Formulation And Evaluation of Herbal Anti-Inflammatory Cream from *Delonix Regia* Seed Oil

[1]Nandini Saxena, [2]Manish Kamble
[1,2]Kamla Nehru College of Pharmacy, Butibori, Nagpur. (M.S.) India-441108
[2]manish.kamble2109@gmail.com

Abstract-Inflammation, a vital immune response to harmful stimuli, can become detrimental when prolonged or excessive, contributing to diseases such as arthritis and cardiovascular conditions. While nonsteroidal anti-inflammatory drugs (NSAIDs) offer effective relief, their long-term use is often limited due to significant adverse effects. In pursuit of safer alternatives, plant-derived therapeutics have gained momentum. This study focuses on *Delonix regia* seed oil, known for its rich phytochemical profile, to develop and evaluate a topical anti-inflammatory cream. The oil was extracted via Soxhlet method and screened for phytochemicals, revealing the presence of flavonoids, steroids, tannins, and volatile oils. Physicochemical analysis confirmed its suitability for topical use, exhibiting optimal viscosity, saponification value, and emollient properties. An oil-in-water (o/w) cream was formulated using standard excipients and evaluated for organoleptic properties, pH, spreadability, irritancy, and stability. The formulation showed excellent skin compatibility, non-greasiness, and stability over time. In vitro anti-inflammatory activity was assessed using the human red blood cell (HRBC) membrane stabilization method. Results demonstrated concentration-dependent membrane protection, with maximum inhibition of hemolysis (33.6%) at 1000 µg/mL, indicating moderate anti-inflammatory activity. While less potent than ibuprofen (85.2% inhibition), the herbal cream displayed promising therapeutic potential with minimal irritancy. The findings support the use of *Delonix regia* seed oil in topical anti-inflammatory applications, particularly for populations seeking natural, low-risk alternatives. This research contributes to the advancement of herbal formulations as effective, economical, and safer options in managing inflammation-related skin conditions.

Index Terms—*Delonix regia*, Seed oil, anti-inflammatory, cream formulation, HRBC assay, phytochemicals.

I. INTRODUCTION

Inflammation is a complex biological response triggered by the body in reaction to harmful stimuli such as pathogens, damaged cells, or irritants. It plays a crucial role in initiating tissue repair and eliminating the causative agents. This defense mechanism is characterized by cardinal signs including redness, heat, swelling, and pain, which arise due to increased vascular permeability and the migration of leukocytes to the affected site [1]. While acute inflammation is beneficial, chronic or excessive inflammation can lead to tissue damage and contribute to various pathologies including arthritis, cardiovascular disorders, and autoimmune diseases [2]. Current therapeutic strategies for managing inflammation primarily rely on nonsteroidal anti-inflammatory drugs (NSAIDs) like diclofenac and aspirin. Although effective, these agents often carry adverse effects such as gastrointestinal erosion, peptic ulcers, nephrotoxicity, leukopenia, and hypersensitivity reactions, especially when used long-term [3]. Hence, there is an urgent need for safer alternatives with better therapeutic profiles. Natural compounds derived from plants have long been used in traditional medicine for their anti-inflammatory potential, and recent pharmacological studies validate many of these traditional claims [4]. Herbal formulations, especially in topical forms like creams and liniments, are increasingly being explored for their localized and safer action.

Numerous botanicals have been studied for their anti-inflammatory effects. For instance, turmeric (Curcuma longa), rich in curcumin, is known to inhibit inflammatory enzymes and cytokines [5]. Similarly, ginger (Zingiber officinale) and boswellia (Boswellia serrata) possess compounds that suppress inflammation and oxidative stress. The Unani system of medicine also includes many anti-inflammatory herbs such as Colchicum luteum (Suranjan) [6], Alpinia galanga (Khulanjan) [7], Matricaria chamomilla (Babuna) [8], Astragalus hamosus (Nakhuna) [9], Withania somnifera (Ashwagandha) [10], Ricinus communis (Arand) [11], Smilax china (Chobchini) [12], Commiphora mukul (Muqil) [13], Zingiber officinale (Zanjabeel) [14], and Datura stramonium (Dhatura) [15]. Among these promising plants, Delonix regia, commonly known as the flamboyant or Gulmohar tree, has recently gained attention not only for its ornamental value but also for its medicinal potential. The seeds of Delonix regia are a source of oil that contains a wide spectrum of phytochemicals, including flavonoids, phenolic acids, tannins, sterols, and essential fatty acids. These constituents are believed to contribute significantly to its anti-inflammatory and antioxidant activities [16]. Preliminary studies suggest that Delonix regia seed oil can down regulate pro-inflammatory cytokines and enzymes, thus modulating inflammatory pathways [17].

The demand for herbal remedies is especially relevant in rural and underserved areas, where access to conventional pharmaceuticals is limited. Herbal formulations like Delonix regia oil-based cream can serve as effective and economical alternatives, with fewer side effects and holistic benefits. Moreover, the synergistic action of various bioactives in the oil can provide broader therapeutic outcomes compared to synthetic agents [18]. Its safety profile, particularly in sensitive populations such as children and the elderly, adds to its appeal. Given these attributes,

this study aims to explore the formulation and evaluation of a topical anti-inflammatory cream using Delonix regia seed oil. The objective is to assess its phytochemical composition, physicochemical properties, and in vitro anti-inflammatory activity using the HRBC membrane stabilization method. The findings may contribute to the development of novel herbal therapeutics with clinical significance in managing inflammatory conditions [19].

II. MATERIALS AND METHODS

2.1 Procurement and Authentication:

Delonix regia seeds were procured from the garden of Kamla Nehru College of Pharmacy and authenticated by Dr. Nitin Dongarwar, Head, Department of Botany, RTMNU, Nagpur. Seeds were collected from mature trees to ensure optimal phytochemical content. Authentication involved morphological comparison with standard botanical references and documentation, including phytosanitary clearances.

2.2 Extraction of Seed Oil:

Seed oil of *Delonix regia* was extracted through soxlation. Alternate methods are cold-press or Soxhlet methods. Cold pressing preserved sensitive phytochemicals, while Soxhlet extraction using hexane or ethanol yielded higher quantities. Oil yield was calculated based on initial and final weights. Extracted oil was stored in amber bottles under refrigeration to protect bioactive compounds [20].

Fig 1: Soxhlet Extraction of Seeds of *Delonix regia*

2.3 Preliminary Phytochemical Screening:

Phytochemical screening of *Delonix regia* seed oil extract was performed using standard qualitative methods to detect alkaloids (Dragendorff's, Mayer's, Hager's, Wagner's tests), saponins (foam test), glycosides (Legal, Baljet, Keller-Killiani, Borntrager's tests), reducing sugars (Molisch's, Fehling's, Benedict's tests), tannins and phenolics (lead acetate, ferric chloride), flavonoids (Shinoda's, NaOH, amyl alcohol), and steroids (Libermann-Burchard, Salkowski).

2.4 Physicochemical Analysis:
Parameters like color, odor, density, viscosity, refractive index, acid value, saponification value, and iodine value were determined using standard protocols to evaluate physical characteristics and chemical stability of the seed oil [21].

2.5 Preparation of o/w Cream Formulation:
The oil-in-water cream was prepared by melting stearic acid and lanolin with seed extract at 70°C. An aqueous phase of water, glycerin, and triethanolamine was warmed separately and mixed with the oil phase under constant stirring. Preservatives and perfume were added for stability and appeal [22].

Table 1: Formula for o/w cream

Sr.no.	Ingredient	Uses	Quantity (Two different Formulations)	
			Formulation 1	Formulation 2
1.	Stearic acid	Emulsifier, Emollient, Stabilizer	6.79 g	6.79 g
2.	Glycerine	Humectant	0.25ml	0.23ml
3.	Extract	Active ingredient	1ml	0.5 ml
3.	Lanolin	Binding property, enhance skin texture, Spread ability	1.13 g	1.13 g
4.	Triethanolamine	pH adjuster, Thickener	1ml	0.08ml
5.	Water	Continuous phase	Q. S	Q. S
6.	Methyl paraben	Preservative	1 g	1g
7.	Perfume	Pleasant fragrance	1ml	1ml

2.6 Evaluation Parameters:
The cream was assessed for pH, viscosity, homogeneity, dye solubility, spread ability, irritancy, smear type, and stability [23]. Organoleptic properties were also noted. Accelerated stability studies were performed over 20 days to monitor consistency and performance.

2.7 Assessment of in-vitro Anti-Inflammatory Activity (HRBC Membrane Stability Method):
Fresh human blood was treated with Alsever's solution and processed to isolate RBCs. A 10% HRBC suspension was exposed to varying concentrations of plant extract and Diclofenac sodium. Hemolysis was induced using hypotonic solution, and hemoglobin release was measured at 560 nm. Percentage protection of RBC membranes was calculated to assess anti-inflammatory activity [24].

III. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

The phytochemical screening of *Delonix regia* seed oil confirmed the presence of various biologically active compounds. The qualitative tests were carried out using standard reagents and methods. The results are summarized below in Table 2:

Table 2: Phytochemical Test Results

Sr. No	Phytochemicals	Test Methods	Observations	Result
1	Flavonoids	Extract + conc. H2SO4	Yellow colour	Positive
2	Polysterol	Ferric chloride test	Blackish green colour	Positive
3	Carbohydrates	Benedict's test	Brick red colour	Positive
4	Steroids	Salkowski test	Red coloured ppt	Positive
5	Tannins	Braymer test	Blue green colour	Positive
6	Volatile oils	Spot test	Spot on filter paper	Positive

Phytochemical screening of the seed extract revealed the presence of various bioactive compounds. Flavonoids were confirmed by the appearance of a yellow color upon reaction with concentrated H_2SO_4. Polysterols produced a blackish green color in the ferric chloride test, while carbohydrates gave a brick red color with Benedict's reagent. The Salkowski test showed red precipitate indicating steroids, and the Braymer test revealed blue-green coloration, confirming tannins. Volatile oils were detected through a spot test, indicated by an oily mark on filter paper.
These findings indicate that the extracted oil is rich in secondary metabolites which may contribute to its anti-inflammatory and therapeutic properties.

3.2 Physicochemical Analysis

The physicochemical properties of the *Delonix regia* seed oil were evaluated to determine its suitability for topical formulation. The results are shown below in Table 3:

Table 3: Physicochemical Parameters

Sr. No	Parameter	Result
1	Colour	Yellow
2	Odour	Blund
3	Nature	Liquid
4	Viscosity (at 30°C)	74.14 cP
5	Saponification value	185 KOH/g

The extracted seed oil appeared yellow, had a bland odor, and maintained a liquid form. At 30°C, its viscosity was recorded as 74.14 cP, indicating moderate flow behavior. With a saponification value of 185 KOH/g, the oil likely contains medium-chain fatty acids. These properties support its appropriateness for use in topical applications. The overall physicochemical profile, including

the relatively high saponification value and moderate iodine content, highlights its potential as a quality emollient base for cream formulation.

3.3 Evaluation of Cream Formulation

Delonix regia seed oil was incorporated into a cream base, and the resulting formulation was evaluated using standard parameters, and shown in Table 4:

Table 4: Evaluation Parameters of Cream Formulation

Sr. No.	Parameter	Result
1	Colour	Pearl white
2	Odour	Pleasant
3	pH	6.61
4	Viscosity (50 rpm)	1900 cP
5	Dye test	o/w emulsion
6	Homogeneity	Homogenous
7	Patch test	No reaction
8	Irritancy	Non-irritant
9	Accelerated stability	Stable
10	Smear test	Non-greasy
11	Spreadability	Spreadable

The formulated cream was pearl white in color with a pleasant odor, making it aesthetically appealing for topical use. It exhibited a pH of 6.61, which falls within the ideal range for skin compatibility. The viscosity at 50 rpm was measured at 1900 cP, indicating a suitable consistency for smooth application. The dye test confirmed an oil-in-water (o/w) emulsion type, and the cream was found to be homogenous. Patch and irritancy tests showed no adverse reactions, confirming the formulation is safe and non-irritant to the skin. Additionally, the cream demonstrated good spreadability, a non-greasy feel, and remained stable under accelerated stability conditions. These observations suggest that the formulated cream is stable, non-irritant, and user-friendly in terms of texture, spreadability, and aesthetic appearance.

3.4 Anti-inflammatory Activity (HRBC Membrane Stabilization Assay)

The anti-inflammatory potential of the formulated cream was evaluated by the HRBC membrane stabilization method. The inhibition of hemolysis indicates the anti-inflammatory potential of the formulation.

Table 5: Anti-inflammatory Activity

Sr. No.	Concentration (ug/mL)	Absorbance (560 nm)	% Hemolysis	% Inhibition
1	Blank	0.0512	-	-
2	50	0.130	97.65%	2.35%
3	100	0.102	89.84%	10.16%

4	250	0.092	78.12%	21.88%
5	500	0.097	76.16%	23.83%
6	1000	0.128	66.40%	33.60%
7	Standard (Ibuprofen) 1000 ug/mL	0.154	14.8	85.2

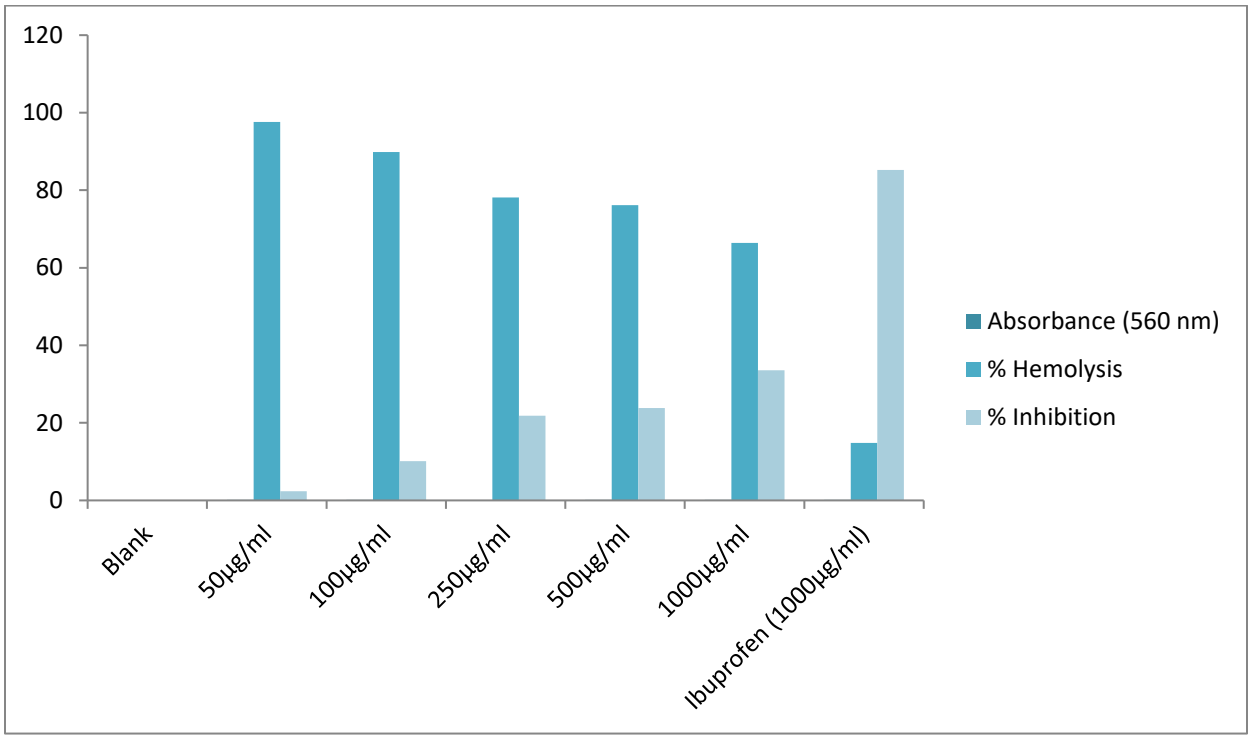

Fig 2: Graph showing HRBC Membrane Stabilization of formulation using Ibuprofen as standard drug.

The anti-inflammatory potential of *Delonix regia* seed oil was assessed in vitro using the HRBC membrane stabilization assay. An increase in absorbance at 560 nm was observed with rising oil concentrations, indicating a concentration-dependent decrease in membrane lysis. At 50 µg/mL, the oil exhibited 2.35% inhibition, which progressively rose to 33.60% at 1000 µg/mL. The highest inhibition at 1000 µg/mL suggests moderate membrane protective effects. In contrast, the standard drug ibuprofen at the same concentration showed 85.2% inhibition, reflecting substantially greater anti-inflammatory efficacy. The blank sample had an absorbance of 0.0512, serving as a reference point for calculating hemolysis. Although the oil's activity was lower than that of the standard, it showed noticeable membrane stabilization at higher doses (Table 5). Overall, *Delonix regia* seed oil demonstrated promising anti-inflammatory activity through concentration-dependent hemolysis inhibition, with peak effectiveness at 1000 µg/mL.

IV. CONCLUSION

The extracted Delonix seed oil was thoroughly analyzed for its phytochemical and physicochemical properties. Phytochemical screening confirmed the presence of flavonoids, polysterols, carbohydrates, steroids, tannins, and volatile oils, all of which contribute to its therapeutic potential. Characteristic color changes during specific tests such as yellow with sulfuric acid (flavonoids) and blackish-green with ferric chloride (polysterols) confirmed these constituents. Physicochemical analysis revealed the oil to be yellow, faintly odorous, and liquid, with a viscosity of 74.14 cP at 30°C. The saponification and iodine values were 185 KOH/g and 88 I_2/100g, respectively, suggesting its suitability for cosmetic formulations like creams. The formulated anti-inflammatory cream exhibited ideal characteristics: pearl-white color, pleasant odor, pH of 6.61, and a viscosity of 1900 cP at 50 rpm. Dye and homogeneity tests confirmed it as a stable oil-in-water (o/w) emulsion. It showed no signs of irritation or adverse effects in patch tests and remained stable under accelerated conditions. Anti-inflammatory activity, evaluated through hemolysis inhibition, showed a concentration-dependent response, with 33.6% inhibition at 1000 µg/ml. These results confirm the formulation's safety, stability, and therapeutic potential as a topical anti-inflammatory agent.

ACKNOWLEDGEMENT

The authors sincerely acknowledge Management and Principal of Kamla Nehru College of Pharmacy, Butibori for providing the necessary facilities and support to carry out this research.

Funding
No financial assistance for the research.

REFERENCES

[1] A. K. Abbas and A. H. Lichtman, *Basic Immunology: Functions and Disorders of the Immune System*, 3rd ed. Philadelphia: Elsevier, 2009.

[2] L. Ferrero-Miliani, O. H. Nielson, P. S. Andersen, and S. E. Girardin, “Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1β generation,” *Clin. Exp. Immunol.*, vol. 147, no. 2, pp. 227–235, 2007.

[3] M. Ziebrou, M. Lompo, N. Ouedraogo, B. Yaro, and I. Guissoun, “Antioxidant, analgesic and anti-inflammatory activities of *Waltheria indica*,” *J. Appl. Pharm. Sci.*, vol. 6, no. 11, pp. 124–129, 2016.

[4] K. M. Anil, “Ethnomedicinal plants as anti-inflammatory and analgesic agents,” *Ethnomedicine*, vol. 10, p. 2673, 2010.

[5] H. M. Ullah *et al*., “In vitro and in vivo anti-inflammatory activity of ethanolic extract of *Curcuma zedoaria*,” *BMC Complement. Altern. Med.*, vol. 14, pp. 1–12, 2014.

[6] V. Nair *et al*., "Anti-inflammatory activity of *Colchicum luteum* Baker," *Inflammation,* vol. 35, pp. 881–887, 2012.
[7] A. K. Ghosh *et al*., "Anti-inflammatory activity of root of *Alpinia galanga*," *Chron. Young Sci.,* vol. 3, no. 2, pp. 139–143, 2011.
[8] G. Hajjaj *et al*., "Anti-inflammatory evaluation of *Matricaria chamomilla*," *World J. Pharm. Res.,* vol. 5, no. 2, pp. 1218–1228, 2013.
[9] A. Shojaii *et al*., "Anti-inflammatory activity of *Astragalus hamosus*," *Iran J. Pharm. Res.,* vol. 14, no. 1, pp. 263–269, 2015.
[10] A. Gupta and S. Singh, "Evaluation of anti-inflammatory effect of *Withania somnifera* on arthritis in rats," *Pharm. Biol.,* 2013.
[11] K. Saini *et al*., "Anti-inflammatory potential of *Ricinus communis* leaf extract," *J. Chem. Pharm. Res.,* vol. 2, no. 5, pp. 690–695, 2010.
[12] I. Khan *et al*., "Anti-inflammatory activities of sieboldogenin from *Smilax china*," *J. Ethnopharmacol.,* vol. 121, pp. 175–177, 2009.
[13] R. Deng, "Therapeutic effects of guggul and guggulsterone," *Cardiovasc. Drug Rev.,* vol. 25, no. 4, pp. 375–390, 2007.
[14] Y. Raji *et al*., "Analgesic and anti-inflammatory properties of *Zingiber officinale*," *Afr. J. Biomed. Res.,* vol. 5, pp. 121–124, 2002.
[15] D. A. Abbas, "Anti-inflammatory effect of *Datura stramonium* leaf extract," *IJRRAS,* vol. 14, no. 1, pp. 193–199, 2013.
[16] S. Singh and N. Kumar, "Introduction to Genus *Delonix*," *WJ Pharm Sci.,* vol. 3, pp. 2042–2055, 2014.
[17] N. Kumar, A. Kaushik, and M. Tuteja, "Evaluation of novel topical polyherbal cream containing *Delonix regia* oil," *J. Pharma Insights Res.,* vol. 2, no. 1, pp. 14–20, 2024.
[18] A. Ahmad, M. A. Khan, and M. S. Hossain, "Therapeutic potential of plant-based anti-inflammatory formulations: A review," *J. Ethnopharmacol.,* vol. 273, p. 113956, 2021.
[19] R. Kumar and V. Singh, "*Delonix regia*: A potential medicinal plant with diverse pharmacological applications," *Phytomedicine Plus,* vol. 1, no. 3, p. 100023, 2020.
[20] J. R. Patel, P. Tripathi, V. Sharma, N. S. Chauhan, and V. K. Dixit, "Extraction and evaluation of herbal oils: A comparative study of cold press and Soxhlet methods," *Int. J. Green Pharm.,* vol. 13, no. 1, pp. 42–47, 2019.
[21] M. S. Rao, L. J. Reddy, and G. Ramesh, "Physicochemical characterization of seed oils of selected Indian medicinal plants," *Indian J. Nat. Prod. Resour.,* vol. 9, no. 2, pp. 123–128, 2018.
[22] V. Sharma, M. Ali, and S. Gupta, "Formulation and evaluation of herbal cream containing *Curcuma longa* extract," *J. Pharmacogn. Phytochem.,* vol. 6, no. 2, pp. 226–229, 2017.
[23] D. M. Patel, B. G. Prajapati, and J. A. Raval, "Formulation and evaluation of herbal cream containing medicinal plant extracts," *Int. J. Pharm. Sci. Res.,* vol. 10, no. 5, pp. 2253–2258, 2019.

[24] M. Kamble, V. Nimbalkar, and J. Baheti, "In-Vitro assay to investigate the anti-inflammatory activity of hydroalcoholic leaves extract of *Acacia auriculiformis* A. Cunn. Ex Benth," *Int. J. Res. Sci. Innov.*, vol. 7, no. 4, pp. 07–09, 2020.