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IN-VITRO ANTI-INFLAMMATORY ACTIVITY OF AN INDIGENOUS PLANT FROM NORTH EAST REGION OF INDIA

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ABSTRACT: Inflammation is essential for the body's defence mechanism but can lead to health issues when chronic inflammation persists over extended periods. *Anemone rivularis* Buch.-Ham. ex DC., native to the West Jaintia Hills of Meghalaya, India, is investigated for its anti-inflammatory potential. This study aim to assess the anti-inflammatory property of *Anemone rivularis* leaf extracts through phytochemical analysis and protein denaturation inhibition assays. Leaves of *Anemone rivularis* were subjected to maceration and hot percolation extraction technique by using methanol. Phytochemical analysis was done to identify bioactive compounds and egg albumin denaturation assay was conducted to evaluate anti-inflammatory activity. Phytochemical analysis revealed flavonoids, saponins, steroids, triterpenoids, tannins, and phenolic compounds in methanolic extracts. The extracts also exhibited significant inhibition of protein denaturation, indicating potential anti-inflammatory activity. So it can be concluded that *Anemone rivularis* shows promise as a natural anti-inflammatory agent with an percentage inhibition for standard 55.45% at a concentration 6.25µg/ml and for extract was 88.86% at a concentration of 0.001µg/ml. Further scientific investigation is necessary to confirm its efficacy and safety for preclinical and clinical use, highlighting its potential as a novel therapeutic option for managing inflammation-related conditions.

Keywords: *Anemone rivularis*, Anti-inflammatory, Egg albumin denaturation, Extraction

1.INTRODUCTION

Inflammation is a crucial aspect of the body's defence mechanism, triggered by various stimuli and involving the immune system's response to remove harmful agents and initiate healing. It presents in two main forms: acute and chronic. Acute inflammation arises rapidly in response to trauma, microbial invasion, or exposure to toxins, characterized by redness, swelling, and heat. Examples include conditions like cellulitis or acute pneumonia. Subacute inflammation bridges the gap between acute and chronic, lasting 2 to 6 weeks. Chronic inflammation, on the other hand, persists over extended periods, often evolving slowly and leading to long-term tissue damage (Zhang et al., 2019). This complex biological response involves a range of changes, including increased blood vessel permeability, protein denaturation, altered blood flow, and the generation of reactive oxygen species. Inflammatory mediators like prostaglandins and leukotrienes play vital roles in modulating these processes. Ultimately, the severity and impact of inflammation depend on factors such as the underlying cause of injury and the body's ability to heal (Abdallah & Esmat, 2017).

Treatment for inflammatory diseases adopts a comprehensive approach, integrating medication, rest, exercise, and sometimes surgery, tailored to individual factors like disease type and overall health. Anti-inflammatory drugs, notably nonsteroidal anti-inflammatory drugs (NSAIDs), play a pivotal role by inhibiting cyclooxygenase (COX) enzymes responsible for prostaglandin production. However, NSAIDs can induce adverse effects such as gastric and renal toxicity, prompting the development of selective COX-2 inhibitors to minimize these drawbacks while targeting inflammation (Vane & Botting, 1998). Despite advancements, these medications still pose potential side effects and limitations. Exploring alternative therapeutic strategies like lifestyle modifications, physical therapy, and dietary changes aims to effectively manage inflammation while reducing reliance on pharmacological interventions. As interest grows in safer alternatives, research into medicinal plants, particularly India's diverse flora, holds promise for uncovering new treatments with fewer drawbacks, highlighting the importance of exploring natural remedies in inflammatory disease management.

Anemone rivularis Buch.-Ham. ex DC., also known as "River anemone" or "River windflower," belonging to the family Ranunculaceae, originates from the Himalayan region of India and holds significant medicinal value in traditional healing practices. Historically utilized in the Indian System of Medicine, this plant has been revered for its diverse therapeutic properties, including its efficacy in treating rheumatoid arthritis, dysentery, stomach pain, malaria, stress, anxiety, cuts, burns, and snake bites. Notably, *Anemone rivularis* has been traditionally employed as a remedy for cancer and inflammation (Rajput et al., 2022). Some phytochemical studies have highlighted its potential anti-cancer activity against several human cancer cell lines (Wang et al., 2014), further underlining its pharmacological importance. With various parts of the plant, including roots, leaves, flowers, seeds, oils and fruits, being utilized for medicinal purposes, *Anemone rivularis* presents a promising avenue for addressing a wide range of health issues. However, further scientific investigation is necessary to substantiate its efficacy and safety in traditional medicine practices, emphasizing the ongoing need for research to unlock the full therapeutic potential of this botanical resource.

2. MATERIALS AND METHODS

2.1. Collection of plant material

The plant *Anemone rivularis* Buch.-Ham. ex DC. was collected from Mihmyntdu, Jowai, nestled within the scenic West Jaintia Hills of Meghalaya and was authenticated at the esteemed Botanical Survey of India, Eastern Regional Centre, Shillong, Meghalaya. Following collection, the leaves were carefully separated from the branches, subjected to thorough washing with tap water and distilled water, and then partially dried under shade and partially under sunlight for around 15 days.

2.2. Preparation of Extract

The dried leaves were further carried out for extraction by using two techniques: maceration and hot percolation.

2.2.1. Maceration

In this method, 300 grams of dried leaves were immersed in 1 litre of methanol for 15 days. Following maceration, the extract underwent filtration and subsequent lyophilization, the percentage yield was calculated.

2.2.2. Hot Percolation

Utilizing a Soxhlet apparatus, an equivalent quantity of dried leaves underwent hot percolation to ensure comprehensive extraction of desired compounds through continuous solvent cycling to produce the percentage yield.

2.3. Preliminary phytochemical tests

The preliminary phytochemical analysis was conducted separately for each of the methanolic extracts obtained through maceration and hot percolation methods, the following methods were used to confirm the presence of different phytochemicals:

2.3.1. Determination of Alkaloids

The procedure involved preparing solvent-free extracts from the methanolic extracts using a water bath. 50g of the resulting extract was then combined with diluted HCl and filtered. The clear filtrate obtained underwent Dragendorff's test, Mayer's test and Wagner's test to detect alkaloids.

2.3.2. Determination of Flavonoids

Using the leaf extracts, alkaline reagent test, concentrated sulfuric acid test and lead acetate test were conducted to determine the presence of flavonoids.

2.3.3. Determination of Saponins

To detect saponins, the froth test was employed. Leaf extract was added to water and vigorously shaken to observe the formation of froth, which indicate the presence of saponins.

2.3.4. Determination of Glycosides

The detection of glycosides involved hydrolyzing a 50g plant extract with concentrated HCl for 2 hours on a water bath, followed by filtration and utilizing the filtrate for Keller-Killiani and Borntrager's tests.

2.3.5. Determination of Carbohydrates

The procedure included dissolving 100mg of solvent-free extract in 5mL of distilled water, followed by filtration. The resulting filtrate was then used for conducting Molisch's, Fehling's, Barfoed's and Benedict's tests.

2.3.6. Determination of Proteins & Amino acids

The plant extract was hydrolyzed with concentrated HCl for 2 hours, filtered, and then tested for proteins and amino acids by performing the Biuret test, Millon's test, Ninhydrin test and Xanthoproteic test.

2.3.7. Determination of Steroids and Triterpenoids

For assessing steroids and triterpenoids, equal parts of chloroform were mixed with the plant extracts, filtered and then used for the Salkowski's test and Sulphur test.

2.3.8. Determination of Tannins and Phenolic compounds

To analyze tannins and phenolic compounds, each extract's residue was mixed with water, gently warmed, and filtered. The resulting filtrate was then tested using Ferric Chloride, Lead Acetate, Gelatin, Bromine and Iodine tests (Shaikh & Patil, 2020).

2.4. Evaluation of in-vitro anti-inflammatory activity

2.4.1. Egg Albumin Denaturation Assay

The anti-inflammatory activity of *Anemone rivularis* Buch.-Ham. ex DC. leaf extract, obtained through extraction technique of maceration and hot percolation, was evaluated using the egg albumin denaturation method with modifications inspired by Madhuranga HDT & Samarakoon DNAW's approach (Madhuranga HDT & Samarakoon DNAW, 2023).1% egg albumin solution was prepared by dissolving 1 ml of egg translucent portion in 100 ml of distilled water. Test samples, including 0.2 ml of egg albumin solution, 1 ml of sample extract, and 2 ml of phosphate buffer solution (pH 7.4), were serially diluted. Controls comprised 2 ml of distilled water, 0.2 ml of egg albumin solution, and 2 ml of phosphate buffer solution. After incubating the mixture at 37°C for 30 minutes and heating it in a water bath at 70°C for 15 minutes, absorbance was measured at 280 nm using a UV/Vis spectrophotometer, with distilled water as the blank. A stock solution of ibuprofen (10 mg in 100 ml of distilled water) served as the standard. Each experiment was conducted in triplicate, and the average was taken.

The percentage inhibition of protein denaturation was calculated using the following formula:

$$Percentage\ Inhibition = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

3. RESULTS AND DISCUSSION:

3.1. Extraction:

The percentage yield for maceration and hot percolation techniques was found to be 26.67% and 20% respectively, calculated based on the weight of the dried leaves used for extraction.

3.2. Preliminary Phytochemical Analysis:

The results of preliminary phytochemical analysis were represented in the following table 1:

Table 1: Preliminary Phytochemical Analysis

Phytoconstituents	Tests	Results	
		Maceration	Soxhlet
Alkaloids	Dragendorff's Test	-	-
	Mayer's Test	-	-
	Wagner's Test	-	-
Flavonoids	Alkaline Reagent Test	+	+
	Sulfuric Test	+	+
	Lead Acetate Test	+	+
Saponin	Froth Test	+	+
Glycosides	Keller-killiani Test	-	-
	Borntrager's Test	-	-
Carbohydrates	Molisch's Test	-	-
	Fehling's Test	+	+
	Barfoed's Test	-	-
	Benedict's Test	+	+
Proteins & Amino Acids	Biuret Test	-	-
	Millon's Test	-	-
	Ninhydrin Test	-	-
	Xanthoproteic Test	+	+
Steroids & Triterpenoids	Salkowski's Test	+	+
	Sulphur Test	+	+
Tannins & Phenolic Compounds	Ferric Chloride Test	+	+
	Lead Acetate Test	-	+
	Gelatin Test	-	+
	Bromine Test	+	+
	Iodine Test	+	+

3.3. Egg Albumin Denaturation Assay:

Protein denaturation is a potent reason for inflammation. Protein denaturation is a process in which proteins lose their tertiary and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when it got denatured. Denaturation of tissue proteins is one of the well documented cause of inflammation(Opie El,1962). The decrease in the absorbance of sample with respect to control indicated stabilization of protein i.e inhibition of protein (albumin) denaturation effect by the extract and the standard drug ibuprofen. The maximum inhibition of 83.86 % was observed at a concentration of 0.001µg/ml for methanol extract. Ibuprofen, a standard anti-inflammatory drug showed the maximum inhibition of 55.45% at the concentration of 6.25 µg/ml compared with control as shown in the Figure 1. Data has been statistically analysed using Graphpad Prism software and represented in the form of a histogram, where yellow histograms indicate the methanol extract of *Anemone rivularis* and green histogram represents standard drug ibuprofen. The yellow bar at the concentration of 0.001 µg/ml represents the increase in the anti-inflammatory activity with the minimum concentration as compared with standard drug which indicates that the leaves of this plant are very potential against inflammatory disorder in a dose dependent manner.

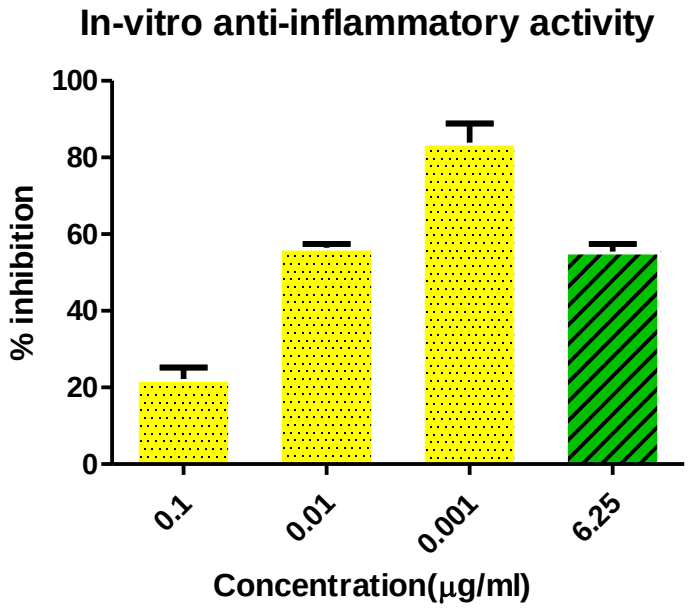

Fig.1 Graphical representation (x = %inhibition, y= concentrations) of the different concentrations of *A. rivularis* extract (yellow histograms) and Standard drug Ibuprofen (Green histogram)

4. CONCLUSIONS:

In present study, results indicate that the methanol extract of *Anemone rivularis* have numerous phytoconstituents like flavonoids, steroids, phenolic compounds, triterpenoids, tannins and saponins, among which flavonoids, steroids and triterpenoids are mainly responsible for having anti-inflammatory property. The extract also possesses anti-inflammatory properties through in-vitro proteinase inhibition. The results of in-vitro study by inhibition of protein denaturation

method showed that methanol extract exhibited significant anti-inflammatory activity by maximum inhibition than the standard drug and therefore it can be used effectively in the management of inflammatory diseases. Our research scientifically validates the traditional believes and practices that paves a direction to all researchers for further extensive investigation designing in-vivo animal models, and molecular basis study.

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6.CONFLICTS OF INTEREST: The author declares no conflict of interest.

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