



Evaluation of *in vitro* anti-inflammatory activity of methanolic extract of *Impatiens bracteata* Colebr. ex wall.

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Abstract:

Background: Inflammation is a complicated process that typically entails pain and involves things like an increase in vascular. Increased protein denaturation, permeability, and membrane modification. When bodily cells are harmed by pathogens, external forces, or chemical agents, the harm takes the shape of stress. Tissue inflammation is a reaction to stress. Redness, pain, heat, swelling, and loss of function in the damaged region characterize this protective reaction. The main objective of this research is to evaluate the *in-vitro* anti-inflammatory activity of methanolic extract of the plant *Impatiens bracteata* colebr. ex. Wall. In order to obtain the anti-inflammatory function, the extract was incubated with egg albumin at various concentrations under strictly regulated experimental circumstances. The standard drug used was diclofenac sodium.

Result: The methanolic extract of *Impatiens bracteata* inhibited protein (albumin) denaturation in the current study in a concentration-dependent manner. When compared to the test extract, diclofenac sodium was shown to have a lower impact.

Conclusion: According to the present study's findings, the plant *Impatiens bracteata's* methanolic extract significantly reduced inflammation *in vitro* and prevented protein denaturation. The presence of polyphenols in the plant extract is likely what caused the impact.

Keywords: Anti-inflammatory, *Impatiens bracteata*, methanolic extract, Protein denaturation.

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1. Introduction:

Inflammation may show up physically as heat, redness, pain, edema, and altered physiological functions. It is the body's response to injury, illness, or devastation. A normal protective response to tissue injury caused by physical trauma, poisonous substances, or pathogenic organisms is inflammation[1 Non-steroidal anti-inflammatory medicines (NSAIDs) are the most widely used therapy for inflammatory disorders, but they have a number of side

effects, particularly gastrointestinal irritation that can lead to stomach ulcers. [2]. The discovery of natural products has considerably benefitted modern medicine. There has been a worldwide evaluation of traditional medicine as a result of recent extensive research on several plant species and their powerful therapeutic properties. The plant kingdom may include a large number of more recent chemicals with significant anti-inflammatory activity. Herbal medicine appears to have three

primary benefits: perceived efficacy, infrequent occurrence of serious side effects, and low cost. [3].

Impatiens bracteata, an annual herb stands 30 to 40 cm tall. More than 1000 species of blooming plants of the genus *Impatiens* may be found all throughout the Northern Hemisphere. The family of this plant is *Balsaminaceae* (fig 1) This family's members include flavonoids, quinones, triterpenes, and phenolic chemicals. more than 307 natural compounds have been isolated and identified from various medicinal parts of plants in the *Balsaminaceae* family [4]. These compounds are divided into various groups, including

flavonoids, naphthoquinones, coumarins, terpenoids, sterols, phenols, fatty acids and their ester, naphthalene derivatives, nitrogen-containing compounds, polysaccharides, and other compounds [5-6]. The main and most typical component is 2-methoxy-1,4-naphthoquinone, one of the naphthoquinones. Balsamina also offers a wide range of complex pharmacological properties, such as antibacterial, antiallergic, antipruritic, anticancer, antioxidant, anti-inflammatory, immunomodulatory, anti-hepatic fibrosis, insecticidal, and anthelmintic properties, among others[7]



Fig1: Plant of *Impatiens bracteata*

2. Methods:

2.1. Plant material

The Botanical Survey of India scientist Shillong authenticated the plant as *Impatiens bracteata* colebr. ex wall. On 11th October 2022. The reference number of the plant authentication is PT /171/22-23. After that throughout the months of August and September, the whole plant was taken away from Cherrapunji (Jaintia Hill), India. The plant was washed, stretched out in the shade at room temperature, and dried for three days in a tray dryer at 30°C. Afterward, a

mechanical grinder was used to powder the raw medication, and it was weighed.

2.2. Drug and chemicals

Diclofenac sodium was available in the Department of Pharmaceutical Technology, University of North Bengal, Darjeeling, West Bengal, India. All other compounds were of the analytical variety and were bought from stores. All of the study's water was double-distilled from an all-glass still.

2.3. Preparation of extract:

Increasingly polar solvents were used in a maceration successive extraction method during the extraction process, including water, petroleum ether, chloroform, ethyl acetate, methanol, and hydroalcoholic. The final extracts were concentrated using a vacuum evaporator (rotary evaporator), and the dried extracts were kept for later research in a desiccator. The concentrated extracts were redissolved in the appropriate solvents and subjected to a range of chemical tests in order to identify the specific constituents. [8-9].

2.4. Phytochemical screening

Impatiens bracteata extracts were tested for their phytochemical composition using conventional techniques using petroleum ether, chloroform, ethyl acetate, methanol, and hydroalcoholic extracts.

2.5. Evaluation of *in vitro* anti-inflammatory activity of methanolic extract:

2.5.1. The Albumin Denaturation Inhibitory Activity

1mL of various concentrations (1.2 µg/ml, 2.5 µg/ml, 5 µg/ml 10 µg/ml in distilled water) was blended with 2ml of egg albumin. and 3 ml of phosphate-buffered saline (PBS, pH 6.5). The mixtures were incubated at 37°C for 15 min. Then, they were heated for 12 min at 65°C, and after cooling, the absorbance was recorded at 660 nm [10]. Diclofenac sodium was used as the standard drug in the same amounts of *Impatiens bracteata* extracts. The percentage inhibition of albumin denaturation was estimated using the following formula

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0] \times 100$$

Where A_0 = The absorbance of the control

A_1 = The absorbance of the extract/standard.

Here, the standard employed was diclofenac. The readings were taken all three times

By graphing the percentage of inhibition with respect to the control versus the treatment concentration, the extract/drug concentration for 50% inhibition (IC50) was discovered.

3. Results:

3.1. Phytochemical screening

Tests	Pet Ether	Chloroform	Ethyl acetate	Methanol	Hydroalcoholic
Test for alkaloid	-	-	-	+	-
Test for Carbohydrate	-	+	-	+	+
Test for Flavonoids	-	-	+	+	+
Test for Tannins	-	-	-	+	+
Test for Glycosides	-	-	-	+	+
Test for Saponins	-	-	+	+	+

‘+’ sign = Positive and ‘-’ sign = Negative

The preliminary phytochemical shows that the maximum number of secondary metabolites was present in the methanolic extract of *Impatiens bracteata* followed by another extract.

3.2. Anti-inflammatory activity

In the current study, the *in vitro* anti-inflammatory activity of *Impatiens bracteata* methanolic extract was assessed against the denaturation of egg albumin using diclofenac as the standard. The graph (fig: 2) provides an overview of the outcome. The protein (albumin) denaturation of methanolic extract

was shown to be concentration-dependently inhibited across the concentration spectrum. The methanolic extract had a maximal inhibition of 58.27% at 50 $\mu\text{g/ml}$. Diclofenac, a common anti-inflammatory medication, showed 56.47% more effectiveness than the control at a concentration of 50 $\mu\text{g/ml}$. When compared to methanolic extract, the impact of diclofenac sodium was shown to be smaller. This was further supported by comparing the IC_{50} values of the methanolic extract and diclofenac sodium. It demonstrates anti-inflammatory properties.

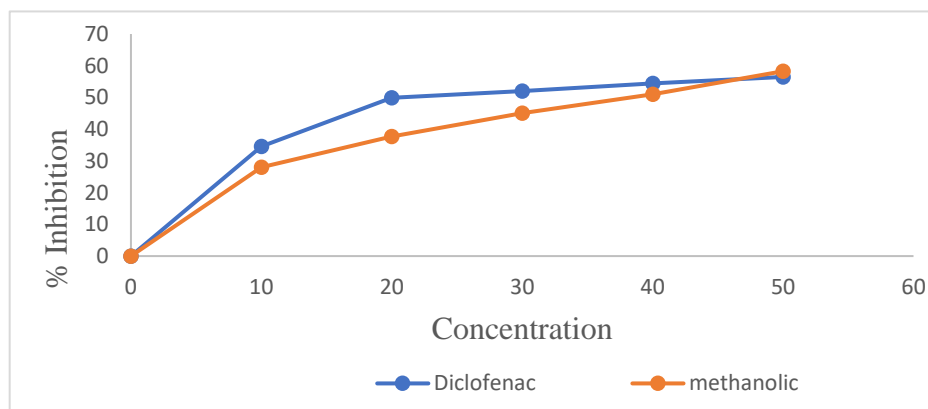


Fig2: The albumin denaturation activity of the methanolic extract

4. Discussion:

There are certain drawbacks to utilizing animals in experimental pharmacological research, including ethical concerns and a lack of justification for their usage when more acceptable alternatives are either already available or may be researched. Since *Impatiens bracteata's* methanolic extract has anti-inflammatory properties, the protein denaturation bioassay was chosen for the current study's *in-vitro* evaluation of this characteristic. One of the well-known

causes of arthritic and inflammatory disorders is the denaturation of tissue proteins. Protein denaturation *in vivo* may be the cause of the production of autoantigens in some arthritic disorders. Therefore, it would be beneficial to create anti-inflammatory drugs that can stop protein denaturation. The methanolic extract was more effective than diclofenac sodium, as shown by the IC_{50} values. The *in vitro* anti-inflammatory action of the methanolic extract of the plant *Impatiens bracteata* in the current

study can be related to its polyphenol content. Instead of just one component, the impact might be the result of a synergistic effect. Numerous non-steroidal anti-inflammatory medicines have been noted to have the potential to stabilize (avoid denaturation) heat-treated albumin at the physiological pH (pH: 6.2–6.5). Accordingly, it can be inferred from the findings of the current preliminary investigation that methanolic extract of the plant *Impatiens bracteata* had a significant *in vitro* anti-inflammatory impact against protein denaturation. To determine the processes and components behind its anti-inflammatory activities, further thorough investigations are required.

5. Conclusion

Numerous plants have been utilized for medicinal reasons from the ancient time. The majority of plant components have been employed as extracts, and they may have anti-inflammatory characteristics that are related to diseases like diabetes, atherosclerosis, neurological disease, or cancer. Additionally, plant extracts with anti-inflammatory properties can control the balance of the gut microbiota. By inhibiting pro-inflammatory cytokines like COX and lowering the translocation of NF- κ B to the nucleus, plants can protect against several diseases whose etiology involves immunological dysfunction or chronic inflammation. From the present investigation, it can be concluded that the different successive extracts of the plant *Impatiens bracteata* showed significant and promising anti-inflammatory activity. The preliminary

phytochemical shows that the maximum number of secondary metabolites was present in the methanolic and ethyl acetate extract of *Impatiens bracteata* followed by another extract. The methanolic extract of *Impatiens bracteata* shows strong anti-inflammatory activity compared to diclofenac sodium.

List of Abbreviations

Not available

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