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In Vitro and in Vivo Anti-Inflammatory Activity of Leaves of Cucumis Melo Linn. And Claytonia Perfoliata Donn Ex Willd.

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Abstract
The present investigation was undertaken to evaluate the in vitro and in vivo anti-inflammatory activity of leaves of Cucumis melo *Linn. and* Claytonia perfoliata *Donn ex Willd., two traditionally used medicinal plants. Ethanolic and aqueous extracts of both plants were prepared and subjected to preliminary phytochemical screening, which revealed the presence of flavonoids, tannins, phenolics, saponins, and glycosides, compounds well known for their anti-inflammatory potential. The in vitro activity was assessed using HRBC method, while in vivo studies were carried out in Wistar albino rats employing carrageenan-induced paw edema. Both extracts exhibited significant inhibition of protein denaturation and stabilization of red blood cell membranes in vitro, with ethanolic extracts demonstrating higher activity. In vivo results revealed dose-dependent inhibition of acute and chronic inflammatory responses, comparable to the standard drug diclofenac. The findings suggest that the leaves of* Cucumis melo *and* Claytonia perfoliata *possess promising anti-inflammatory properties, validating their ethnomedicinal use and providing a scientific basis for further exploration as potential natural alternatives for the management of inflammatory disorders.*
Key-words: *Plant Extract, HRBC, Inflammation*

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INTRODUCTION

Inflammation is a complex biological response of the body's immune system to harmful stimuli such as pathogens, damaged cells, or irritants, and while it is an essential defense mechanism, uncontrolled or chronic inflammation contributes to the pathogenesis of several diseases, including arthritis, cardiovascular disorders, neurodegenerative conditions, and cancer. Conventional anti-inflammatory drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, though effective, are often associated with adverse effects like gastric irritation, renal toxicity, and immunosuppression. This has intensified the search for safer and more effective alternatives from natural sources, particularly medicinal plants rich in bioactive phytoconstituents. [1-3]

Cucumis melo Linn. (Cucurbitaceae), commonly known as musk melon, has been traditionally used for its diuretic, antioxidant, and anti-inflammatory properties. Its leaves contain flavonoids, triterpenes, and phenolic compounds, which may contribute to its pharmacological activity. [4-5] Similarly, Claytonia perfoliata Donn ex Willd. (Montiaceae), commonly referred to as miner's lettuce, is a lesser-known ethnomedicinal plant valued for its nutritional and therapeutic attributes, including anti-inflammatory, wound healing, and antioxidant effects. Despite their traditional applications, scientific validation of the anti-inflammatory efficacy of these plants, particularly their leaves, remains limited. [6]

The present study was therefore designed to systematically evaluate both in vitro and in vivo anti-inflammatory activity of ethanolic and aqueous extracts of Cucumis melo and Claytonia perfoliata leaves. By employing established experimental models, the work aims to provide a pharmacological basis for their ethnomedicinal claims and to explore their potential as natural anti-inflammatory agents that may serve as safer alternatives to synthetic drugs.

MATERIAL AND METHODS

Selection, Collection and Authentication of Medicinal Plants

Based on the folklore and traditional claims the leaves of Cucumis melo Linn. and Claytonia perfoliata Donn ex Willd. were selected for the present investigation and were collected in the month of August-2024 from local areas of Bhopal and Dehradun. The collected plant materials were authenticated by the Botanist and Voucher specimen J/Bot./CML-15 & J/Bot./CPL-16 were allocated.

Drying of Powdered Plant Material

The collected plant material i.e., leaves of Cucumis melo Linn. and Claytonia perfoliata Donn ex Willd. was dried under sun.

In vitro anti-inflammatory activity (HRBC Method)

Human red blood cell membrane stabilization method

Preparation of drug

Standard drug (Indomethacin, 2.5 mg/ml) and extract (6.0 mg/ml) was prepared in isosaline (0.85% NaCl) to final the concentration.

Preparation of Suspension (10% v/v) of Human Red Blood cell

The blood sample was collected from healthy human volunteer who has not taken any NSAID for 2 weeks prior to the experiment and transferred to heparinized centrifuge tube. Blood samples were centrifuged at 3000 rpm at room temperature for 15 min. The supernatant (plasma and leucocytes) were carefully removed while the packed red blood cell was washed with fresh normal saline (0.85% w/v NaCl). The process of washing and centrifugation were repeated five times until the supernatants were clear. Then, Human erythrocytes suspension (10% v/v) were prepared.

Assay of Membrane stabilizing activity

The HRBC membrane stabilizing activity assay was carried out as per reported method using 10% (v/v) Human erythrocyte suspension while Indomethacin was used as standard drugs. The assay mixtures consisted of 2 ml of hyposaline (0.25% w/v) sodium chloride, 1.0 ml of 0.15 M sodium phosphate buffer, pH 7.4, 0.5 ml of 10% (v/v) human erythrocyte suspension, 1.0 ml of drugs (standard and extracts) and final reaction mixtures were made up to 4.5 ml with isosaline. To determine the anti-inflammatory activity by HRBC membrane stabilization method, the following solutions were used. [7-9]

Test solution (4.5ml) consists of 2ml of hypotonic saline (0.25%w/v) ,1ml of phosphate buffer (pH7.4), 1ml of test extract (6 mg/ml) in normal saline and 0.5ml of 10% w/v human red blood cells in isotonic saline.

Test control (4.5ml) consists of 2ml of hypotonic saline (0.25%w/v) 1ml of phosphate buffer (7.4pH) and 1ml of isotonic saline and 0.5ml of 10%w/v human red blood cells in isotonic saline.

Standard solution (4.5ml) consists of 2ml of hypotonic saline (0.25%w/v) 1ml of phosphate buffer (7.4pH) and 1ml of Indomethacin (2.5mg/ml) and 0.5ml 10%w/v human red blood cells in isotonic saline.

Drug was omitted in the blood control, while the drug control did not contain the erythrocyte suspension. The reaction mixtures were incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant solution was measured spectrophotometrically at 560 nm. Each experiment was carried out in triplicate and the average was taken. The percentage inhibition of haemolysis or membrane stabilization was calculated using the following equation.

% Inhibition of haemolysis = 100 x (A_1-A_2/A_1)

Where: A_1 = Absorption of hypotonic buffered saline solution alone; A_2 = Absorption of test sample in hypotonic solution

In vivo anti-inflammatory activity (Carrageenan induced)

Animals

Wistar rats (200-250 gm) were procured and maintained under ideal feeding and management practices in the laboratory. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water ad libitum. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The experimental protocols were approved by Institutional Animal Ethics Committee after scrutinization.

Study Design

The animals were divided into 10 groups each containing six animals. Group I served as vehicle control (2% Tween 80), Group II served as standard (diclofenac, 10 mg/kg, p.o.) and others group (Group III to X) were treated with different doses of ethanolic and aqueous leave extract of Cucumis melo Linn. and Claytonia perfoliata Donn ex Willd.

Anti-inflammatory Screening

The ethanolic and aqueous leave extract of Cucumis melo Linn. and Claytonia perfoliata Donn ex Willd. and standard drug diclofenac were administered in prescribed doses. Control received 0.1 ml of 1% carrageenan in 2% Tween 80. The administration of extract and drug was 30 min prior to injection of 0.1 ml of 1% carrageenan in the right hind paw subplatar of each rat. The paw volume was measured plesthysmometrically (model 7140, Ugo Basil, Italy). Prior to injection of carrageenan, the average volume of the right hind paw of each rat was calculated. At 1, 2, 3, 4, 5 and 6 hr after injection paw volume was measure. Reduction in the paw volume compared to the vehicle-treated control animals was considered as anti-inflammatory response. [10-12] Then percentage of inhibition of edema was calculated for each group with respect to the control group as follows,

% **Inhibition of paw edema = (VC-VT/VC) 100**

Where, VC and VT represent average paw volume of control and drug treated animals respectively

Statistical analysis

All the values ware statistically analyzed by one-way analysis of variance (ANOVA) followed Bonferroni's post hoc test. Comparison between control and drug treated groups were considered to be significant (*P<0.01). All values are expressed as mean ± SEM.

RESULTS AND DISCUSSION

The in-vitro anti-inflammatory activity of ethanolic and aqueous extracts of leaves of Cucumis melo Linn. and Claytonia perfoliata Donn ex Willd. was evaluated using the **r**ed blood cell (RBC) membrane stabilization method**,** a reliable model for assessing anti-inflammatory potential. This method is based on the principle that the stabilization of the RBC membrane mirrors the stabilization of lysosomal membranes, a key mechanism in preventing the release of inflammatory mediators. The results demonstrated that both ethanolic and aqueous extracts exhibited significant membrane stabilization activity in a concentration-dependent manner, indicating their ability to protect erythrocyte membranes against hypotonic-induced lysis. Notably, the ethanolic extracts of both plants showed higher protective effects compared to the aqueous extracts, which may be attributed to the presence of lipophilic phytoconstituents like flavonoids and phenolics in greater concentrations. These findings suggest that the leaf extracts, particularly the ethanolic fractions, possess promising anti-inflammatory properties that could be further explored for therapeutic applications. The results were given in table 1.

Table 1: In-vitro anti-inflammatory activity of extract by red blood cell membrane stabilization method

S/No.	Treatment	% Inhibition
1.	Control	-
2.	Indomethacin	83.19
3.	EECML	78.46
4.	AECML	64.29
5.	EECPL	76.15
6.	AECPL	61.41

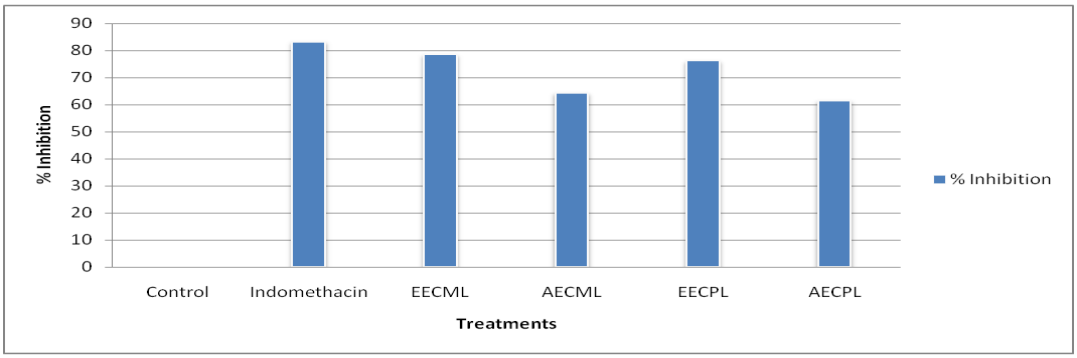

Fig. 1: Percentage Inhibition of Inflammation

The in vivo anti-inflammatory activity of ethanolic and aqueous extracts of leaves of Cucumis melo Linn. and Claytonia perfoliata Donn ex Willd. was evaluated using the carrageenan-induced paw edema model in rats, a well-established method for assessing acute inflammation. In this model, inflammation is induced by subplantar injection of carrageenan, leading to the release of pro-inflammatory mediators such

as histamine, serotonin, and prostaglandins. The administration of both ethanolic and aqueous extracts at selected doses significantly reduced paw edema in treated animals compared to the control group. The ethanolic extracts of both plants exhibited greater anti-inflammatory effects, suggesting a stronger inhibition of mediator release or activity, likely due to higher concentrations of bioactive compounds such as flavonoids, phenolics, and other anti-inflammatory phytoconstituents. The aqueous extracts also demonstrated notable activity, though to a lesser extent. These results indicate that bothplant extracts, particularly in ethanolic form, possess significant anti-inflammatory potential and support their traditional use in managing inflammatory conditions. The results were given in table 5.19 and percentage inhibition was mentioned in table 2.

Table 2: Effect of extract on change in paw edema volume on carrageenan induced inflammation in experimental animals

S/No.	Group	Dose in mg/kg bw	Change in paw edema volume (%) at Hour		
			1	3	5
1.	Control	-	1.22 ± 0.06	3.06 ± 0.05	4.94 ± 0.07
2.	Standard	10	1.06 ± 0.02+	2.76±0.06**	3.70 ± 0.06***
3.	EECML	250	1.09± 0.04+	2.84± 0.02**	3.84± 0.22***
4.	EECML	500	1.08± 0.02***	2.82± 0.07**	3.79± 0.04**
5.	AECML	250	1.02± 0.08*	3.24± 0.22+	4.20± 0.05**
6.	AECML	500	1.94± 0.20*	3.08± 0.24*	4.02± 0.07***
7.	EECPL	250	1.47± 0.29*	3.02± 0.04+	4.99± 0.08***
8.	EECPL	500	1.58± 0.04*	2.98± 0.09**	4.95± 0.09**
9.	AECPL	250	1.22± 0.02+	3.56± 0.08*	4.54± 0.20***
10.	AECPL	500	1.90± 0.22*	3.47± 0.02+	4.44± 0.04***

Data are expressed as mean ± S.E.M.; n=6 rats per group. Two way ANOVA followed by Bonferroni's post hoc test when compared with carrageenan control *P<0.05, **P<0.01, ***P<0.001, +NS

Table 3: Percentage Inhibition of Paw edema on Carrageenan induced inflammation in experimental animal

S/No.	Group	Dose in mg/kg bw	% Inhibition
1.	Control	-	-
2.	Standard	10	32.07
3.	EECML	250	29.10
4.	EECML	500	28.18
5.	AECML	250	19.18
6.	AECML	500	20.15
7.	EECPL	250	26.13
8.	EECPL	500	15.16
9.	AECPL	250	11.10
10.	AECPL	500	11.78

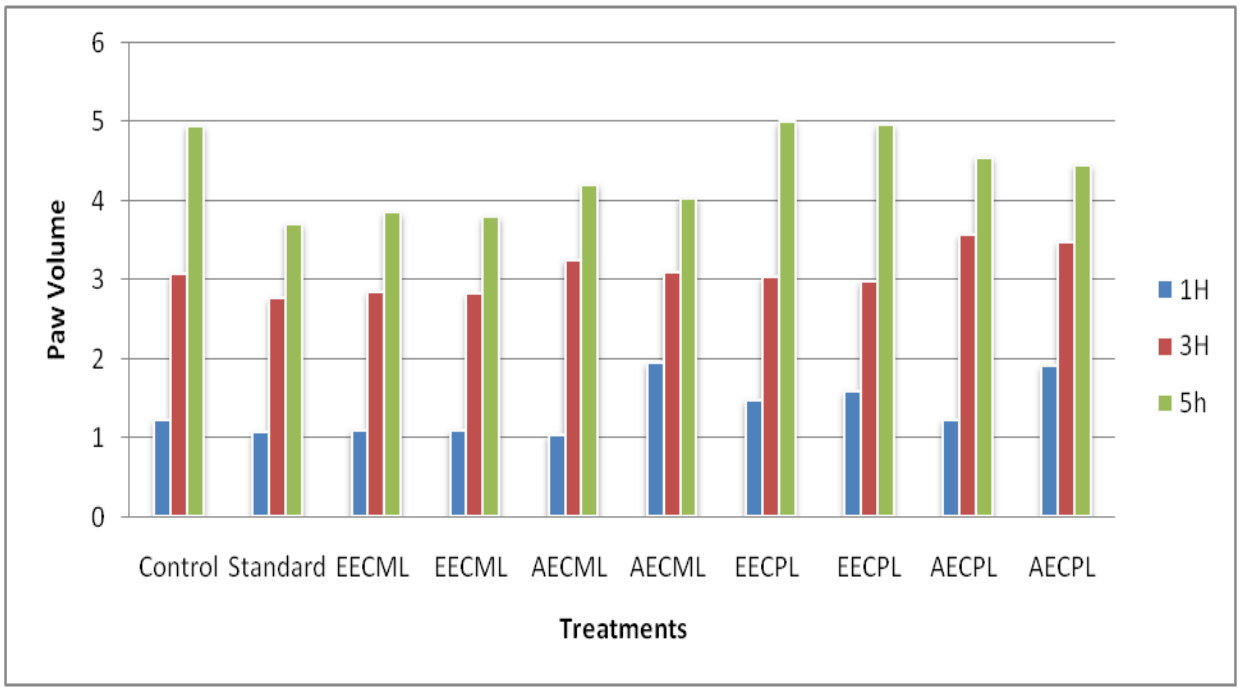

Fig. 2: Paw Volume of groups in Carrageenan induced inflammation in experimental animal

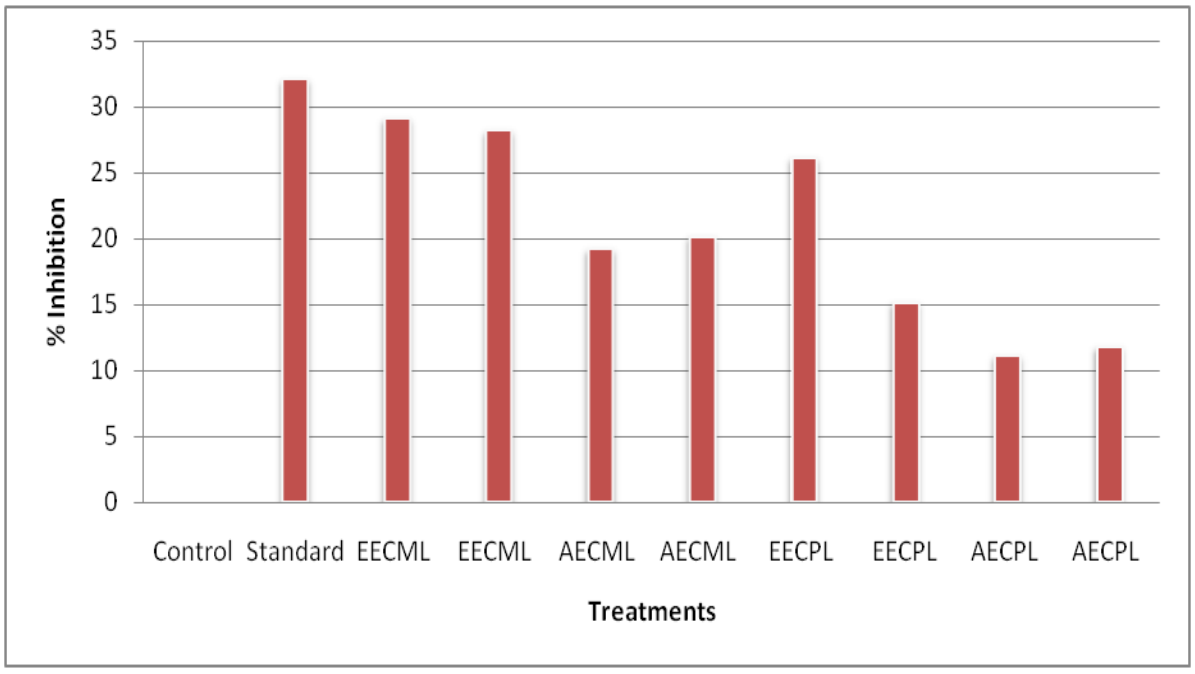

Fig. 3 Percentage Inhibition in Carrageenan induced inflammation in experimental animal

CONCLUSION

The present study demonstrated that the ethanolic and aqueous extracts of Cucumis melo Linn. and Claytonia perfoliata Donn ex Willd. leaves possess significant anti-inflammatory activity in both in vitro and in vivo experimental models. The extracts effectively inhibited protein denaturation and stabilized red blood cell membranes in vitro, while also producing marked suppression of carrageenan-induced paw edema in vivo. Among the tested extracts, the ethanolic extracts exhibited comparatively higher activity, which may be attributed to the better solubility and extraction of flavonoids, tannins, phenolics, and other bioactive constituents. These findings strongly support the traditional use of Cucumis melo and Claytonia perfoliata in the management of inflammatory conditions and suggest that the plants could serve as potential sources of safe and effective anti-inflammatory agents. However, further studies involving isolation and characterization of specific phytoconstituents, elucidation of underlying molecular mechanisms, and clinical validation are necessary to fully establish their therapeutic potential.

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