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Original Article

Anti-Inflammatory and Analgesic Effects of *Bixa orellana* Chloroform Extract

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ABSTRACT

Background: Inflammation is a critical defence mechanism employed by the body in response to injury, infection, or harmful stimuli. While Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) are commonly used to manage pain and inflammation, their prolonged use is often associated with adverse effects, including gastrointestinal, cardiovascular, and renal complications. Consequently, there is a growing interest in exploring alternative therapeutic strategies. Among these, plant-derived natural compounds have garnered significant attention due to their rich content of bioactive constituents with potent analgesic and anti-inflammatory properties. **Objectives:** This study aimed to evaluate the preliminary phytochemicals, anti-inflammatory, and analgesic activities of the Chloroform Extract of *Bixa orellana* leaves (CEBO). **Materials and Methods:** Leaves were extracted using cold maceration with chloroform. Preliminary Phytochemical screening was performed to identify key bioactive compounds. Anti-inflammatory activity was assessed using the carrageenan-induced paw edema model. Analgesic activity was evaluated using Eddy's hot plate and tail-flick methods. Statistical analysis was done using two-way ANOVA followed by Tukey's multiple comparison test. **Results:** Phytochemical analysis revealed the presence of flavonoids, alkaloids, terpenoids and phytosterols. Inflammation was significantly reduced in a dose-dependent manner in the carrageenan-induced paw edema model. The extract at 200 mg/kg showed comparable efficacy to indomethacin. CEBO also significantly increased pain threshold in both hot plate and tail-flick tests. **Conclusion:** The chloroform extract of *Bixa orellana* leaves exhibited significant anti-inflammatory and analgesic activities. These findings support its traditional use and suggest the potential for developing Phyto-therapeutic agents. Further studies are recommended to isolate active constituents and explore their mechanisms of action.

Keywords: *Bixa orellana*, Carrageenan, Anti-inflammatory, Analgesic, Phytochemicals, Tail-flick test.

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INTRODUCTION

Inflammation is a fundamental biological process triggered by injury or infection, and it is often accompanied by pain as a key symptom. Inflammatory pain arises because of chemical mediators like prostaglandins, bradykinin, and cytokines, which sensitize nociceptors and intensify pain signaling. Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) are widely used to manage inflammatory pain by inhibiting Cyclooxygenase (COX) enzymes, thereby reducing prostaglandin production. Despite their effectiveness, prolonged NSAID use is linked to gastrointestinal, renal, and cardiovascular complications, which emphasizes the need for safer therapeutic alternatives (Vane and Botting, 1998).

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Pain itself is a complex protective phenomenon, but it can become a clinical issue when it persists or becomes chronic. Various drug classes-including NSAIDs, opioids, anticonvulsants, and antidepressants-are utilized to address different pain mechanisms. However, concerns regarding side effects, tolerance, and dependence continue to push research toward safer and more targeted analgesic therapies (Scholz and Woolf, 2002).

Conventional treatment strategies, especially synthetic analgesics and anti-inflammatory agents, play an essential role in controlling pain and inflammation. Drugs like NSAIDs and opioids remain the mainstay due to their quick and significant relief. However, long-term use often brings notable adverse effects, including gastrointestinal irritation, renal impairment, cardiovascular risks, and the risk of addiction and tolerance associated with opioids (Lanas and Chan, 2017; Benyamin *et al.*, 2008). These challenges have encouraged exploration of alternative therapies, especially those derived from medicinal plants.

Phytochemicals-bioactive compounds present in plants-have shown promising anti-inflammatory and analgesic effects, often

with fewer side effects. Compounds like flavonoids, alkaloids, terpenoids, and polyphenols are being extensively studied for their capacity to modulate inflammatory responses and influence pain perception, without the toxicity commonly observed in synthetic drugs (Calixto, 2000). As a result, there is a growing interest in integrating phytochemical-based medicine as complementary or alternative options for managing pain and inflammation.

Bixa orellana, widely known as the lipstick plant, is traditionally used in several medicinal systems. Native to tropical regions of the Americas (Neotropics), it is now cultivated across tropical and subtropical zones worldwide, including India, Southeast Asia, the Caribbean, and parts of Africa (Prasad and Kashyap, 2013). This small evergreen tree or shrub typically grows 6-8 M tall, with simple, ovate leaves, entire margins, and fragrant bisexual flowers. The plant's fruit is a spiny capsule containing many seeds covered with a vivid orange-red aril rich in the pigment bixin (Prasad and Kashyap, 2013; Vilar *et al*., 2010).

Different parts of *Bixa orellana*, particularly the leaves and seeds, are abundant in phytochemicals such as carotenoids (notably bixin and norbixin), flavonoids, phenolic compounds, glycosides, tannins, saponins, steroids, and alkaloids (Rivera *et al*., 2017; Calixto, 2000). Bixin, the main pigment, is widely utilized as a natural food colorant (E160b) and also in cosmetics and textiles. Traditionally, the plant has been employed to address ailments including fever, sore throat, gastrointestinal disorders, jaundice, hypertension, diabetes, snakebite, and skin infections. In many cultures, it is valued for its anti-inflammatory, antimicrobial, antipyretic, analgesic, and anticonvulsant activities. Decoctions or infusions made from the leaves are often consumed orally or used for gargling, particularly in the treatment of respiratory and digestive conditions (Mbah *et al*., 2012). The present study was conducted to evaluate the anti-inflammatory and analgesic activities of *Bixa orellana* Chloroform leaves Extract (CEBO), administered orally, in animal models of inflammation and pain to validate ethnopharmacological claims.

MATERIALS AND METHODS

Drugs and chemicals

All necessary chemicals, reagents, and laboratory facilities required for the experimental procedures were generously provided by Shri Guru Ram Rai University, Dehradun, India. The chemicals used in the study included Chloroform, Carrageenan, Indomethacin (standard anti-inflammatory drug), Ethanol, Vegetable oil, Carboxy Methyl Cellulose (CMC), Tween 80, and Distilled Water. All reagents and solvents were of analytical grade.

Collection and extraction of plant material

Bixa orenalla, leaves samples were obtained in the month of January 2025 from SGRRU Herbal garden Dehradun. S. K. Singh of BSI, Dehradun authenticate and granted a certificate under reference number BSI/ NRC/ Herb (Ident.)/ 2024-25/997.

The coarsely powdered leaves of *Bixa orellana* was subjected to cold maceration using chloroform as the solvent. Approximately 100 g of dried leaves of *Bixa orellana* powder was immersed and macerated in 500 mL of chloroform in a stopped conical flask and kept at room temperature for 72 hr with intermittent stirring to facilitate maximum extraction of bioactive compounds. After maceration, the mixture was filtered through Whatman No. 1 filter paper. The obtained filtrate was then concentrated using a rotary evaporator under reduced pressure at 45 °C. The crude extract was stored in a well-closed container and protected from light (Bhatt *et al*., 2022; Morris *et al*., 2016; Syamkumar *et al*., 2023).

Phytochemical Screening

Phytochemical screening of the chloroform extract of *Bixa orellana* leaves (CEBO) was performed as per Shaikh and Patil (2020) to identify different phytoconstituents like flavonoids, alkaloids, phenols, carbohydrate, tannins, phytosterols, and terpenoids (Shaikh *et al*., 2020).

Animal and Ethical Aspects

Wistar albino rats (150-180 g) were used as experimental models, with six rats per group. They were given a 7-day acclimatization period to the laboratory environment and were provided with food and water ad libitum. The work protocol was approved by the Institutional Animal Ethics Committee (IAEC No. 264/ CPCSEA/IAEC/2025/08), and the study was conducted in the School of Pharmaceutical Sciences, Shri Guru Ram Rai University, Dehradun, in 2025 (Nyiramugisha and Odoma, 2021; Hassan *et al*., 2024; Yong *et al*., 2013).

Anti-Inflammatory Activity

Carrageenan Induced paw edema

The anti-inflammatory activity of the chloroform extract of *Bixa orellana* leaves was investigated using the carrageenan-induced paw edema model in rats. The animals were randomly assigned into six groups (n=6 per group): Group A, the normal control, received 0.9% saline orally at a dose of 10 mL/kg; Group B served as the carrageenan control without any treatment; Group C was administered indomethacin at 10 mg/kg orally as the reference anti-inflammatory agent; and Groups D, E, and F received the *Bixa orellana* chloroform extract orally at doses of 50, 100, and 200 mg/kg respectively. Inflammation was induced by injecting 0.1 mL of carrageenan (prepared as a 1% suspension in sterile normal saline) into the rats' left hind paws to cause edema. All treatments were given orally 1 hr prior to the carrageenan injection. Paw volume was measured with a plethysmometer at intervals of 0, 2, 4, 6, and 24 hr after injection to evaluate edema and the extract's anti-inflammatory effects (Morris, 2003).

Analgesic Activity

Eddy's hot plate

Rat of either sex with an initial weight of 150-180 g are used for each dose. The hot plate, which is commercially available, consists of an electrically heated surface. The temperature was maintained at 55±0.2°C. The animals are placed on the hot plate and the time until either licking or jumping occurs is recorded by a stopwatch. The latency time is recorded before and after 0, 30, 60, 90 and 120 min (Mian *et al.*, 2025; Savla *et al.*, 2025).

Tail flick test

Radiant heat was applied to a single spot on the proximal third of the tail using an analgesiometer. The time taken for the animal to withdraw (flick) its tail was recorded as the reaction time. The standard drug or test substance was administered after measuring the baseline reaction times at intervals of 0, 30, 60, 90 and 120 min (Kaushik *et al.*, 2025; Cecchi *et al.*, 2008).

Statistical Analysis

The results were analysed using the statistical program GraphPad, with two-way ANOVA employed for group comparisons. A p-value of less than 0.05 ($p<0.05$) was considered statistically significant. Data are presented as Mean±S.E.M. (Standard Error of the Mean).

RESULTS

Phytochemical Screening

In extracts from chloroform extract of *Bixa orellana* leaves, a variety of metabolites were discovered (Table 1). Preliminary phytochemical study of the chloroform extract of *Bixa orellana* leaves, showed the presence of alkaloids, flavonoids, phytosterols and terpenoids.

Anti-Inflammatory Activity- Carrageenan Induced Paw edema

The anti-inflammatory activity of the Chloroform Extract of *Bixa orellana* leaves (CEBO), evaluated in rats using carrageenan-induced paw edema, and compared with the standard anti-inflammatory drug indomethacin (10 mg/kg) summarizes in Table 2 and Figure 1. The changes in paw thickness observed at various time points demonstrated that the Chloroform Extract of *Bixa orellana* leaves (CEBO) produced a dose-dependent anti-inflammatory effect. Notably, the 200 mg/kg dose exhibited significantly greater efficacy compared to the lower doses. These findings suggest that CEBO possesses promising anti-inflammatory properties, potentially supporting its use in the development of plant-based therapeutic agents.

Analgesic Activity

Eddy's hot plate

The Chloroform Extract of *Bixa orellana* leaves (CEBO) produced a significant, dose-dependent increase in reaction time, indicative of central analgesic activity, as presented in Table 3 and Figure 2. Among the extract-treated groups, CEBO at 50 mg/kg exhibited a moderate analgesic effect, while higher doses (100 mg/kg and 200 mg/kg) produced more pronounced responses. Notably, the 200 mg/kg dose showed analgesic efficacy comparable to the standard drug, Indomethacin.

Tail-flick test method

As summarized in Table 4 and Figure 3, the Chloroform Extract of *Bixa orellana* leaves (CEBO) showed significant, dose-dependent analgesic activity in rats. These results demonstrate that CEBO produces a significant, dose-dependent increase in pain threshold, indicating strong central analgesic activity comparable to the standard drug at higher doses.

DISCUSSION

The present study was utilize to determine the preliminary phytochemical analysis, anti-inflammatory and analgesic activities of the chloroform extract of *Bixa orellana* leaves (CEBO). The qualitative phytochemical analysis revealed the presence of alkaloids, flavonoids, phytosterols and terpenoids compounds-each known for distinct pharmacological actions, particularly in relation to inflammation and pain modulation. The presence of key bioactive compounds are well documented for their medicinal properties (Pietta, 2000; Russo, 2008; Gertsch *et al.*, 2008).

CEBO exhibited dose-dependent anti-inflammatory effects in the carrageenan-induced paw edema model. At 100 mg/kg and 200 mg/kg, CEBO significantly inhibited paw swelling with efficacy comparable to the reference drug indomethacin. This anti-inflammatory activity may be attributed to inhibition of pro-inflammatory mediators such as prostaglandins and leukotrienes, membrane stabilization by phytosterols, and antioxidative effects of flavonoids mitigating reactive oxygen

Table 1: Preliminary Phytochemical Screening of chloroform extract of *Bixa orellana* leaves.

Test	Observation
Alkaloids	Present
Flavonoids	Present
Phenolic Compounds	Absent
Carbohydrate	Absent
Tannins	Absent
Phytosterols	Present
Terpenoids	Present

Table 2: **Effect of chloroform extract of *Bixa orellana* leaves carrageenan-induced paw edema.**

Group	Treatment	Dose	Paw volume(mL) at different time interval				
			0 Hr	2 Hr	4 Hr	6 Hr	24 Hr
Group A (Control)	0.9% Saline	10 mL/kg	0.961±0.005	0.960±0.011	0.968±0.007	0.966±0.008	0.960±0.008
Group B (Positive Control)	Carrageenan (1% w/v)	-	0.970±0.010	1.976±0.004 a***	1.993±0.005 a***	1.966±0.005 a***	1.952±0.004 a***
Group C (Standard)	Indomethacin	10 mg/kg	0.968±0.007	1.396±0.008 a***,b***	1.416±0.007 a***,b***	1.297±0.011 a***,b***	1.059±0.009 a***,b***
Group D (CEBO)	*Bixa orellana* Extract	50 mg/kg	0.958±0.011	1.760±0.014 a***,b***, c***	1.769±0.013 a***,b***, c***	1.589±0.003 a***,b***, c***	1.209±0.005 a***,b***, c***
Group E (CEBO)	*Bixa orellana* Extract	100 mg/kg	0.956±0.004	1.662±0.021 a***,b***, c*,d*	1.679±0.013 a***,b***, c***,d*	1.459±0.012 a***,b***, c***,d***	1.161±0.013 a***,b***, c**
Group F (CEBO)	*Bixa orellana* Extract	200 mg/kg	0.966±0.018	1.436±0.007 a***,b***, c*,d***, e***	1.453±0.006 a***,b***, c*,d***,e***	1.320±0.006 a***,b***, d***,e***	1.113±0.004 a***,b***, c*,d***

Values are mean±S.E.M, * Significance Indicators: a*=vs. Group A (Control), b*=vs. Group B (Positive Control), c*=vs. Group C (Standard), d*=vs. Group D (CEBO 50 mg/kg), e*=vs. Group E (CEBO 100 mg/kg), *p<0.05, **p<0.01, ***p<0.001

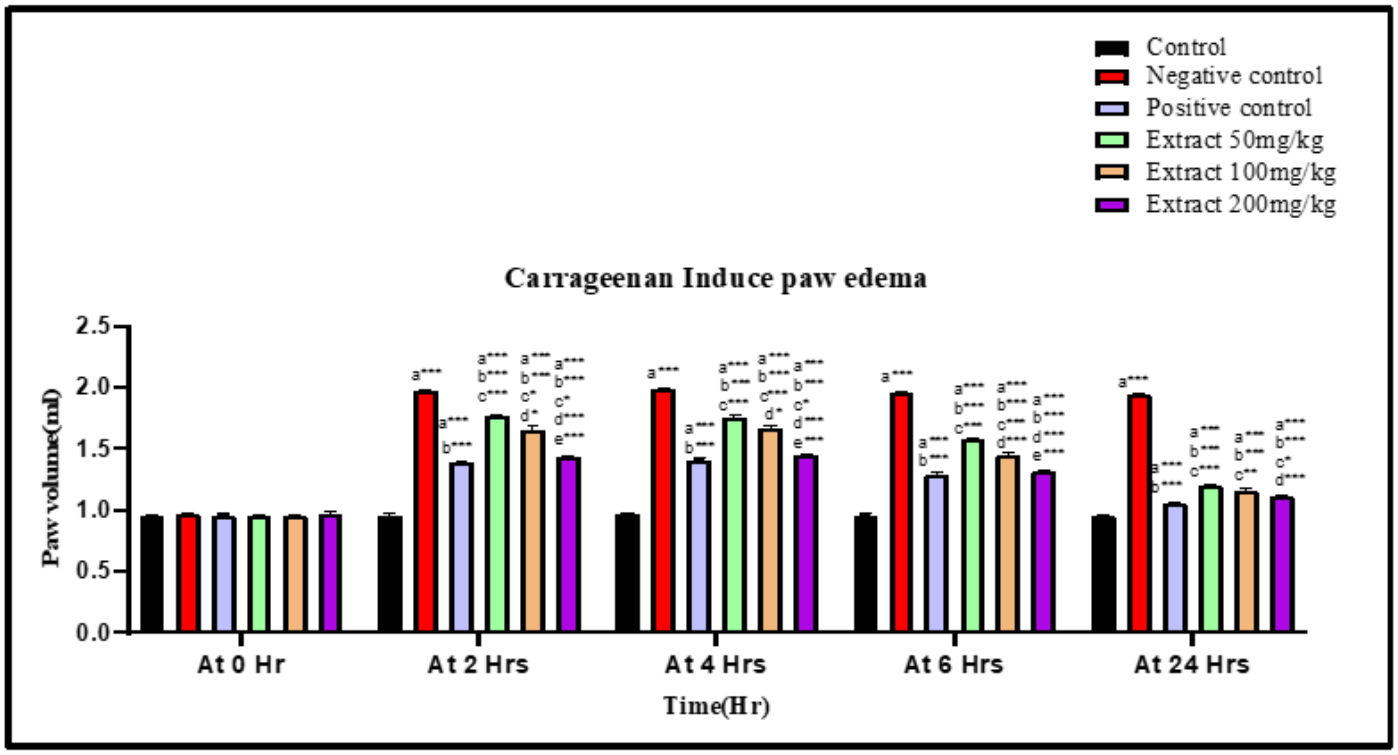

Figure 1: Graphical representation of effect of chloroform extract (doses 50, 100 and 200 mg/kg) of *Bixa orellana* leaves carrageenan induced hind paw edema. Indomethacin (10 mg/kg) was used as a standard drug. Values are Mean±S.E.M, * Significance Indicators: a*=vs. Group A (Control), b*=vs. Group B (Positive Control), c*=vs. Group C (Standard), d*=vs. Group D (CEBO 50 mg/kg), e*=vs. Group E (CEBO 100 mg/kg), *p<0.05, **p<0.01, ***p<0.001.

Table 3: Effect of chloroform extract of *Bixa orellana* leaves by hot plate test, all the values are in second.

Sl. No.	Treatment and Dose	Reaction Time(sec)				
		0 Min	30 Min	60 Min	90 Min	120 Min
1.	Control	3.542 ±0.069	3.8±0.042	3.818±0.112	4.044±0.036	3.93±0.025
2.	Standard	3.4±0.043	7.624±0.123 a***	10.11±0.285 a***	12.963±0.282 a***	11.311±0.172 a***
3.	CEBO 50mg/kg	3.235 ±0.067	5.932±0.066 a***,b***	6.837±0.148 a***, b***	8.653±0.159 a***,b***	7.209±0.277 a***,b***
4.	CEBO 100mg/kg	3.337 ±0.113	6.912±0.093 a***,b**,c***	8.181±0.294 a***, b**, c*	9.989±0.235 a***,b***,c**	8.579±0.353 a***,b**
5.	CEBO 200mg/kg	3.555 ±0.140	7.770±0.074 a***,c***,d***	9.593±0.331 a***, c***	12.437±0.318 a***,c***,d**	10.650±0.227 a***,c***,d**

Values are Mean±S.E.M. Significance Indicators: a*=vs. Group A (Control), b*=vs. Group B (standard), c*=vs. Group C (CEBO 50mg/kg), d*=vs. Group D (CEBO 100 mg/kg), *p<0.05, **p<0.01, ***p<0.001

Table 4: Effect of chloroform extract of *Bixa orellana* leaves by tail-flick test, all the values are in second.

Sl. No.	Treatment and Dose	Reaction Time (sec)				
		0 Min	30 Min	60 Min	90 Min	120 Min
1.	Control	2.216±0.130	2.34±0.325	2.42±0.212	2.36±0.095	2.36±0.198
2.	Standard	2.3±0.141	5.68±0.185 a***	7.5±0.178 a***	10.24±0.151 a***	8.927±0.164 a***
3.	CEBO 50mg/kg	2.249±0.117	3.77±0.060 a*,b***	4.917±0.054 a***,b***	5.72±0.304 a***,b***	5.522±0.099 a***,b***
4.	CEBO 100mg/kg	2.356±0.152	4.606±0.091 a**,b**,c***	6.018±0.070 a***,b**,c***	7.83±0.150 a***,b***,c**	6.9±0.135 a***,b***,c***
5.	CEBO 200mg/kg	2.361±0.128	5.303±0.139 a***,c***,d*	7.309±0.144 a***,c***d***	9.761±0.229 a***,c***d***	8.060±0.032 a***,b*,c***,d**

Values are Mean±S.E.M. Significance Indicators: a*= vs. Group A (Control), b* = vs. Group B (standard), c* = vs. Group C (CEBO 50mg/kg), d* = vs. Group D (CEBO 100 mg/kg), *p<0.05, **p<0.01, ***p<0.001

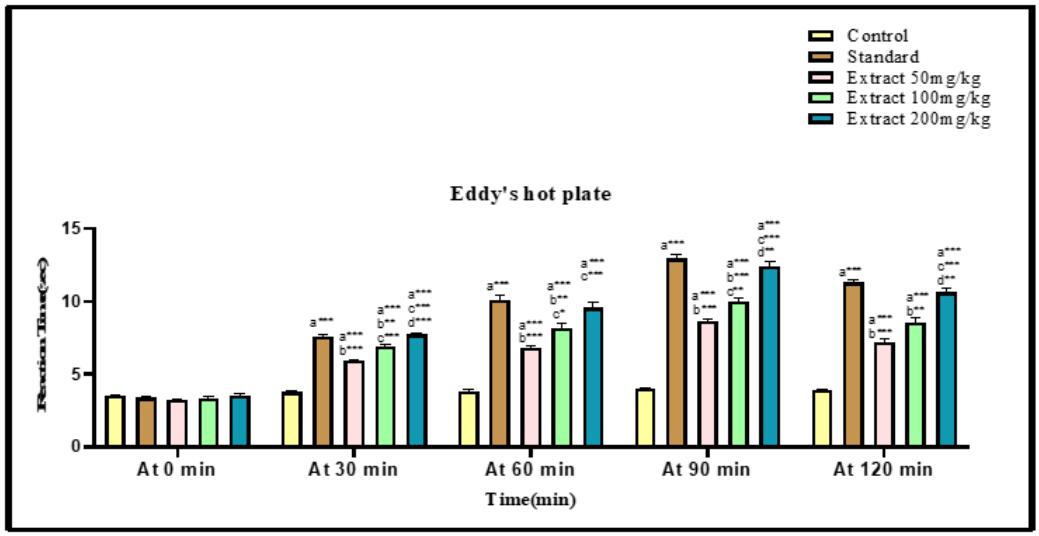

Figure 2: Graphical Representation of Effect of chloroform extract of *Bixa orellana* leaves (doses 50, 100 and 200mg/kg) using Hot Plate. Indomethacin (10 mg/kg) used as a standard. All values are expressed as Mean±SEM. Significance Indicators: a*=vs. Group A (Control), b*=vs. Group B (standard), c*=vs. Group C (CEBO 50mg/kg), d*=vs. Group D (CEBO 100 mg/kg), *p<0.05, **p<0.01, ***p<0.001.

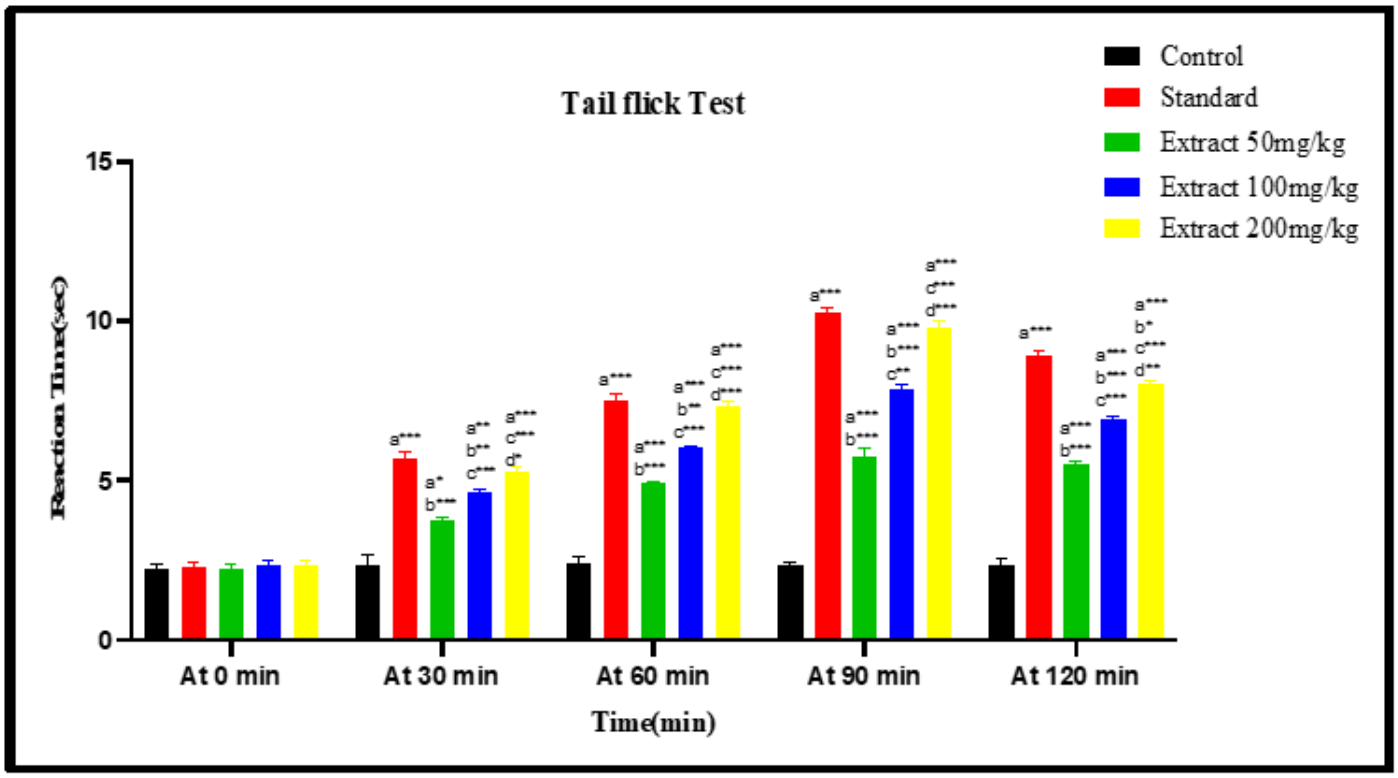

Figure 3: Graphical Representation of Effect of chloroform extract of *Bixa orellana* leaves (doses 50, 100 and 200mg/kg) using Tail Flick Method. Indomethacin (10 mg/kg) used as a standard. Values are Mean±S.E.M.Significance Indicators: a*=vs. Group A (Control), b*=vs. Group B (standard), c*=vs. Group C (CEBO 50mg/kg), d*=vs. Group D (CEBO 100 mg/kg), *p<0.05, **p<0.01, ***p<0.001.

species (Pietta, 2000; Bouic and Lamprecht, 1999; Middleton *et al.*, 2000).

Analgesic activity was demonstrated by increased latency in Eddy's hot plate and tail-flick tests, indicating central and peripheral analgesic mechanisms. The highest dose (200 mg/kg) showed analgesic effects approaching those of indomethacin. Terpenoids in CEBO may exert analgesic effects through CB2 receptor activation, while flavonoids likely modulate TRPV1 channels involved in pain transmission (Gertsch *et al.*, 2008; Vriens *et al.*, 2008; Wagner and Elmadfa, 2003; Paduch *et al.*, 2007; Gupta, 1980).

These findings corroborate traditional claims regarding *Bixa orellana* and highlight its potential as a promising source of natural anti-inflammatory and analgesic agents. Further studies are warranted to isolate the active constituents and elucidate the underlying mechanisms of action.

CONCLUSION

The chloroform extract of *Bixa orellana* leaves contains a diverse array of phytochemicals that have demonstrated notable anti-inflammatory and analgesic activities. However, further pharmacological investigations are essential to elucidate the precise mechanisms of action and explore the full spectrum of biological activities associated with this plant. The present study reinforces the potential of plant-based therapeutics and underscores the importance of integrating traditional knowledge with modern scientific approaches to develop safe and effective medicines.

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ABBREVIATIONS

CEBO: Chloroform extract of *Bixa orellana* leaves; **NSAIDs:** Nonsteroidal anti-inflammatory drugs; **COX:** Cyclooxygenase; **CMC:** Carboxy Methyl Cellulose; **g:** Gram; **mL:** Millilitre; **IAEC:** Institutional Animal Ethics Committee; **kg:** Kilogram; **S.E.M.:** Standard Error of the Mean; **CB2 Receptor:** Cannabinoid Receptor 2; **TRPV1 Channels:** Transient Receptor Potential Vanilloid-1.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

This study evaluated the anti-inflammatory and analgesic potential of the Chloroform Extract of *Bixa orellana* leaves (CEBO). Phytochemical screening revealed the presence of flavonoids, alkaloids, terpenoids, and phytosterols. Anti-inflammatory activity was assessed using the carrageenan-induced paw edema model, while analgesic effects were evaluated through hot plate and tail-flick methods. CEBO showed significant, dose-dependent reduction in inflammation and increased pain threshold, with 200 mg/kg demonstrating comparable efficacy to indomethacin. The findings support the traditional use of *Bixa orellana* and highlight its potential as a source of phytotherapeutic agents. Further studies are recommended to isolate and characterize its active constituents.

REFERENCES

Benyamin, R., Trescot, A. M., Datta, S., Buenaventura, R., Adlaka, R., Sehgal, N., Glaser, S. E., & Vallejo, R. (2008). Opioid complications and side effects. Pain Physician, 11(2)(Suppl.), S105–S120. https://doi.org/10.36076/ppj.2008/11/s105

Bhatt, B., Chaurasia, H., Singh, R., & Kaushik, S. (2022). Phytochemical profile and *in vitro* sun-protective activity of *Polyalthia longifolia* (Sonn.) Thwaites bark extracts. Tropical Journal of Natural Product Research, 6(8), 1174–1177. https://doi.org/10.26538/tjnpr/v6i8.1

Bouic, P. J. D., & Lamprecht, J. H. (1999). Plant sterols and sterolins: A review of their immune-modulating properties. Alternative Medicine Review, 4(3), 170–177.

Calixto, J. B. (2000). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Brazilian Journal of Medical and Biological Research, 33(2), 179–189. https://doi.org/10.1590/S0100-879X2000000200004

Cecchi, M., Capriles, N., Watson, S. J., & Akil, H. (2008). Differential responses to morphine-induced analgesia in the tail-flick test. Behavioural Brain Research, 194(2), 146–151. https://doi.org/10.1016/j.bbr.2008.06.034

Gertsch, J., Leonti, M., Raduner, S., Rácz, I., Chen, J.-Z., Xie, X.-Q., Altmann, K.-H., Karsak, M., & Zimmer, A. (2008). β-caryophyllene is a dietary cannabinoid. Proceedings of the National Academy of Sciences of the United States of America, 105(26), 9099–9104. https://doi.org/10.1073/pnas.0803601105

Gupta, M. B., Nath, R., Srivastava, N., Shanker, K., Kishor, K., & Bhargava, K. P. (1980). Anti-inflammatory and antipyretic activities of β-sitosterol. Planta Medica, 39(2), 157–163. https://doi.org/10.1055/s-2008-1074919

Hassan, M., Zahra, N., Shafi, A., Shahzadi, S., Moustafa, A., & Kloczkowski, A. (2024). Investigation of anti-inflammatory, antipyretic and analgesic activities of *Citrullus colocynthis* in albino rats through *in vivo* and pharmacoinformatics studies. Recent Advances in Anti-infective Drug Discovery, 19(2), 119–136. https://doi.org/10.2174/2772434418666230412105317

Kaushik, S., Bhandari, P., Bhandari, A., Bhatt, B., Mittal, H., & Babbar, A. (2025). Analgesic and anti-inflammatory properties of *Oxalis dehradunensis* Raizada in rats. Pharmacognosy Research, 17(1), 185–191. https://doi.org/10.5530/pres.20251985

Lanas, A., & Chan, F. K. L. (2017). Peptic ulcer disease. The Lancet, 390(10094), 613–624. https://doi.org/10.1016/S0140-6736(16)32404-7

Mbah, C. J., Udeh, N. E., & Okechukwu, P. N. (2012). Ethnobotanical survey and pharmacological evaluation of Bixa orellana used in traditional medicine in Africa. African Journal of Traditional, Complementary and Alternative Medicines, 9(4), 500–506.

Mian, S. S., Shuaib, M., Zehra, A., Ansari, M. S., Husain, I., Begam, B., & Asif, M. (2025). Synergistic analgesic effects of ginger, colchicum, and nux vomica extracts in a Unani polyherbal combination. South Eastern European Journal of Public Health, S1, 894–906. https://doi.org/10.70135/seejph.vi.3756

Middleton, E., Jr., Kandaswami, C., & Theoharides, T. C. (2000). The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. Pharmacological Reviews, 52(4), 673–751. https://doi.org/10.1016/S0031-6997(24)01472-8

Morris, C. J. (2003). Carrageenan-induced paw edema in the rat and mouse. Methods in Molecular Biology, 225, 115–121. https://doi.org/10.1385/1-59259-374-7:115

Morris, S. S., Jeyabalan, G., Jha, A. K., Verma, S., & Swarnkar, Y. (2016). Phytochemical screening and *in vitro* anthelmintic activity of seed extracts of plants Carum carvi of family Apiaceae. Asian Journal of Research in Pharmaceutical Science, 6(4), 246–254. https://doi.org/10.5958/2231-5659.2016.00036.9

Nyiramugisha, D., & Odoma, S. (2021). Analgesic activity of methanol leaf extract of *Momordica foetida* (Schumach) in albino rats. KIU Journal of Health Sciences, One(One), 31–37. https://doi.org/10.59568/KJHS-2021-1-1-005

Paduch, R., Kandefer-Szerszeń, M., Trytek, M., & Fiedurek, J. (2007). Terpenes: Substances useful in human healthcare. Archivum Immunologiae et Therapiae Experimentalis, 55(5), 315–327. https://doi.org/10.1007/s00005-007-0039-1

Pietta, P. G. (2000). Flavonoids as antioxidants. Journal of Natural Products, 63(7), 1035–1042. https://doi.org/10.1021/np9904509

Prasad, S., & Kashyap, R. S. (2013). *Bixa orellana*: A plant of multiple uses. Journal of Medicinal Plants Studies, 1(5), 14–20.

Rivera-Mondragón, A., Castañeda-Hernández, G., Martínez-Cruz, M. *et al.* (2017). Chemical composition and pharmacological activities of *Bixa orellana*: A review.* Phytotherapy Research, 31(8), 1215–1228.

Russo, E. B. (2008). Cannabinoids in the management of difficult to treat pain. Therapeutics and Clinical Risk Management, 4(1), 245–259. https://doi.org/10.2147/TCRM.S1928

Savla, S. R., Oza, M. J., Laddha, A. P., & Kulkarni, Y. A. (2025). Anti-nociceptive activity of biochanin A-an important isoflavone from natural source. Pharmacognosy research-Natural products, 100157.

Scholz, J., & Woolf, C. J. (2002). Can we conquer pain? Nature Neuroscience, 5(Suppl.), 1062–1067. https://doi.org/10.1038/nn942

Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies, 8(2), 603–608. https://doi.org/10.22271/chemi.2020.v8.i2i.8834

Syamkumar, T. S., S, G., & Augustine, A. (2023). Study of pharmacological profile of chloroform leaf extract of *Ludwigia perennis*-A wetland plant. European Journal of Medicinal Plants, 34(8), 58–70. https://doi.org/10.9734/ejmp/2023/v34i81154

Vane, J. R., & Botting, R. M. (1998). Mechanism of action of anti-inflammatory drugs. International Journal of Tissue Reactions, 20(1), 3–15.

Vilar, D. D. A., Rodrigues, J. A. G., Dos Santos, S. C., & da Silva, F. M. R. (2010). Morphological and anatomical characterization of *Bixa orellana* L. (Bixaceae). Revista Brasileira de Farmacognosia, 20(6), 889–893.

Vriens, J., Nilius, B., & Vennekens, R. (2008). Herbal compounds and toxins modulating TRP channels. Current Neuropharmacology, 6(1), 79–96. https://doi.org/10.2174/157015908783769644

Wagner, K.-H., & Elmadfa, I. (2003). Biological relevance of terpenoids: Overview focusing on mono-, di- and tetraterpenes. Annals of Nutrition and Metabolism, 47 (3–4), 95–106. https://doi.org/10.1159/000070030

Yong, Y. K., Zakaria, Z. A., Kadir, A. A., Somchit, M. N., Lian, E. C., G., & Ahmad, Z. (2013). Chemical constituents and antihistamine activity of *Bixa orellana* leaf extract. BMC Complementary and Alternative Medicine, 13, 1–7.

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