

Formulation and Evaluation of a Polyherbal Topical Anti-inflammatory Cream

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ABSTRACT: The study evaluates the characteristics of herbal cream in comparison to the commercially available synthetic anti-inflammatory cream. Synthetic formulations show side effects. Herbal creams are considered a safer alternative due to their better biocompatibility, reduced adverse effects and the synergistic therapeutic activity of phytoconstituents. Therefore, an attempt was made to formulate and evaluate a polyherbal topical anti-inflammatory cream using extracts of *Curcuma longa* Linn., *Vitex negundo* Linn. and *Moringa oleifera* Lam. as these plants are well known for their anti-inflammatory and antioxidant properties. Three cream formulations (F1, F2 and F3) containing different concentrations of the herbal extracts were prepared using a cold cream base. The formulations were evaluated for physicochemical parameters including appearance, pH, viscosity, homogeneity, spreadability, washability, greasiness, skin irritancy and phase separation. All formulations showed acceptable physicochemical properties and good stability, with pH values compatible with skin. In-vitro antioxidant activity was assessed by the DPPH radical scavenging assay and formulation F1 exhibited the highest antioxidant potential with a comparatively lower IC₅₀ value. In-vitro anti-inflammatory activity of F1 was evaluated using the protein denaturation assay, which showed significant inhibition comparable to standard diclofenac sodium.

KEYWORDS: Anti-inflammatory, *Curcuma longa*, *Vitex negundo*, *Moringa oleifera*, topical cream.

I. INTRODUCTION

Inflammation is a complex protective response of the body to harmful stimuli such as infection, injury, chemical agents or tissue damage and it plays a crucial role in eliminating the initial cause of cell injury and initiating tissue repair. The major causes of inflammation include microbial infections, physical or chemical injury, immune reactions and chronic exposure to irritants. Inflammation is

mediated through several biochemical pathways including the cyclooxygenase (COX) pathway, the lipoxygenase (LOX) pathway, the NF-κB pathway, the MAPK pathway and the JAK-STAT pathway.^[1]

An herbal formulation is a dosage form consisting of one or more specified herbs or processed herbs in specified quantities to provide specific supplementary, therapeutic, nutritional and cosmetic benefits.^[2] Herbal medicines have different dosage forms which are safe and are formulated in different forms of herbal product. Herbal formulations are going to be the most influential elements that are fundamental for success and welfare for the people of nations. Many of the modern medicines are produced indirectly from a variety of medicinal plants.^[3]

Long-term use of NSAIDs and corticosteroids in chronic inflammation is associated with significant side effects. Herbal anti-inflammatory formulations offer safer, plant-based alternatives with multi-targeted mechanisms of action.^[4]

Creams are widely used in the pharmaceutical and cosmetic industries for their ability to deliver active ingredients to the skin effectively. Herbal extracts have gained significant attention due to their perceived safety and potential therapeutic benefits. Incorporating herbal extracts into creams offers a natural alternative to synthetic ingredients, aligning with the growing consumer demand for green and sustainable products.

Numerous herbal drugs offer potent anti-inflammatory activity and can be used for their specific effects in the treatment of various conditions. For example, turmeric (*Curcuma longa*), containing the active compound curcumin, is widely used to reduce systemic inflammation and alleviate joint pain in arthritis. Similarly, ginger (*Zingiber officinale*) with its gingerols, is effective in soothing muscular soreness and digestive inflammation. *Boswellia (Boswellia serrata)* is specifically targeted for its ability to improve mobility and reduce swelling in osteoarthritis and inflammatory

bowel diseases. Some other examples of anti-inflammatory herbal drugs include green tea, licorice, basil, sage, rosemary, aloe vera, garlic, oregano, etc. These herbs offer a natural way to manage inflammatory conditions.

Herbal anti-inflammatory preparations offer several advantages, including better safety profiles, fewer side effects with long-term use, multi-targeted action and improved patient acceptance, making them valuable alternatives or adjuncts to synthetic anti-inflammatory drugs.^[5]

Rationale of Herbal Drug Selection

The three plant ingredients were selected based on their complementary and synergistic therapeutic profiles. *Curcuma longa* Linn. serves as the primary anti-inflammatory agent through curcumin-mediated inhibition of cyclooxygenase, lipoxygenase and pro-inflammatory cytokines, while also providing strong antioxidant activity that reduces oxidative stress and promotes tissue repair. *Vitex negundo* Linn. complements this action by inhibiting prostaglandin synthesis via its flavonoids, iridoid glycosides and volatile oils, making it particularly effective against pain and musculoskeletal inflammation. *Moringa oleifera* Lam. was incorporated for its broad-spectrum bioactive compounds quercetin, kaempferol and essential vitamins which reinforce the anti-inflammatory and antioxidant effects, while additionally contributing skin nourishment, hydration, tissue regeneration and antimicrobial protection against secondary infections at inflamed sites. Together, these three botanicals offer a multitargeted approach to topical inflammation management, addressing not only the inflammatory cascade but also oxidative damage, pain and skin integrity.

II. PLANT PROFILE

Curcuma longa Linn.

Turmeric consists of dried as well as fresh rhizomes of the plant known as *Curcuma longa* Linn. (*C. domestica*), belonging to the family Zingiberaceae. It should contain not less than 1.5 per cent of curcumin.^[6]

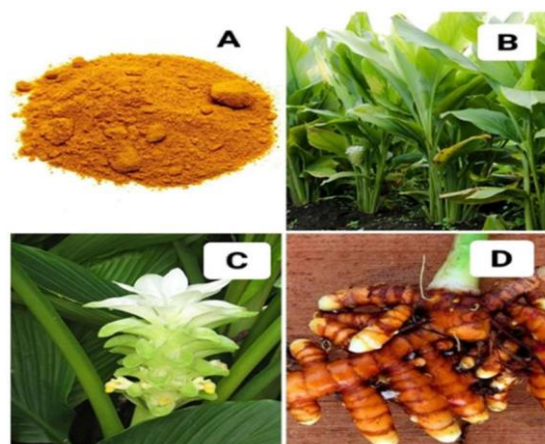


Figure 1

- A. Dried rhizome powder of *Curcuma longa* Linn.
- B. *Curcuma longa* Linn. plant
- C. Flower of *Curcuma longa* Linn.
- D. Fresh rhizome of *Curcuma longa* Linn.

Vitex negundo Linn.

Vitex negundo Linn., an aromatic shrub growing to a small tree, belongs to the family Verbenaceae.^[6]

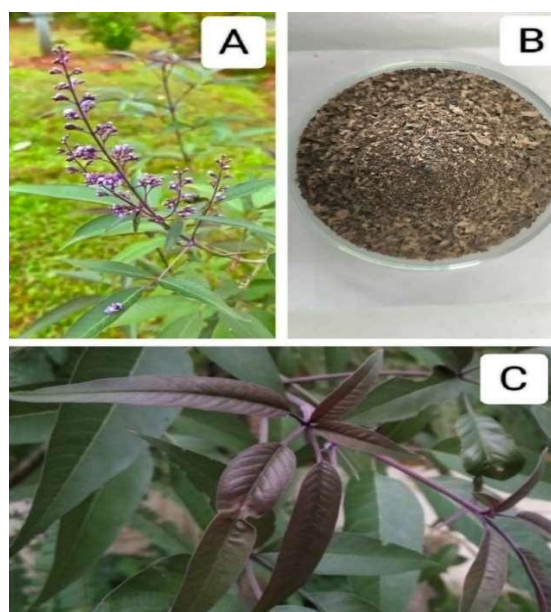


Figure 2:

- A. Aerial part showing inflorescence of *Vitex negundo* Linn.
- B. Dried leaf powder of *Vitex negundo* Linn.
- C. Leaves of *Vitex negundo* Linn.

Moringa oleifera Lam.

Moringa consists of the dried leaves, as well as the seeds, pods and other parts, of the plant known as *Moringa oleifera* Lam., belonging to the family Moringaceae.^[7]

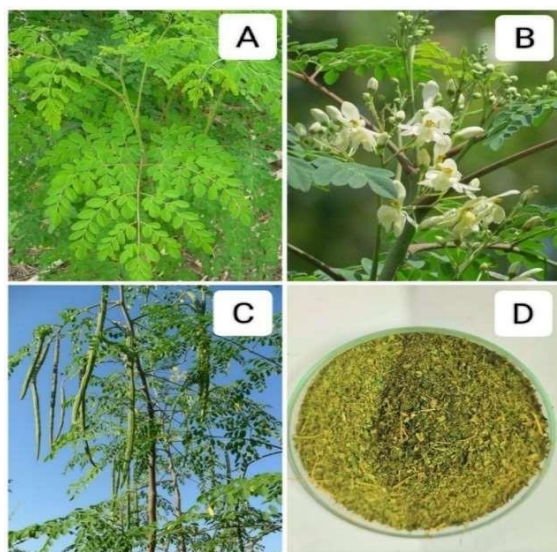


Figure 3

- A. Leaves of *Moringa oleifera* Lam.
 B. Flowering twig of *Moringa oleifera* Lam.
 C. Plant bearing elongated pods
 D. Dried leaf powder of *Moringa oleifera* Lam.

III. MATERIALS AND METHODS

Extraction of plant materials

50 g of dried, coarsely powdered leaves of *Vitex negundo* Linn. and 16 g of *Moringa oleifera* Lam. were extracted using a Soxhlet apparatus with ethyl alcohol (95%) and water in the ratio 3:2 and 50 g of dried, coarsely powdered rhizomes of *Curcuma longa* Linn. were extracted using a Soxhlet apparatus with 95% ethyl alcohol. The specified temperatures were maintained throughout the extraction processes. To ensure the complete extraction process, exhaustive extraction was continued for about 6-8 cycles. The extracts were filtered and collected separately in dried, tared, clean porcelain evaporating dishes and evaporated using a water bath. [8]

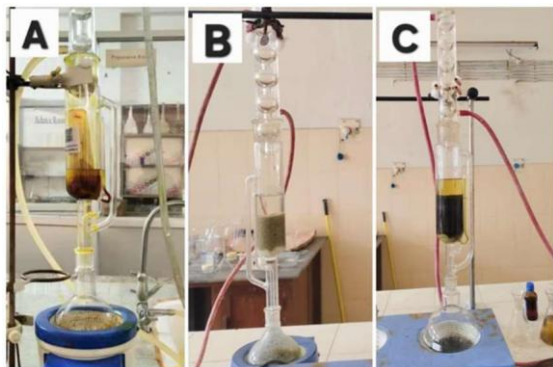


Figure 4:

- A. Soxhlet extraction of *Curcuma longa* Linn.

rhizome powder

B. Soxhlet extraction of *Moringa oleifera* Lam. leaves

C. Soxhlet extraction of *Vitex negundo* Linn. Leaves

Preliminary phytochemical

screening Preliminary phytochemical screening of the extracts was carried out to identify different classes of phytoconstituents present in extracts, such as carbohydrates, proteins, lipids, flavonoids, tannins, glycosides, alkaloids, essential oil etc. All the extracts were subjected to preliminary phytochemical screening as per the standard procedures [9]

Formulation of polyherbal topical anti-inflammatory cream

Cream is characterized as a sort of semisolid emulsion that is meant to be applied externally. It can be either water in oil (w/o) or oil in water (o/w). Cold cream is a water-in-oil (w/o) emulsion, a semi-solid preparation used for skin soothing and cleansing, often containing ingredients like beeswax, mineral oil and borax known for its moisturizing and protective properties. The purpose of cold cream, an emulsion-based moisturizer, is to protect, soothe and hydrate the skin.

We are preparing a polyherbal topical anti-inflammatory cream using herbal extracts of powdered rhizomes of *Curcuma longa* Linn., leaves of *Vitex negundo* Linn. and leaves of *Moringa oleifera* Lam. that are incorporated into a cold cream base.

Main ingredients are:

- Beeswax: In cold creams beeswax acts as a thickening agent and also as the base for the cream. Beeswax also forms a part of the emulsifier. Beeswax also has additional protective effects as it is a humectant and aids in the healing and softening of the skin.
- Liquid paraffin: It is used as an emollient to soften and moisturize the skin in topical formulations and it forms a protective layer on the skin surface to prevent moisture loss and improve skin hydration.
- Borax: In cold cream, borax serves primarily as an emulsifier and a preservative helping to stabilize the mixture of oil and water and preventing bacteria and fungi thus extending the cream's shelf life and it is also responsible for the whitening of cold cream.
- Methyl paraben: It is used as a preservative to prevent the growth of bacteria and fungi in pharmaceutical and cosmetic products and also helps extend the shelf life of formulations by maintaining

product stability and safety.^[10]

5.5.1. Formulation of the cream

This study focused on the preparation of a topical polyherbal anti-inflammatory cream using plant extracts known for their therapeutic potential. Therefore, formulations (F1, F2 and F3) were prepared using different concentration of selected plant extracts. The extracts of rhizomes of *Curcuma longa* Linn., leaves of *Moringa oleifera* Lam. and leaves of *Vitex negundo* Linn., are incorporated into a standard cold cream base. Using the same base for all formulations ensures uniformity and allows for comparative assessment of their physical characteristics and potential anti-inflammatory effectiveness. The ingredients and their respective quantities used in the preparation of three cream formulations (F1, F2, and F3) are shown in Table 1.

Procedure

- Preparation of oil phase: Accurately weighed 22.5 ml of liquid paraffin and 7.5 g of beeswax were transferred into a clean, tared china dish and heated to melt at a temperature of 70°C. The contents were stirred continuously to ensure uniform mixing. The temperature was maintained at a constant temperature of 70°C and it was regularly monitored using a thermometer throughout the process.
- Preparation of aqueous phase: Accurately weighed 0.075 g of methylparaben and 0.0375 g of borax were transferred into a clean china dish and dissolved in 7.5 ml of purified water with continuous stirring. The solution was then heated to 70°C using a water bath. The temperature was maintained at 70°C and regularly monitored with a thermometer, while the contents were uniformly stirred using a glass rod to ensure complete dissolution.
- Preparation of cream base: When the specified temperature (70°C) was reached, the contents of the oil phase were transferred into a clean, dry mortar. Then contents of the aqueous phase were gradually added to the oil phase with continuous and uniform stirring using a pestle. Stirring was carried out in one direction to ensure proper mixing and to avoid incorporation of air. Stirring was continued until a smooth, uniform cream was produced.
- Preparation of herbal anti-inflammatory creams: For formulation F1, *Curcuma longa* Linn. extract (0.3 g), *Vitex negundo* Linn. extract (0.3 g) and *Moringa oleifera* Lam. extract (0.15 g) were accurately weighed and incorporated into the cooled cream base with continuous trituration until a smooth and homogeneous cream was obtained. For

formulation F2, *Curcuma longa* Linn. extract (0.6 g), *Vitex negundo* Linn. extract (0.6 g) and *Moringa oleifera* Lam. extract (0.3 g) were incorporated into the cream base in the same manner as described in the preparation of formulation F1 and stirring was continued until a smooth cream was formed. For formulation F3, *Curcuma longa* Linn. (0.9 g), *Vitex negundo* Linn. extract (0.9 g) and *Moringa oleifera* Lam. extract (0.45 g) were added to the cream base and mixed thoroughly until a smooth cream was formed. For aroma, rose oil (q.s.) was added to all three formulations and mixed well.^[11]

Table 1: Composition of different cream formulations (30g).^[12]

Sl No	Ingredients	F1	F2	F3
1	<i>Curcuma longa</i> Linn. extract	0.3 g	0.6 g	0.9g
2	<i>Vitex negundo</i> Linn. extract	0.3 g	0.6 g	0.9g
3	<i>Moringa oleifera</i> Lam. extract	0.15g	0.3 g	0.45g
4	Beeswax	7.5 g	7.5 g	7.5g
5	Liquid paraffin	22.5 ml	22.5 ml	22.5 ml
6	Borax	0.375 g	0.375 g	0.375g
7	Methylparaben	0.075 g	0.075 g	0.075 g
8	Water	7.5ml	7.5ml	7.5ml
9	Rose oil	q.s.	q.s.	q.s.

Evaluation of cream

The evaluation of creams is an important process in pharmaceutical and cosmetic science to ensure their quality, safety and effectiveness. Through proper evaluation, creams can be standardized for consistency, therapeutic performance and consumer acceptability, ensuring they meet required regulatory and quality standards.

1. Organoleptic Evaluation

The formulated creams were evaluated by observing their colour, odour, texture and state.^[13]

2. pH

The pH of a topical anti-inflammatory cream is a critical parameter, as it should be compatible with the normal skin pH to avoid irritation and to ensure

formulation stability and therapeutic effectiveness. The pH of the formulation was determined using a digital pH meter that was previously calibrated with standard buffer solutions. About 0.5 g of the cream was accurately weighed and dispersed in 50.0 mL of distilled water, and the electrode was immersed in the prepared dispersion. The pH value was recorded after the reading stabilized. The average of three readings was taken as the final pH.^[14]

3. Viscosity

Viscosity is significant as it influences the spreadability, stability, consistency, drug release, patient acceptability and overall quality of the formulation, making it an essential parameter in pharmaceutical evaluation.

The viscosity of the cream formulations was determined using a Brookfield viscometer fitted with spindle S-64 at 20 rpm, with the sample maintained at $25 \pm 1^\circ\text{C}$. An adequate amount of formulation was placed in a beaker, the spindle was immersed without touching the walls and readings were recorded after stabilization. The average of three readings was taken as the final viscosity.^[14]

4. Thermal stability test

The BOD incubator was set to 60–70% relative humidity and maintained at a temperature of $45 \pm 1^\circ\text{C}$. Using a spatula, the cream samples were transferred into clean, dry petri dishes and gently tapped to allow uniform settling of the samples at the bottom. The petri dishes were filled to one-third of their capacity and the lids were securely closed.

The prepared petri dishes were then placed in the incubator and maintained at $45 \pm 1^\circ\text{C}$ for 48 hours. After the completion of the test period, the samples were removed and examined for any signs of physical instability such as phase separation, creaming, cracking, or changes in appearance.^[14]

5. Homogeneity

The different cream formulations were tested for homogeneity by visual appearance and by touch.^[15]

6. Spreadability

The spreadability was expressed in terms of time thesecondstaken by two slides to slip off from the cream, placed in between the slides, under a

certain load. The lesser the time taken for separation of the two slides, the better the spreadability. Two sets of glass slides of standard dimension were taken. Then one slide of suitable dimension was taken and the cream formulation was placed on that slide. Then the other slide was placed on top of the formulation. Then a weight or certain load was placed on the upper slide so that the cream between

the two slides was pressed uniformly to form a thin layer. Then the weight was removed and excess formulation adhering to the slides was scraped off. The upper slide was allowed to slip off freely by the force of weight tied to it. The time taken by the upper slide to slip off was noted.^[16]

Spreadability = $m \times l / t$

Where,

m = Standard weight which is tied to or placed over the upper slide (50g)

l = length of glass slide (5cm) t = time taken in seconds.

7. Irritancy test

Marked an area (1 sq.cm) on the dorsal surface of the hand. The different cream formulations were applied to the specified area and the time was noted. The treated area was observed at regular intervals for up to 24 hours for any signs of redness, edema, inflammation or irritation and the observations were recorded.^[13]

8. Washability

A small amount of different cream formulations was applied on the dorsal surface of the hand and it was then washed with tap water.^[13]

9. Greasiness

The cream formulations were applied to the skin in the form of a smear and the texture of the smear was examined to determine whether it exhibited an oily or greasy nature.

10. Phase separation

Prepared cream formulations were kept in a closed container at room temperature away from light. Then phase separation was checked for 24 hours for 30 days. Any change in the phase separation was observed/checked.^[15]

11. In-vitro Antioxidant Activity DPPH radical scavenging assay

Reagents:

A 0.1 mM DPPH solution was prepared by dissolving 4 mg of DPPH in 100 ml of methanol.

Procedure:

A 0.1 mM solution of DPPH in methanol was prepared and it was protected from light influence by maintaining the dark condition and also by covering the test tube with aluminium foil. 3 ml of this solution were added to 1 ml of various concentrations (25–250 $\mu\text{g/ml}$) of formulations and a standard solution of ascorbic acid (6.25–100 $\mu\text{g/ml}$). Absorbance was taken after 30 minutes at 517 nm. The percentage inhibition activity was calculated.^[17]

Statistical analysis:

Statistical analysis was done by one-way ANOVA

followed by Tukey's multiple comparison test using GraphPad Prism Version 9.5.1.

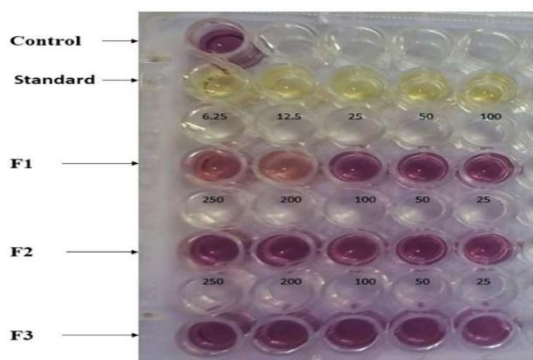


Figure 6: DPPH radical scavenging activity of standard Ascorbic acid and formulations (F1, F2 and F3) at different concentrations.

12. In-vitro Anti-inflammatory Activity Protein Denaturation Using Bovine Serum Albumin

Reagents:
 0.5% Bovine Serum Albumin (BSA), Phosphate Buffer Saline pH 6.3.

Procedure:
 0.05 ml of various concentrations (31.25, 62.25, 125, 250 and 500 µg/ml) of the cream formulation F1 and the standard drug Diclofenac sodium (20, 40, 60, 80 and 100 µg/ml) were taken respectively. Then 0.45 ml (0.5% w/v) BSA was mixed in all tubes. The samples were incubated at 37°C for 20 minutes and the temperature was increased to keep the samples at 57°C for 3 minutes.

After cooling, added 2.5 ml of phosphate buffer to the above solutions. The absorbance was measured using a UV-Visible spectrophotometer at 660 nm. The control represents 100% protein denaturation. The results were compared with Diclofenac sodium. [18, 19]

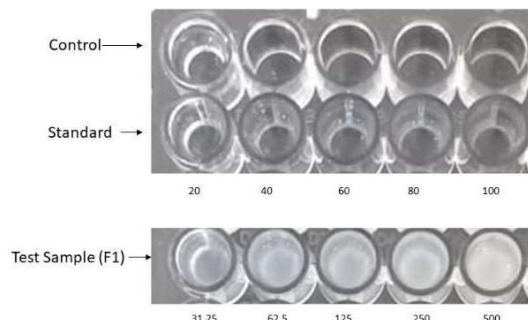


Figure 7: Anti-inflammatory activity of standard Diclofenac sodium and formulation F1 evaluated by protein denaturation assay at different

concentrations.

Statistical analysis:

Statistical analysis was done by one-way ANOVA followed by Tukey's multiple comparison test using GraphPad Prism Version 9.5.1.

IV. RESULTS AND DISCUSSION

Phytochemical Studies

Preliminary phytochemical screening revealed that the alcoholic extract of *Curcuma longa* Linn. contained carbohydrates, alkaloids, glycosides, flavonoids, tannins and phenolic compounds, and steroids and triterpenoids, while the hydroalcoholic extracts of *Vitex negundo* Linn. and *Moringa oleifera* Lam. showed the presence of all the above constituents except steroids and triterpenoids. The common presence of flavonoids, tannins and phenolic compounds across all three extracts supports their antioxidant and anti-inflammatory potential.

Evaluation of Cream

1. Physical appearance

The results of the physical evaluation of the different formulations of cream were shown in Table 2.

Table 2: Physical evaluation parameters of the different formulations of cream F1, F2 and F3.

Formula	Colour	Odour	Texture	State
F1	Orange-brown	Pleasant	smooth	Semi-solid
F2	Orange-brown	Pleasant	smooth	Semi-solid
F3	Orange-brown	Pleasant	smooth	Semi-solid

The creams were found to be smooth, semisolid, and showed good aesthetic appeal and uniform blending of ingredients.

2. pH

The pH values of the different cream formulations are presented in Table 3.

Table 3: Evaluation of pH of different formulations of cream.

Sl.No.	Formulation	pH
1	F1	6.5±0.05
2	F2	6.5±0.05

3	F3	6.5±0.10
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Values were expressed as Mean±SD, n=3.

The pH of the formulated creams was found to be 6.5, which is good for skin pH.

3. Viscosity

The viscosity of the formulations was determined and the results are shown in Table 4.

Table 4: Viscosity of formulated creams.

Sl. No.	Formulation	Viscosity (cP)
1	F1	12600±200
2	F2	12300±200
3	F3	12500±200

Values were expressed as Mean±SD, n=3

The viscosity values of the formulations indicate that the creams exhibit good consistency, with adequate thickness to ensure stability, optimal spreadability and effective retention on the skin.

4. Homogeneity

Visual inspection of the formulations was carried out. The formulations were found to be homogeneous, with no lumps or gritty particles, confirming their smooth texture and absence of particulate impurities.

5. Thermal stability

Formulations F1, F2 and F3 passed the thermal stability test and showed no evidence of phase separation or oil separation.

6. Spreadability

The results of the spreadability tests were as shown in Table 5.

Table 5: Spreadability values (g.cm/s) of prepared formulations F1, F2 and F3.

Sl. no.	Formulation	Spreadability (g.cm/s)
1	F1	9.75± 0.02
2	F2	9.64±0.02.
3	F3	9.46± 0.03

Values were expressed as Mean±SD, n=3.

The spreadability of the formulated creams was determined in triplicate and found as 9.75 g·cm/s, 9.64 g·cm/s and 9.46 g·cm/s, respectively.

The mean spreadability value was calculated as 9.62 ± 0.085 g·cm/s, indicating satisfactory spreading characteristics of the formulation.

7. Irritancy test

Formulations F1 and F2 exhibited no signs of redness, edema, inflammation or irritation during the skin irritancy studies and were therefore considered suitable for topical application. In contrast, formulation F3 produced mild skin irritation upon topical application and was thus deemed unsuitable for topical use.

8. Washability

The prepared cream formulations showed moderate washability when washed with running water. Upon application to the skin, the creams formed a smooth, uniform layer and required mild rubbing for complete removal.

9. Greasiness

The greasiness test demonstrated that the prepared water-in-oil (w/o) topical cream formulations produced a smooth and uniform smear upon application to the skin. The formulations left slightly oily and greasy films after spreading, which is characteristic of w/o creams. The smears remained on the skin for a longer duration, indicating enhanced retention and prolonged contact with the skin surface.

10. Phase separation

Phase separation and stability of formulations F1, F2, and F3 were evaluated after storage at room temperature for 30 days, as summarized in Table 6. Table 6: Phase separation study of different cream formulations.

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

In-vitro antioxidant activity of all the formulations was evaluated by the DPPH assay method. The percentage inhibitions and IC₅₀ values of different formulations of creams were compared to the percentage inhibition and IC₅₀ values obtained with the standard ascorbic acid and depicted in Table 7.

The values were statistically analyzed by the One-way ANOVA Tukey's multiple comparison test. *** P value < 0.001. Therefore, considered as significant

Sl. no.	Formulation	Observation	Inference
1	F1	No phase separation	Stable
2	F2	No phase separation	Stable

3	F3	No phase separation	Stable
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The phase separation study revealed that all the prepared formulations (F1, F2, and F3) remained homogeneous throughout the observation period. No evidence of phase separation was observed when the creams were stored in closed containers at room temperature for 30 days. All formulations retained

their original consistency and appearance, indicating good physical stability.

11. In-vitro antioxidant activity

Table 7: Mean absorbance, percentage inhibition and IC50 value of standard Ascorbic acid and different formulations of cream such as F1, F2 and F3.

Sample	Concentration (µg/ml)	Absorbance at 517 nm	Percentage inhibition (%)	IC50 value (µg/ml)
Control	-	1.392±0.0004	-	
Standard ascorbic acid	6.25	0.844±0.0003	39.35	10.2
	12.5	0.612±0.0006	56.03	
	25	0.488±0.0007	64.94	
	50	0.236±0.0009	83.08	
	100	0.119±0.0010	91.47	
Formulation 1	25	1.243±0.0005	10.70	207.07
	50	1.176±0.0007	15.52	
	100	0.998±0.0008	28.30	
	200	0.802±0.0012	42.39	

Formulation 2	250	0.507±0.0004	63.58	243.13
	25	1.186±0.0005	14.80	
	50	1.129±0.0007	18.89	
	100	1.037±0.0007	25.50	
	200	0.878±0.0009	36.93	
	250	0.614±0.0011	55.89	
Formulation 3	25	1.239±0.0003	10.99	268.91
	50	1.197±0.0006	14.01	
	100	1.117±0.0004	19.76	
	200	0.897±0.0008	35.56	
	250	0.699±0.0012	49.78	

Values were expressed as Mean ± SEM, n=3.

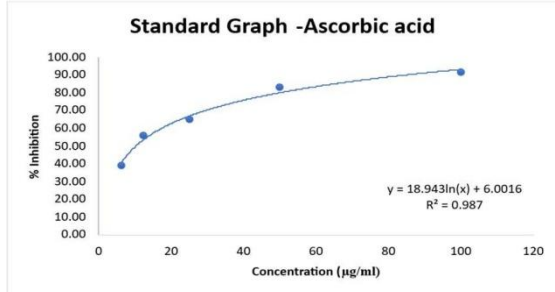


Figure 8: Percentage inhibition of standard Ascorbic acid

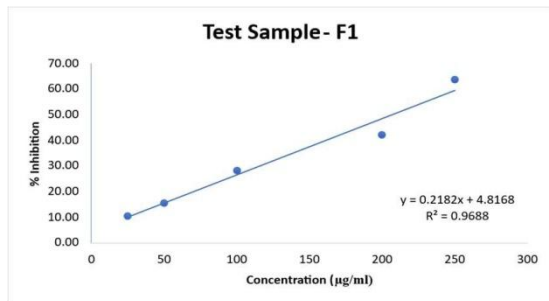


Figure 9: Percentage inhibition of formulation F1.

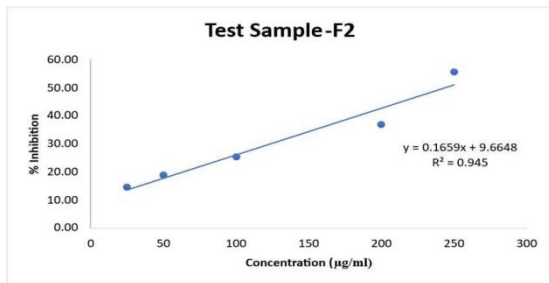


Figure 10: Percentage inhibition of formulation F2.

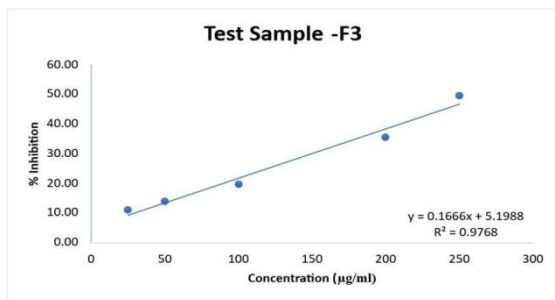


Figure 11: Percentage inhibition of formulation F3.

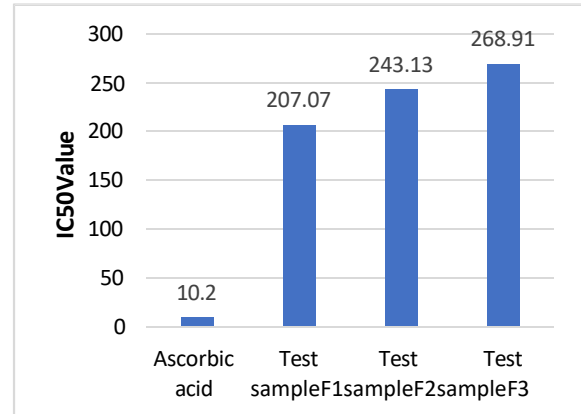


Figure 12: Comparison of IC₅₀ values of formulations F1, F2 and F3 with standard ascorbic acid.

The antioxidant activity of the formulations showed an inverse relationship between percentage inhibition and IC₅₀ values. Formulation F1 exhibited the highest percentage inhibition with the lowest IC₅₀ value, followed by F2 and F3, indicating superior antioxidant activity. Although F3 showed antioxidant potential, it exhibited mild skin irritation. Therefore, considering both efficacy and skin compatibility, formulation F1 was selected as the optimized formulation for further in-vitro anti-inflammatory studies.

12. In-vitro anti-inflammatory assay Protein Denaturation Assay

In-vitro anti-inflammatory activity of Formulation 1 was evaluated by Protein Denaturation Assay. The results of the protein denaturation assay shown in the table 8 and figure 13, 14 and 15.

Table 8: Mean absorbance, percentage inhibition and IC50 value of standard Diclofenac sodium and formulation F1.

Sample	Concentration (µg/ml)	Absorbance at 660 nm	Percentage Inhibition (%)	IC50 value (µg/ml)
Control	-	0.192± 0.0009	-	
Standard Diclofenac	20	0.145± 0.0050	24.47	48.46
	40	0.101± 0.0018	47.32	
	60	0.073± 0.0027	62.21	
	80	0.055± 0.0038	71.26	
	100	0.022± 0.0009	88.76	
Control	-	1.202± 0.0006	-	
Formulation F1	31.25	0.995± 0.0066	17.26	80.84
	62.5	0.614± 0.0068	48.91	
	125	0.355± 0.0026	70.44	
	250	0.256± 0.0110	78.70	
	500	0.127± 0.0029	89.44	

Values were expressed as Mean ± SEM, n=3, ***P value < 0.001, when compared to control.

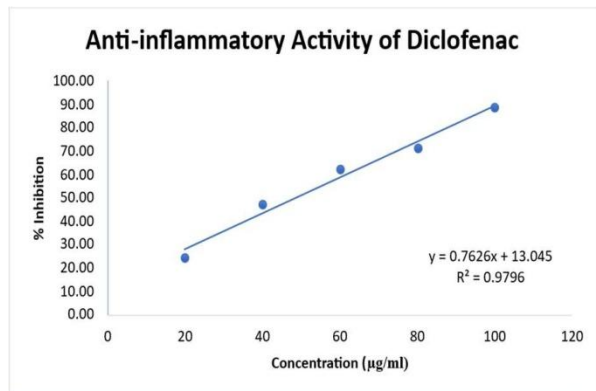


Figure13:CalibrationcurveofstandardDiclofenac sodium.

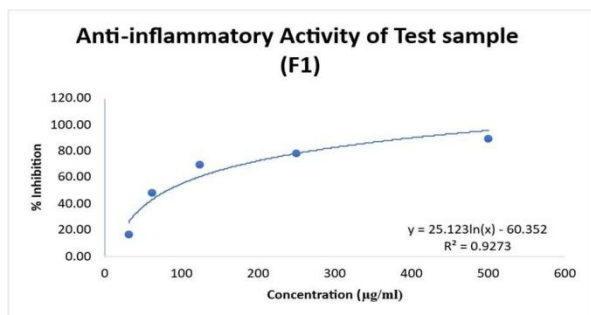


Figure14:CalibrationcurveofformulationF1.

The anti-inflammatory activity of formulation (F1) was evaluated by protein denaturation inhibition assay in a concentration range (31.25–500 µg/ml) and for standard Diclofenac sodium in a concentration range (20–100 µg/ml).

Formulation (F1) and standard Diclofenac sodium inhibited protein denaturation in a concentration dependent manner and figure (20) depicts the inhibitory effect of different concentration (31.25–500 µg/ml) of formulation (F1) and standard Diclofenac sodium.

Values were statistically analysed by One-way ANOVA Tukey's multiple comparison test, *** P value<0.001whencomparedtocontroland

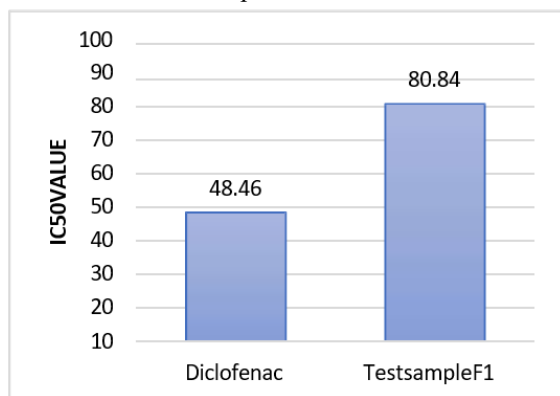


Figure15:ComparisonofIC50Valueofformulation F1 with standard Diclofenac sodium.

comparable to the effect of standard Diclofenac sodium and was found to be significant.

Packaging and Labelling

The prepared polyherbal anti-inflammatory creams were filled into clean and dry containers and properly sealed to prevent contamination. The formulations were stored at room temperature. Each container was labelled with the formulation code, ingredients, date of preparation, storage condition

and the statement. "For external use only".



Figure16:Packagedandlabelledpolyherbal topical anti-inflammatory cream



Figure17:Product label and antioxidant properties. Plant materials were subjected to alcoholic and hydroalcoholic Soxhlet

V. SUMMARY AND CONCLUSION

The present study aimed to formulate and evaluate a polyherbal topical anti-inflammatory cream using extracts of *Curcuma longa* Linn., *Vitex negundo* Linn., and *Moringa oleifera* Lam., well-documented in traditional medicine for their anti-inflammatory

extraction. Phytochemical screening confirmed the presence of flavonoids, phenolic compounds, alkaloids, glycosides, tannins and steroids. Three formulations (F1, F2, F3) were prepared using a cold cream base with varying extract concentrations and evaluated for physicochemical parameters, all of which showed acceptable results with skin-compatible pH, good spreadability and stability. DPPH antioxidant assay identified F1 as having the highest antioxidant activity, which was further subjected to anti-inflammatory evaluation by BSA protein denaturation assay, demonstrating significant activity compared to Diclofenac sodium as standard. Statistical analysis was performed using one-way ANOVA followed by Tukey's test. Overall, F1 was identified as the optimal formulation based on its physicochemical properties, stability, safety and biological activity, while F3 showed mild skin irritation. The study concludes that formulation F1 is safe, stable and effective, offering a promising natural alternative to synthetic anti-inflammatory preparations, though further in-vivo studies, clinical trials and long-term stability assessments are

recommended.

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