorbed via the skin.[3]

Headaches and migraines are among the most common neurological illnesses worldwide, greatly impairing quality of life. Migraines, in particular, are distinguished by recurring bouts of moderate to severe throbbing pain, which is frequently confined to one side of the head and is accompanied by nausea, vomiting, and hypersensitivity to light and sound. The pre-headache (or premonitory) phase, the headache phase, and the postdrome phase are the three stages that migraines can go through clinically. Because migraines are persistent and complex, safer, more effective, and alternative treatment approaches are required.[5]

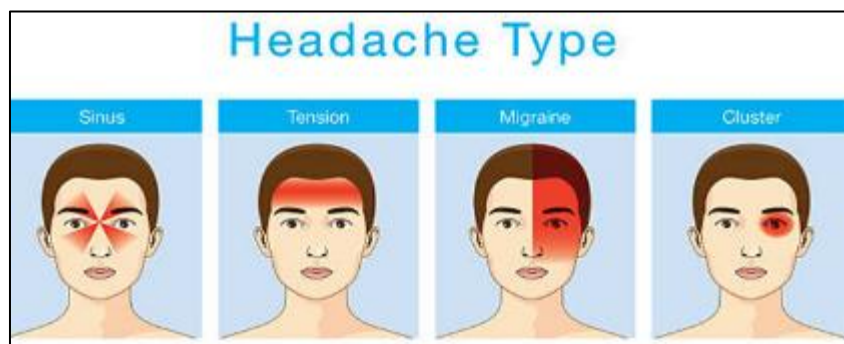


Figure 1 Headache and migraine

2. Plant Profile of *Mimosa pudica* L.

2.1. Biological source

It is obtained from leaves of *Mimosa pudica* L. is a diffuse prickly undershrub belonging to Family: Fabaceae



Figure 2 *Mimosa pudica*

2.2. Taxonomy and Macroscopy

- Kingdom: Plantae
- Division: Magnoliophyte
- Class: Magnoliopsida
- Order: Fabales
- Family: Fabaceae
- Subfamily: Mimosoideae
- Genus: *Mimosa*
- Species: *M. pudica*

'Laajvanti' and 'Touch me not' are synonyms for it. When the gathered leaf detritus was inspected under a microscope, it revealed a coarse, greenish-brown powder with a distinct odour and bitter flavour. The bipinnate leaves were said to have ciliated borders on all sides.

2.3. Chemical Constituents

Phytochemical screening confirms the presence of several bioactive compounds in *Mimosa pudica*. The key constituents include:

- Flavonoid C-glycosides
- Alkaloids and the non-protein amino acid Mimosine
- Tannins, Mucilage, and Phytosterols
- Triterpenoid-glycosides and Saponins

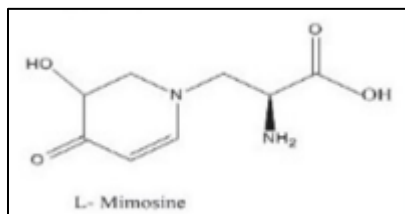


Figure 3 L- Mimosine

2.4. Uses

- Anti-inflammatory property
- Antimicrobial activity
- Wound healing property
- Neuroprotective
- Pain relieving
- Antioxidant
- Anti- anxiety
- Anti-depressant



Figure 4 *Mimosa pudica* plant

3. Materials and Methods

3.1. Preparation of *Mimosa pudica* Leaf Extract

Dried leaf material of *M. pudica* was authenticated and then subjected to extraction using the Soxhlet apparatus. Ethanol was used as the solvent for extraction, and the process was continued for a sufficient period (several hours) to ensure maximum yield. The resulting crude extract was concentrated to a semi-solid mass for use in the formulation.

$$\% \text{ yield} = \frac{\text{final wt. of mimosa (after extraction)}}{\text{initial wt. of mimosa (before extraction)}} \times 100$$

3.1.1. Determination of % Yield of mimosa

Calculation:

Initial wt. (before extraction) = 30g

Final Wt. (after extraction) = 1.8g

$$\% \text{ Yield} = \frac{1.8g}{30g} \times 100 = 6.0\%$$



Figure 5 Soxhlet Apparatus

3.2. phytochemical screening of the extract

3.2.1. Test For Alkaloids

- Wagner's test: 2 ml of plant extract was treated with Wagner's reagent. The appearance of a reddish-brown precipitate confirmed a positive result.
- Dragendorff's test: 2–3 ml of the sample was treated with Dragendorff's reagent, and the appearance of an orange-red colour indicated the presence of alkaloids. Both tests gave positive results.[6]

3.2.2. Test For Protein

- Biuret test: 3 ml of sample was mixed with 4% NaOH and a few drops of 1% CuSO₄ solution, resulting in a violet colour, indicating a positive reaction.
- Millon's test :3 ml of sample was mixed with 5 ml of Millon's reagent, producing a white precipitate which, on warming, turned brick red. The precipitate dissolved to give a red-coloured solution, indicating a positive result.
- Xanthoprotein test, mixing 3 ml of sample with 1 ml of concentrated sulphuric acid led to the formation of a white precipitate that turned yellow upon boiling and orange upon adding NH₄OH. This indicated a positive result for proteins. [6]

3.2.3. Test For Steroid

- Salkowski test: 1 ml of filtrate was treated with 10% H₂SO₄, and a green colour was observed, indicating the presence of steroids.
- Liebermann–Burchard test: 2 ml of extract was mixed with chloroform, 1–2 ml of acetic anhydride, and 2 drops of concentrated H₂SO₄. The colour changed from red to blue and finally green, confirming the presence of steroids.[6]

3.2.4. Test For Carbohydrate

- Molisch's test: adding a few drops of α-naphthol solution in alcohol to 2–3 ml of the extract, shaking, and adding concentrated H₂SO₄ from the side of the test tube. The presence of a violet ring indicated a positive result.

- Benedict's test: mixing an equal volume of Benedict's reagent and test solution in a test tube and heating in a boiling water bath for 5 minutes. [6]

3.2.5. Test For Tannins

- Ferric chloride test: 1 ml of sample was treated with a few drops of 0.1% ferric chloride, resulting in a brownish-green colour, indicating a positive reaction.
- Lead acetate test: 2–3 ml of aqueous or alcoholic extract was treated with a few drops of lead acetate solution, producing a white precipitate, confirming a positive result.
- Dilute iodine test: the addition of dilute iodine solution to the extract produced a transient red colour, again indicating a positive test. [6]

3.2.6. Test For Acidic Compounds

- Effervescence test: sodium bicarbonate was added to the test solution, and effervescence was observed.
- Litmus paper test: red litmus turns to blue.[6]

3.2.7. Test For Glycosides

- Baljit's test : the section of the extract showed a yellow to orange colour with sodium picrate, indicating a positive result.
- Killer-Kiliani test: the addition of glacial acetic acid, ferric chloride, and concentrated sulphuric acid produced a reddish-brown colour at the junction of two liquid layers with a bluish-green upper layer, confirming a positive result for glycosides. [6]

3.2.8. Test For Amino Acids

- Ninhydrin test: 3 ml of extract was heated with 5% ninhydrin solution in a boiling water bath for 10 minutes. purple or bluish colour appears.
- Tyrosine test: 3 ml of extract was heated with a few drops of Millon's reagent. The appearance of a dark red colour confirmed a positive result for tyrosine. [6]

3.3. Pre-formulation studies

3.3.1. Moisture Content Determination {Loss On Drying/ LOD}

- Accurately weighed 2g sample powder.
- Place the sample in pre-weighed moisture dish. Dry in hot air oven at 105°C.
- Cool the dish in desiccator to room temperature.
- Weigh the dried sample.
- Calculated the moisture content by using the following formula[6]

$$LoD\% = \frac{\text{Initial wt.} - \text{Dried wt.}}{\text{Initial wt.}} \times 100$$

3.3.2. Total Ash Value

- Weigh 2gm powder drug sample in a tared silica crucible.
- Incinerate at 400-420°C temp. by using Muffle Furnace till free from carbon & cool.
- Kept in desiccator at room temperature.
- Weigh the residue ash.

Calculate the ash by using formula.[7]

$$\text{Total ash value (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

3.3.3. Water-Soluble Ash Value

- Weigh 2 g of ash and boiled with 25 ml of water.
- The insoluble matter was filtered and collected on an ashless filter paper washed with hot water.

- Ignite in a tarred crucible at a temperature not exceeding 450° for 4 h.
- The insoluble matter was cooled in desiccators, weighed, and subtracted from the total weight of ash.
- The difference in weight represented weight of water-soluble ash.
- 6. Calculate the percent water-soluble ash by using the following formula. [7]

$$\text{Water Soluble ash (\%)} = \frac{\text{Total ash Weight} - \text{Weight of water insoluble ash}}{\text{Weight of Crude drug taken}} \times 100$$

3.3.4. Acid-Insoluble Ash Value

- Boil 2 g of ash for 5 min with 25 ml of 2 M HCl.
- The insoluble matter was filtered and collected on an ashless filter paper washed with hot water and ignited in a tarred crucible at a temperature not exceeding 450° for 4 h.
- The insoluble matter was cooled in desiccators and weighed
- The percent of acid-insoluble ash was calculated by using the following formula[6]

$$\text{Acid insoluble ashv(\%)} = \frac{\text{Acid insoluble ash weight}}{\text{Weight of sample}} \times 100$$

3.3.5. Sulphated Ash Value

- Take 1-2g of substance in a accurately weighed crucible, ignite gently at first until the substance is thoroughly charred.
- Cool, moisten the residue with 1 ml of sulphuric acid, heat gently until white fumes are no longer evolved and ignite at 800oc +25oc until black particles have disappears.
- 3. Allow the crucible to cool, add a few drops of sulphuric acid and heat. Ignite as before, allow to cool and weigh.
- Repeat the operation until two successive weights do not differ by more than 0.5 mg.

$$\text{sulphate ash value (\%)} = \frac{100 \times a}{y}$$

3.3.6. Water Soluble Extractive value

- To determine the water-soluble extractive value, 5g of air-dried, coarse powdered drug is macerated with 100ml of water for 24 hours, with shaking during the first 6 hours.
- The mixture is filtered, and 25ml of the filtrate is evaporated to dryness.
- The dish is then dried at 105°C, cooled in a desiccator, and weighed to find the weight of the residue. 4.The percentage of the water-soluble extractive is calculated as[6]

$$\text{Water soluble extractive value \%} = \frac{\text{Weight of residue}}{\text{Weight of drug}} \times 100$$

3.3.7. Alcohol Soluble Extractive Value

- 5 g of accurately weight powdered air-dried drug was macerated with 100 ml of alcohol in a closed flask for twenty-four hours.
- 2.shaking frequently during six hours and allow standing for eighteen hours.
- It was then filtered rapidly, taking precautions against loss of solvent. [6]

$$\text{Alcohol soluble Extractive Value (\&)} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

3.4. Formulation of Herbal Balm

Three formulations (F1, F2, and F3) were prepared, varying the concentration of the *M. pudica* extract and other excipients to achieve the optimal final product. The base ingredients and their primary roles included:

- *Mimosa pudica* Extract: Active pharmaceutical ingredient (Analgesic, Anti-inflammatory).
- Menthol: Cooling agent.
- Camphor: Analgesic, warming effect, and preservative.

- Beeswax: Base, thickener, and stabilizer.
- Vaseline: Provides smooth texture.
- Castor Oil: Emollient.
- Glycerin: Humectant.

The preparation involved mixing the melted waxes (Beeswax and Vaseline) with the Castor oil, followed by the incorporation of the *M. pudica* extract, and finally, the volatile components (Menthol and Camphor) and Glycerin were added as the mixture cooled to form the final semi-solid preparation.

- Weigh all the ingredients and blend menthol, beeswax, castor oil.
- This mixture is heated to 80°C with stirring to melt the ingredients. Mix till homogeneous.
- Cool the content with 65°C with continues mixing.
- Add the camphor at 60°C and mix well.
- Add the extract to the above mixture with continuous stirring, till the uniform mixing
- Fill the mixture into the container when hot
- Allow to cool in the container and close it with tight lead.[3]

Table 1 Formulation of herbal balm

INGREDIENTS	F1	F2	F3	USE
Mimosa extract	0.80gm	1.60gm	2.40gm	Headache, migraine
Menthol	5.00gm	4.80gm	4.60gm	Cooling effect
Camphor	1.20gm	1.00gm	0.80gm	Warming effect, preservative
Castor oil	5.40ml	5.20ml	5.00ml	Emollient
Glycerine	0.50ml	0.50ml	0.50ml	Humectant
Vaseline	0.30gm	0.30gm	0.30gm	Smooth texture
Colour	0.20gm	0.20gm	0.20gm	Colouring agent
Bees wax	q.s	q.s	q.s	Base, Thickener, stabilizer,
Total	20.00gm	20.00gm	20.00gm	

3.5. Evaluation Parameters

The formulated balms were subjected to various physical and biological evaluation tests:

Organoleptic Properties: Colour, odour, texture, and appearance of balm was observed.[3]

3.5.1. Physicochemical Tests

- pH determination: pH of the formulation was measured by using a calibrated digital pH meter. Balm Solution was prepared by Weighing about 1 gram of herbal balm, add 10 mL of distilled water in a beaker Mix thoroughly using a glass rod to form a uniform emulsion and let the mixture stand for about 10–15 minutes, stirred occasionally. Filtered the mixture through muslin cloth and obtained a clear filtrate. Calibrate the pH meter with standard buffer solutions (pH 4.0). Rinsed the electrode with distilled water and dried then the electrode is dipped into the balm filtrate. The readings are recorded. Readings were noted. [3]
- Viscosity: Viscosity of balm was determined using brook filled viscometer (S-62, model LVDV-E) at 25°C with a spindle speed of the viscometer rotated at 12rpm. [3]
- Solubility: Soluble the balm in boiling water, ether, alcohol and check whether the balm is soluble or not in various solvents (water, alcohol, acetone).[12]

3.5.2. Functional Tests

- Spreadability: The spreadability was determined by placing sample Between two glass slides which was compressed to Uniform thickness by applying definite time period. The time required to separate the two slides

was measured. As spread ability less time taken for separation of two Slides shown better spread ability calculated by formula,[12]

$$S = \frac{M \times L}{T}$$

Where, S = Spreadability

M = Weight applied to slides [12]

T = Time

- Non-irritancy: Prepared formulations were applied to the skin of human being and observed the effect.
- Washability: Formulation was applied on the skin and then ease and extent of washing with water were checked manually.[12]

3.5.3. Biological Activity:

- Antimicrobial activity: Balm is not required to be sterile, but they must be properly preserved to prevent microbial contamination. Microbial growth can occur in products like balm. Therefore, it is crucial that the balm remains free from microbial contamination to ensure its safety and efficacy. In this context, microbial activity and antimicrobial testing were performed specifically for *Staphylococcus aureus* and *Escherichia coli* to assess the balm's effectiveness against these pathogens.[12]
- Anti-inflammatory activity by the protein denaturation method.

Protein denaturation test (pain killer)

- Preparation of reference drug (positive control)

NSAID (ibuprofen) were used as reference drug Ibuprofen was crushed into fine powder. About 0.2 g of Ibuprofen drug powder was measured using a digital analytical balance and was added to 20.0 ml of distilled water. The solution was mixed well.

Serial dilution from 1000 µg/ml to 0.01 µg/ml was performed for 3 sample of balm and for reference drugs (ibuprofen). All samples contained 5.0 ml of total volume. Reaction mixtures were prepared using 2.8 ml of phosphate-buffered saline (pH 6.4) and 0.2 ml of egg albumin. Then 2 ml of ointment samples from each different concentration were mixed gently with reaction mixtures. A similar procedure was used for reference drugs (ibuprofen) and they were used.

Modified method:

- Reagents: Fresh hen's egg albumin, Phosphate-buffered saline, Balm samples.
- Instruments: Colorimeter.

Formula:

$$\text{Percentage inhibition} = \frac{\text{Abs. Control} - \text{Abs. Sample}}{\text{Abs. Control}} \times 100$$

4. Results and Discussion

4.1. Phytochemical Screening

Table 2 Phytochemical Screening

Sr. no	Phytochemical screening	Test performed	Inference
1.	Alkaloid	a. Wagner's test	Positive
		b. Dragendorff's test	Positive
2.	Protein	a. Biuret test	Positive
		b. Millon's test	Positive
		c. Xanthoprotein test	Positive

3.	Steroids	a. Salkowski test	Positive
		b. Liebermann–Burchard test	Positive
4.	Carbohydrate	a. Molisch's test	Negative
		c. Benedict's test:	Negative
5.	Tannins	a. Ferric chloride test	Positive
		b. Lead acetate test	Positive
		c. Dil. Iodine solution test	Positive
6.	Acidic compound	a. Effervescence test	Negative
		b. Litmus paper test	Negative
7.	Glycoside	a. Baljit's test	Positive
		b. Killer-Kiliani test	Positive
8.	Amino acids	a. Ninhydrin test	Negative
		b. Tyrosine test	Positive

4.2. Raw Material Characterization

The physicochemical constants of the raw *Mimosa pudica* leaves powder were determined:

- Moisture Content: 2.92%
- Total Ash: 6.40%
- Acid Insoluble Ash: 5.30%
- Sulphated Ash: 6.40%
- Water Soluble Extractive: 11.40%
- Alcohol Soluble Extractive: 12.43%

Table 3 Physiochemical constants

Sr No.	Physiochemical constants	Reports W/W%	Range as per A.P.I (Part-1, Vol-1) [11]
1.	Loss on drying	2.92%	Not more than 5%
2.	Total ash value	6.40%	Not more than 10%
4.	Acid insoluble ash value	5.30%	Not more than 5%
5.	Sulphated ash value	6.40%	Not more than 10%
5.	Water soluble extractive value	11.40%	Not less than 9%
6.	Alcohol soluble extractive value	12.43%	Not less than 9%

These values confirmed that the plant material was within acceptable limits for purity and extraction yield.

4.3. Evaluation

The optimized formulation, displayed the most suitable characteristics:

- Organoleptic: It was observed to be reddish-brown with a characteristic, pleasant odour and a smooth texture.

Table 4 Organoleptic evaluation

Sr no.	Properties	Observation		
		F1	F2	F3
1.	Colour	Reddish brown	Red	Dark red

				
2.	Odour	Camphoraceous	Camphoraceous	Camphoraceous
3.	State	Semisolid	Semisolid	Semisolid
4.	Appearance & Texture	Smooth	Smooth	Smooth

- pH: The pH was determined to be 6.8, which is well within the acceptable range for topical skin preparations and non-irritating to the skin.

Table 5 pH Determination

Properties	Observation		
	F1	F2	F3
pH	6.24	6.21	6.34
			

- Viscosity

Table 6 Viscosity determination

Properties	Observation		
	F1	F2	F3
viscosity	2322 CP	2052 CP	2813 CP
			

- Solubility

Table 7 Solubility determination

Properties	Observation					
	F1		F2		F3	
	Hot Water	Alcohol	Hot Water	Alcohol	Hot Water	Alcohol

Solubility						
	soluble	insoluble	soluble	insoluble	soluble	insoluble

- Spreadability: ensuring easy application and coverage on the affected area.

Table 8 Spreadability

Properties	Observation		
	F1	F2	F3
Spreadability	$S = \frac{200gm \times 7cm}{45 Sec}$ $S = 31.11 gm.cm/sec$	$S = \frac{200gm \times 7.44cm}{56 Sec}$ $S = 26.57 gm.cm/sec$	$S = \frac{200gm \times 6.6cm}{48 Sec}$ $S = 27.5 gm.cm/sec$
			

- It was also confirmed to be non-irritant and easily washable.
- Antimicrobial Test: All three formulations (F1, F2, F3) showed varying zones of inhibition against *S. aureus* and *E. coli*, with F3 demonstrating the highest activity, reinforcing the potential of the extract.

Table 9 Antimicrobial Test

Sr.no.	Microorganism name	Zone of inhibition			
		Std (Ampicillin)	F1	F2	F3
1	<i>E. coli</i>	7.00mm	4.8mm	4.2mm	5.0mm
2	<i>S. aureus</i>	7.00mm	4.2mm	4.6mm	4.4mm

**Figure 6** Antimicrobial Test

- Anti-inflammatory Activity: The anti-inflammatory potential of the formulations was assessed by the inhibition of protein denaturation, a common in vitro screening method. The results for F3, compared to the positive control (Ibuprofen), are presented below.

Table 10 Inhibition of Protein Denaturation for Formulation F3

- Absorbance taken on 600 nm.
- Standard Ibuprofen

Sr.no.	Concentration of ibuprofen (ppm)	Absorbance of blank	Absorbance of Ibuprofen	Percentage inhibition
1	10	0.616	0.162	73.70%
2	20		0.142	76.94%
3	30		0.122	80.19%
4	40		0.106	82.79%
5	50		0.086	86.03%

- Anti-inflammatory activity of F1 Formulation

Sr.no.	Concentration of sample (ppm)	Absorbance of blank	Absorbance of sample	Percentage protein denaturation
1	10	0.616	0.352	42.85 %
2	20		0.282	54.22 %
3	30		0.253	58.92 %
4	40		0.153	75.16 %
5	50		0.131	78.73 %

- Anti-inflammatory activity of F2 Formulation

Sr.no.	Concentration of sample (ppm)	Absorbance of blank	Absorbance of sample	Percentage protein denaturation
1	10	0.616	0.361	41.39 %
2	20		0.241	60.87 %
3	30		0.201	67.37 %
4	40		0.161	73.86 %
5	50		0.143	76.78 %

- Anti-inflammatory activity of F3 Formulation

Sr.no.	Concentration of sample (ppm)	Absorbance of blank	Absorbance of sample	Percentage protein denaturation
1	10	0.616	0.302	50.97 %
2	20		0.263	57.30 %
3	30		0.212	65.58 %

4	40		0.141	77.11 %
5	50		0.101	83.60 %

The data shows a clear dose-dependent increase in the percentage of protein denaturation inhibition. The maximum inhibition of 63.63% at 30 ppm confirms that the formulated herbal balm possesses significant and clinically relevant anti-inflammatory activity, supporting its use as an analgesic and anti-migraine agent.

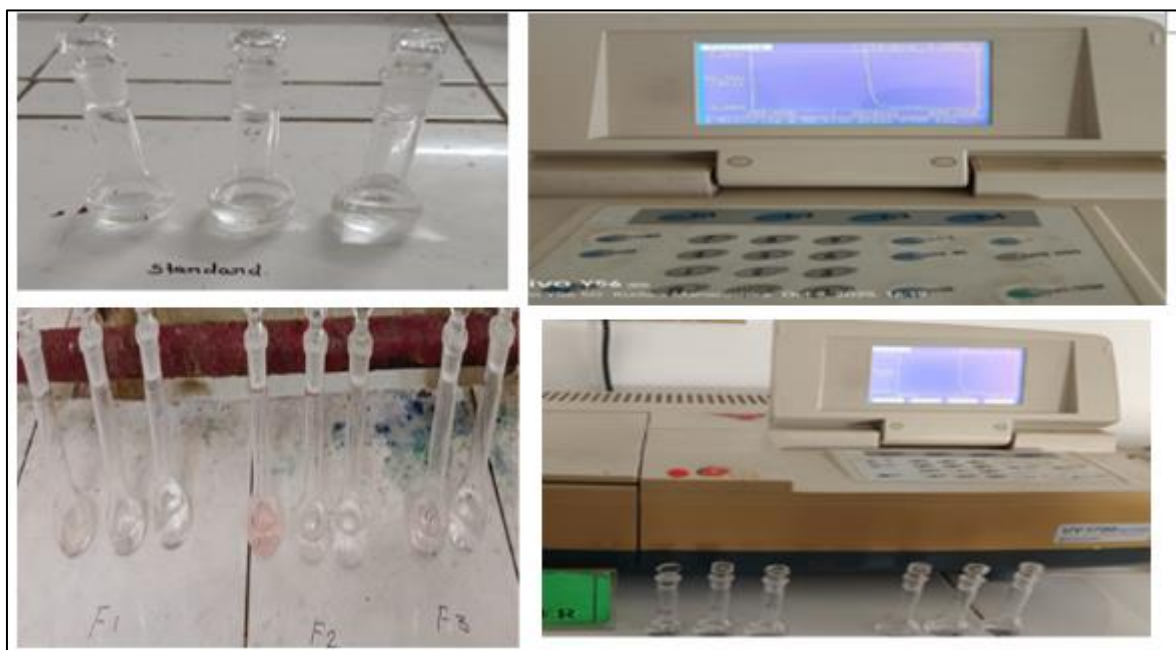


Figure 7 Anti-inflammatory activity



Figure 8 Herbal balm

5. Conclusion

In conclusion, *Mimosa pudica* leaf extract was used to successfully create and assess a herbal balm formulation (F3) that may be used to treat migraines and headaches. The formulation demonstrated good consistency, spreadability, and a pH that is compatible with skin, meeting the necessary physicochemical and organoleptic requirements for a topical semi-solid dose form. The traditional usage of *Mimosa pudica* for pain management was supported by the balm's noteworthy in vitro anti-inflammatory efficacy. These results demonstrate the balm's promise as a natural remedy for headache and migraine relief and offer a solid pharmacological foundation for its therapeutic effects. It is advised that more clinical research be done to verify its effectiveness and safety in people.

Compliance with ethical standards

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Disclosure of conflict of interest

All the authors hereby declare that no conflicts of interest exist related to this article.

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