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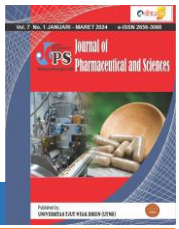

Dose–Response Effect of the Ethanolic Leaf Extract of *Cayratia trifolia* Linn. on Carrageenan-Induced Paw Edema in Rats

Efek Dosis–Respons Ekstrak Etanol Daun *Cayratia trifolia* Linn. pada Edema Kaki Tikus Terinduksi Karagenan

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Abstract

Introduction: *Leaves of Cayratia trifolia Linn.* contain several secondary metabolites with potential anti-inflammatory activity. However, evidence regarding the dose–response relationship of their ethanolic extract in acute inflammation remains limited. **Objective:** To evaluate the anti-inflammatory activity and dose–response trend of *Cayratia trifolia* leaf ethanolic extract (CTLEE) in a carrageenan-induced rat paw edema model. **Methods:** Twenty-five male rats (*Rattus norvegicus*) were divided into five groups (n=5): a 0.5% Na-CMC negative control, a 0.2% diclofenac sodium positive control, and CTLEE treatment groups receiving 100, 200, or 300 mg/kg body weight. Treatments were administered orally, followed 30 min later by an intraplantar injection of 0.1 mL of 1% carrageenan. Paw volume was measured every 60 min for 360 min using a digital plethysmometer. Data were expressed as mean ± SD and analyzed using one-way ANOVA followed by Tukey's post hoc test ($p<0.05p<0.05p<0.05$). **Results:** CTLEE reduced paw oedema relative to the negative control and showed a trend toward greater activity as the dose increased. At 360 min, edema percentages were 38.25 ± 11.88%, 29.21 ± 5.36%, and 24.43 ± 6.60% for CTLEE at 100, 200, and 300 mg/kg, respectively, compared with 74.83 ± 13.77% in the negative-control group. The corresponding inhibition values were 48.74 ± 12.10%, 60.24 ± 8.57%, and 65.43 ± 13.96%. Significant differences in paw edema were observed among groups at all assessment times ($p<0.001p<0.001p<0.001$). **Conclusion:** CTLEE demonstrated anti-inflammatory activity with a dose-related trend. The 300 mg/kg dose produced the greatest edema reduction and inhibition among the tested extract doses; however, therapeutic equivalence with diclofenac sodium was not established.

Keywords: Cayratia trifolia; anti-inflammatory activity; carrageenan; paw edema; dose–response.

Abstrak

Pendahuluan: Daun *Cayratia trifolia* Linn. mengandung berbagai metabolit sekunder yang berpotensi sebagai antiinflamasi. Namun, bukti mengenai hubungan dosis–respons ekstrak etanol daun tersebut pada model inflamasi akut masih terbatas. **Tujuan:** Mengevaluasi aktivitas antiinflamasi dan kecenderungan dosis–respons *ekstrak etanol daun Cayratia trifolia* (CTLEE) pada edema kaki tikus yang diinduksi karagenan. **Metode:** Sebanyak 25 ekor tikus putih jantan (*Rattus norvegicus*) dibagi menjadi lima kelompok (n=5), yaitu kontrol negatif Na-CMC 0,5%, kontrol positif natrium diklofenak 0,2%, serta CTLEE dengan dosis 100, 200, dan 300 mg/kg BB. Perlakuan diberikan secara oral, diikuti oleh induksi intraplantar 0,1 mL karagenan 1% setelah 30 menit. Volume kaki diukur setiap 60 menit selama 360 menit menggunakan pletismometer digital. Data disajikan sebagai rerata ± SD dan dianalisis menggunakan ANOVA satu arah yang dilanjutkan dengan uji Tukey ($p < 0,05$). **Hasil:** CTLEE menurunkan edema kaki dibandingkan dengan kontrol negatif dan menunjukkan kecenderungan peningkatan efek seiring meningkatnya dosis. Pada menit ke-360, persentase edema pada CTLEE dosis 100, 200, dan 300 mg/kgBB masing-masing sebesar 38,25 ± 11,88%, 29,21 ± 5,36%, dan 24,43 ± 6,60%, dibandingkan dengan 74,83 ± 13,77% pada kontrol negatif. Persentase inhibisi pada ketiga dosis tersebut masing-masing mencapai 48,74 ± 12,10%, 60,24 ± 8,57%, dan 65,43 ± 13,96%. Perbedaan edema antarkelompok bermakna pada seluruh waktu pengamatan ($p<0,001$). **Kesimpulan:** CTLEE menunjukkan aktivitas antiinflamasi dengan kecenderungan dosis-respons. Dosis 300 mg/kg BB menghasilkan penurunan edema dan inhibisi tertinggi di antara dosis ekstrak yang diuji, tetapi belum dapat dinyatakan setara secara terapeutik dengan natrium diklofenak.

Kata kunci: Cayratia trifolia; antiinflamasi; karagenan; edema kaki; dosis–respons.

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Introduction

Inflammation is a protective physiological response of the body to various external and internal stimuli, including foreign-body invasion, tissue damage, mechanical trauma, harmful chemicals, and physical factors. [1–4]. This complex process involves vascular reactions that increase vascular permeability, allowing fluids, blood components, and chemical mediators to accumulate at the site of injury to neutralise harmful agents and repair damaged tissues. [4–6]. The clinical manifestations of inflammation are generally characterised by the classical signs of redness (rubor), heat (calor), pain (dolour), swelling (tumour), and loss of function (*functio laesa*). These stimuli trigger the release of inflammatory mediators such as histamine, serotonin, bradykinin, and prostaglandins, which intensify the inflammatory response, increase vascular permeability, and cause the accumulation of fluids and leukocytes at the site of injury. [4,6,7].

The current management of inflammation generally relies on modern anti-inflammatory drugs, including steroidal and nonsteroidal anti-inflammatory drugs (NSAIDs) [8]. However, the long-term use of these synthetic drugs is often associated with serious adverse effects, including gastrointestinal toxicity such as gastric ulceration, impaired cardiac function, and renal damage. Therefore, there is an urgent need to explore alternative anti-inflammatory agents derived from natural sources that may offer more favourable safety profiles and fewer adverse effects.

One plant that communities in Indonesia and other countries have traditionally used is lakum or galing (*Cayratia trifolia* Linn.) [9–12]. This member of the Vitaceae family is a climbing plant widely distributed in tropical and subtropical regions, particularly in moist habitats such as riverbanks. [9–13]. Traditionally, the leaves of *Cayratia trifolia* have been used in herbal preparations for postpartum women, the treatment of boils and high fever, and as an antidote for snakebites. Various studies have demonstrated that *C. trifolia* possesses a broad spectrum of pharmacological activities, including antioxidant, antibacterial, antifungal, anticancer, antidiabetic, immunomodulatory, and hepatoprotective activities. [9–14].

The therapeutic potential of *Cayratia trifolia* leaves is supported by their abundant secondary metabolites, including alkaloids, flavonoids, tannins, phenolic compounds, saponins, terpenoids, and steroids. Flavonoid compounds, particularly resveratrol found in *Cayratia trifolia* leaves, have been reported to exert potent anti-inflammatory activity through the inhibition of cyclooxygenase-2 (COX-2) and prostaglandin biosynthesis. [15–17]. In addition, tannins and saponins may contribute to anti-inflammatory activity by reducing oxidant production and inhibiting increases in vascular permeability during inflammation. [15,18].

Evidence of the anti-inflammatory activity of *C. trifolia* has been reported in both in vitro and in vivo studies. The ethyl acetate fraction of the ethanolic stem extract of *C. trifolia* at a concentration of 1,000 ppm produced 91.81% erythrocyte membrane stabilisation in an in vitro assay [15,16]. A previous study in male mice reported that ethanolic lakum leaf extract administered at doses of 50, 100, and 150 mg/kg body weight produced inflammatory inhibition rates of 32%, 42%, and 51.36%, respectively, with the highest activity observed at 150 mg/kg body weight. [15,16]. However, studies specifically evaluating the dose–response effect of the extract in rats over a wider dose range remain limited.

Therefore, this study aimed to evaluate the anti-inflammatory activity of the ethanolic leaf extract of *C. trifolia* in male rats (*Rattus norvegicus*) using a carrageenan-induced paw oedema model. Carrageenan was selected as the inflammatory inducer because it produces a stable acute inflammatory response, does not cause permanent tissue damage, and is highly sensitive to anti-inflammatory agents. The findings of this study are

expected to provide a strong scientific basis for determining the optimal dose of *Cayratia trifolia* leaves as a potential herbal anti-inflammatory agent.

Methods

Materials and Instruments

The instruments used in this study included a drying cabinet, beakers, graduated cylinders, a percolator, aluminum foil, a blender, an analytical balance, an animal weighing balance, an oral gavage needle, a water bath, a digital plethysmometer (Ugo Basile, Cat. No. 7140), a rotary evaporator, syringes, a microscope, rat cages, a mortar and pestle, and a stopwatch. The plant material used in this study was Cayratia trifolia (L.) Domin leaves. The chemicals and reagents included 70% ethanol, distilled water, sodium carboxymethyl cellulose (Na-CMC), 0.9% sodium chloride solution, carrageenan, and diclofenac sodium.

Preparation and Characterisation of Plant Material

Leaves of *Cayratia trifolia* Linn. were collected using purposive sampling from Aceh, Indonesia. Young, fresh, and visibly undamaged leaves were selected. Botanical identification was performed to confirm the taxonomic identity of the plant material and prevent errors in species selection.

The collected leaves were cleaned to remove adhering dirt and foreign materials, cut into small pieces, and dried in a drying cabinet at approximately 40°C until completely dry. The dried material was subsequently ground into powder, weighed, and stored in a properly labelled, tightly closed container under dry conditions. Organoleptic characterisation was conducted by evaluating the appearance, colour, odour, and taste of the powdered plant material.

The moisture content was determined using a thermogravimetric method. Briefly, 5 g of the powdered plant material was weighed and dried in an oven at 100–105°C for 6 h. The sample was subsequently cooled in a desiccator and reweighed. The moisture content was calculated based on the difference between the sample weights before and after drying using the following equation:

$$\text{Moisture content (\%)} = [(W_1 - W_2) / W_1] \times 100$$

where $W_1$is the sample weight before drying and W_2 is the sample weight after drying.

Preparation of *Cayratia trifolia* Leaf Ethanolic Extract

A total of 500 g of powdered *Cayratia trifolia* leaves was moistened with 70% ethanol and allowed to stand for 3 h. The moistened plant material was gradually transferred into a percolator, and sufficient 70% ethanol was added to immerse it, forming a fully saturated solvent layer. The percolator was covered with aluminium foil and allowed to stand for 24 h [10,15].

Percolation was performed at a flow rate of approximately 20 drops/min. An additional 70% ethanol was continuously added at a rate corresponding to the percolate flow to maintain a constant solvent layer above the plant material. Percolation was terminated when the resulting percolate appeared clear. [10,15]. The collected percolate was concentrated under reduced pressure using a rotary evaporator at a temperature not exceeding 50°C until a concentrated extract was obtained. The extract was subsequently freeze-dried, weighed, and stored in a tightly closed glass container. [19]. The resulting extract was designated as *Cayratia trifolia* leaf ethanolic extract (CTLEE) [10,15]..

Experimental Animals and Treatment Groups

Twenty-five male rats (*Rattus norvegicus*), aged 2–3 months and weighing 200–300 g, were used in this study. The animals were acclimatised for two weeks in adequately ventilated cages maintained under hygienic conditions. Only healthy animals exhibiting normal growth, active movement, and no apparent signs of illness were included. [20–23]. The animals were allocated into five groups, with five rats in each group. The negative-control group received 0.5% sodium carboxymethyl cellulose (Na-CMC) suspension, whereas the positive-control group received 0.2% diclofenac sodium suspension. The three treatment groups received 2.5% CTLEE suspension at doses of 100, 200, and 300 mg/kg body weight, respectively. All treatments were administered orally. [20–23].

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Preparation of Test Suspensions and Inflammatory Inducer

The 0.5% Na-CMC suspension was prepared by dispersing 1 g of Na-CMC in 75 mL of hot distilled water in a mortar. The mixture was covered and allowed to stand for 15 min, until a transparent, gel-like mass formed. It was then triturated until homogeneous, and the final volume was adjusted to 200 mL with distilled water [24–28]. The 2.5% CTLEE suspension was prepared by triturating 2.5 g of the extract in a mortar. Na-CMC gel was gradually added while continuously triturating until a homogeneous suspension was obtained. The final volume was adjusted to 100 mL with distilled water [29].

The 0.2% diclofenac sodium suspension was prepared using 0.1 g of diclofenac sodium, equivalent to four tablets containing 25 mg each. The tablets were finely powdered, and Na-CMC gel was gradually added while triturating until a homogeneous mixture was obtained. The final volume was adjusted to 50 mL.[24–27]. The 1% carrageenan solution was prepared by triturating 50 mg of carrageenan with 0.9% sodium chloride solution until homogeneous. The mixture was transferred into a 5 mL volumetric flask and diluted to the calibration mark with 0.9% sodium chloride solution. The carrageenan solution was incubated at 37°C for 24 h before use as the inflammatory inducer. [24–27].

Evaluation of Anti-Inflammatory Activity

Anti-inflammatory activity was evaluated using the carrageenan-induced rat paw edema model. Before the experiment, the rats were fasted for 18 h but were allowed free access to drinking water. On the day of the experiment, each animal was weighed and marked on the tail and left hind paw for identification. [30,31]. The baseline volume of the left hind paw was measured using a digital plethysmometer. The paw was immersed in the measuring fluid up to a predetermined mark at the ankle joint. The value displayed on the instrument was recorded as the initial paw volume (V_0) before treatment administration and carrageenan induction. [30,31].

Following baseline measurement, the animals received the respective treatments orally. Thirty minutes after treatment administration, each rat was injected intraplantarly with 0.1 mL of 1% carrageenan solution into the plantar region of the left hind paw. Paw volume was measured every 60 min for 6 h using the digital plethysmometer. [30–32].

Throughout the measurement period, the volume of the measuring fluid and the depth of paw immersion were maintained consistently at each observation time. Paw oedema was determined from the increase in paw volume following carrageenan induction relative to the baseline volume. [30–32]..

The percentage of paw oedema was calculated using the following equation:

$$\text{Paw edema (\%)} = [(V_t - V_0) / V_0] \times 100$$

where V_t is the paw volume at a specified observation time, and V_0 is the baseline paw volume before carrageenan induction.

The percentage of oedema inhibition was calculated using the following equation:

$$\text{Edema inhibition (\%)} = [(a - b) / a] \times 100$$

where a is the mean percentage of paw oedema in the negative-control group and b is the mean percentage of paw oedema in the CTLEE-treated or positive-control group.

Statistical Analysis

Data were analysed using SPSS version 24 and expressed as mean values. Differences among the treatment groups were evaluated using one-way analysis of variance, followed by Tukey's post hoc test. Differences were considered statistically significant at the 95% confidence level when $p < 0.05$.

Results and Discussion

Plant Identification Results

The identification results confirmed that the plant material used in this study was the leaves of *Cayratia trifolia* Linn. The plant was classified under Kingdom Plantae, Division Magnoliophyta, Class Magnoliopsida, Order Rhamnales, Family Vitaceae, and Genus *Cayratia*. Plant identification is an essential stage in natural product research to ensure the accuracy of the species used and to prevent errors in sample selection. Accurate plant identification is also important because differences among species may affect the types and concentrations of secondary metabolites, potentially resulting in different pharmacological activities. Based

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on these findings, the plant material used in this study was confirmed to be the leaves of *Cayratia trifolia* Linn. belonging to the family Vitaceae.

Plant Material and Extract Characteristics

Botanical identification confirmed that the plant material used in this study consisted of leaves of *Cayratia trifolia* Linn. (Vitaceae). Macroscopic examination showed green leaf blades with waxy surfaces, a relatively soft texture, serrated margins, pointed apices and bases, and palmate venation. These characteristics provided an initial quality-control assessment to support authentication of the plant material and reduce the risk of species misidentification before extraction.

A total of 4 kg of fresh leaves yielded 600 g of dried leaf material, corresponding to a drying yield of 15.0%. Subsequently, 500 g of powdered dried material was extracted by percolation with 70% ethanol, yielding approximately 4 L of percolate and 110 g of extract. The extract yield was therefore 22.0% relative to the weight of the powdered material. Seventy per cent ethanol was selected because it can extract compounds across a relatively broad polarity range, whereas percolation provides continuous contact between fresh solvent and the plant matrix. In this article, the resulting extract is referred to as *Cayratia trifolia* leaf ethanolic extract (CTLEE).

Moisture Content of the Dried Plant Material

The moisture content of the powdered dried leaf material, determined by thermogravimetry, was 1.4%. This value was below the 10% limit used in the study as an acceptance criterion. Low moisture content can help restrict microbial growth, enzymatic activity, and degradation reactions that may alter the chemical composition of plant materials during storage. Therefore, the dried material was considered to be in a condition that supported stability before extraction. [33–35].

Anti-Inflammatory Activity of CTLEE

Anti-inflammatory activity was evaluated using the carrageenan-induced rat paw edema model. Paw oedema was assessed every 60 min for up to 360 min. All means and standard deviations presented in the following tables and figures were recalculated from the individual data of five animals in each group. This recalculation was performed to resolve inconsistencies between several values in the previous summary tables and the individual data reported in the study appendices.

Paw Oedema Percentage

Table 1. Paw oedema percentages after carrageenan injection

Group	Time after carrageenan injection (min)					
	60	120	180	240	300	360
0.5% Na-CMC	40.62 ± 4.96	74.20 ± 12.62	98.47 ± 9.13	91.29 ± 8.67	82.82 ± 10.42	74.83 ± 13.77
Diclofenac sodium	22.97 ± 4.89	32.60 ± 3.44	40.33 ± 5.95	31.33 ± 4.31	22.30 ± 5.69	10.79 ± 3.92
CTLEE 100 mg/kg BW	35.41 ± 3.93	61.72 ± 11.65	75.49 ± 12.79	61.53 ± 9.57	49.45 ± 10.27	38.25 ± 11.88
CTLEE 200 mg/kg BW	33.44 ± 4.09	56.46 ± 5.45	67.90 ± 7.90	52.37 ± 7.60	40.78 ± 7.19	29.21 ± 5.36
CTLEE 300 mg/kg BW	28.10 ± 5.63	49.68 ± 7.78	59.00 ± 3.80	49.05 ± 5.52	36.53 ± 8.10	24.43 ± 6.60

Data are presented as mean ± SD (n = 5). SD, standard deviation; CTLEE, *Cayratia trifolia* leaf ethanolic extract.

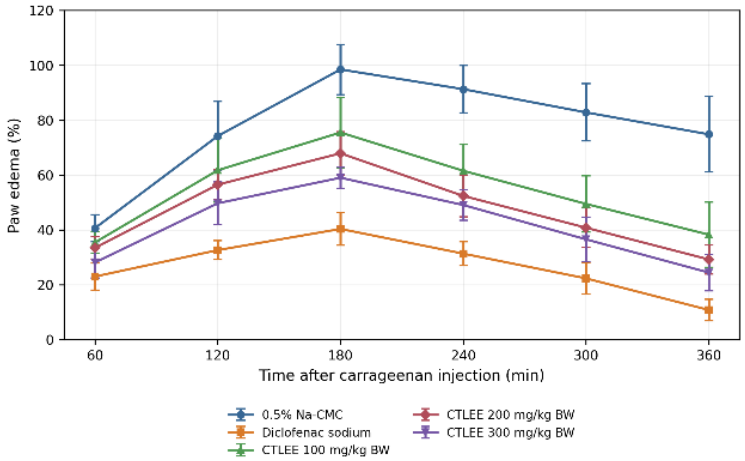

Figure 1. Paw oedema profile after carrageenan injection.

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The negative control group showed the greatest oedema response. Paw oedema increased from 40.62 ± 4.96% at 60 min to a peak of 98.47 ± 9.13% at 180 min, followed by a gradual decline; however, oedema remained high at 74.83 ± 13.77% at 360 min. This pattern indicates that carrageenan injection successfully produced an acute inflammatory response that persisted throughout the observation period.

Diclofenac sodium produced the lowest oedema percentages at all observation times. At 180 min, oedema in this group was 40.33 ± 5.95% and subsequently decreased to 10.79 ± 3.92% at 360 min. This response is consistent with the role of diclofenac sodium as an anti-inflammatory positive control, which suppresses prostaglandin formation by inhibiting the cyclooxygenase pathway.

CTLEE reduced edema relative to the negative control and showed a tendency toward a stronger response with increasing dose. At 180 min, the oedema percentages at 100, 200, and 300 mg/kg BW were 75.49 ± 12.79%, 67.90 ± 7.90%, and 59.00 ± 3.80%, respectively. At 360 min, these values decreased to 38.25 ± 11.88%, 29.21 ± 5.36%, and 24.43 ± 6.60%, respectively. Thus, CTLEE at 300 mg/kg BW consistently produced the lowest mean oedema among the extract-treated groups.

One-way ANOVA indicated significant differences among groups at all observation times ($p < 0.001$). Based on Tukey's post hoc analysis of the individual data, CTLEE at 300 mg/kg BW differed significantly from the negative control at every observation time. Compared with diclofenac sodium, CTLEE at 300 mg/kg BW showed no significant difference at 60, 300, and 360 min, whereas significant differences remained at 120, 180, and 240 min. These findings indicate that 300 mg/kg BW produced the strongest effect among the extract doses, particularly during the later phase of observation. Nevertheless, the absence of a statistically significant difference at selected time points should not be interpreted as evidence of therapeutic equivalence because the study was not designed as an equivalence trial.

Edema Inhibition

Table 2. Oedema inhibition percentages after carrageenan injection

Group	Time after carrageenan injection (min)					
	60	120	180	240	300	360
Diclofenac sodium	42.04 ± 17.15	54.78 ± 10.57	58.40 ± 9.26	65.30 ± 6.51	72.40 ± 9.15	84.57 ± 8.45
CTLEE 100 mg/kg BW	18.00 ± 10.23	21.87 ± 13.49	27.25 ± 15.41	32.29 ± 11.63	40.07 ± 11.67	48.74 ± 12.10
CTLEE 200 mg/kg BW	16.70 ± 13.14	21.82 ± 16.13	30.66 ± 9.11	42.15 ± 10.51	50.11 ± 11.05	60.24 ± 8.57
CTLEE 300 mg/kg BW	31.18 ± 8.10	31.82 ± 13.57	39.68 ± 6.51	46.03 ± 6.54	54.59 ± 13.58	65.43 ± 13.96

Data are presented as mean ± SD (n = 5). Values were recalculated from individual data in the study appendices. SD, standard deviation; CTLEE, *Cayratia trifolia* leaf ethanolic extract.

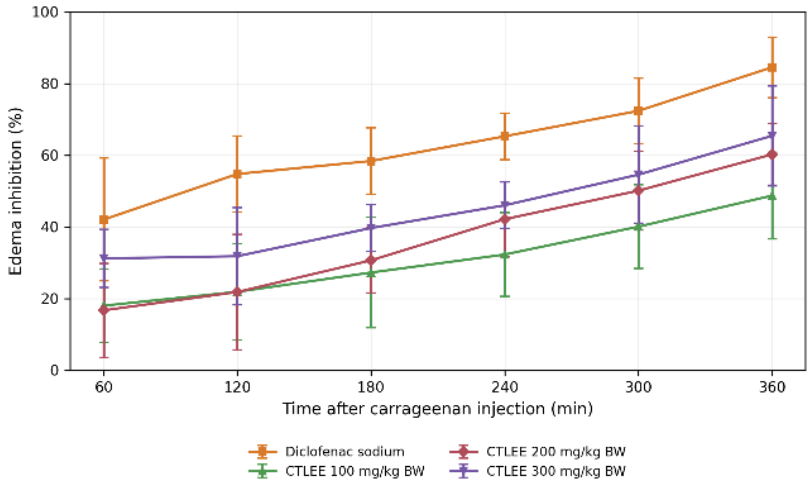

Figure 2. Oedema inhibition profile after carrageenan injection.

Oedema inhibition increased over time in all treatment groups. At 360 min, diclofenac sodium produced 84.57 ± 8.45% inhibition. At the same time point, CTLEE at 100, 200, and 300 mg/kg BW produced inhibition values of 48.74 ± 12.10%, 60.24 ± 8.57%, and 65.43 ± 13.96%, respectively. These data indicate that CTLEE at 300 mg/kg BW produced the greatest inhibitory effect among the three extract doses.

Differences in oedema inhibition among the groups were significant at 60 min ($p = 0.018$), 120 min ($p = 0.004$), 180 min ($p = 0.001$), 240 min ($p < 0.001$), 300 min ($p = 0.003$), and 360 min ($p < 0.001$). Tukey's test showed

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that the inhibitory effect of CTLEE at 300 mg/kg BW was not significantly different from that of diclofenac sodium at 60, 120, 180, 300, and 360 min. In contrast, a significant difference remained at 240 min. Although mean inhibition increased from 100 to 300 mg/kg BW, differences among the three CTLEE doses were not statistically significant at individual time points. Therefore, the findings are more appropriately interpreted as a dose-response trend rather than definitive evidence that each increase in dose produced a statistically distinct effect.

Discussion

The findings of this study are consistent with previous reports describing the pharmacological potential of *C. trifolia*. Kumar *et al*. [11,13] reported the presence of phenolic and stilbenoid compounds, including resveratrol and viniferin, as well as stilbenoid such as quercetin, kaempferol, and myricetin. These compounds are generally associated with modulation of inflammatory processes and oxidative stress. Resveratrol should be classified as a stilbenoid rather than a stilbenoid; therefore, its chemical class should be described accurately. [12].

Supporting evidence was also reported by Ilyas *et al*. [15], who evaluated erythrocyte membrane stabilisation in vitro using fractions of an ethanolic stem extract of *C. trifolia*. The ethyl acetate fraction at 1,000 ppm produced 91.81% membrane stabilisation. This finding supports the possible presence of anti-inflammatory constituents in the plant. However, it cannot be directly compared with the present study because the plant part, preparation method, concentration, and experimental model differed.

Nefertiti *et al*. [36] reported modulation of COX-2 expression by flavonoids from *C. trifolia* in a different biological model. This provides a biological basis for the possible involvement of the cyclooxygenase pathway. However, the present study did not measure COX-2, prostaglandins, cytokines, oxidative stress markers, or histopathological changes. Consequently, the reduction in oedema caused by CTLEE cannot be attributed directly to a single mechanism. Cyclooxygenase inhibition, membrane stabilisation, reduced vascular permeability, and antioxidant activity should be regarded as plausible mechanisms supported by the literature rather than mechanisms demonstrated in this study. [12].

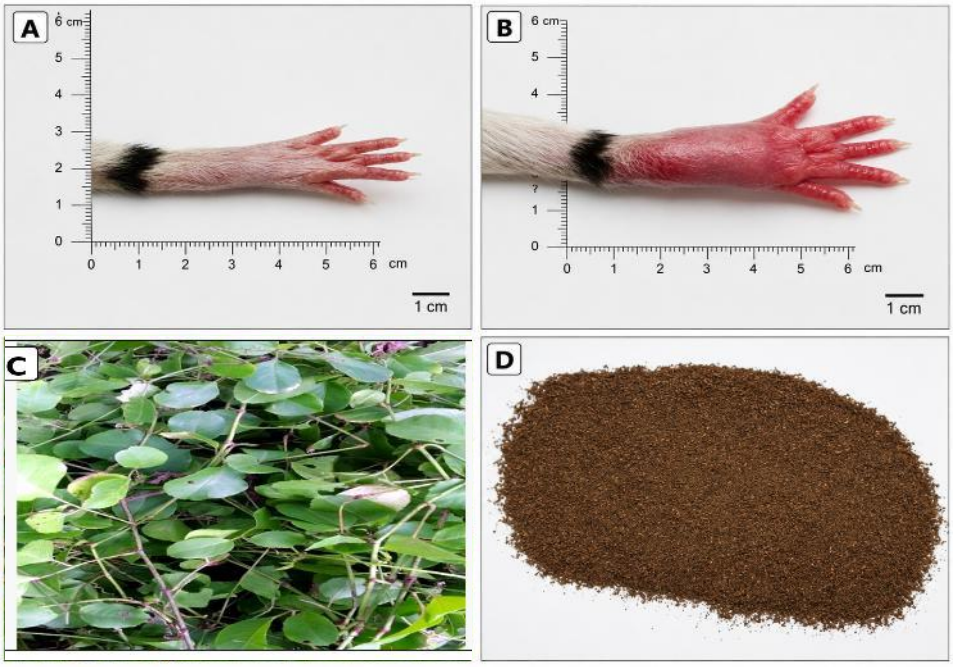

Figure 3. Plant materials and the rat paw oedema model: (A) rat paw before carrageenan induction; (B) rat paw after carrageenan induction; (C) leaves of *Cayratia trifolia* Linn.; and (D) powdered crude drug of *Cayratia trifolia* Linn. leaves.

The immunomodulatory potential of *C. trifolia* has also been reviewed by Siregar *et al*. [37]. Nevertheless, the relationship between immunomodulatory activity and reduced acute oedema in the carrageenan model requires confirmation through biomarker assessment and characterisation of the active constituents. Thus, the present in vivo results most strongly support a phenotypic effect, namely reduced paw oedema, whereas the molecular explanation remains hypothetical.

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CTLEE reduced carrageenan-induced paw oedema, with a tendency toward a stronger effect at higher doses. The 300 mg/kg BW dose produced the lowest oedema percentage and the greatest inhibition among the extract-treated groups. Although several comparisons with diclofenac sodium showed no statistically significant difference, these findings are insufficient to establish equivalent efficacy. Scientifically, the results provide promising preliminary evidence of anti-inflammatory activity and support further standardised studies to identify the active constituents, more clearly characterise the dose-response relationship, and evaluate safety and the mechanism of action.

The inhibitory mechanism of flavonoids against cyclooxygenase-2 (COX-2)

As illustrated in Figure 4, the anti-inflammatory activity of *Cayratia trifolia* is hypothesised to involve modulation of the arachidonic acid pathway. Cell membrane phospholipids are initially hydrolysed by phospholipase, resulting in the release of arachidonic acid, which is subsequently metabolised by cyclooxygenase-2 (COX-2) into prostaglandins and other eicosanoid mediators. Increased prostaglandin production contributes to vasodilation, enhanced vascular permeability, pain, and oedema formation. Flavonoids present in the extract are thought to suppress cyclooxygenase pathway activity, thereby reducing prostaglandin synthesis and attenuating the inflammatory response. In addition, flavonoids have been reported to exert membrane-stabilising effects that may limit the release of proteolytic enzymes from lysosomes and reduce further tissue damage during inflammation.

Resveratrol present in *C. trifolia* should be classified as a stilbenoid rather than a flavonoid. Theoretically, this compound may contribute to anti-inflammatory activity by modulating COX-2 expression or activity and reducing the biosynthesis of pro-inflammatory mediators. The combined effects of flavonoids, resveratrol, and other phenolic compounds may have contributed to the reduction in paw oedema observed following CTLEE administration. However, the present study did not directly measure COX-2 activity, prostaglandin levels, lysosomal membrane stabilisation, or other inflammatory mediators. Therefore, the mechanism depicted in the figure should be interpreted as a biologically plausible mechanism supported by the literature rather than one directly demonstrated in this study.

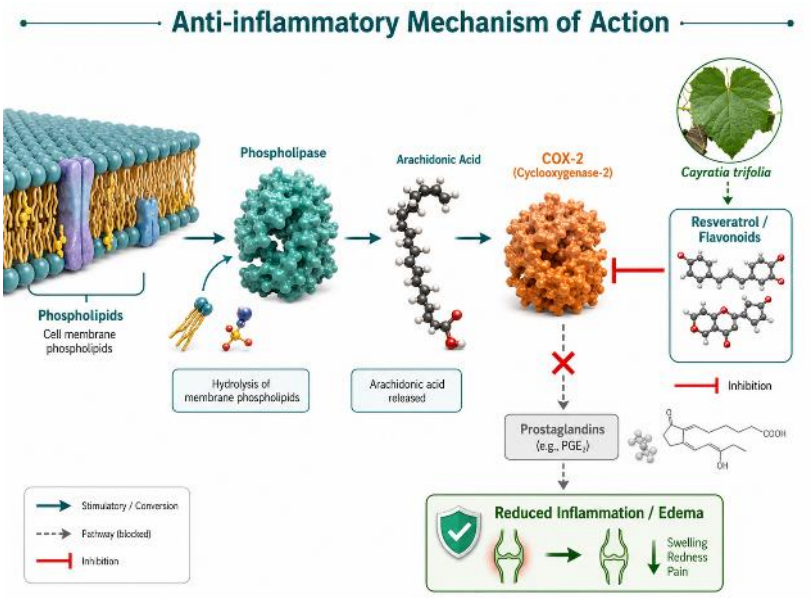

Figure 4. Proposed anti-inflammatory mechanism of *Cayratia trifolia* leaf ethanolic extract (CTLEE) through modulation of the arachidonic acid–COX-2–prostaglandin pathway.

Implications and Study Limitations

CTLEE demonstrated anti-inflammatory activity in the carrageenan-induced paw oedema model, with 300 mg/kg BW yielding the most favourable response among the tested doses. During the later phase of observation, the response at this dose approached that of diclofenac sodium in both the percentage of oedema and the degree of inhibition. These findings support the potential of *C. trifolia* leaves as a source of natural anti-inflammatory agents and provide a basis for further investigation.

Interpretation of the results should consider the small sample size of 5 animals per group and the biological variability, as reflected by relatively large standard deviations at several observation times. In

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addition, one-way ANOVA was performed separately at each time point even though repeated measurements were obtained from the same animals. A two-way repeated-measures ANOVA or a mixed-effects model would be more appropriate for evaluating the effects of treatment group, time, and the group × time interaction.

The extract was not standardised using a marker compound, and the study did not include inflammatory biomarkers, histopathological evaluation, toxicity assessment, or long-term safety testing. Future studies should use a standardised extract, determine the number of animals using a formal sample-size calculation, document randomisation and blinding procedures, apply repeated-measures statistical analyses, and quantify inflammatory mediators to strengthen the pharmacological evidence and clarify the mechanism of action of CTLEE.

Conclusions

Cayratia trifolia leaf ethanolic extract (CTLEE) demonstrated anti-inflammatory activity in the carrageenan-induced rat paw edema model. Among the tested doses, CTLEE at 300 mg/kg body weight produced the greatest reduction in paw oedema and the highest percentage of oedema inhibition, indicating a dose-related trend in anti-inflammatory activity. Although the response at this dose approached that of diclofenac sodium at several observation times, the findings do not establish therapeutic equivalence. Further studies using standardised extracts, larger sample sizes, repeated-measures statistical analyses, inflammatory biomarkers, histopathological assessments, and toxicity testing are required to confirm the active constituents, mechanism of action, efficacy, and safety of CTLEE.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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