



Passiflora edulis Sims leaves ameliorate inflammation and prevent cartilage degradation in osteoarthritic rats by suppressing cytokines, nitric oxide, and matrix metalloproteinase-9

[Las hojas de *Passiflora edulis* Sims mejoran la inflamación y previenen la degradación del cartílago en ratas osteoarthriticas al suprimir las citocinas, el óxido nítrico y la metaloproteína de matriz-9]

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Abstract

Context: Treatment for osteoarthritis focuses on controlling pain, lowering inflammation, delaying cartilage deterioration, and minimizing impairment.

Aims: To evaluate the bioactivity of leaf extract 'markisa' (*Passiflora edulis*, MLE) as an *in vitro* radical scavenger, and an *in vivo* edema reducer and pro-inflammatory mediators in an osteoarthritis rat model.

Methods: MLE was prepared by an ultrasonication method with ethanol. First, the *in vitro* antioxidant activity of MLE was assessed with ABTS reagent, and the total phenolics (TPC) and flavonoid (TFC) contents were determined. Furthermore, *in vivo* tests were conducted. Wistar rats were induced osteoarthritis by intraarticular injections of sodium iodoacetate (13.5 mg/kg BW) and observed for one month. MLE was administered orally to animal models in doses of 100, 200, and 400 mg/kg BW every day for two weeks. Body weight and knee edema enlargement were observed every week. At week 6th, all animals were sacrificed for evaluation of spleen weight, hematology analysis (leukocyte and lymphocyte number), and pro-inflammatory mediators assay (NO, IL-6, TNF- α , and MMP-9).

Results: This study showed that MLE has antioxidant capacity $13.26 \pm 0.7 \mu\text{mol TE/g}$, TPC $8.17 \pm 0.03 \text{ mg GAE/g}$, and TFC $3.28 \pm 0.3 \text{ mg QE/g}$. *In vivo* tests showed that MLE-treated rats had reduced knee edema, hematological improvement (decreased NO, IL-6, and TNF- α), spleen enlargement, and protection against cartilage degradation by decreasing MMP-9.

Conclusions: These findings suggest that MLE therapy has the potential to restore the state of osteoarthritic rats to normal, with varying degrees of dose-dependent improvement.

Keywords: antioxidant; IL-6; NO; pro-inflammatory mediators; sodium iodoacetate; TNF- α .

Resumen

Contexto: El tratamiento de la osteoartritis se centra en controlar el dolor, reducir la inflamación, retrasar el deterioro del cartílago y minimizar el deterioro.

Objetivos: Evaluar la bioactividad del extracto de hoja de 'markisa' (*Passiflora edulis*, MLE) como eliminador de radicales *in vitro*, y como reductor de edemas y mediador proinflamatorio *in vivo* en un modelo de rata con osteoartritis.

Métodos: El MLE se preparó mediante un método de ultrasonificación con etanol. Primero, se evaluó la actividad antioxidante *in vitro* del MLE con el reactivo ABTS y se determinó el contenido total de fenoles (TPC) y flavonoides (TFC). Además, se realizaron pruebas *in vivo*. Se indujo osteoartritis a ratas Wistar mediante inyecciones intraarticulares de yodoacetato de sodio (13,5 mg/kg de peso corporal) y se las observó durante un mes. Se administró MLE por vía oral a modelos animales en dosis de 100, 200 y 400 mg/kg de peso corporal todos los días durante dos semanas. Se observó el peso corporal y el aumento del edema de rodilla cada semana. En la semana 6, todos los animales fueron sacrificados para la evaluación del peso del bazo, análisis hematológico (número de leucocitos y linfocitos) y ensayo de mediadores proinflamatorios (NO, IL-6, TNF- α y MMP-9).

Resultados: Este estudio mostró que MLE tiene una capacidad antioxidante de $13,26 \pm 0,7 \mu\text{mol TE/g}$, TPC $8,17 \pm 0,03 \text{ mg GAE/g}$ y TFC $3,28 \pm 0,3 \text{ mg QE/g}$. Las pruebas *in vivo* mostraron que las ratas tratadas con MLE tuvieron una reducción del edema de rodilla, una mejoría hematológica (disminución de NO, IL-6 y TNF- α), un aumento del bazo y protección contra la degradación del cartílago al disminuir la MMP-9.

Conclusiones: Estos hallazgos sugieren que la terapia MLE tiene el potencial de restaurar el estado de las ratas osteoarthriticas a la normalidad, con diversos grados de mejoría dependiente de la dosis.

Palabras Clave: antioxidante; IL-6; mediadores proinflamatorios; NO; TNF- α ; yodoacetato de sodio.

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Abbreviations: ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); BW: body weight; COX: cyclooxygenase; COX-2: cyclooxygenase-2; FCR: Folin-Ciocalteu reagent; IL-1 β : interleukin-1 β ; IL-6: interleukin-6; iNOS: inducible nitric oxide synthase; LPX: lipoxygenase; MLE: *Passiflora edulis* leaf extract; MMP: matrix metalloproteinase; NF- κ B: nuclear factor *kappa* B; NO: nitric oxide; NOS: nitric oxide synthase; OA: osteoarthritis; PGE2: prostaglandin E2; TFC: total flavonoid compounds; TNF- α : tumor necrosis factor- α ; TPC: total phenolic compounds; CMC Na: sodium carboxymethylcellulose; GAE: gallic acid equivalent; QE: quercetin equivalent; Neg.: negative group; Pos.: positive group.

INTRODUCTION

Osteoarthritis (OA) is a degenerative musculoskeletal condition; it is becoming more common and causes chronic pain, stiff joints, and impairment (Fang et al., 2022). According to Indonesia Primary Health Research data in 2018, OA is a significant threat to social and public health, where its prevalence is estimated to be 7.3%. It is unclear how OA develops from the beginning. Due mainly to articular cartilage degeneration, subchondral osteosclerosis, synovial lesions, and chondrocyte destruction, this syndrome is characterized by chronic inflammation (Guo et al., 2022). In OA, proinflammatory cytokines such as interleukin-1 β (IL-1 β), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and nerve growth factor (NGF) are produced and released into joint fluid, along with chemokines, and this triggers a series of events. Pain can occur due to the impact of cytokines on sensory nerves that increase peripheral sensitivity (Yu et al., 2022). The advancement of OA has been linked to several transduction pathways, most notably the increase in the nuclear factor *kappa*-light chain enhancer of activated B cells (NF- κ B). When stimulated, NF- κ B transfers to the nucleus from the cytosol by activating I κ B α kinase phosphorylating the inhibitor of the *kappa*-B protein, which causes the expression of cyclooxygenase-2 (COX-2), prostaglandin E2 (PGE2) synthesis, IL-1 β , IL-6, TNF- α , and inducible nitric oxide synthase (iNOS) (Gong et al., 2020). An important rise in pro-inflammatory mediators and cytokines accelerates the destruction of the extracellular matrix, which causes pain, inflammation, and worsened OA development (Guowei et al., 2022). Inhibiting pro-inflammatory cytokines (such as IL, TNF- α), nitric oxide (NO), and matrix metalloproteinase (MMP) activity may help reduce or stop the progression of OA by lowering chondrocyte inflammation and cartilage resorption (Han et al., 2020).

It was once thought that tissue damage or inflammation was the source of OA pain. OA symptoms, such as pain, inflammation, stiffness, and decreased mobility, are the main causes of patients seeking medical attention and therapy. Chronic discomfort that lasts for a long time will interfere with daily activities, sleep, mental health, and social interactions (Yu et al., 2022). However, the nociceptive etiology of OA is not fully understood; the current method of managing OA nociceptive pain by painkillers has not been successful, and it is known that the use of painkillers for

a prolonged period of time can have serious side effects and toxicity. Steroids and non-steroidal anti-inflammatory medications are the usual treatments for OA. Numerous skeletal disorders, such as osteoporosis and joint deformity, are caused by long-term use of steroids. Numerous pharmacological methods for treating OA have been studied to date, including the introduction of stem cells, protease inhibitors, biological agents, and anti-inflammatory medications. People would also prefer to use herbal remedies to treat OA and enhance their quality of life (Fang et al., 2022). Important new perspectives for the development of safer and more effective pain medicines by investigating processes by which natural substances contribute to the inhibition of pain and inflammation in OA.

The plant that is believed to provide anti-inflammatory effects is *Passiflora edulis* Sims (family *Passifloraceae*). The *P. edulis* plant has been reviewed to provide a variety of medicinal benefits that can be utilized to treat conditions such as OA, including anti-inflammatory, anticonvulsant, anticancer, antibacterial, antidiabetic, antihypertensive, and anti-sedative. The leaves are frequently used as an anti-inflammatory (Rai et al., 2022). The ethanol extract of *P. edulis* leaves contains some saponin, polyphenolic, flavonoid, terpenoid, and steroid (He et al., 2020; Urrego et al., 2021). Flavonoids have many mechanisms of action, such as inhibiting enzymes that regulate inflammatory responses by reducing the production of arachidonic acid and prostaglandins, leukotrienes, and NO by inhibiting the enzymes phospholipase A2, cyclooxygenase (COX), lipoxygenase (LPX) and nitric oxide synthase (NOS) (Chanput et al., 2016). *P. edulis* leaves are a well-known source of flavonoids and contain 158.0 mg/mL total amount of flavonoids, 0.42 mg/g of quercetin, and 16.2 mg/mL of isoorientin (He et al., 2020).

Despite existing OA treatments that focus on pain management, reducing inflammation, delaying cartilage damage, and minimizing disability, there is an urgent need for more effective and natural therapies that can provide better results with minimal side effects. This study aims to explore the potential bioactivity of *P. edulis* leaf extract as a potential therapy that could offer an alternative approach to the treatment of OA. The ethnomedicinal use of plants such as *P. edulis* is often based on traditional knowledge passed down through generations. In this study, the

antioxidant and anti-inflammatory properties of *Passiflora edulis* leaf extract (MLE) were scientifically evaluated to demonstrate its effectiveness in addressing OA. These findings not only support the ethnomedicinal use of passion fruit but also strengthen the scientific understanding of its bioactive mechanisms, such as reducing edema, protecting against cartilage degradation, and modulating immune responses, which are critical in managing OA.

In this current study, *P. edulis* leaves were extracted using ultrasonic extraction to increase cell wall permeability, thus obtaining a higher content of secondary metabolites in a relatively short time (Zhang et al., 2018). However, there are several intriguing effects of *P. edulis* in the research of OA treatment, including its anti-inflammatory function, which is still unknown. In this research, intraarticular injection into the rat knee is used to model animal inflammatory responses and pain to induce OA models in rats. We sought to determine the regulatory effects of MLE on inflammatory pathways in knee tissues of OA model rats and to test whether MLE can reduce NO and cytokines (IL-6 and TNF- α), and protect cartilage degradation by lowering MMP-9.

MATERIAL AND METHODS

Authentication of plant specimens and reagents

The leaf of *P. edulis* Sims (*Passifloraceae*) was obtained from Bogor, Indonesia (6.5° S, 106° E), and authenticated at the Universitas Indonesia with determination number 867/UN2.F3.11/PDP.02.00/2022. Sodium iodoacetate, Folin-Ciocalteu reagent (FCR), gallic acid, quercetin, Trolox, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma-Aldrich (Darmstadt, Germany), USP ketamine HCl injection (Hospira, Inc., USA), Xylazine (Interchemie, Holland), sodium diclofenac 25 mg/tablet (PT Novell Pharmaceutical Laboratories, Indonesia), Quantikine TM ELISA kits (R&D Systems, Minneapolis, USA): NO (KGE001), IL-6 (R6000B), TNF- α (RTA00), and MMP-9 (RMP900). Ethanol, NaCl, sodium carboxymethylcellulose (CMC Na), distiller water (Brataco Chemical, Indonesia).

Osteoarthritis animal models

Male Wistar rats (two months old) weighing 200 to 250 g were obtained from the Balitbangkes Laboratory, the Ministry of Health of the Republic of Indonesia. The animals were housed in cages, according to the Pharmacological Research Facilities of the Faculty of Pharmacy of Pancasila University, in a controlled environment of $24 \pm 2^\circ\text{C}$, with a relative humidity of 60 to 70% and illumination cycle 12 h per day (07:00-19:00). Any treatment given to experimental animals

was carried out according to the ethical standards established by the Ethics Committee for Health Research (KET-1386/UN2.F1/ETIK/PPM.00.02/2022), Faculty of Medicine, Universitas Indonesia. Food intake was regularly given every day, and body weight was assessed weekly during the research period.

Preparation of *P. edulis* leaf extract (MLE)

The dry powder of *P. edulis* leaf was extracted using the ultrasonication method (frequency 40 kHz, 30°C , 45 minutes) (GT SONIC-P13, Guangdong, China) with 70% ethanol. Extraction was carried out two times, and then the filtrate was concentrated by rotavapor to obtain the MLE).

Analysis of the total phenolic content (TPC) and total flavonoid content (TFC)

TPC assay was performed using FCR and gallic acid as a standard (Bobo-García et al., 2015). A series of gallic acid solutions were prepared in ethanol (final concentration 12.5-200 $\mu\text{g/mL}$) was prepared. 20 μL of gallic acid was pipetted into a 96-well microplate, 100 μL of FCR solution (1:4) was added and allowed to stand for 4 minutes, then 80 μL of sodium carbonate (10%) was added. The solution was incubated for 120 minutes in a light-protected location, and the absorbance was measured at 750 nm using a microplate reader (Multiskan, Thermo Fisher Scientific Inc., USA). A linear regression equation was made. The 3000 $\mu\text{g/mL}$ extract solution in ethanol was prepared in a similar way. The TPC of the extract was represented as mg GAE/g.

The determination of the total flavonoid content using a 2% AlCl_3 solution as reagent and quercetin as standard (Chatatikun and Chiabchalard, 2017). Quercetin was dissolved in methanol (final concentration 12.5-200 $\mu\text{g/mL}$). 50 μL of each sample was pipetted, and 50 μL of 2% AlCl_3 reagent was added. The solution was incubated for 15 minutes in a light-protected place, and the absorbance was measured at 435 nm using a microplate reader (Multiskan, Thermo Fisher Scientific Inc., USA). The 3000 $\mu\text{g/mL}$ extract solution in ethanol was treated similarly. The total flavonoid content was expressed as mg QE/g of extract.

2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging capacity

Antioxidant capacity assay according to the description of Thring et al. (2009), with modifications. A solution of ABTS 7 mM (in distilled water) was added to 2.45 mM $\text{K}_2\text{S}_2\text{O}_8$ (in distilled water) and incubated for 12-16 hours. The mechanism is that ABTS is oxidized by $\text{K}_2\text{S}_2\text{O}_8$, which converts it into the colored ABTS radical cation (ABTS $^{\cdot+}$). This radical cation ab-

sorbs light at 734 nm. When an antioxidant is introduced, it will reduce ABTS-⁺ back to its colorless form, thus causing a decrease in absorbance. This change is used to measure the antioxidant capacity of the sample. This makes the ABTS assay a versatile and widely used method for evaluating antioxidant capacity. The ABTS solution was pipetted 1 mL and the volume was sufficient to 5 mL with ethanol. The Trolox solution was made in distilled water until a final concentration of 5-25 µg/mL was obtained. The extract was processed into a series of concentrations until a final concentration of 20-100 µg/mL was obtained. 20 µL of the test solution and 180 µL of ABTS solution were pipetted on Nunc 96 well plates, then incubated at room temperature and protected from light for 30 minutes. The absorbance was measured at 734 nm in a microplate reader (Multiskan, Thermo Fisher Scientific Inc., USA). The antioxidant capacity of the extract was expressed as equivalence to Trolox in µmol TE/g.

In vivo experimental design

The test animals were divided into six groups (4 rats/group). Group 1 was a normal and healthy group given standardized feed and water. The remaining five groups, groups 2 to 6, on day 1, received an intraarticular injection in the knee with 0.25 mL of sodium iodoacetate with a sodium iodoacetate concentration of 10 mg/mL. Before injection of sodium iodoacetate, rats were anesthetized using a combination of xylazine at a dose of 8.8 mg/kg BW and ketamine (75 mg/kg BW) intraperitoneally. After induction with intraarticular injection of sodium iodoacetate on the first day, groups 2 to 6 were monitored until they developed OA within one month. Group 2 was the negative group (Neg.), OA rat models without any treatment. Groups 3 to 6 were OA rat models and then treated with diclofenac sodium (13.5 mg/kg BW) for the positive control group (group 3: Pos.) and *P. edulis* leaf extract once daily orally at doses of 100 (group 4: MLE-100), 200 (group 5: MLE-200), and 400 (group 6: MLE-400) mg/kg BW for 14 days. Sodium diclofenac and MLE suspension were prepared by suspending crushed diclofenac tablets or MLE in 0.5% Na-CMC. Throughout the research period, the diameter of the knee was measured using a micrometer screw. At the end of the study (end of week 6), all rats were sacrificed. Blood, spleen, and knee tissue were taken for analysis.

Evaluation of cytokines of spleen weight, hematology, and knee tissue

In the current investigation, the rat's spleen organs were weighed and then the average of each group

was calculated (Sandhiutami et al., 2023). Rasio spleen weights per body weight of each rat were determined. Rat blood was taken and put in a clean tube, and K₃EDTA was administered. Leukocytes and lymphocytes were counted using the Sysmex XS-1000i hematology analyzer using the flow cytometry method. The levels of the IL-6, TNF-α, NO, and MMP-9 from knee tissue were evaluated using accessible rat ELISA kits, according to the manufacturer's protocol, in triplicate, and the results were interpreted appropriately.

Statistical analysis

The mean and standard deviation were used to express all the results data. Statistical analysis was performed using SPSS 20.0 (SPSS for Windows, USA). The one-way ANOVA hypothesis test followed by post hoc Least Square Difference (LSD) determines the differences between each group to indicate the statistical significance difference using a confidence interval at p<0.05.

RESULTS

TPC, TFC, and antioxidant determination

The leaves of *P. edulis* were reported to contain flavonoids, polyphenols, triterpenoids, and other compounds that play an important role in their bioactivity. Some studies also show that *Passiflora* leaves have strong antioxidant activity (da Silva et al., 2013; He et al., 2020).

In the flavonoid assay, the standard compound quercetin was used. The standard curve can be seen in Fig. 1A. The polyphenolic assay uses a standard gallic acid compound. The calibration curve can be seen in Fig. 1B. *In vitro* antioxidant activity test with ABTS reagent using Trolox as a comparative compound. The standard curve can be seen in Fig. 1C. The results of the examination of the flavonoid and polyphenol content and the *in vitro* antioxidant test of the extracts are presented in Table 1.

Table 1. Determination of the total flavonoid and phenolic content and the *in vitro* antioxidant test of the MLE.

Extract data	Results
Extract yield	29 ± 0.2%
TFC	3.28 ± 0.3 mg QE/g extract
TPC	8.17 ± 0.03 mg GAE/g extract
ABTS	13.26 ± 0.7 µmol TE/g extract

Data are expressed as mean ± SD (n = 3). TFC: Total flavonoid compounds; TPC: Total phenolic compounds; QE: Quercetin equivalent; GAE: Gallic acid equivalent; TE: Trolox equivalent; MLE: *Passiflora edulis* leaf extract.

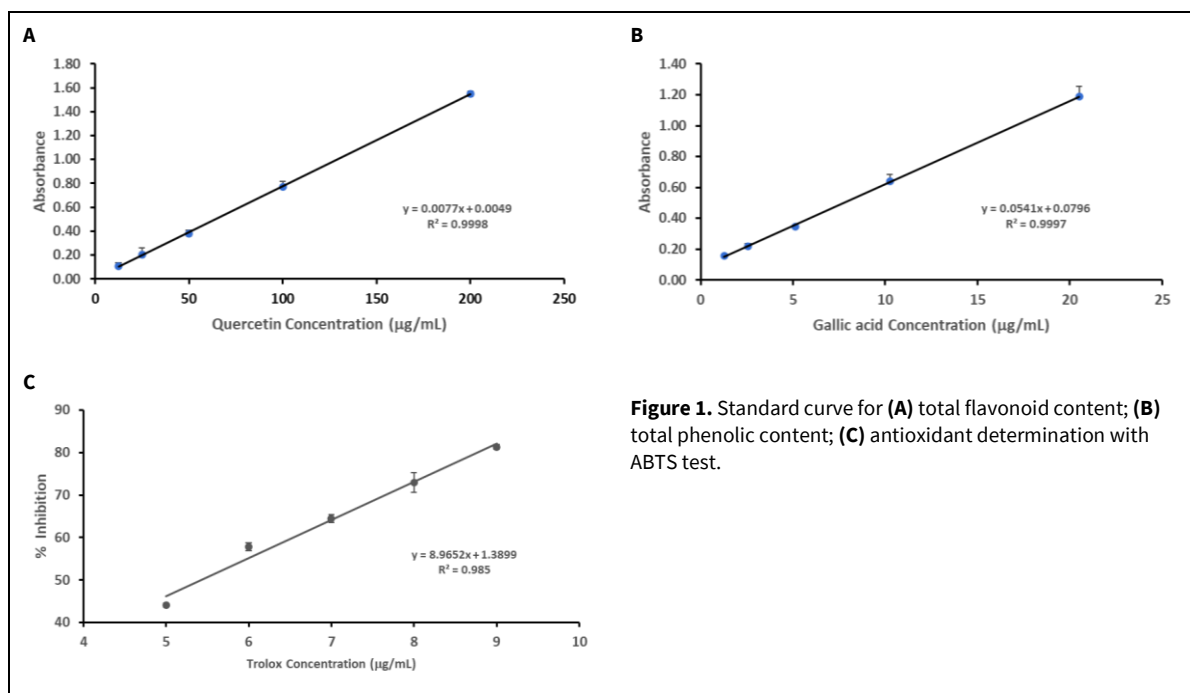


Figure 1. Standard curve for (A) total flavonoid content; (B) total phenolic content; (C) antioxidant determination with ABTS test.

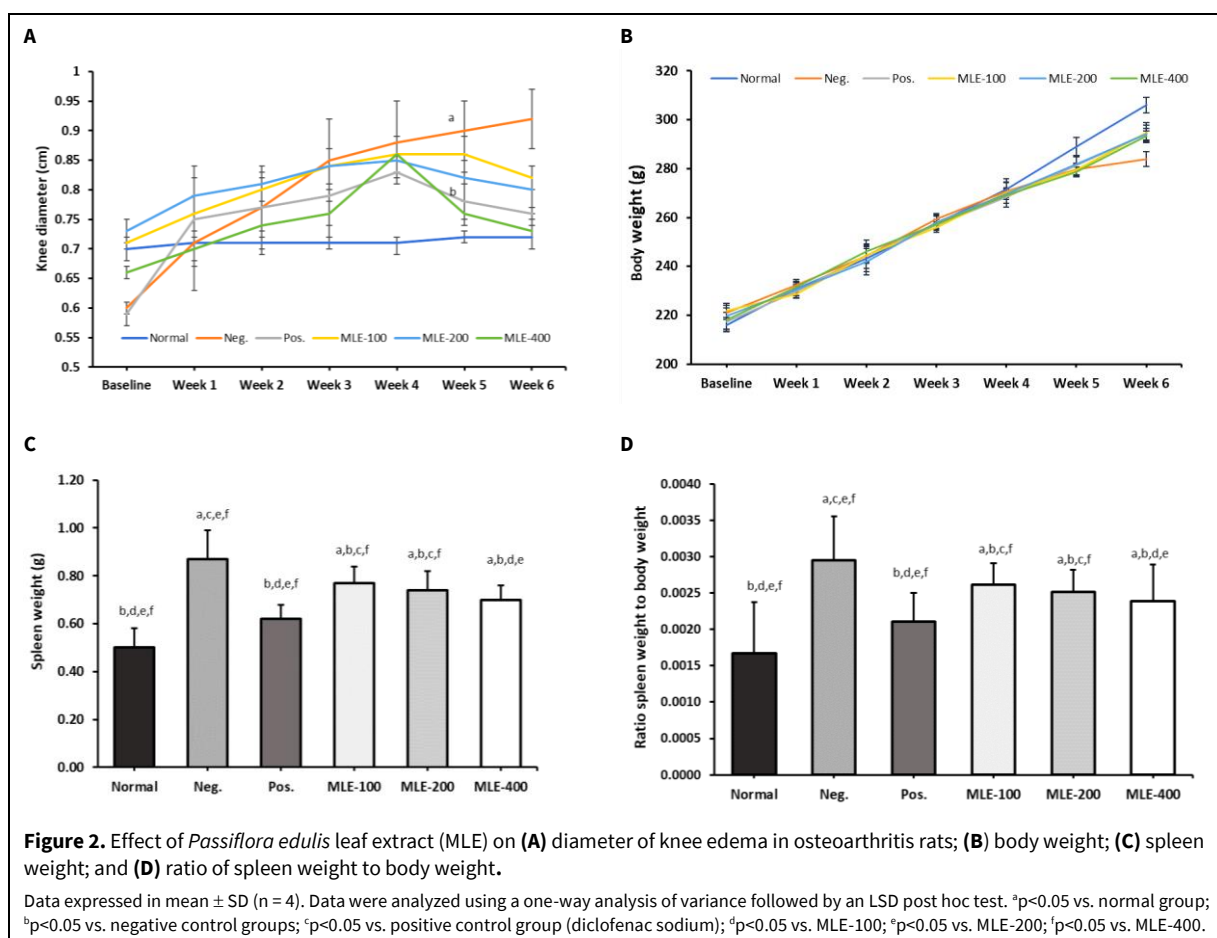


Figure 2. Effect of *Passiflora edulis* leaf extract (MLE) on (A) diameter of knee edema in osteoarthritis rats; (B) body weight; (C) spleen weight; and (D) ratio of spleen weight to body weight.

Data expressed in mean $\pm$ SD (n = 4). Data were analyzed using a one-way analysis of variance followed by an LSD post hoc test. *p<0.05 vs. normal group; ^bp<0.05 vs. negative control groups; ^cp<0.05 vs. positive control group (diclofenac sodium); ^dp<0.05 vs. MLE-100; ^ep<0.05 vs. MLE-200; ^fp<0.05 vs. MLE-400.

Sodium iodoacetate-induced osteoarthritis rats

To investigate the effectiveness of a drug or natural ingredient in reducing OA symptoms, an experimental animal model that develops OA is needed. As demonstrated in Fig. 2A, OA-induced rats with sodi-

um iodoacetate exhibited a larger diameter of knee edema compared to normal rats. The OA model in rats was successfully performed four weeks after intraarticular injection in the rats' knees. Interestingly, MLE administration to OA rat models significantly

reduced the width of knee edema ($p < 0.05$) compared to the negative control group (vehicle group). Treatment with MLE for two weeks at doses of 100 and 200 mg/kg BW could decrease the diameter of knee edema. There was a significant difference ($p < 0.05$) between the negative group versus the positive control of the 400 mg/kg BW dose, but the administration of MLE doses of 100 and 200 mg/kg BW did not have statistically significant differences compared to the negative group.

Effect of MLE on body weight, spleen weight, and spleen weight ratio

In this study, there were no significant differences in body weight between the normal group and the MLE-treated group (Fig. 2B). Meanwhile, in the normal group, the body weight of the rats increased the most, whereas the lowest weight gain occurred in the negative group. In the negative group, rats not given any treatment experienced more severe OA symptoms such as enlargement of the knee diameter and pain due to the formation of inflammation, so the rats seemed to have difficulty in activity and decreased appetite.

In inflammatory conditions in sodium iodoacetate-induced OA rats, there was an increase in the weight and ratio of the spleen (Fig. 2C-D). When comparing the normal group with the negative group, the spleen weight and the ratio of spleen weight to body weight significantly increased in the negative group. MLE with a dose of 100 and 200 mg/kg BW did not have a significant effect on spleen weight, while attenuation of spleen weight by treatment with a dose of 400 mg/kg BW had a significant effect with $p < 0.05$. Similarly, with the ratio of spleen weight to body weight.

Effect of MLE on leukocytes and lymphocytes

As demonstrated in Fig. 3A-B, the rat model of OA exhibited inflammation symptoms in the form of a large number of leukocytes and lymphocytes compared to the normal group. Compared to the negative group, MLE-treated rats decreased significantly ($p < 0.05$) in the 400 mg/kg BW dose, but not in doses of 100 and 200 mg/kg BW. However, there was a trend for leukocyte and lymphocyte levels to decline after MLE treatment at doses of 100 and 200 mg/kg BW, but compared to the negative group, the difference was not statistically significant.

Effect of MLE on pro-inflammatory mediators (NO, IL-6, and TNF- α)

To investigate the role of MLE in the suppression

of leukocyte activation in the release of pro-inflammatory mediators and cartilage degradation, the concentration of NO was assessed (Fig. 4A). Induction of sodium iodoacetate in rats to develop an OA model was shown to significantly increase NO levels in blood serum versus the normal group ($p < 0.05$). Statistical testing indicated that the chemical mediator NO in all doses of the MLE-treated group was considerably lower than the negative group ($p < 0.05$). In rats in the normal group versus the negative group, MLE treatment reduced the pro-inflammatory cytokines IL-6 and TNF- α (Fig. 4B-C).

Effect of MLE on preventing cartilage degradation (MMP-9)

To investigate the role of MLE in inhibiting cartilage degradation, the concentration of MMP-9 was measured. Elevated levels of MMP-9 in OA caused by pro-inflammatory cytokines such as TNF- α and IL-6 stimulate chondrocytes to manufacture MMP-9. As shown in Fig. 4D, a significant increase in MMP-9 was observed in the negative group compared to those of the normal group ($p < 0.05$). However, MLE at doses of 100, 200, and 400 mg/kg BW given to OA model rats showed a significantly lower level of MMP-9, along with a reduction in IL-6 and TNF- α at all treatment doses of MLE. The percentage of effectiveness of MLE in reducing NO, IL-6, TNF- α , and MMP-9 is shown in Table 2.

DISCUSSION

The quantity of secondary metabolite compounds in plants is directly related to their bioactivity and will be influenced by geography, temperature, and growing location (da Silva et al., 2013). In this research, *Passiflora edulis* leaves from Bogor, Indonesia, were used. Flavonoids and polyphenolic compounds are the largest group of secondary metabolite compounds in this plant. Several studies have shown that flavonoids and polyphenolic compounds have strong activity in inhibiting enzymes and as antioxidants. Therefore, quantitative and qualitative evaluations of flavonoids and polyphenolics represent the main targets of antioxidant and other bioactivity research. FCR is a general reagent used in the quantitative assay of polyphenolic compounds in extracts. FCR is a mixture of phosphotungstate and phosphomolybdate, which will oxidize polyphenolic compounds into blue complex compounds so that they can be measured colorimetrically. The results of the TPC test of the extract obtained 8.17 ± 0.03 mg GAE/g of extract, which is in line with other studies (Carmona-Hernandez et al., 2021; da Silva et al., 2013).

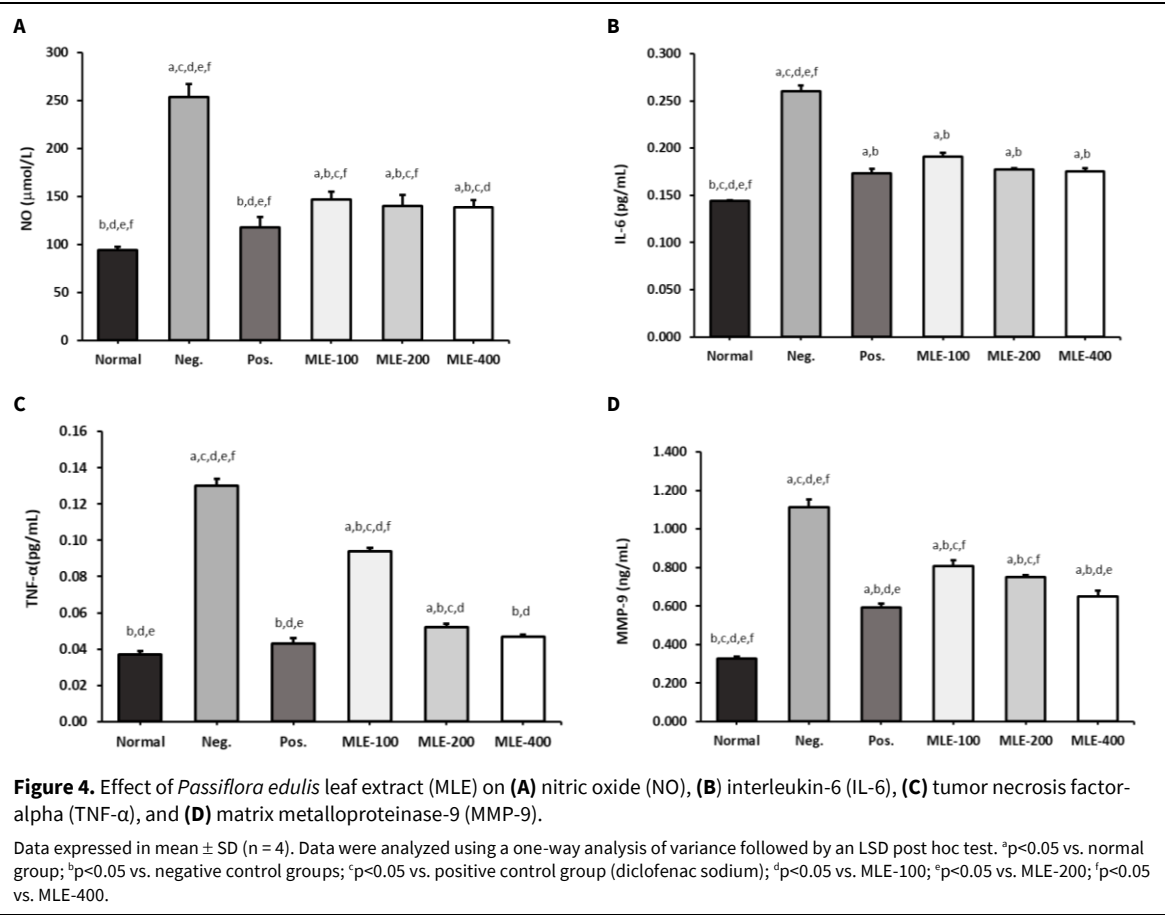
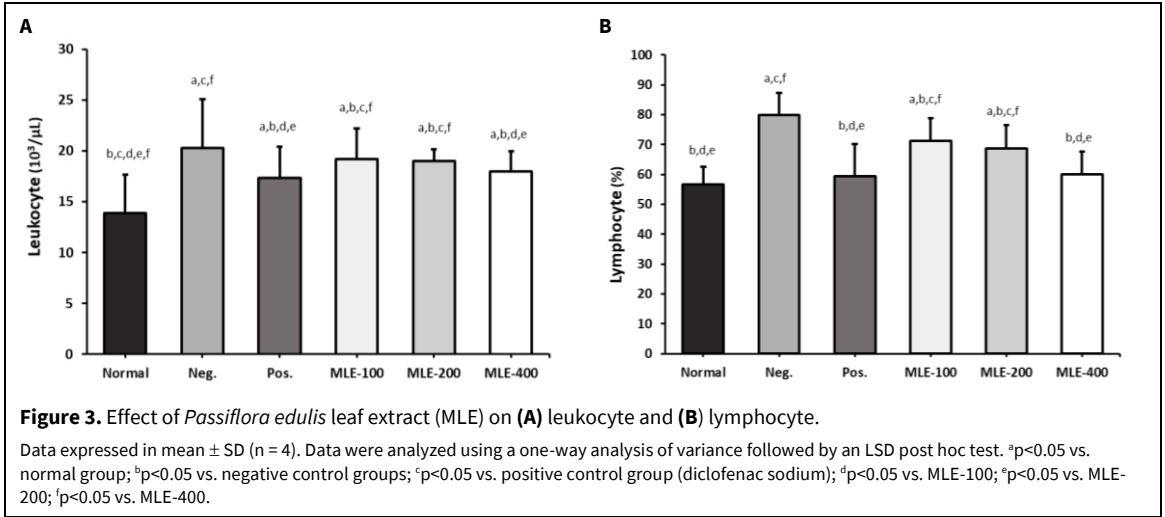


Table 2. The percentage of effectiveness of *Passiflora edulis* leaf extract (MLE) (n = 4).

Groups	Percentage of effectiveness (%)			
	NO	IL-6	TNF- α	MMP-9
MLE-100	78.75	79.28	41.37	58.42
MLE-200	83.68	93.39	89.65	69.34
MLE-400	84.89	97.69	95.39	89.09
Diclofenac sodium	91.62	98.62	97.71	93.92

NO: nitric oxide; IL-6: interleukin-6; TNF- α : tumor necrosis factor-alpha; MMP-9: matrix metalloproteinase-9.

As potent antioxidants, flavonoids are also known to have the potential to reduce fibrosis or tissue damage. As a result, several *in vivo* research using experimental animals have discovered that flavonoids may prevent the start of inflammation and the progression of inflammatory disorders (Al-Khayri et al., 2022). Other studies have shown that the *P. edulis* extract contains several flavonoids, such as vitexin, isovitexin, orientin, and isoorientin. These compounds play a role in anxiolytic activity (De-Paris et al., 2002). In this study, the total flavonoid content of the extract was 3.28 ± 0.3 mg QE / g of extract. This is consistent with the report on the total flavonoid content in various organs of *Passiflora cincinnata*, and the highest value was obtained in the leaf extract (Leal et al., 2020).

The antioxidant assay using ABTS reagent is based on the principle of light scavenging by ABTS radicals. Antioxidants with the ability to donate hydrogen atoms will quench stable free radicals, a process associated with changes in absorption, which can be observed spectrophotometrically. ABTS activity was quantified as the percentage inhibition of ABTS free radical cation by antioxidants in each sample. The extract ABTS radical scavenging assay of the extract was 13.26 ± 0.7 μ mol TE/g extract, while another study showed ABTS antioxidant activity of 19.2 ± 0.5 μ mol TE/g extract (da Silva et al., 2013).

In OA, there is a thinning of the cartilage in the joints that causes the bones to friction with each other, resulting in pain, rigor, loss of joint function, and disturbance of motion. Degradation of the cartilage matrix triggers an inflammatory process in the synovium (synovitis). The high incidence and clinical manifestations that hamper patient activities cause *in vivo* tests in the exploration of agents, especially natural materials, to reduce OA symptoms. In this study, an intraarticular injection into the rat knee with sodium iodoacetate was used to model OA. Four weeks after the intraarticular injection of sodium iodoacetate was administered, the results showed OA parameters, including edema, redness, and increased temperature in the knees of rats until the rats had difficulty walking and doing activities. These changes were confirmed by an increase in the width of the rat knee in all groups of OA rat models versus knee rats under normal conditions. According to previous studies, intraarticular induction of sodium iodoacetate into the knee can produce a two-way response characterized by an initial reaction of inflammation, that is, the breakdown of all cartilage that is felt after 14 days. Intraarticular induction of sodium iodoacetate generates a significant inflammatory response and swelling of the joints caused by the infiltration of monocytes and neutrophils (Abdel-Rahman et al., 2020).

Sodium iodoacetate is a glyceraldehyde-3-phosphate dehydrogenase inhibitor that works by stopping glycolysis, increasing chondrocyte death, and decreasing proteoglycans, fibrillation, and cyst and osteophyte formation. The main early signs of sodium iodoacetate poisoning are shrinkage, degeneration, and death of chondrocytes, with synovial edema and mononuclear cell proliferation. A week after sodium iodoacetate induction, there was osteoblast accumulation and increased osteoclast activation, indicating increased bone remodeling. After the injection of iodoacetate sodium, several significant pro-inflammatory genes increased, in line with decreased proteoglycan production. This model manifests pain about ten days after injection, making it a model used regularly to measure pain behavior, as well as to test drug therapies for pain in animals (Gundogdu et al., 2020). The sodium iodoacetate-induced test animal model is thought to be minimally invasive, rapid, easy to apply, and comparable to human OA conditions (Barry et al., 2023).

In this present research, the body weight of all groups of rats increased over time. The standard weight gain occurred in the normal group, while the lowest weight increase was owned by rats in the negative group. Enlargement in the diameter of the knee and pain due to inflammation formation resulted in difficulty for the rat to participate in activity and decreased appetite. This is consistent with previous research. In the group of test animals induced OA with monosodium iodoacetate, there was a decrease in body weight due to decreased appetite (Li et al., 2021). However, across all treatment groups, the increase in weight was consistent with the normal group.

Spleen weight measurements were taken at the end of this study. The spleen is an organ connected to the immune system. It produces mature lymphocytes and white blood cells and sends them to the rest of the body through the lymph nodes. In OA conditions, it is known to increase spleen size (splenomegaly); therefore, the spleen weight ratio is one of the indicators that can be used to detect the absence of inflammatory activity in monosodium iodoacetate-induced OA rats (Ekambaram et al., 2020). OA can cause the spleen to continue to produce white blood cells as an immune response. In OA, chronic inflammation involves an adaptive immune response mediated by lymphocytes. Numerous lymphocytes are required to combat this persistent inflammation and must first be activated in the spleen, as explained. This leads to hyperplasia of the splenic white pulp to facilitate the activation of these large numbers of lymphocytes. Therefore, white pulp hyperplasia results in the enlargement of the spleen or splenomegaly (Crane et al.,

2021). In the rat OA model, the average weight of the spleen was greater than in normal rats. Likewise, the results of this experiment showed that there was an elevation in the spleen weight ratio in the sodium iodoacetate-induced group. In the group of rats given MLE ethanol extract at a dose of 100, 200, and 400 mg/kg BW, there was a decrease in spleen weight; this can be caused by flavonoid compounds in MLE to inhibit splenomegaly. Previous research using *Passiflora* fruit peel extract containing flavonoids showed nonspecific immune effects as anti-inflammatory by suppressing immune cell activation and suppressing macrophage and lymphocyte activation, so it can be concluded that OA affects spleen weight because it is closely related to lymphocyte proliferation in case of inflammation (Cazarin et al., 2016).

Induction with monosodium iodoacetate brings an inflammatory response characterized by an increase in leukocytes and lymphocytes (Alsharif et al., 2022). The high level of lymphocytes retrieved the formation of an adaptive immune response, which is an immune response that is not caused immediately or slowly because lymphocytes must first be activated by being exposed to a specific antigen in the spleen organ. Following antigen recognition, lymphocytes will specialize and multiply into many so that the number increases (Abbas et al., 2019). After treatment for 14 days, the results showed that the 400 mg/kg BW dose group had been able to reduce the number of blood lymphocytes even though it had not reached a normal state. On the contrary, the difference is not significant in the dose groups of 100 and 200 mg/kg BW with normal and positive, so it can be interpreted that the effect of induction of monosodium iodoacetate and the treatment with diclofenac sodium and MLE has not had enough systematic effect on the immune system. This is in line with previous studies; active substances in plants, such as flavonoid-rich extracts or fractions, can delay the production of pro-inflammatory cytokines that may enhance the diffusion of inflammatory cells to the site of inflammation that has occurred and can effectively reduce leukocyte count because it contains flavonoid quercetin, which is able to decrease the migration flow of leukocytes consisting of macrophages, lymphocytes, granulocytes, and monocytes to inflammatory sites by inhibiting the formation of leukocytes into proteins that can suppress the inflammatory process (Ożarowski and Karpiński, 2021).

Worsening OA conditions result in an imbalance between its breakdown and its synthesis of the cartilage matrix. Matrix degradation products are recognized as autoantigens by immune cells and trigger an inflammatory process in the synovium (synovitis). In this state, chondrocytes and synovial cells secrete

inflammatory cytokines, namely, IL-8 and TNF- α , which inhibit collagen formation and stimulate catabolic mediators and inflammatory compounds such as MMPs, PGE₂, IL-6, IL-8, and NO. If this happens continuously, it can cause chondrocyte apoptosis and cartilage loss. As a result, the distance between the joints narrows, causing discomfort and changes in joint shape. Adjacent subchondral bones will rub against each other so that they become stiff and brittle and experience a decrease in their ability to support weight. The subchondral bone will also release vasoactive peptides and MMPs, enzymes that will damage cartilage, exacerbating the cartilage injury (DiPiro et al., 2021; Stefik et al., 2021). In the current study, we observed that the chemical mediator NO increased in the negative group than in the normal group ($p < 0.05$). This means that MIA induction causes inflammation in OA rats, which is characterized by an abundance of NO chemical mediators. This is consistent with research by Min et al. (2021), where the induction of OA with MIA can cause the release of the inflammatory mediator NO.

NO is a chemical mediator that is important in the inflammatory process and the breakdown of the articular cartilage. NO relaxes the smooth muscle of the vascular system, causing vasodilation that increases blood flow and leads leukocytes to the site of inflammation. Subsequently, inflammatory mediators such as histamine and leukotrienes increase the permeability of vascular endothelial cells, allowing the exudation of plasma proteins and leukocytes from the circulation to the tissue where the inflammation occurs (Xian Bo et al., 2022). The function of NO in the inflammatory process is to stimulate leukocytes to release IL-1 and TNF- α . Meanwhile, the role of NO in the degradation of articular cartilage is by inducing apoptosis, inhibiting chondrocyte proliferation, collagen and proteoglycan synthesis, synthesizing IL-1 receptor antagonists, and stimulating MMPs that will further degrade articular cartilage. According to the previous study, MIA induction in rats was shown to significantly increase NO levels in blood serum (Lee et al., 2022a; 2022b). Treatment with MLE resulted in a significant and dose-level-dependent decrease in NO. In line with that, previous research has shown that MLE contains polyphenolic compounds in the form of flavonoids that have anti-inflammatory activity (Sunny et al., 2020). Al-Khayri et al. (2022) showed that flavonoids can reduce NO and inhibit iNOS expression. According to previous study showed that isovitexin, a C-glycosyl flavonoid compound in the aqueous leaf extract of passion fruit, can inhibit the expression of NO mRNA induced by lipopolysaccharide-activated macrophages (Ożarowski and Karpiński, 2021).

In this current study, intra-articular injection of sodium iodoacetate significantly elevated pro-inflammatory cytokines, including IL-6 and TNF- α , significantly as compared to rats in the normal group. IL-6 is one of several pro-inflammatory cytokines present in the synovial fluid of patients with a clinical diagnosis of OA. The introduction of monosodium iodoacetate causes IL-6 to increase dramatically. Increased levels of IL-6 in the knee circulation will result in cartilage loss; additionally, higher levels of IL-6 in the serum of OA also correspond to the course of the disease or the severity of cartilage pathology (Wiegertjes et al., 2020). The pathophysiology of OA involves inflammatory conditions triggered by articular deterioration and the production of pro-inflammatory cytokines. In this study, MLE administration for two weeks dramatically reduced IL-6 levels, particularly in the group that received a dose of 400 mg/kg BW; besides that, TNF- α levels also decreased significantly in rats given MLE doses of 100, 200, and 400 compared to the negative group. MLE can also worsen the process of inflammation by reducing pro-inflammatory cytokines such as IL-6 and TNF- α . Many studies have reported that *P. edulis* extract contains flavonoids and can inhibit inflammation, which has many mechanisms of action, including inhibiting enzymes that control inflammatory responses and lowering the production of arachidonic acid, and prostaglandins, leukotrienes, and NO by inhibiting COX and LOX enzymes so that they can prevent the development of OA (Urrego et al., 2021). Prior research has also shown that flavonoids can prevent enzymes from controlling cytokines implicated in inflammation. Flavonoids, known as strong antioxidants, have the capacity to reduce fibrosis or tissue deterioration. In passion fruit leaves, specific compounds are found, isoorientin and vitexin, which can suppress IL-1, IL-6, IL-33, and TNF- α , which are pro-inflammatory cytokines linked to hyperalgesia and cartilage damage caused by the induction of monosodium iodoacetate induction (Cazarin et al., 2015).

One of the most significant symptoms of OA is proteolytic MMP-9 against collagen in synovial fluid. MMP-9 plays a role in the degradation of cartilage. MMP-9 can also be expressed by chondrocytes. MMPs can degrade the extracellular matrix of articular cartilage, which is mainly made up of collagen and proteoglycans. The MMP-9 level is elevated in monosodium iodoacetate-induced animals that cause OA. Expression of MMP-9 gelatinase has been shown to be associated with the pathogenesis of OA. MMP-9 is responsible for the removal of the extracellular matrix, cytokines, and chemokines, thus increasing their activity. MMP-9 levels are elevated in inflamed synovial tissue compared to non-inflamed tissue (Chow

and Chin, 2020). These study results revealed that the level of MMP-9 rose dramatically in the group of rats that received only monosodium iodoacetate. The increase in MMP-9 in animal models of OA is caused by several circumstances, in this case, caused by the induction of monosodium iodoacetate, which causes inflammation directly in the joint, chondrocyte death, osteophyte formation, and degradation of articular cartilage degradation (Permatasari et al., 2019). In contrast, the group of rats treated with doses of diclofenac sodium and *P. edulis* leaf extract (MLE) of 100, 200, and 400 mg/kg BW resulted in a significant decrease in MMP-9 levels but had not reached normal levels. These results are supported by research showing that the administration of monosodium iodoacetate causes elevated levels of cytokines. However, the administration of sodium diclofenac and the chemical component of passion fruit leaf extract, namely quercetin, can dramatically reduce the value of pro-inflammatory parameters in OA rat model animals, which reduces the value of MMP-9 (Permatasari et al., 2019). This can be attributed to the anti-inflammatory effects of flavonoids; particularly, quercetin can reduce MMP-9 in healing OA damage. Quercetin inhibits COX and LOX and then reduces the formation of inflammatory metabolites, especially MMPs (Wang et al., 2022), so the quercetin content in MLE can prevent inflammation and TNF- α and MMP-9 expression by preventing proteoglycan breakdown.

The flavonoids contained in MLE reduce inflammation by inhibiting the regulation of key enzymes involved in arachidonic acid metabolism by suppressing COX activity and can also directly control chondrocytes. The anti-inflammatory activity of quercetin and other flavonoids can reduce the tendency to oxidative stress in OA. Treatment with conventional therapy should help osteoarthritic patients with OA lessen their need for non-steroidal anti-inflammatory drugs (Akbari et al., 2022). An indicator of the success of an ingredient as a potential cure for inflammation is its ability to reduce pro-inflammatory parameters. The results of this study showed a decrease in MMP-9 levels after the administration of passion fruit leaf extract, so it can be said that *P. edulis* leaf extract has the ability to lower the levels of pro-inflammatory parameters caused by knee tissue damage in monosodium iodoacetate-induced OA rat models.

CONCLUSION

In view of all these present findings, it can be concluded that sodium iodoacetate-induced OA in rats could potentially increase inflammatory processes in knee rats. MLE containing flavonoid and polyphenolic compounds has antioxidant activity and significantly attenuated signs of OA: weight gain, knee edema,

ratio of spleen weight, number of leukocytes and lymphocytes. Furthermore, MLE shows inhibitions of inflammation through NO and inflammatory cytokines such as TNF- α and IL-6, as well as inhibition of proteolytic MMP-9, thus inhibiting cartilage degradation. This study also has some limitations; the duration of MLE treatment is still a relatively short period of time, and extracts will be developed in nanoparticles to provide maximum absorption, bioavailability profile, and target organ efficacy.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Sandhiutami NMD	Desmiaty Y	Fahleni	Muslimawati R	Sari F
Concepts or ideas	x	x	x		
Design	x	x			
Definition of intellectual content	x	x	x		
Literature search	x	x			
Experimental studies				x	x
Data acquisition			x	x	x
Data analysis	x	x		x	x
Statistical analysis			x	x	x
Manuscript preparation	x	x			
Manuscript editing	x	x	x		
Manuscript review	x	x	x	x	x

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