Passiflora edulis Sims leaves nanoparticle alleviates inflammation and osteoarthritis by suppressing pro-inflammatory cytokines production and matrix metalloproteinase-9: A combined in-silico, in-vitro, and in-vivo models

Ni Made Dwi Sandhiutami
Universitas Pancasila Fakultas Farmasi
Yesi Desmiaty
Universitas Pancasila Fakultas Farmasi
Febby Nurdiya Ningsih
research center for vaccine and drugs development, national research and innovation agency (BRIN)
Rika Sari Dewi
Universitas Pancasila Fakultas Farmasi
Diah Kartika Pratami
Universitas Pancasila Fakultas Farmasi
Syahirah Syahirah
Universitas Pancasila Fakultas Farmasi
Wafda Nurtaqwa Zahara
Universitas Pancasila Fakultas Farmasi
Francis Xavier
fxavi3r@gmail.com

Universitas Pancasila Fakultas Farmasi https://orcid.org/0009-0001-3025-2749

Research Article

Keywords: Passiflora edulis Sims, nanoparticle, osteoarthritis, inflammatory biomarker, molecular docking

Posted Date: June 12th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-6833708/v1

Abstract

Osteoarthritis (OA) is a musculoskeletal disease characterized by cartilage degradation and chronic pain, affecting 7.6% of the global population in 2021. *Passiflora edulis* leaf contains bioactive compounds with potential for OA medication. Formulating *P. edulis* into chitosan nanoparticles can enhance absorption, bioavailability, and efficacy. This study aims to evaluate anti-inflammation and anti-OA properties of *P. edulis* leaf nanoparticles (PLEN). PLEN was evaluated for its anti-inflammation activity on LPS-induced RAW 264.7 cells and anti-OA monosodium iodoacetate (MIA) induced rats OA model. Furthermore, *P. edulis* leaves compounds identified from previous studies were subjected to molecular docking against iNOS (PDB ID: 3E6T), TNF-α (PDB ID: 7KP8), IL-6 (PDB ID: 7DC8), and MMP-9 (PDB ID: 4XCT) receptors. PLEN treatment demonstrated anti-inflammation activity by inhibiting NO production by 43.68–60.58%, iNOS concentration by 11.98–38.83%, and decreasing TNF-α concentration by 7.74–16.92%. In-vivo test showed that PLEN increased body weight, reduced knee oedema, spleen weight, inflammation, and cartilage degradation. Molecular docking of *P. edulis* bioactive compounds revealed that cyclopassifloside II exhibited the highest iNOS inhibition with a docking score of -113.083 kcal/mol, N-cis-feruloyltyramine inhibited TNF-α with a docking score of -115.021 kcal/mol, cyclopassifloside XII inhibited IL-6 with a docking score of -116.875 kcal/mol, and Luteolin-6-C-fucoside inhibited MMP-9 with a docking score of -122.91 kcal/mol. In conclusion, this study demonstrated that PLEN exerts anti-inflammation and anti-OA activities and suggesting different groups of *P. edulis* bioactive compounds targeting distinct anti-inflammation and anti-OA pathways.

1. Introduction

Osteoarthritis (OA) is a musculoskeletal disease characterized by cartilage degradation and chronic pain, manifested from persistent low-grade inflammation (Han et al. 2024). The global prevalence of OA in 2021 shows 149.08 million males and 236.39 million females in 2021 suffer from OA, with projections suggesting a continuously rising trend to 235.41 million males and 365.97 million females suffer from OA in. OA affects approximately 7.6% of the global population, and its global burden significantly impairs health and overall quality of life (Steinmetz et al. 2023; Li et al. 2024; Ren et al. 2025). Current OA treatments including oral nonsteroidal anti-inflammatory drugs (NSAIDs), intra-articular hyaluronic acid injection, and surgical interventions, fail to halt OA progression (Han et al. 2024). Several major risk factors that cause OA include age, knee trauma history, female sex, and obesity (Dong et al. 2023; Courties et al. 2024). While several guidelines recommended NSAIDs as the first line medication for acute OA, their use is widely associated with gastrointestinal, cardiovascular, and renal adverse effects, which bring limitation to NSAIDs therapy (Curtis et al. 2019; Reginster et al. 2019). Furthermore, prolonged NSAIDs use for over 4 to 5 years has been proven to aggravate knee OA symptoms (Salis and Sainsbury 2024). Conventional OA medication safety and cost concerns have spurred interest in herbal medication as complementary or alternative therapies. Several plants based treatments have demonstrated clinical efficacy for OA treatment while showing safety, making them promising candidates to developed as complementary or alternative OA treatment (Lindler et al. 2020).

OA progression has been associated with low-grade joint inflammation development (Panichi et al. 2024). This inflammatory state induces pro-inflammatory cytokine production, such as TNF-α, IL-1β, IL-6, and IL-8 by chondrocytes, osteoblasts, and synoviocytes. Pro-inflammatory cytokines led to extracellular matrix (ECM) breakdown and cartilage degradation through matrix metalloproteinase (MMP) activation. IL-1β and TNF-α stimulate NO, iNOS, COX-2, and PGE synthase production, creating a positive feedback loop that further upregulates the production of IL-1β and TNF-α (Molnar et al. 2021; Yao et al. 2023). Targeting the inflammation cascade, proinflammatory cytokines, NO, and MMP activity could mitigate or stop OA pathogenesis by reducing chondrocyte inflammation and cartilage resorption (Sandhiutami et al. 2025a).

Passion fruit (*Passiflora edulis* Sims), a member of Passifloraceae family, is a native Brazilian plant cultivated worldwide, particularly in tropical and subtropical regions (He et al. 2020; Weyya et al. 2024). The leaves of *P. edulis* is a major by-product that empirically used for various ailments, including antiinflammation and antiOA purposes (Farid et al. 2010; Fonseca et al. 2022; Sandhiutami et al. 2024). It contains antiinflammation compounds with potential as a complementary antiOA medication. Saponin fraction, flavonoid fraction, cyclopassifloside IX, and cyclopassifloside XI have demonstrated anti-inflammation effects by suppressing myeloperoxidase and nitric oxide synthase activity (Urrego et al. 2021). *P. edulis* administration on colitis rats significantly reduced the level of IL-1β, IL-6, and TNF-α in colon tissue (Cazarin et al. 2015). *P. edulis* ethanol extract exhibited anti-inflammation through inhibition of NO and inflammatory cytokines such as TNF-α, IL-6 production, along with suppression of MMP-9 mediated cartilage degradation (Sandhiutami et al. 2025a).

Although flavonoids, triterpenoids, and saponins exhibited anti-inflammation activity, their oral administration remains a challenge. Flavonoid suffer from poor aqueous solubility, intensive metabolism, and low systemic absorption (Yuan et al. 2024). Triterpenoid properties of high hydrophobicity, poor permeability, poor absorption, and rapid metabolism hampered its oral bioavailability (Nistor et al. 2023). Saponin poor membrane permeability, prone gastric degradation, and undergoes colonic metabolism (Navarro del Hierro et al. 2018). The mentioned physicochemical properties of *P. edulis* compounds seriously hinder the efficacy of oral administration for OA management. Nanoparticle delivery systems is one of the promising strategies to enhance absorption, bioavailability, and efficacy (Sandhiutami et al. 2025a). Previous study has demonstrated that *P. edulis* chitosan nanoparticle showed no toxicity and adverse effects. It also enhanced analgesic and anti-inflammatory to carrageenan-induced oedema (Fahleni et al. 2024; Sandhiutami et al. 2025b). Chitosan as a nanoparticle polymer also offers additional therapeutic advantages, as its biodegradation yields glucosamine. Glucosamine has been proven clinically to reduce pain in knee OA patient without adverse effect (Vo et al. 2023). Previous studies also confirmed the safety of glucosamine for anti-OA, turning chitosan into an ideal polymer for nanoparticle formulation (Nagaoka et al. 2019; Takeuchi et al. 2024).

This study aims to evaluate anti-inflammation and anti-OA properties of *P. edulis* leaf nanoparticles (PLEN) through various model. *In-vitro* anti-inflammation assay using RAW 264.7 macrophage cell line to assess anti-inflammation cytokines regulation (iNOS, TNF-α, and IL-6) and *in-vivo* to monosodium

iodoacetate (MIA) induced rats OA model. Furthermore, reported *P. edulis* compounds were subjected to molecular docking against iNOS, TNF-α, IL-6, and MMP-9 receptors to identify the bioactive compounds responsible for anti-inflammation and anti-OA activity.

2. Materials and Methods

2.1. Materials

2.1.1. Plant material

Passiflora edulis leaves were obtained from Balitro, Bogor, Indonesia (6.5767° S, 106.7858° E). The plant material was identified and deposited at the Department of Biology, University of Indonesia, with specimen voucher 992/UN2.F3/PDP.02.00/2023.

2.1.2. Chemical reagents

Fetal bovine serum Premium (FBS) (Gibco), Dulbecco's modified Eagle's medium High Glucose (DMEM) (Gibco), 1% penicillin-streptomycin-Amphotericin B solution (Gibco). DMSO (Merck), MTT (CSH Protoc), Griess reagent, lipopolysaccharide (LPS), Biotinylated detection ab (Elabscience), HRP conjugate (Elabscience), Dulbecco's Phosphate Buffered Saline (DPBS) (Elabscience), Substrate reagent (Elabscience), Stop solution (Elabscience), Standard solution (Elabscience), ethanol 96%, distilled water, TNF-α ELISA kit (Elabscience) dan iNOS ELISA kit (Elabscience). Monosodium iodoacetate (Sigma Aldrich), Sodium diclofenac tablet (PT. Novell Pharmaceutical), NaCl 0.9% (PT Widarta Bhakti), distilled water (Brataco), CMC (Brataco), Ketamine (Guardian Pharmatama).

2.2. *P. edulis* extract preparation

As much as 100 g of dried *P. edulis* leaf powder was decocted with 1000 mL boiling distilled water for 30 minutes. The extract was then filtered and dried in a dehydrator at 40°C to obtain dried extract.

2.3. *P. edulis* extract nanoparticle preparation

P. edulis extract nanoparticle (PLEN) was prepared by chitosan/sodium tripolyphosphate ionic gelation method as described in the previous study (Fahleni et al. 2024). Briefly, the *P. edulis* extract was dissolved in a mixture of DMSO, Tween 80, propylene glycol, and distilled water to achieve good solubility. The solution was added with 0.2 % chitosan solution nd mixed using magnetic stirrer at 500 rpm for 10 minutes. Subsequently, sodium tripolyphosphate 0.1% solution was added dropwise with a drop rate of 1 drop every 3 seconds through a burette and stirred at 500 rpm for 1 hour until homogenous nanoparticle was formed.

2.4. *In-vitro* anti-inflammation assay

2.4.1. Cell culture

Murine macrophage RAW 264.7 cells (ECACC 91062702) were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin-Amphotericin B solution. Cells were thawed and subcultured until reached 70–80% confluency.

2.4.2. MTT assay

Cell viability was assessed using MTT method. As much as 100 µl of RAW 264.7 cells with a density of 10^5 cells/100 µl were seeded in a 96-well plate, then incubated for 24 hours at temperature of 37°C and humidity of 5% CO_2. After incubation, 100 µl of PLEN at concentration ranging from 1.56 to 200 µg/mL was added, then incubated under the same condition for 24 hours. After treatment, 100 µl of cells and PLEN mixture was added with 20 µl of MTT solution, then incubated for 4 hours under the same condition. The formed formazan crystals were dissolved in 150 µl DMSO to terminate the reaction. Cell viability absorbances were measured at 540 nm using a microplate reader (Rahmawati et al. 2024).

2.4.3. NO production assay

NO production assay was performed using Griess method according to the kit manufacturer procedure (E-BC-K035-M, Elabscience). The RAW 264.7 cells were pretreated with PLEN (12.5, 25, and 50 µg/mL) for 1 hour incubation time. After incubation, the mixture was induced by 1 µg/mL of LPS, then incubated for 20 hours. After LPS induction, 100 µL of supernatant was transferred to 96-well microplate and added with 100 µL of Griess reagent. The solutions were incubated for 10 minutes and the absorbances were measured at 540 nm.

2.4.4. iNOS production assay

iNOS production assay was performed according to the kit manufacturer procedure (E-EL-M0696, Elabscience). Briefly, 100 µL of standard or sample solutions were added to 96-well microplate, then incubated for 90 minutes at 37°C. The solution was then discarded and immediately added 100 µL of Biotinylated Detection Ab, three times. One hundred microliters of HRP conjugate was added and incubated for 30 minutes at 37°C, and the plate was aspirated and washed using DPBS for five times. As much as 90 µL substrate reagent was added, then the solutions were incubated for 15 minutes at 37°C. Fifty microliters of stop solution was added and the absorbances were measured at 450 nm.

2.4.5. TNF-α production assay

TNF-α production assay was performed according to the kit manufacturer procedure (E-UNEL-M0103, Elabscience). Briefly, 100 µL standard and sample solutions were added to 96-well microplate, then incubated for 90 minutes at 37°C. The solution then discarded and immediately added 100 µL of Biotinylated Detection Ab, then incubated for 1 hour at incubated for 1 hour at 37°C. The plate was aspirated and washed using DPBS for three times. One hundred microliters of HRP conjugate was added and incubated for 30 minutes at 37°C, then the plate was aspirated and washed using DPBS five times. As much as 90 µL substrate reagent was added, then the solutions were incubated for 15 minutes at 37°C. Fifty microliters of stop solution was added and the absorbances were measured at 450 nm.

2.5. *In-vivo* anti-inflammation assay

2.5.1. Experimental animal

In this study, 24 male Wistar rats (*Rattus Norvegicus*) aged 2–3 months and weighing 250–300 g were obtained from Balitbangkes, Indonesian Ministry of Health. Prior to experiments, all animals were acclimatized for 7 days under laboratory conditions (24 ± 2°C, humidity 50–60%, 12 hours light/dark cycle). All procedures were approved by Faculty of Medicine, University of Indonesia (KET-395/UN2.F1/ETIK/PPM.00.02/2024).

2.5.2. MIA-Induced joint osteoarthritis experiment

Twenty four rats were divided into 6 groups, each consisting of 4 rats, as follows: (1) Normal group (without MIA-induced and PLEN), (2) negative group (MIA-induced and without PLEN), (3) positive control (MIA-induced and sodium diclofenac treatment), (4) PLEN 50 (MIA-induced and PLEN 50 mg/kg treatment), (5) PLEN 100 (MIA-induced and PLEN 100 mg/kg treatment), and (6) PLEN 200 (MIA-induced and PLEN 200 mg/kg treatment). On the 1st day, groups 2 to 6 were anesthetized using ketamine (75 mg/kg) and xylazine (8.8 mg/kg) intraperitoneally, then received an intra-articular injection of 0.25 mL of MIA (10 mg/mL) on the left knee, then observed until the 28th day. On the 29th to 42nd day, all groups received the treatment orally once a day. Knee diameter was measured on the 7th, 14th, 21st, 28th, 35th, and 42nd day using a digital caliper at the largest joint circumference. At the end of the experiment, all rats were euthanized for spleen weight and knee tissue histopathology analysis.

2.5.3. Histopathological analysis

The knee joint tissues were fixated in 10% neutral formaldehyde buffer, followed by decalcification in 20% nitric acid for 10–12 days. The decalcified tissues were trimmed and dehydrated using a graded ethanol (70–100%). The tissues were washed in xylene, embedded in paraffin wax, sliced using microtome, and stained with Haematoxylin and Eosin (H&E). The tissues were evaluated its inflammation and cartilage damage according to Osteoarthritis Research Society International (OARSI) scoring system (Glasson et al. 2010).

2.6. Molecular Docking simulation

Reported *P. edulis* leaves compounds from previous studies were subjected to molecular docking against iNOS (PDB ID: 3E6T), TNF-α (PDB ID: 7KP8), IL-6 (PDB ID: 7DC8), and MMP-9 (PDB ID: 4XCT) receptors (Yoshikawa et al. 2000b, a; Zucolotto Stella; Montanher Ana B.; Reginatto Flávio Henrique; Schenkel Eloir Paulo; Fröde Tânia Silvia 2009; Wang et al. 2013; Yuan et al. 2017; Gadioli et al. 2018; Urrego et al. 2021). Docking simulation carried out using Molegro Virtual Docker. Ligands preparation were carried out by minimizing energy using Chem3D. Protein was prepared by removing water molecules and cofactors, followed by internal validation to obtain RMSD value less than 2. The scoring function, algorithm, and coordinate of each protein as follows: iNOS moldock score - moldock se, x = 123.29, y = 114,53, z = 35,94, radius = 10 Å. TNF-α moldock score grid-iterated simplex x = -24.81, y = 18.59, z = -0.32, radius = 9 Å. IL-6 plant score grid-moldock optimizer, x = 10.54, y = 23.20, z = 20.34, radius = 9 Å. MMP-9 moldock score grid-moldock optimizer, x = 18.15, y = -18.16, z = 19.12, radius = 10 Å.

2.7. Statistical Analysis

Data were analyzed using SPSS V.27.0.1.0. One Way Analysis of Variance (ANOVA) analysis was performed, followed by Least Significant Difference (LSD) with significant value $p < 0.05$. All data were presented as mean ± SD.

3. Results and discussion

3.1. Cell viability of PLEN

Cell viability assay conducted using MTT assay to determine PLEN suitable concentration that does not possess toxicity to RAW 264.7 cells. As shown in Fig. 1, PLEN exhibited more than 80% cell viability across all tested concentrations, indicating PLEN non-toxic properties up to 200 µg/mL on RAW 264.7 cells (López-García et al. 2014).

3.2. Effect of PLEN on inflammatory biomarker

The *in-vitro* anti-inflammation activity of PLEN was evaluated by NO, iNOS, and TNF-α concentration on LPS-induced RAW 264.7 cells. As shown in Fig. 2, LPS stimulated the production of NO, iNOS, and TNF-α significantly higher compared to the normal group. This response occurs through LPS binding to Toll-like receptor 4 (TLR4), which activates NF-κB signaling (Liu et al. 2017). NF-κB activation upregulates both iNOS/NO pathway and proinflammatory cytokines secretion, such as TNF-α, IFN-I, IL-1, and IL-6 (Guo et al. 2024a, b). PLEN treatment demonstrated anti-inflammation activity by reducing NO, iNOS, and TNF-α concentration. Figure 2A shows the NO concentration of PLEN ranged from 6.39 ± 0.05 to 9.13 ± 0.03 mg/mL, inhibiting NO production from 43.68 to 60.58 %, significantly lower compared to th negative control with NO concentration of 16.21 ± 0.04 mg/mL. Figure 2B shows the iNOS concentration of PLEN ranging from 1.00 ± 0.01 to 1.45 ± 0.00 ng/mL, inhibiting iNOS production from 11.98 to 38.83 %, significantly lower compared than egative control with iNOS concentration of 1.64 ± 0.03 ng/mL.

Figure 2C shows the TNF-α concentration of PLEN ranging from 6105 ± 26 to 6780 ± 59 ng/mL, inhibiting TNF-α production from 7.74 to 16.92%, significantly lower compared than negative control with TNF-α concentration of 7349 ± 85 ng/mL. TNF-α is a proinflammatory cytokine produced by synovial and chondrocytes in OA. It stimulates osteoclast differentiation and enhances the expression of other proinflammatory cytokines (Zhu et al. 2024). TNF-α inhibition has been reported to have effect on OA by improving osteochondral viability and proliferation, chondrogenesis and chondroprotection, and recovery of osteochondral lesion (Chisari et al. 2020). It has also been clinically proven that TNF-α inhibition could reduce pain in knee OA (Ohtori et al. 2015).

Oxidative stress is another factor that contributes to chronic inflammation. It activates cellular signal transduction pathways and changes transcription factors like NF-κB. These signals upregulate inflammatory cytokines including IL-6 and TNF-α, growth factors, chemokines, and proinflammatory

enzymes, including LOX-5 and COX-2. This pathway recruit additional inflammatory cells to inflammation site and generates more reactive species (Rahmawati et al. 2024). NF-κB activation due to oxidative stress and ROS accumulation accelerates chondrocyte cell senescence, chondrocyte cell apoptosis, cartilage damage, and ECM degradation and synthesis inhibition (Ansari et al. 2020; He et al. 2024). Previous studies have demonstrated *P. edulis* leaf decoction exhibited strong *in-vitro* antioxidant capacity, improving endogenous antioxidants such as glutathione and glutathione reductase, and reducing lipid peroxidation. *P. edulis* extraction at 100°C showed the highest antioxidant capacity compared to lower temperatures as it extracts more flavonoid, measured at 16.38 mg QE/g (da Silva et al. 2013; Cazarin et al. 2015; Desmiaty et al. 2025).

3.3. Effect of PLEN on MIA-induced OA rats

In this study, *In-vivo* OA model was successfully established through intra-articular MIA injection. MIA induces OA by inhibiting glyceraldehyde-3-phosphate dehydrogenase and disrupting cellular glycolysis in the knee joint, leading to cartilage degradation that mimics pathological features of human OA (de Sousa Valente 2019). The results showed that the normal group gained the most body weight by 74.05 g, while MIA-induced rat groups (groups 2–6) showed an increase only by 23.55–48.60 g. This study showed normal group gained the most body weight. MIA-induced rats showed OA symptoms such as knee oedema, pain, and discomfort that decreased the movement and appetite of rats. The anti-OA effects of PLEN were evaluated using MIA-induced OA rat model. As depicted in Fig. 3B, intra-articular injection of MIA successfully induced knee oedema on the 28th day. Oral administration of PLEN 100 dan PLEN 200 for 14 days to OA rats significantly reduced knee diameter in OA rats compared to negative control. Additionally, PLEN 200 knee diameter does not significantly difference to normal group, indicating PLEN 200 completely ameliorates knee oedema in OA rats. Spleen is a lymphoid organ that plays role in the immune system, which increase in size under inflammation conditions (Abd El-Aziz et al. 2018). As shown in Fig. 3C and Fig. 3D, spleen weight and spleen to body weight ratio significantly increase in negative control compared to normal group. Administration of PLEN at all dose ameliorates spleen weight increase and shows no significant difference to normal group.

This study demonstrated that PLEN exhibited superior anti-OA activity than *P. edulis* ethanol extract from previous study. PLEN significantly reduced knee oedema at a dose of 100 mg/kg, whereas *P. edulis* ethanol extract required 400 mg/kg to reduce knee oedema. Several factors influenced this result, such as extraction method, antioxidant capacity, and nanoparticle formulation. *P. edulis* leaf extraction using boiling water exhibited higher TPC, TFC, and antioxidant capacity than ethanol extract (Sandhiutami et al. 2025a; Desmiaty et al. 2025). Chitosan nanoparticles also play a major role in oral bioavailability by improving water solubility, increasing intestinal mucosal adhesion, and slowing metabolism and elimination, thus accelerating and increase absorption, along with higher and prolonged plasma concentration (Arozal et al. 2020). Similarly, *P. edulis* selenium demonstrated high penetration through semi-permeable membrane and exhibited controlled release properties (Namdevrao et al.). Besides its bioavailability advantage, chitosan and glucosamine, as its biodegradation product, exhibited anti-

inflammation properties by modulating pro-inflammatory cytokines, chondroprotective, antioxidant, and tissue regeneration (Al-Saadi et al. 2019; Novy et al. 2025).

3.4. Effect of PLEN to histopathological change of MIA-induced OA rats

Histopathological analysis of knee tissue inflammation and cartilage damage was observed through H&E staining post-treatment. As depicted in Fig. 4, the normal group exhibited no inflammation and cartilage damage. In contrast, the negative control group exhibited pronounced inflammatory cell infiltration, synovial hyperplasia, high cell density, and moderate to severe cartilage surface necrosis. The positive control group showed mild to moderate inflammatory cell infiltration, higher cell density around joint tissue, and mild to moderate cartilage surface damage. Oral administration of PLEN 50, PLEN 100, and PLEN 200 reduced inflammatory cell infiltration and cartilage damage compared to negative group. Based on OARSI scoring system, PLEN administration reduced inflammation score, with only PLEN 200 significantly reducing cartilage degradation score. Furthermore, PLEN 200 alleviated inflammation and cartilage degradation more effectively than the positive control and showed no significant difference on cartilage degradation compared to the normal group (Table 1).

Histopathological analysis of MIA-induced OA revealed synovial inflammation and cartilage degeneration, featuring both early phase and advanced of human OA symptoms. Joint cartilage degradation causes bones surface to rub against each other, resulting in stiffness, pain, loss of joint function, and impaired mobility. This process also further promotes inflammatory cell infiltration in the synovium, leading to synovitis (Sandhiutami et al. 2025a). NSAIDs medication, such as diclofenac have disadvantage, as they only suppress inflammation on early phase OA (Sasaki et al. 2024). Table 1 shows that diclofenac reduces inflammation score, but has no effect on cartilage degradation score. In contrast, *P. edulis* reported to reduce *in-vivo* pro-inflammatory cytokines and MMP-9 levels (Sandhiutami et al. 2025a). MMP-9 is a gelatinase enzyme that further degrades cartilage after collagenase cleavage. Degradation of both proteoglycans and collagen cartilage by MMP-9 is the final stage of synovial joint degradation in OA (Mukherjee and Das 2024). *P. edulis* MMP-9 inhibition properties for advanced OA symptoms synergize with chitosan, which has been reported to alleviate chondrocytes degeneration and recover cartilage fibers in MIA-induced OA rats (Hamza et al. 2020).

Table 1
Effect of PLEN to inflammation and cartilage degradation score to MIA-induced rats

Group	Inflammation score	Cartilage degradation score
Normal	0 ± 0 [a]	0,25 ± 0,5 [a]
Negative control	3 ± 0 [b]	2,5 ± 0,58 [b]
Positive control	1,75 ± 0,5 [c]	1,5 ± 0,58 [b]
PLEN 50	1,75 ± 0,5 [c]	2 ± 0 [b]
PLEN 100	1,25 ± 0,5 [cd]	2 ± 0 [b]
PLEN 200	1 ± 0 [d]	1 ± 0,81 [ac]

3.5. Molecular docking

Molecular docking simulation was conducted to support *in-vitro* and *in-vivo* anti-inflammation and anti-OA potential of *P. edulis* extract. Table 2 shows *P. edulis* saponin compounds, such as cyclopassifloside II with a docking score of -113.083 kcal/mol, followed by cyclopassifloside VI, cyclopassifloside VII, cyclopassifloside XII, cyclopassifloside VIII, and cyclopassifloside V exhibited the highest binding affinity to iNOS compared to other compounds. Molecular docking against iNOS aligns with a prior study that demonstrated saponin fraction, cyclopassifloside XI, and cyclopassifloside XII inhibit NOS activity by 92.2%, 71.4%, and 87.0%, respectively. These findings strongly suggest that saponin compounds are the primary *P. edulis* bioactive compounds responsible for iNOS inhibition (Urrego et al. 2021). Besides iNOS inhibition, cyclopassifloside XII also demonstrated the highest binding affinity to IL-6 with a docking score of -116.875 kcal/mol. Two alkaloid compounds, N-cis-feruloyltyramine and N-trans-feruloyltyramine exhibited the highest binding affinity to TNF-α with docking scores of -115.021 kcal/mol and – 111.883 kcal/mol, respectively. Luteolin glucosides, such as luteolin-6-C-fucoside and luteolin-6-C-chinovoside, exhibited the high MMP-9 binding affinity with a docking score of -122.91 kcal/mol and – 117.741 kcal/mol, respectively. Furthermore, triterpenoid compounds such as cyclopassifloic acids G, cyclopassifloic acids F, and cyclopassifloic acids B exhibited high MMP-9 inhibition as well. From docking results, different compound groups work synergically for anti-inflammation and anti-OA, as it responsible for different pathways.

Cyclopassifloside II hydroxyl group at C-1, C-3, C-24, and sugar moiety formed hydrogen bonds, in addition to hydrophobic interaction of methyl groups at C-16 and C-21, forming a strong complex with iNOS (Fig. 5a). N-cis-feruloyltyramine and TNF-α complex mainly formed by hydrophobic interactions from aromatic group with Tyr C59, Try A59, and Leu A57, complemented by hydrogen bonds with Tyr A118 and Tyr B118 (Fig. 5b). Cyclopassifloside XII and IL-6 complex formed mainly from ring B formed hydrophobic interactions with Leu B98 and His A56 and the methyl group at C-21 formed hydrophobic interactions with Tyr B58 and Tyr B95. C-31 OH group and sugar moiety of cyclopassifloside XII formed

hydrogen bonds that enhance cyclopassifloside XII and IL-6 binding (Fig. 5c). Luteolin-6-C-fucoside OH groups at C-5 and ring B catechol group play a major role in inhibition of MMP-9, as they form hydrogen bonds with Ala A189, Arg A249, and Tyr A245. The His A226 amino acid also hydrophobic complex with all flavonoid aromatic and heterocyclic rings (Fig. 5d).

Table 2
Docking score of *P. edulis* identified compounds to iNOS (PDB ID: 3E6T), TNF-α (PDB ID: 7KP8), IL-6 (PDB ID: 7DC8), and MMP-9 (PDB ID: 4XCT) receptors.

No	Compounds	Docking score (kcal/mol)			
		iNOS	TNF-α	IL-6	MMP-9
	Alkaloids				
1	N-cis-feruloyltyramine	-90.9819	-115.021	-100.7910	-109.3840
2	N-trans-feruloyltyramine	-90.8523	-111.883	-98.6799	-111.1070
	Flavonoids				
3	Apigenin	-73.5237	-93.5301	-88.6014	-96.9775
4	Isoorientin	-88.4441	-80.3403	-87.9199	-105.0750
5	Isovitexin	-83.8686	-72.1222	-89.6604	-99.6747
6	Lucenin-2	-90.2682	-85.8574	-63.3698	-95.9013
7	Luteolin	-75.8022	-96.8362	-91.4134	-102.1360
8	Luteolin-6-C-chinovoside	-93.8321	-85.8289	-102.604	-117.7410
9	Luteolin-6-C-fucoside	-96.8287	-92.9105	-102.564	-122.9100
10	Orientin	-83.7753	-102.601	-84.9891	-106.569
11	Spinosin	-82.2063	-9.3082	-78.6028	-99.2773
12	Vicenin-2	-95.6676	13.0512	-99.2633	-78.4288
13	Vitexin	-89.1870	-97.8761	-79.7366	-103.2340
	Phenolic acids				
14	p-hydroxybenzoic acid	-48.6855	-57.7127	-61.066	-63.6000
15	Syringaresinol	-99.0079	-91.6669	-105.443	-95.8086
16	Syringic acid	-57.8868	-73.9144	-80.8387	-69.9615
17	Vanillic acid	-59.0033	-63.2478	-75.3486	-73.4200
	Saponins				
18	Cyclopassifloside I	-95.5079	135.294	-14.2797	-110.192
19	Cyclopassifloside II	-113.083	-26.5054	-54.4354	-86.7743
20	Cyclopassifloside III	-96.9097	34.6066	-34.1552	-47.1514
21	Cyclopassifloside IV	-70.124	80.9145	-19.0944	-77.3749

No	Compounds	Docking score (kcal/mol)			
		iNOS	TNF-α	IL-6	MMP-9
22	Cyclopassifloside V	-106.415	19.7535	-82.5816	-2.3922
23	Cyclopassifloside VI	-108.642	20.0981	-18.9932	-100.191
24	Cyclopassifloside VII	-107.838	118.649	-34.3194	-79.307
25	Cyclopassifloside VIII	-107.518	-22.5164	-50.3822	-95.6667
26	Cyclopassifloside IX	-91.8629	32.4461	134.309	-55.1647
27	Cyclopassifloside X	-67.3459	15.3451	-69.7081	-62.1292
28	Cyclopassifloside XI	-89.9422	33.4087	246.765	-48.6833
29	Cyclopassifloside XII	-107.651	-8.0844	-116.875	-98.606
30	Cyclopassifloside XIII	-76.4999	-44.7007	-67.9698	-40.1778
31	Passiflorin	-81.9545	74.8499	-68.9694	-101.916
	Triterpenoids				
32	Cyclopassifloic acids A	-89.4088	-7.7767	-36.8161	-80.8199
33	Cyclopassifloic acids B	-90.1246	-17.6881	-92.0734	-112.5140
34	Cyclopassifloic acids C	-97.5567	15.2334	-90.8527	-87.7751
35	Cyclopassifloic acids D	-93.5099	0.4841	-83.3214	-106.0410
36	Cyclopassifloic acids E	-90.8634	-20.3792	-77.6977	-97.4845
37	Cyclopassifloic acids F	-89.4544	-6.9952	-100.745	-114.1050
38	Cyclopassifloic acids G	-102.377	12.1909	-90.4586	-121.2700
39	Passifloric acid	-88.5963	-8.093	-65.4489	-106.696
40	Diclofenac (Positive control)	-69.7683	-90.3853	-100.505	-81.4435

4. Conclusion

In conclusion, the present study showed that PLEN exerts anti-inflammation and anti-OA activity. In LPS-induced RAW 264.7 cells, PLEN reduced proinflammatory biomarkers such as NO, iNOS, and TNF-α concentration. In MIA-induced OA rats, PLEN alleviated OA signs by increasing body weight, reducing knee oedema and spleen weight, inflammatory cell infiltration, and cartilage degradation. Further molecular docking simulation revealed that saponins, flavonoids, and alkaloids work synergistically to target distinct anti-inflammation and anti-OA pathways.

Abbreviations

OA : Osteoarthritis

PLEN : *Passiflora edulis* leaves extract nanoparticle

MIA : Monosodium iodoacetate

ECM : Extracellular matrix

MMP : Matrix metalloproteinase

NSAID : Nonsteroidal anti-inflammatory drugs

H&E : Haematoxylin and Eosin

Declarations

Funding

This work was supported by The Ministry of Education, Culture, Research, and Technology, The Republic of Indonesia under the Fundamental Regular Research 2024 program (0034/LPPM/UP/VI/2024)

Conflict of interest

The authors declare that they have no conflict of interest.

Authorship contribution

NMDS: Conceptualization, methodology, investigation, resources, writing - review & editing, supervision, visualization, project administration, funding acquisition. YD: Investigation, methodology, supervision, writing - review & editing. FNN: Investigation, methodology, supervision, writing - review & editing. RSD: Investigation, methodology, supervision, writing - review & editing. DKP: Investigation, methodology, supervision, writing - review & editing. SS: Investigation, writing – original draft. WNZ: Investigation, writing – original draft. FX: Investigation, writing – original draft.

Data Availability

All data supporting the findings of this study are included in the published article. Raw data will be made available upon reasonable request

Ethics approval

The animal experimental study was granted ethical approval by Faculty of Medicine, University of Indonesia (KET-395/UN2.F1/ETIK/PPM.00.02/2024).

References

1. Abd El-Aziz R, Naguib M, Rashed LA (2018) Spleen size in patients with metabolic syndrome and its relation to metabolic and inflammatory parameters. Egypt J Intern Med 30:78–82. https://doi.org/10.4103/ejim.ejim_86_17
2. Al-Saadi HM, Pang K-L, Ima-Nirwana S, Chin K-Y (2019) Multifaceted Protective Role of Glucosamine against Osteoarthritis: Review of Its Molecular Mechanisms. Sci Pharm 87. https://doi.org/10.3390/scipharm87040034
3. Ansari MY, Ahmad N, Haqqi TM (2020) Oxidative stress and inflammation in osteoarthritis pathogenesis: Role of polyphenols. Biomed Pharmacother 129:110452. https://doi.org/https://doi.org/10.1016/j.biopha.2020.110452
4. Arozal W, Louisa M, Rahmat D et al (2020) Development, Characterization and Pharmacokinetic Profile of Chitosan-Sodium Tripolyphosphate Nanoparticles Based Drug Delivery Systems for Curcumin. Adv Pharm Bull 11:77–85. https://doi.org/10.34172/apb.2021.008
5. Cazarin CBB, da Silva JK, Colomeu TC et al (2015) Intake of Passiflora edulis leaf extract improves antioxidant and anti-inflammatory status in rats with 2,4,6-trinitrobenzenesulphonic acid induced colitis. J Funct Foods 17:575–586. https://doi.org/https://doi.org/10.1016/j.jff.2015.05.034
6. Chisari E, Yaghmour KM, Khan WS (2020) The effects of TNF-alpha inhibition on cartilage: a systematic review of preclinical studies. Osteoarthritis Cartilage 28:708–718. https://doi.org/10.1016/j.joca.2019.09.008
7. Courties A, Kouki I, Soliman N et al (2024) Osteoarthritis year in review 2024: Epidemiology and therapy. Osteoarthritis Cartilage 32:1397–1404. https://doi.org/https://doi.org/10.1016/j.joca.2024.07.014
8. Curtis E, Fuggle N, Shaw S et al (2019) Safety of Cyclooxygenase-2 Inhibitors in Osteoarthritis: Outcomes of a Systematic Review and Meta-Analysis. Drugs Aging 36:25–44. https://doi.org/10.1007/s40266-019-00664-x
9. da Silva JK, Cazarin CBB, Colomeu TC et al (2013) Antioxidant activity of aqueous extract of passion fruit (Passiflora edulis) leaves: In vitro and in vivo study. Food Res Int 53:882–890. https://doi.org/https://doi.org/10.1016/j.foodres.2012.12.043
10. de Sousa Valente J (2019) The Pharmacology of Pain Associated With the Monoiodoacetate Model of Osteoarthritis. Front Pharmacol Volume. https://doi.org/10.3389/fphar.2019.00974. 10-2019
11. Desmiaty Y, Sandhiutami NMD, Fahleni F et al (2025) Temperature-Dependent Variations in Antioxidant Activities and Phytochemical Profiles of Passiflora edulis Leaf Extracts. Trends Sci 22:9772. https://doi.org/10.48048/tis.2025.9772
12. Dong Y, Yan Y, Zhou J et al (2023) Evidence on risk factors for knee osteoarthritis in middle-older aged: a systematic review and meta analysis. J Orthop Surg Res 18:634. https://doi.org/10.1186/s13018-023-04089-6

13. Fahleni F, Desmiaty Y, Dewi RS et al (2024) The Development of a Novel Nanoherbal Passionfruit (Passiflora edulis) Leaves with Safety and Analgesic Effect. Trends Sci 21:8412. https://doi.org/10.48048/tis.2024.8412
14. Farid R, Rezaieyazdi Z, Mirfeizi Z et al (2010) Oral intake of purple passion fruit peel extract reduces pain and stiffness and improves physical function in adult patients with knee osteoarthritis. Nutr Res 30:601–606. https://doi.org/https://doi.org/10.1016/j.nutres.2010.08.010
15. Fonseca AMA, Geraldi MV, Junior MRM et al (2022) Purple passion fruit (Passiflora edulis f. edulis): A comprehensive review on the nutritional value, phytochemical profile and associated health effects. Food Res Int 160:111665. https://doi.org/https://doi.org/10.1016/j.foodres.2022.111665
16. de Gadioli IL Sá Barreto da Cunha M, de Carvalho MVO et al (2018) A systematic review on phenolic compounds in Passiflora plants: Exploring biodiversity for food, nutrition, and popular medicine. Crit Rev Food Sci Nutr 58:785–807. https://doi.org/10.1080/10408398.2016.1224805
17. Glasson SS, Chambers MG, Van Den Berg WB, Little CB (2010) The OARSI histopathology initiative – recommendations for histological assessments of osteoarthritis in the mouse. Osteoarthritis Cartilage 18:S17–S23. https://doi.org/https://doi.org/10.1016/j.joca.2010.05.025
18. Guo Q, Jin Y, Chen X et al (2024a) NF-κB in biology and targeted therapy: new insights and translational implications. Signal Transduct Target Ther 9:53. https://doi.org/10.1038/s41392-024-01757-9
19. Guo Q, Jin Y, Chen X et al (2024b) NF-κB in biology and targeted therapy: new insights and translational implications. Signal Transduct Target Ther 9:53. https://doi.org/10.1038/s41392-024-01757-9
20. Hamza RZ, Al-Salmi FA, El-Shenawy NS (2020) Chitosan and Lecithin Ameliorate Osteoarthritis Symptoms Induced by Monoiodoacetate in a Rat Model. Molecules 25. https://doi.org/10.3390/molecules25235738
21. Han Z, Wang K, Ding S, Zhang M (2024) Cross-talk of inflammation and cellular senescence: a new insight into the occurrence and progression of osteoarthritis. Bone Res 12:69. https://doi.org/10.1038/s41413-024-00375-z
22. He F, Wu H, He B et al (2024) Antioxidant hydrogels for the treatment of osteoarthritis: mechanisms and recent advances. Front Pharmacol Volume. https://doi.org/10.3389/fphar.2024.1488036. 15-2024
23. He X, Luan F, Yang Y et al (2020) Passiflora edulis: An Insight Into Current Researches on Phytochemistry and Pharmacology. https://doi.org/10.3389/fphar.2020.00617. Front Pharmacol 11-2020
24. Li H-Z, Liang X-Z, Sun Y-Q et al (2024) Global, regional, and national burdens of osteoarthritis from 1990 to 2021: findings from the 2021 global burden of disease study. Front Med (Lausanne) 11–2024. https://doi.org/10.3389/fmed.2024.1476853
25. Lindler BN, Long KE, Taylor NA, Lei W (2020) Use of Herbal Medications for Treatment of Osteoarthritis and Rheumatoid Arthritis. Medicines 7. https://doi.org/10.3390/medicines7110067

26. Liu T, Zhang L, Joo D, Sun S-C (2017) NF-κB signaling in inflammation. Signal Transduct Target Ther 2:17023. https://doi.org/10.1038/sigtrans.2017.23
27. López-García J, Lehocký M, Humpolíček P, Sáha P (2014) HaCaT Keratinocytes Response on Antimicrobial Atelocollagen Substrates: Extent of Cytotoxicity, Cell Viability and Proliferation. J Funct Biomater 5:43–57. https://doi.org/10.3390/jfb5020043
28. Molnar V, Matišić V, Kodvanj I et al (2021) Cytokines and Chemokines Involved in Osteoarthritis Pathogenesis. Int J Mol Sci 22. https://doi.org/10.3390/ijms22179208
29. Mukherjee A, Das B (2024) The role of inflammatory mediators and matrix metalloproteinases (MMPs) in the progression of osteoarthritis. Biomaterials Biosystems 13:100090. https://doi.org/https://doi.org/10.1016/j.bbiosy.2024.100090
30. Nagaoka I, Tsuruta A, Yoshimura M (2019) Chondroprotective action of glucosamine, a chitosan monomer, on the joint health of athletes. Int J Biol Macromol 132:795–800. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2019.03.234
31. Namdevrao AP, Shaikh MS, Rajjak PR et al Formulation and Optimization of Selenium Nanoparticles Using Passiflora edulis Leaf Extract: Permeation and in Vitro Release Kinetic Study. Chem Biodivers n/a:e202500730. https://doi.org/https://doi.org/10.1002/cbdv.202500730
32. del Navarro J, Herrera T, Fornari T et al (2018) The gastrointestinal behavior of saponins and its significance for their bioavailability and bioactivities. J Funct Foods 40:484–497. https://doi.org/https://doi.org/10.1016/j.jff.2017.11.032
33. Nistor M, Rugina D, Diaconeasa Z et al (2023) Pentacyclic Triterpenoid Phytochemicals with Anticancer Activity: Updated Studies on Mechanisms and Targeted Delivery. Int J Mol Sci 24. https://doi.org/10.3390/ijms241612923
34. Novy TCT, Made I, Ronny J L, et al (2025) Chitosan Nanoparticles as an Alternative Therapeutic Approach for Knee Osteoarthritis Treatment: A Systematic Review. Int J Nanomed 20:6187–6203. https://doi.org/10.2147/IJN.S503829
35. Ohtori S, Orita S, Yamauchi K et al (2015) Efficacy of Direct Injection of Etanercept into Knee Joints for Pain in Moderate and Severe Knee Osteoarthritis. Yonsei Med J 56:1379–1383
36. Panichi V, Costantini S, Grasso M et al (2024) Innate Immunity and Synovitis: Key Players in Osteoarthritis Progression. Int J Mol Sci 25. https://doi.org/10.3390/ijms252212082
37. Rahmawati SI, Indriani DW, Ningsih FN et al (2024) Dual anti-inflammatory activities of COX-2/5-LOX driven by kratom alkaloid extracts in lipopolysaccharide-induced RAW 264.7 cells. Sci Rep 14:28993. https://doi.org/10.1038/s41598-024-79229-x
38. Reginster J-Y, Bruyère O, Conaghan PG et al (2019) Importance of Safety in the Management of Osteoarthritis and the Need for Updated Meta-Analyses and Recommendations for Reporting of Harms. Drugs Aging 36:3–6. https://doi.org/10.1007/s40266-019-00659-8
39. Ren J-L, Yang J, Hu W (2025) The global burden of osteoarthritis knee: a secondary data analysis of a population-based study. Clin Rheumatol 44:1769–1810. https://doi.org/10.1007/s10067-025-07347-6

40. Salis Z, Sainsbury A (2024) Association of long-term use of non-steroidal anti-inflammatory drugs with knee osteoarthritis: a prospective multi-cohort study over 4-to-5 years. Sci Rep 14:6593. https://doi.org/10.1038/s41598-024-56665-3
41. Sandhiutami N, Desmiaty Y, Fahleni F et al (2025a) Passiflora edulis Sims leaves ameliorate inflammation and prevent cartilage degradation in osteoarthritic rats by suppressing cytokines, nitric oxide, and matrix metalloproteinase-9. J Pharm Pharmacogn Res 13:356–368. https://doi.org/10.56499/jppres24.2047_13.2.356
42. Sandhiutami N, Desmiaty Y, Fahleni, Fakhran M (2024) Antinociceptive and anti-inflammatory effect of Passiflora edulis leaves extract in acetic acid-induced pain and carrageenan-induced paw oedema in vivo. Pharm Educ 24:78–85. https://doi.org/10.46542/pe.2024.242.7885
43. Sandhiutami NMD, Desmiaty Y, Fahleni F et al (2025b) Enhanced Anti-Inflammatory Activity of Passiflora edulis Leaf Extract Nanoparticle Gel in Carrageenan-Induced Rat Model. Sci Pharm 4:32–39. https://doi.org/https://doi.org/10.58920/sciphar0401301
44. Sasaki Y, Kijima K, Yoshioka K (2024) Validity evaluation of a rat model of monoiodoacetate-induced osteoarthritis with clinically effective drugs. BMC Musculoskelet Disord 25:975. https://doi.org/10.1186/s12891-024-08083-9
45. Steinmetz JD, Culbreth GT, Haile LM, et al (2023) Global, regional, and national burden of osteoarthritis, 1990–2020 and projections to 2050: a systematic analysis for the Global Burden of Disease Study 2021. Lancet Rheumatol 5:e508–e522. https://doi.org/10.1016/S2665-9913(23)00163-7
46. Takeuchi T, Oyama M, Hatanaka T (2024) Effect of Chitosan Degradation Products, Glucosamine and Chitosan Oligosaccharide, on Osteoclastic Differentiation. https://doi.org/10.3390/biotech13010006. BioTech 13:
47. Urrego N, Sepúlveda P, Aragón M et al (2021) Flavonoids and saponins from Passiflora edulis f. edulis leaves (purple passion fruit) and its potential anti-inflammatory activity. J Pharm Pharmacol 73:1530–1538. https://doi.org/10.1093/jpp/rgab117
48. Vo NX, Le NNH, Chu TDP et al (2023) Effectiveness and Safety of Glucosamine in Osteoarthritis: A Systematic Review. https://doi.org/10.3390/pharmacy11040117. Pharmacy 11:
49. Wang C, Xu F-Q, Shang J-H et al (2013) Cycloartane triterpenoid saponins from water soluble of Passiflora edulis Sims and their antidepressant-like effects. J Ethnopharmacol 148:812–817. https://doi.org/https://doi.org/10.1016/j.jep.2013.05.010
50. Weyya G, Belay A, Tadesse E (2024) Passion fruit (Passiflora edulis Sims) by-products as a source of bioactive compounds for non-communicable disease prevention: extraction methods and mechanisms of action: a systematic review. Front Nutr Volume. https://doi.org/10.3389/fnut.2024.1340511. 11-2024
51. Yao Q, Wu X, Tao C et al (2023) Osteoarthritis: pathogenic signaling pathways and therapeutic targets. Signal Transduct Target Ther 8:56. https://doi.org/10.1038/s41392-023-01330-w

52. Yoshikawa K, Katsuta S, Mizumori J, Arihara S (2000a) New Cycloartane Triterpenoids from Passiflora edulis. J Nat Prod 63:1377–1380. https://doi.org/10.1021/np000182a
53. Yoshikawa K, Katsuta S, Mizumori J, Arihara S (2000b) Four Cycloartane Triterpenoids and Six Related Saponins from Passiflora edulis. J Nat Prod 63:1229–1234. https://doi.org/10.1021/np000126+
54. Yuan D, Guo Y, Pu F et al (2024) Opportunities and challenges in enhancing the bioavailability and bioactivity of dietary flavonoids: A novel delivery system perspective. Food Chem 430:137115. https://doi.org/https://doi.org/10.1016/j.foodchem.2023.137115
55. Yuan TZ, Kao CL, Li WJ et al (2017) Chemical Constituents of Leaves of Passiflora edulis. Chem Nat Compd 53:1165–1166. https://doi.org/10.1007/s10600-017-2227-5
56. Zhu R, Fang H, Wang J et al (2024) Inflammation as a therapeutic target for osteoarthritis: A literature review of clinical trials. Clin Rheumatol 43:2417–2433. https://doi.org/10.1007/s10067-024-07042-y
57. Zucolotto Stella, Montanher Ana B, Reginatto, Silvia SMG (2009) Bioassay-Guided Isolation of Anti-Inflammatory C-Glucosylflavones from Passiflora edulis. Planta Med 75:1221–1226. https://doi.org/10.1055/s-0029-1185536

Figures

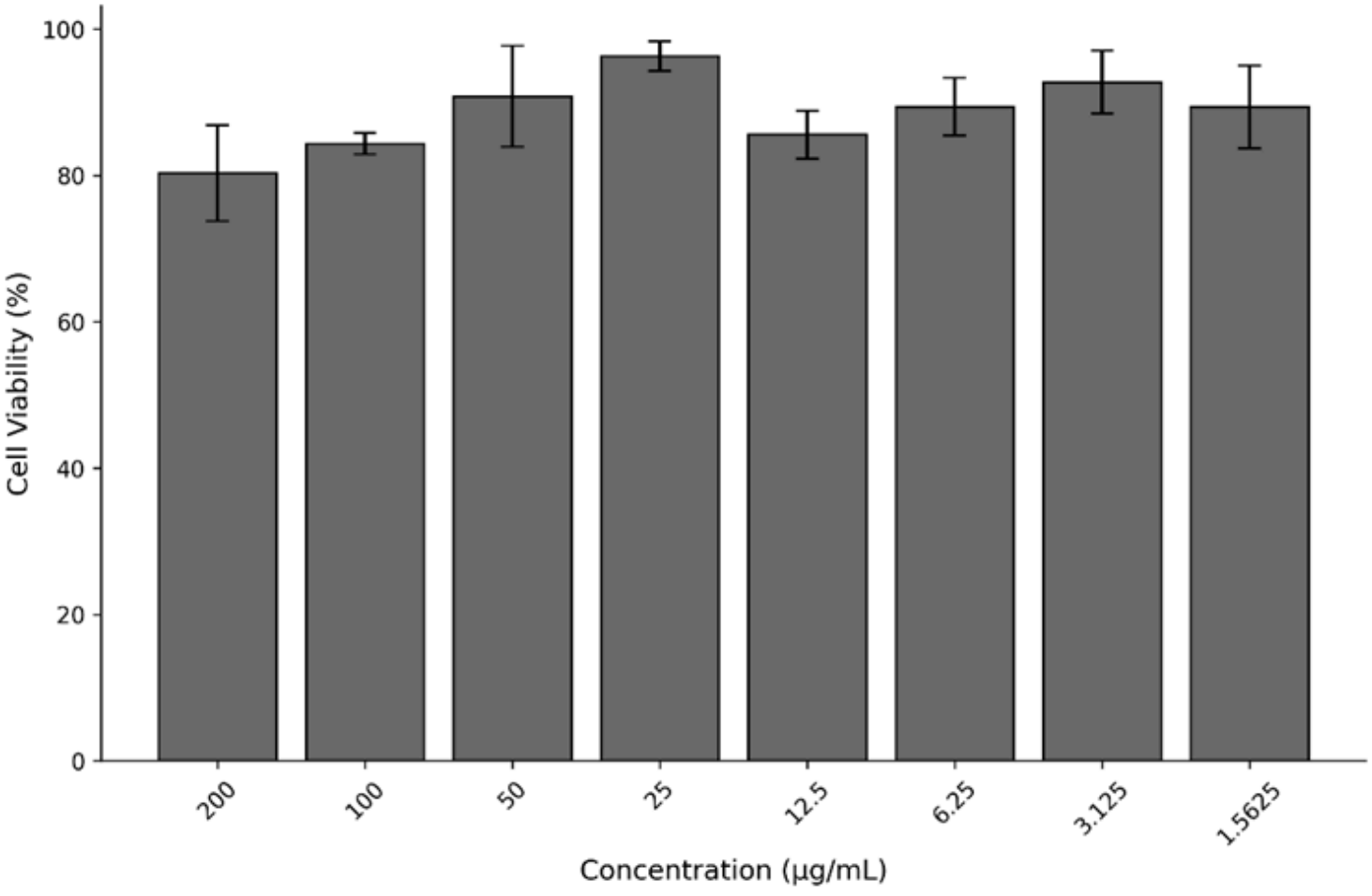


Figure 1

Cell viability of Passiflora edulis leaf extract nanoparticle (PLEN) against RAW 264.7 cells. The results are displayed as mean ± SD.

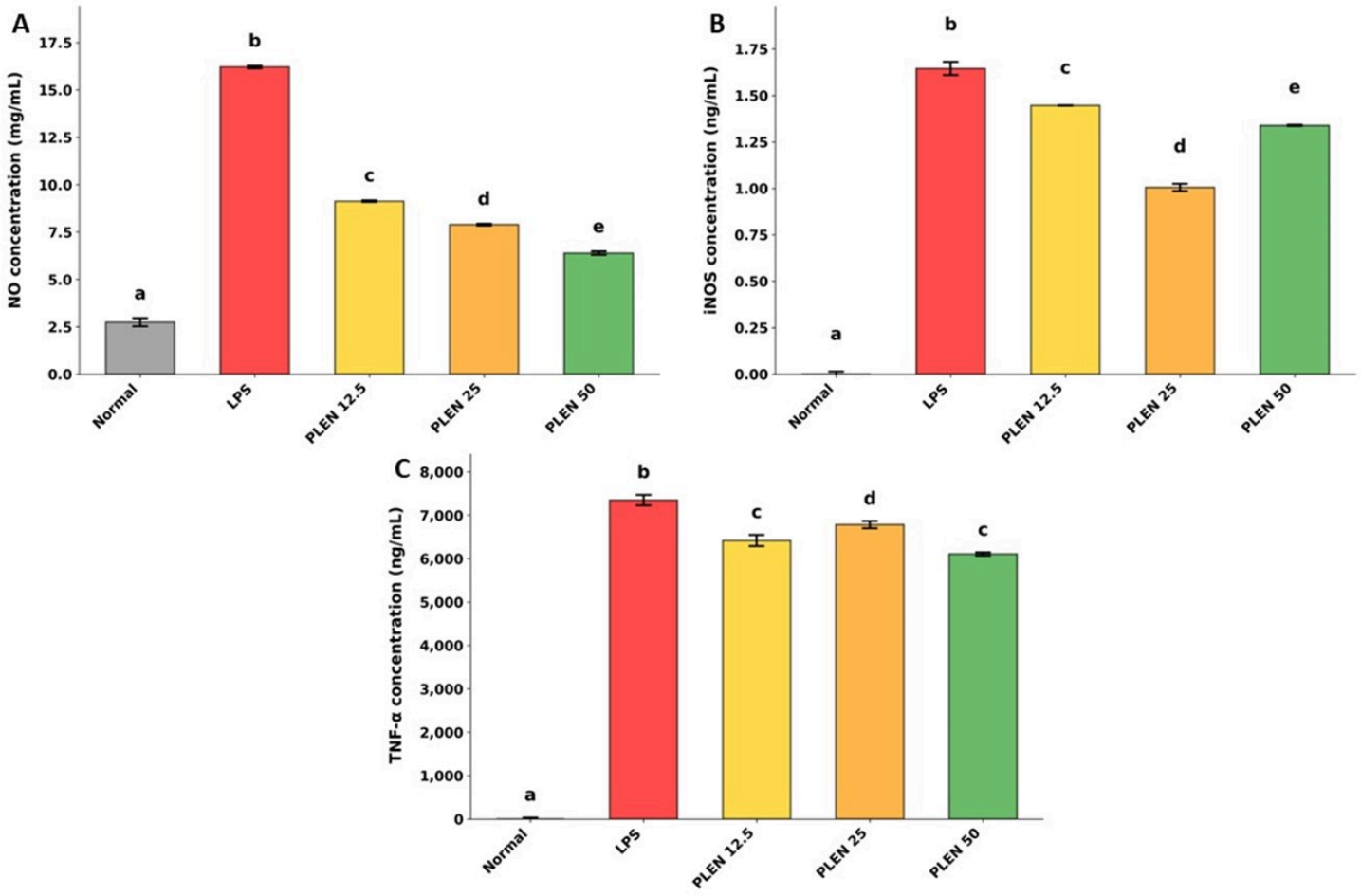


Figure 2

Inflammatory biomarker assessment of Passiflora edulisleaf extract nanoparticle (PLEN) against LPS-induced RAW 264.7 cells. (A) NO concentration, (B) iNOS concentration, and (C) TNF-α concentration. The results are displayed as mean ± SD. Different letters indicate significant difference ($p < 0.05$).

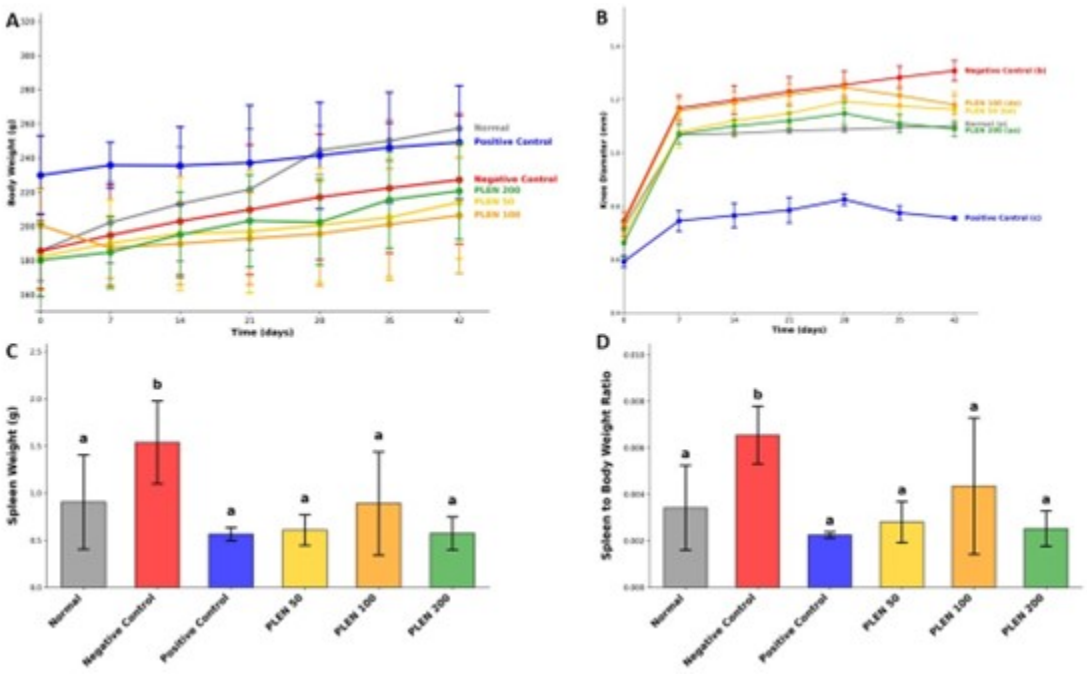


Figure 3

Passiflora edulis leaf nanoparticle (PLEN) effect on (A) Body weight, (B) knee diameter of osteoarthritis rats, (C) spleen weight, and (D) spleen to body weight ratio. The results are displayed as mean ± SD. Different letters indicate significant difference ($p < 0.05$).

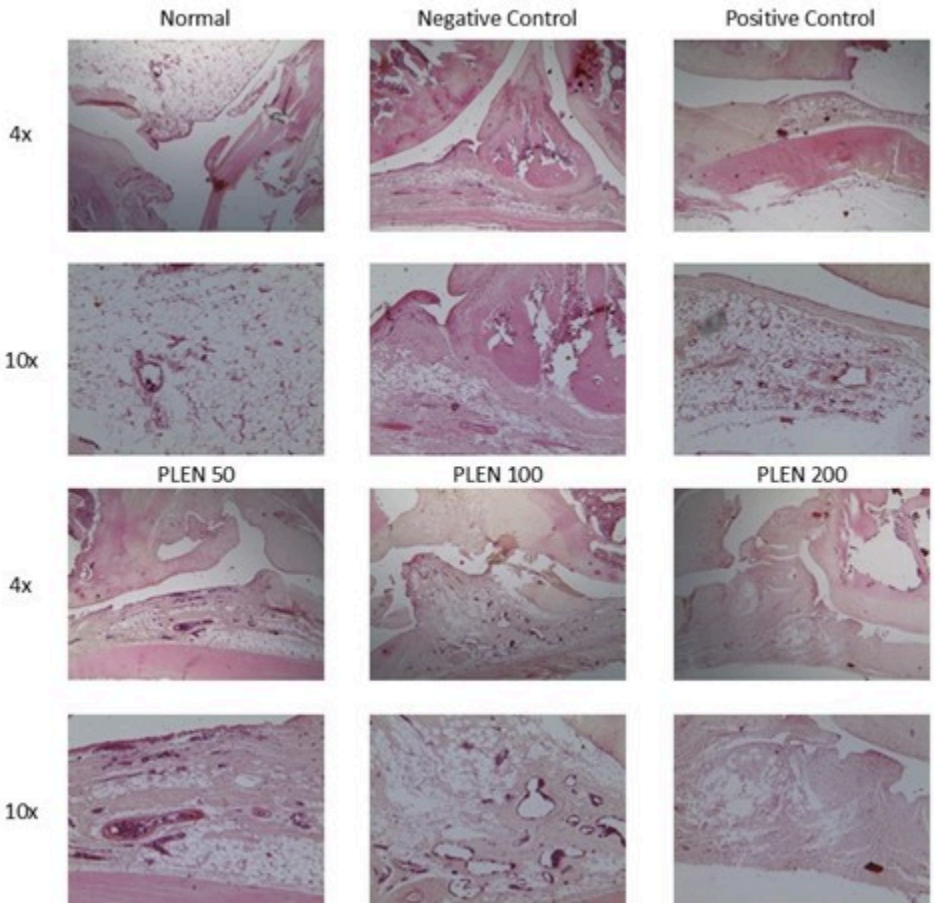
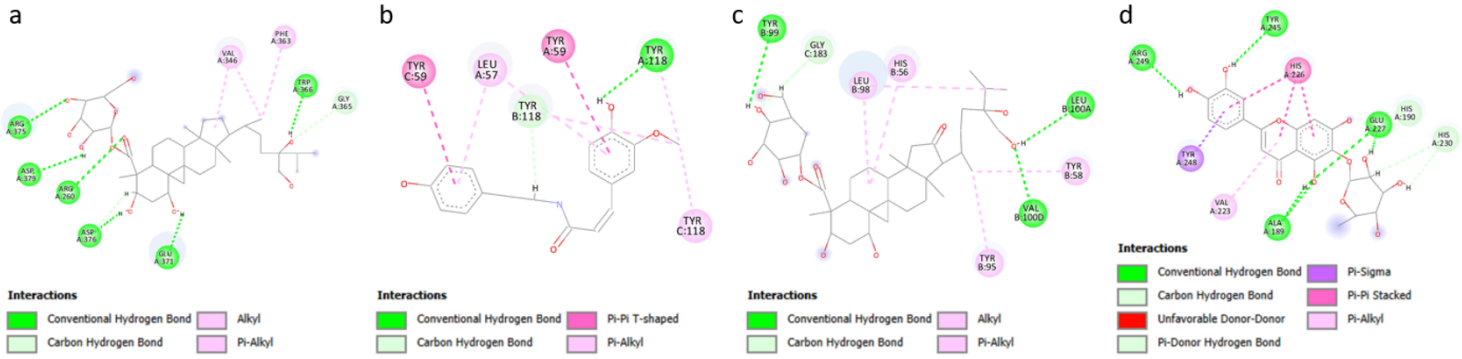


Figure 4

Effect of PLEN on histopathological change of knee tissue using H&E staining with 4x and 10x magnification

Figure 5



Docking visualization of (a) iNOS and cyclopassifloside II complex, (b) TNF-α and N-cis-feruloyltyramine complex, (c) IL-6 and cyclopassifloside XII, and (d) MMP-9 and luteolin-6-C-fucoside