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COMPARATIVE EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF *PHYLLANTHUS FRATERNUS* AND *IMPATIENS BALSAMINA* ETHANOLIC EXTRACTS

Pratibha S. Gavarkar * and Rajashree S. Chavan

Department of Pharmaceutical Chemistry, Seth Govind Raghunath Sable College of Pharmacy, Saswad, Punrander, Pune - 412301, Maharashtra, India.

Keywords:

Phyllanthus fraternus, *Impatiens balsamina*, Analgesic activity, Anti-inflammatory activity, HRBC assay, Carrageen an paw edema

Correspondence to Author:
Pratibha S. Gavarkar

Research Scholar,
Department of Pharmaceutical Chemistry, Seth Govind Raghunath Sable College of Pharmacy, Saswad, Punrander, Pune - 412301, Maharashtra, India.

E-mail: pratibhaadnaik@gmail.com

ABSTRACT: The current research was conducted to compare the acute oral toxicity, analgesic and anti-inflammatory properties of ethanolic extracts of *Phytanthus fraternus* and *Impatiens balsamina*, two medicinal plants that have been used traditionally in treating pain and inflammation. The acute toxicity was evaluated in Swiss albino mouse based on the OECD guideline 423 at a dose of 300 mg and 2000 mg/kg. There were no mortalities, abnormal behaviour or adverse clinical effects, which categorised the two extracts as the Globally Harmonised System (GHS) Category 5. To assess analgesic activity, the acetic acid-induced writhing test was used on mice, whereas anti-inflammatory potential has been assessed using both *in-vitro* and *in-vivo* models: the human red blood cell (HRBC) membrane stabilisation assay and carrageenan-induced paw oedema in rats, respectively. Findings revealed that both extracts had marked, dose-dependent analgesic and inflammatory effects, with the extract of *P. fraternus* having a uniformly higher potency than *I. balsamina*. Their stabilising behaviour against hypotonicity-induced haemolysis, an indicator of membrane-stabilising and anti-inflammatory activities, was confirmed with the HRBC membrane stabilisation test. In a similar manner, oedema was severely suppressed in the carrageenan-induced paw oedema model, which was another indication of their *in-vivo* anti-inflammatory effect. Comprehensively, these results offer pharmacological support to the ethnomedicinal claims of both plants and indicates that, in particular, the plant of interest, *P. fraternus,* is even better positioned to be developed into a new series of plant-based analgesic and anti-inflammatory compounds.

INTRODUCTION: Inflammation and pain are complex biological processes fundamental to host defense and tissue repair. However, dysregulation of these responses contributes to the development of several chronic diseases, including rheumatoid arthritis, cardiovascular disorders, neuro-degenerative conditions, and cancer [1]. Analgesic and anti-inflammatory drugs, particularly non-steroidal anti-inflammatory drugs (NSAIDs), continue to represent the cornerstone of pharmacotherapy [2]. Nevertheless, prolonged NSAID use is often associated with adverse effects such as gastrointestinal irritation, renal dysfunction, and elevated cardiovascular risk [3]. These safety concerns have stimulated increasing interest in safer, plant-derived alternatives with improved therapeutic indices [4].

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Medicinal plants have long served as invaluable sources of bioactive compounds, with nearly one-quarter of currently approved prescription drugs originating from natural products [5]. Within this context, members of the Euphorbiaceae and Balsaminaceae families have been traditionally employed for the management of inflammation and pain [6].

Phyllanthus fraternus Webster (Euphorbiaceae), commonly known as Bhoiamli, is a tropical and subtropical species traditionally utilized for the treatment of jaundice, digestive ailments, and inflammatory disorders [7]. Phytochemical investigations reveal that *P. fraternus* contains lignans, flavonoids, and polyphenolic compounds such as phyllanthin, hypophyllanthin, and naphthoquinones, which collectively exhibit strong antioxidant and anti-inflammatory activities [8, 9]. Similarly, *Impatiens balsamina* Linn. (Balsaminaceae), or garden balsam, is extensively used in Asian traditional medicine for treating rheumatism, wound healing, and microbial infections [10]. Its bioactive constituents-including lawsone, anthocyanins, flavonoids, and phenolic acids-have been reported to display remarkable antimicrobial, antioxidant, and anti-inflammatory properties [11]. Despite their widespread ethnomedicinal utilization, comprehensive comparative studies evaluating the pharmacological efficacy and safety profiles of these two plants remain scarce. Therefore, the present study aims to comparatively assess the analgesic and anti-inflammatory potential of ethanolic extracts of *Phyllanthus fraternus* and *Impatiens balsamina* through validated *in-vitro* and *in-vivo* experimental models, and to establish their safety according to OECD guidelines for acute oral toxicity [12].

MATERIALS AND METHODS:

Plant Material and Preparation of Extracts: Whole plant of *Phyllanthus fraternus* and leaves of *Impatiens balsamina* were collected at Gaganbavada, Western Ghats, Maharashtra, India. The plant material was authenticated by a botanist at The New College, Kolhapur and voucher specimens were deposited in the departmental herbarium for future reference. Plant material was washed, dried in shade at ambient temperatures between 25° and 28°C and powdered in a mechanical grinder. Ethanolic extracts were prepared by Soxhlet extraction over 48 h, concentrated at reduced pressure at 40 °C using rotary evaporator and stored at 4 °C in airtight containers until use [13, 14].

Experimental Animals: Swiss albino mice (20–25 g) and Wistar rats (150–180 g) of either sex were maintained under standard laboratory conditions (12 h light/dark cycle, 25 ± 2 °C, 55 ± 5% humidity) with free access to standard pellet diet and water. Experiments were conducted in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, India, and approved by the Institutional Animal Ethics Committee.

Acute Oral Toxicity Study: Acute toxicity was evaluated according to OECD Guideline 423 (OECD, 2001). Swiss albino mice (n = 6 per group) were orally administered extracts at doses of 300 mg/kg and 2000 mg/kg (suspended in 0.5% CMC). Animals were observed for 14 days for mortality, behavioural changes, neurotoxic symptoms, and body weight fluctuations. Classification was performed according to the Globally Harmonized System (GHS) of chemical safety.

Analgesic Activity:

Acetic Acid-Induced Writhing Test: Analgesic activity was evaluated using the acetic acid-induced writhing test in mice, a validated model for peripheral analgesia based on the method of Koster *et al* [15] and applied in recent studies [16]. Mice were divided into groups (n = 6): Control (0.5% CMC), Standard (Aspirin 100 mg/kg, p.o.), and Test groups (*P. fraternus* and *I. balsamina* extracts at 200 and 400 mg/kg, p.o.). Thirty minutes after treatment, writhing was induced by intraperitoneal injection of 0.6% acetic acid (10 mL/kg). The number of writhes (abdominal constrictions) was recorded for 20 minutes, and % inhibition of writhing was calculated relative to control.

Anti-Inflammatory Activity:

***In-vitro:* HRBC Membrane Stabilization Assay:** The method of hypotonic solution-induced HRBC lysis was employed to evaluate *in-vitro* anti-inflammatory activity, following the protocol of Shinde *et al* [17]. (1999) as applied in recent studies [18]. Fresh human blood was collected in heparinized tubes, centrifuged at 3000 rpm for 10 min, and washed thrice with normal saline. A 10% HRBC suspension was prepared in isotonic buffer. Reaction mixtures contained 0.5 mL of HRBC suspension, 1 mL of test extract (100–500 µg/mL in phosphate buffer, pH 7.4) or standard drug (Diclofenac sodium, 10 µg/mL), and 2 mL of hypotonic saline. After incubation at 37 °C for 30 min, the mixtures were centrifuged, and supernatant absorbance was measured at 560 nm. % inhibition of hemolysis was calculated.

***In-vivo* Carrageenan-Induced Paw Edema:** Anti-inflammatory activity *in-vivo* was determined using carrageenan-induced paw edema in rats, a standard acute inflammation model established by Winter *et al.* (1962) [19] and widely used in recent studies [20]. Rats were divided into groups (n = 6): Control (0.5% CMC), Standard (Diclofenac sodium 10 mg/kg, p.o.), and Test groups (*P. fraternus* and *I. balsamina* extracts at 200 and 400 mg/kg, p.o.). One hour after treatment, acute inflammation was induced by subplantar injection of 0.1 mL of 1% carrageenan into the right hind paw. Paw volume was measured using a plethysmometer at 0, 1, 2, 3, and 4 h post-carrageenan injection. The % inhibition of paw edema was calculated relative to control.

Statistical Analysis: All data were expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test, with $p < 0.05$ considered statistically significant (GraphPad Prism version X).

RESULTS:

Acute Oral Toxicity: Administration of *Phyllanthus fraternus* and *Impatiens balsamina* ethanolic extracts at doses of 300 mg/kg and 2000 mg/kg produced no mortality or observable toxic signs in Swiss albino mice throughout the 14-day observation period. Behavioral responses, grooming, feeding activity, and locomotion remained normal across groups. Body weight progression was comparable to controls, with no significant differences ($p > 0.05$) **Table 1.**

TABLE 1: ACUTE ORAL TOXICITY OUTCOMES OF ETHANOLIC EXTRACTS IN SWISS ALBINO MICE

Group	Dose (mg/kg)	Mortality (n=3)	Clinical Signs	Mean Body Weight Change (gm, Day 0–14)
Control	—	0	None	+6.2 ± 0.7
P. fraternus	300	0	None	+6.0 ± 1.1
P. fraternus	2000	0	None	+5.8 ± 1.2
I. balsamina	300	0	None	+6.3 ± 1.0
I. balsamina	2000	0	None	+6.1 ± 1.2

Both extracts were thus classified as GHS Category 5 (low hazard) according to OECD 423 guidelines.

Analgesic Activity:

Acetic Acid-Induced Writhing Test: Both ethanolic extracts significantly reduced the number of writhes induced by acetic acid in mice in a dose-dependent manner ($p < 0.05$ vs. control). *P. fraternus* showed stronger inhibition compared to *I. balsamina* at equivalent doses, though both were less potent than aspirin (100 mg/kg).

TABLE 2: EFFECT OF DIFFERENT EXTRACTS ON ACETIC ACID-INDUCED WRITHING IN MICE

Group	Dose (mg/kg)	Mean Writhes (± SEM)	% Inhibition
Control (CMC)	—	62.3 ± 2.1	—
Aspirin	100	18.4 ± 1.2*	70.5
P. fraternus	200	32.6 ± 1.5*	47.7
P. fraternus	400	24.1 ± 1.3*	61.3
I. balsamina	200	36.8 ± 1.7*	41.0
I. balsamina	400	27.9 ± 1.4*	55.2

*Values are mean ± SEM, n=6. $p < 0.05$ vs. control (ANOVA followed by Dunnett's test).

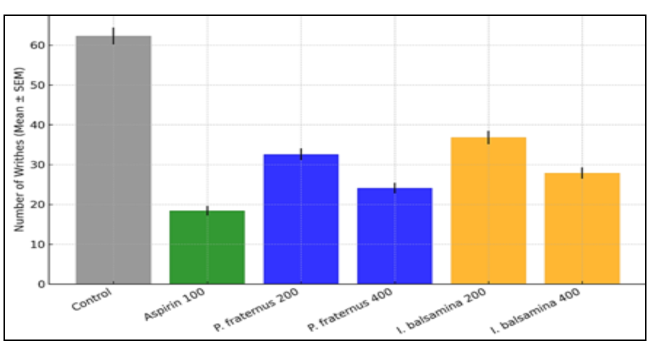

FIG. 1: EFFECT OF DIFFERENT EXTRACTS ON ACETIC ACID-INDUCED WRITHING IN MICE

Anti-Inflammatory Activity:

***In-vitro:* HRBC Membrane Stabilization Assay:** Both extracts demonstrated significant membrane stabilization activity, reducing hemolysis in a concentration-dependent manner. *P. fraternus* was more effective, with maximum protection (73.5%) at 500 µg/mL, compared to *I. balsamina* (68.2%). Diclofenac sodium produced the highest inhibition (82.1%).

TABLE 3: HRBC MEMBRANE STABILIZATION BY EXTRACTS AND STANDARD DRUG

Concentration (μg/mL)	% Inhibition)		
	Diclofenac	*P. fraternus*	*I. balsamina*
100	42.5	30.2	28.7
200	55.6	44.1	39.4
300	68.3	57.2	51.6
400	75.9	66.4	60.8
500	82.1	73.5	68.2

***In-vivo* Carrageenan-Induced Paw Edema:** Carrageenan challenge caused a marked increase in paw volume in control rats, peaking at 3 h post-injection.

Diclofenac sodium significantly reduced edema at all time points ($p < 0.01$). Both extracts produced dose-dependent inhibition of paw edema, with *P. fraternus* (400 mg/kg) showing the highest effect (56.8% at 3 h), followed by *I. balsamina* (400 mg/kg, 51.2%).

TABLE 4: EFFECT OF EXTRACTS ON CARRAGEENAN-INDUCED PAW EDEMA IN RATS (3 H POST-INJECTION)

Group	Dose (mg/kg)	Paw Edema (mL)	% Inhibition
Control (CMC)	—	1.25 ± 0.06	—
Diclofenac	10	0.42 ± 0.03*	66.4
P. fraternus	200	0.72 ± 0.04*	42.4
P. fraternus	400	0.54 ± 0.05*	56.8
I. balsamina	200	0.81 ± 0.05*	35.2
I. balsamina	400	0.61 ± 0.04*	51.2

*Values are mean ± SEM, n=6. $p < 0.05$ vs. control.

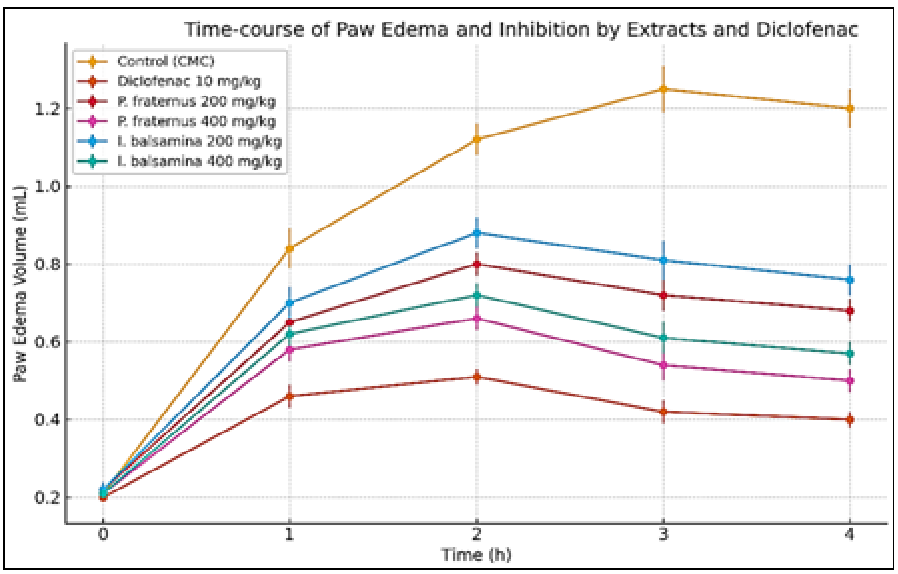

FIG. 2: TIME-COURSE OF PAW EDEMA DEVELOPMENT AND INHIBITION BY EXTRACTS AND DICLOFENAC SODIUM IN CARRAGEENAN-INDUCED PAW EDEMA MODEL

Both extracts were safe up to 2000 mg/kg and showed significant analgesic and anti-inflammatory effects, with *Phyllanthus fraternus* outperforming Impatiens balsamina though neither matched the potency of standard drugs.

DISCUSSION: The present study provides experimental evidence supporting the safety, analgesic, and anti-inflammatory potential of ethanolic extracts of *Phyllanthus fraternus* and *Impatiens balsamina*. Acute toxicity testing revealed no mortality or adverse clinical signs up to 2000 mg/kg, indicating that both extracts fall into GHS Category 5 (low hazard). These results agree with previous studies reporting the safety of Phyllanthus species, such as *P. amarus* and *P. niruri*, which are considered relatively non-toxic even at high doses [21-22]. Similarly, *Impatiens balsamina* extracts have been shown to exhibit low acute toxicity in rodents, supporting its ethnomedicinal use [23-25]. Analgesic activity was confirmed using the acetic acid-induced writhing model, which is widely accepted for evaluating peripheral analgesics. This model reflects increased levels of prostaglandins (mainly PGE_2 and $PGF_{2}\alpha$)

and lipoxygenase products in peritoneal fluids following acetic acid administration [26]. Both extracts significantly reduced writhing, with *P. fraternus* showing stronger inhibition than *I. balsamina*. The observed effects are likely due to inhibition of prostaglandin synthesis or suppression of inflammatory mediators, consistent with reports of analgesic activity in other *Phyllanthus* species [27].

Stabilization of erythrocyte membranes is analogous to the stabilization of lysosomal membranes, thereby preventing the release of lysosomal enzymes such as proteases and phospholipases that exacerbate inflammation [28]. Similar *in-vitro* protective effects of polyphenol-rich extracts on erythrocyte membranes have been reported in recent studies, reinforcing the antioxidant and membrane-stabilizing properties of phenolic compounds [29-30].

Recent research continues to validate the use of the carrageenan-induced paw edema model as a gold standard for evaluating acute inflammation *in-vivo*. Studies confirm the biphasic release of mediators: histamine and serotonin dominate the early phase (0–2 h), while prostaglandins, nitric oxide, and cytokines mediate the late phase (3–4 h), closely mirroring the mechanism described by Di Rosa *et al.* (1971) [31]. The carrageenan-induced paw edema model remains the gold standard for evaluating acute inflammation, as it mimics biphasic mediator release: histamine and serotonin in the early phase (0–2 h), and prostaglandins, nitric oxide, and cytokines in the late phase (3–4 h) [32-33]. Both extracts significantly inhibited paw edema in a dose-dependent manner, with *P. fraternus* showing higher efficacy than *I. balsamina*. The activity in both phases suggests that the extracts may inhibit multiple mediators of inflammation. These findings are consistent with reports of anti-inflammatory activity of lignans, flavonoids, and naphthoquinones present in *Phyllanthus* species [34-35] and lawsone derivatives in *Impatiens balsamina* [36].

Interestingly, *P. fraternus* consistently outperformed *I. balsamina* in both analgesic and anti-inflammatory assays. This may be attributed to the higher content of lignans such as phyllanthin and hypophyllanthin, which have been shown to modulate cyclooxygenase and lipoxygenase pathways [35, 37]. In contrast, *I. balsamina* is rich in lawsone and anthocyanins, which exert moderate antioxidant and anti-inflammatory effects but may be less potent than lignans in inhibiting prostaglandin pathways [38].

Collectively, these findings validate the ethnomedicinal use of both plants in treating pain and inflammation, while also suggesting that *P. fraternus* may be a more promising candidate for further phytochemical and pharmacological development. Future work should focus on isolating and characterizing the active constituents, elucidating their mechanisms at molecular targets (e.g., COX-2, NF-κB, TNF-α pathways), and assessing chronic toxicity and efficacy in chronic inflammation models.

CONCLUSION: The current research has indicated that ethanolic extract of *Phytanthus fraternus* and *Impatiens balsamina* are safe up to 2000mg/kg of body weight (GHS Category 5, low hazard) and have strong analgesic and anti-inflammatory properties in their proven experimental models. The two extracts had similar effects of dose dependence, with *P. fraternus* always having higher efficacy than *I. balsamina*.

The results of these studies can be scientifically justified to their use as ethnomedicines in pain and inflammatory disorders and stimulate their use as substitutes or complements to non-steroidal anti-inflammatory drugs. In conclusion, *Phyllanthus fraternus* appears to be a more promising candidate for further drug development, while *Impatiens balsamina* may serve as a complementary source of moderate analgesic and anti-inflammatory activity. Collectively, these results can broaden the pharmacological foundation of traditional medicine and provide an opportunity to create plant-based therapeutics with better safety profiles.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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