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RESEARCH ARTICLE

Antinociceptive and anti-inflammatory effect of *Passiflora edulis* leaves extract in acetic acid-induced pain and carrageenan-induced paw oedema in vivo

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Abstract

Background: In Indonesia, people commonly treat pain and inflammation with herbs. *Passiflora edulis* leaves extract (PLE) is known to contain many chemical compounds that are useful as analgesic and anti-inflammatory treatments. However, there is very limited scientific literature that reports the results of P. *edulis* in reducing pain and inflammation. **Objective:** This research aims to evaluate the antinociceptive and anti-inflammatory effects of the PLE. **Method:** PLE was extracted by Ultrasonic-Assisted Extraction (UAE). The PLE at doses of 20, 40 and 80 mg/200g BW (PLE-20, PLE-40, and PLE-80) and diclofenac sodium (Dic. sod. at a dose of 2.7 mg/200g BW as a positive control group was tested for antinociceptive and anti-inflammatory effects in experimental animal models (n=5). The effect of antinociceptive was evaluated by the wriggling in mice by acetic acid-induced, and the effect of acute anti-inflammatory was evaluated by carrageenan-induced in rats. **Result:** PLE at doses of 20, 40, and 80 mg/200g BW showed a significant antinociceptive effect at acetic acid-induced wriggling and inhibition of the oedema. Higher doses lead to more pronounced effects. The greater effect of antinociceptive was observed at PLE-80 (64.55%), and inhibition of the oedema was observed at PLE-80 (34.33%). **Conclusion:** PLE significantly reduced pain and acute inflammation in the animal-tested models.

Introduction

Many ailments people experience today, like osteoarthritis, back pain, and headaches, are frequently brought on by our way of life and work-related risks. Long-term, non-treated pain is the most common condition that may cause psychological problems, resulting in loss of function due to absences from work or social activities (Yimer *et al.*, 2020). Even though there are many medications available, soreness and inflammation are the hardest to treat among the health issues that impact 80% of people globally. Non-resolving inflammation also promotes the development of serious inflammatory disorders. People rely on painkillers to help them get through the pain. Due to its importance in practically all human disorders, inflammation has been the topic of much scientific investigation on a global scale (Salmerón-Manzano *et al.*, 2020). The use of NSAIDs increases the risk of cardiovascular and gastrointestinal effects. Additionally, opioid-class analgesics are associated with several toxicities and undesirable side effects, including respiratory problems, fatigue, vomiting and nausea, burning sensation, constipation, disturbance of hormonal balance, diminished hearing, compassion, and psychological and physical dependence. Considering the socio-economic impact of pain and inflammation, the search for promising drugs to reduce pain and inflammation with few side effects, especially from traditional plants for medicinal purposes, needs to be continuously developed. Current research is also increasingly recognising that herbal medicine has good prospects. Due to this, research into herbs allegedly useful in treating pain and inflammation has to be intensified (Kim *et al.*, 2018).

In Indonesia, people commonly treat pain and inflammation with herbs (Nisa *et al.*, 2022). For a very long time, people have used plants as natural resources to treat illnesses. Research on pharmacology and screening of phytochemicals that provide benefits continues to grow, especially on herbs that have been used empirically. Many herbal plants have been studied by researchers and are known to have effects on inflammation and pain, including plants that consist of many components so that they work synergistically with various targets of action or isolation of active compounds from plants so that specific target pathways can be known (Nunes *et al.*, 2020).

Passiflora edulis belongs to the Passifloraceae family. P. *edulis* is famous for its fruit flesh and leaves for treatment. The leaves are known to contain many chemical compounds that are useful for treatment. P. *edulis* leaves contain alkaloids, flavonoids, and steroids/triterpenoids, which are useful as pain relievers (antinociceptive) and anti-inflammatory. Alkaloids act as painkillers, flavonoids have phenolic content as anti-inflammatories, tannins act as antioxidants and anti-inflammatories (He *et al.*, 2020). Alkaloids and flavonoids have been shown to inhibit prostaglandins and leukotrienes, which are products of arachidonic acid, which will later cause leukocyte chemotaxis (Araújo *et al.*, 2020). There are several studies that state anti-inflammatory activity in *Passiflora* species (He *et al.*, 2020). Empirically, P. *edulis* leaves have been used and its antinociceptive and anti-inflammatory properties are regularly mentioned. However, there is very limited scientific literature that reports the results of P. *edulis* in reducing pain and inflammation. Therefore, it was considered appropriate to scientifically examine the antinociceptive and anti-inflammatory properties of the P. *edulis* leaves. In order to turn an herbal medicine into a nutraceutical that is safe and proven to be effective for treating pain and inflammation that commonly occurs in osteoarthritis, back pain, and headaches, a preclinical test was carried out on P. *edulis* leaves. The aim of this study was to examine the antinociceptive and anti-inflammatory effects of 70% ethanol leaf extract of P. *edulis* in an animal model.

Methods

P. *edulis materials*

Fresh *Passiflora edulis* leaves were purchased from BALITRO and identified in Herbarium Depokensis, Biota Collection Room Universitas Indonesia, Indonesia (867/UN2.F3.11/PDP.02.00/2022). The determination showed that they were *Passiflora edulis* Sims. from the Passifloraceae family.

Extraction and phytochemical screening test of P. *edulis leaves (PLE)*

P. *edulis* leaf simplicia powder was weighed to approximately 100 g of dried simplicia. Then, it was extracted by Ultrasonic-Assisted Extraction (UAE) method for 45 minutes at a temperature of 30˚C and wave frequency of 40kHz using a total of 70% ethanol solvent as much as 1000 mL (1:10). Extraction was carried out two times with details of the volume of solvent: for the first extraction 500 mL was used, the residue from the first extraction was extracted again using 500 mL of solvent. A vacuum rotary evaporator was used to thicken the collected filtrate until a thick extract was produced. Phytochemical screening assays were conducted to standard testing procedures used in this assay (Desmiaty *et al*., 2019; Kumar *et al*., 2021; Irawan *et al.*, 2022).

Chemical and drugs

Diclofenac sodium (Novell), sodium carboxymethyl cellulose (CMC) (Brataco Chemical), carrageenan (Sigma-Aldrich Chemical Company, Steinheim, Germany), Acetic acid (Brataco Chemical), Aquadest (Brataco Chemical). All chemicals and drugs used were of the highest purity and analytical grade.

Animals

In this study, 25 male Sprague Dawley rats (200-250 grams) and 25 male DDY mice (20-30 grams), each aged eight weeks, were used, which were obtained from the Non Ruminant and Wildlife Laboratory, Faculty of Animal Husbandry IPB. The animals used were acclimatised for one week. Rats and mice were maintained under laboratory temperature (24± 2) °C and constant air humidity 60 ± 5%, with 12 h light/ 12 h dark cycles, were given standard food and allowed access to water.

In vivo experimental design for antinociceptive and anti-inflammatory test

In vivo assays were conducted after getting approval from the Ethics Committee, Faculty of Medicine, Universitas Indonesia (KET-242/UN2.F1/ETIK/PPM.00.02/2023). The study was performed at the Department of Pharmacology and Toxicology, Faculty of Pharmacy, Pancasila University, South Jakarta, Indonesia. Twenty-five mice and rats, respectively, were each divided into five groups (n = 5). Five major groups, i.e. the control negative group received sodium carboxymethyl cellulose (CMC), the

control positive group were administered with diclofenac sodium (2.7 mg/200g BW), while three different doses of P. *edulis* leaves extract, i.e. 20, 40 and 80 mg/200g BW (PLE-20; PLE-40; PLE-80).

Antinociceptive activity test in mice

The antinociceptive effect against acetic acid-induced wriggling in mice (Sandhiutami *et al.*, 2023) method was used to preferentially evaluate the possible peripheral effect of PLE. A total of 25 mice were divided into five groups (n = 5). Group 1: The negative group received CMC. Group 2: positive control received Dic. sod. 2.7 mg/200g BW. Meanwhile groups 3, 4 and 5 were administered p.o with PLE-20; PLE-40; PLE-80 respectively. Thirty minutes after treatment, the mice were injected 0.2 mL/20 g BW of acetic acid (3%) intraperitoneally to induce the characteristic wrigglings. After 5 minutes, the mice were observed in a transparent box. Record the amount of wriggling response shown by the mice. The antinociceptive effect of PLE was assessed by counting the number of wriggling every 5 minutes for 60 minutes. Wriggling was assessed by stretching the mice's limbs backwards accompanied by contraction of the abdomen. The data obtained from each group was presented in a graph, and the Area Under Curve (AUC) and the percentage of activity were calculated. A reduction in the value of AUC as compared to the negative control group was regarded as evidence for the antinociceptive potential of the extract, and it was expressed as follows:

Antinociceptive activity (%) =

$$\frac{\text{AUC of wriggling (in negative–test group)}}{\text{AUC of wriggling in negative group}} \times 100\%$$

The percentage effectiveness of analgesia PLE was calculated by comparison with Dic. sod.

Anti-inflammatory test in rats

The anti-inflammatory screening test for PLE was carried out by adopting the carragenenan method. (Sandhiutami *et al.*, 2023; Sianipar & Jap, 2023). Carrageenan injected in rat paws to induce oedema is a well-established model of acute inflammation for screening agents that have potential as anti-inflammatories. Oedema was debuted by injecting 1% carrageenan in the amount of 0.2 ml into the subplantar side of the right hind paw until oedema formed in the rat paw. Prior to induction of inflammation, the paw of each rat was marked so that it could be immersed at the same height on the plethysmometer. The paw volume of rats in normal conditions was measured before induction. Then, the rat groups were given a standard drug (diclofenac sodium) and three different doses of PLE-20, PLE-40, and PLE-80. Thirty minutes after the administration of each treatment, the induction was done with carrageenan. Inflammation was quantified in mL every 1 hour for 5 hours (at 1st, 2nd, 3rd, 4th, and 5th hour) using a plethysmometer. The data obtained from each group was presented in a graph, and the AUC and the percentage of activity were calculated. A reduction in the value of AUC as compared to the negative control group was regarded as evidence for the anti-inflammatory potential of the extract, and it was expressed as follows:

Oedema inhibition (%) =

$$\frac{\text{AUC of paw oedema (negative – test group)}}{\text{AUC of paw oedema in negative group}} \times 100\%$$

The percentage effectiveness of anti-inflammatory PLE was calculated by comparison with Dic. sod.

Statistical analysis

The data were presented in the mean ± standard deviation (SD) of responses. Analysis of variance and significant differences ($p < 0.05$) among means were tested by one-way ANOVA; then, the analysis will be continued to determine whether there is a difference between each group using Tukey post hoc multiple comparison test with a significance level of 5% (0.05).

Results

Phytochemical screening

In the phytochemical screening of PLE, Phytoconstituents from PLE were polyphenols, flavonoids, saponins, alkaloids, tannin, and triterpenoids (Table I). These phytoconstituents will provide answers about the possible mechanisms of PLE in providing effects such as antinociceptives and anti-inflammatories.

Table I: Phytochemical screening of *Passiflora edulis* leaves extract

Active compounds	Test results
Alkaloids	+
Polyphenol	+++
Hydrolysed tannins	-
Condensed tannins	++
Saponins	++
Flavonoids	++
Steroids	+++
Triterpenoids	+

Antinociceptive activity of PLE on acetic acid-induced wriggling in mice

In this study, PLE-20; PLE-40; PLE-80 when compared to the negative control, showed significantly different antinociceptive effects ($p < 0.05$). The increase in effectiveness was in line with the increase in dose. Dic. sod. and all three test doses of PLE can reduce the number of wriggles when compared to the negative control group, with the maximum inhibition given by the group given dic. sod. and the maximum dose (PLE-80) ($p < 0.05$). A significant decrease in the number of wriggles ($p < 0.05$) was given by the highest dose (PLE-80) with a percent inhibition of 64.55%, the middle dose (PLE-40) with a percent inhibition of 52.11% and the lowest dose (PLE-20) with a percent inhibition of 45.70%, respectively ($p < 0.05$). PLE-80 was comparable to with 2.7 mg/200g BW dic. sod (65.41%) (Table IIa and b).

Table IIa: Effect of *Passiflora edulis* leaves extract on acetic acid-induced wriggling in mice from 5 to 30 minutes

	Change in the number of wriggling on mice (Mean ± SD)					
Treatment groups	**5 min**	**10 min**	**15 min**	**20 min**	**25 min**	**30 min**
Negative control	3.6±0.9	9.0±1.6	12.8±2.2	15.4±0.5	16.6±0.5	18.4±1.1
Dic. sod.	2.0±0.6	3.8±1.3	7.8±0.8	6.8±1.7	5.6±1.5	4.6±1.3
PLE-20	2.6±0.5	7.6±1.6	8.6±1.8	11.6±1.1	9.4±1.1	8.0±0.7
PLE-40	2.0±1.2	6.2±1.9	7.4±2.5	10.2±1.9	7.8±1.7	6.6±1.5
PLE-80	4.4±1.5	7.4±1.5	9.0±1.5	7.8±0.4	5.8±1.3	4.8±0.4

Number of wriggles are expressed as mean ± SD. n = 5. PLE: *Passiflora edulis* leaves extract. min: minute

Table IIb: Effect of *Passiflora edulis* leaves extract on acetic acid-induced wriggling in mice from 35 to 60 minutes

	Change in the number of wriggling on mice (Mean ± SD)					
Treatment groups	**35 min**	**40 min**	**45 min**	**50 min**	**55 min**	**60 min**
Negative control	15.8±0.8	13.2±1.1	10.4±1.8	7.6±1.9	5.2±0.8	3.2±0.4
Dic. sod.	3.4±1.1	2.8±1.0	2.4±0.5	1.8±0.8	1.4±0.5	1.0±0.0
PLE-20	6.4±1.1	5.0±1.0	4.4±0.5	3.4±0.5	2.8±0.4	1.8±0.4
PLE-40	5.8±1.0	5.2±1.6	4.2±1.4	3.4±1.1	2.4±1.1	2.0±0.7
PLE-80	3.6±2.0	3.0±1.7	2.6±0.8	2.2±1.3	1.8±0.8	1.4±0.5

Number of wriggles are expressed as mean ± SD. n = 5. PLE: *Passiflora edulis* leaves extract. min: minute

Assessing the effectiveness of antinociceptive drugs, can also be seen from the AUC value of wriggling in mice. Based on statistical tests, the results showed that there was a significant difference between the negative group and the positive control group, PLE-20; PLE-40; PLE-80. In this research, it can be concluded that dic. sod. and the three test doses have antinociceptive effects because the AUC of the negative control (639.0 ± 35.66) is higher than the AUC of dic. sod. (221.0 ± 29.98) and the three test doses. PLE-80 had the lowest AUC value (226.5 ± 48.9) compared to PLE-20 (347.0 ± 27.13) and PLE-40 (306.0 ± 24.05) and PLE-80 was equivalent to the AUC of dic. sod. Comparison of AUC values can be seen in Figure 1. There is a decrease value of AUC in antinociceptive test on mice (no. of wriggling.minute) after treatment with dic. sod. and PLE-20, PLE-40, PLE-80.

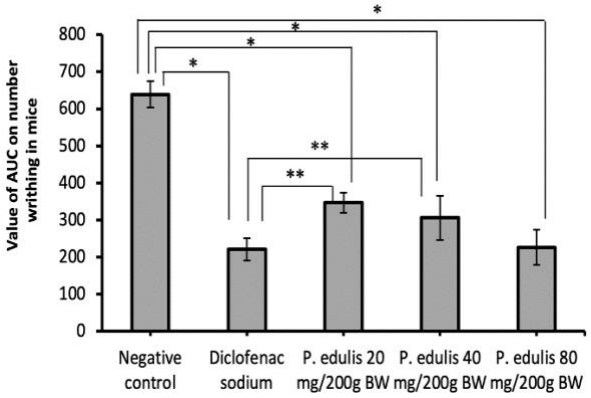

Figure 1: Comparison of AUC values (*) *p* value < 0.05 compared to negative control group; (**) *p* value < 0.05 compared to positive control group (dic. sod.); PLE: *Passiflora edulis* leaves extract

In Table III showed the percentage antinociceptive effectivenesss on acetic acid-induced wriggling in mice. The PLE-80 showed comparable % inhibition of the numbers of wriggling with the standard drug (dic. sod. 2.7 mg/200g BW) with respective percentage values of 64.55% and 65.41%.

Table III: Percentage analgesic effectivenesss on acetic acid-induced wriggling in mice

Groups	Mean area under curve of wriggling ± SD	% Inhibition	% Effectiveness analgesic compared to diclofenac sodium
Negative control	639.0 ± 35.66[b,c,d]	---	---
Dic. sod.	221.0 ± 29.98[a,c]	65.41	---
PLE-20	347.0 ± 27.13[a,b,d]	45.70	69.86
PLE-40	306.0 ± 24.05[a,b,d]	52.11	79.66
PLE-80	226.5 ± 48.90[a,c]	64.55	98.69

Values are expressed as mean ± SD. n=5. Decreased value of AUC in analgesic test on mice (no. of writhing.minute) after treatment with diclofenac sodium and three dose of extracts (a) *p* value < 0.05 compare to negative control group; (b) *p* value < 0.05 compare to positive control group (dic. sod); (c) *p* value < 0.05 compared to PLE-20 (d) *p* value < 0.05 compare to PLE-40; (e) *p* value < 0.05 compare to PLE-80. PLE: *Passiflora edulis* leaves extract

Anti-Inflamatory activity of PLE on carrageenan-induced paw oedema model in rats

In this study, an acute inflammation model was successfully established. Injection of 1% carrageenan as sub plantar 0.2 ml in the hind paw of rats has resulted in a progressive increase in the thickness of the rat paw. Oedema in the negative control reached its maximum value 4 hours after induction (Table IV). The administration of dic. sod., PLE-20; PLE-40; PLE-80) significantly inhibited paw thickness compared to the negative control starting from 1 hour ($p < 0.05$) and the effect persisted until the fifth hour of observation ($p < 0.05$) after carrageenan induction.

Table IV: Effect of *Passiflora edulis* leaves extract on carrageenan-induced paw oedema model in rats

	Change in oedema (mL) (Mean ± SD)					
Treatment groups	**0 hr**	**1 hr**	**2 hr**	**3 hr**	**4 hr**	**5 hr**
Negative control	1.28±0.02	1.96±0.02	2.24±0.04	2.40±0.01	2.55±0.07	2.35±0.12
Dic. sod.	1.19±0.07	1.31±0.08	1.40±0.07	1.33±0.08	1.28±0.08	1.24±0.09
PLE-20	1.23±0.04	1.53±0.07	1.70±0.07	1.64±0.06	1.57±0.06	1.48±0.05
PLE-40	1.28±0.06	1.50±0.09	1.68±0.10	1.61±0.10	1.50±0.05	1.43±0.05
PLE-80	1.25±0.02	1.48±0.07	1.57±0.05	1.49±0.05	1.41±0.04	1.35±0.03

Volume of oedema are expressed as mean ± SD. n=5. PLE: *Passiflora edulis* leaves extract

Inhibition of oedema by dic. sod. and PLE-20, PLE-40, PLE-80 was observed throughout the study time with percentage values of 40.51%, 28.83%, 30.29% and 34.33% respectively. The percentage inhibition increased as the dose of PLE increased. In this study, administration of the highest dose of PLE i.e. PLE-80 maximum dose (PLE-80) resulted in higher inhibition of paw oedema compared to PLE-20 and PLE-40 ($p < 0.05$), however, the oedema inhibitory effect of PLE-80 was not comparable to the positive control (dic sod 2.7 mg/200 g BW) (Table V).

Table V: Percentage anti-inflammatory effectivenesss of PLE: *Passiflora edulis* leaves extract on carrageenan-induced paw oedema model in rats

Groups	Mean± SD Area Under Curve (AUC) of inflammation	% Inhibition	% Effectiveness anti-inflammatory compared with diclofenac sodium
Negative control	$10.96 \pm 0.14^{b,c,d,e}$	---	---
Dic. sod.	$6.52 \pm 0.36^{a,c,d,e}$	40.51	---
PLE-20	$7.80 \pm 0.27^{a,b,d}$	28.83	71.17
PLE-40	$7.64 \pm 0.35^{a,b,d}$	30.29	74.77
PLE-80	$7.23 \pm 0.18^{a,b,c,d}$	34.33	84.74

Values are expressed as mean ± SD. n=5. Decreased value of AUC in anti-inflammatory test on rats (volume of oedema.hour) after treatment with diclofenac sodium and three dose of extracts (a) *p* value < 0.05 compare to negative control group; (b) *p* value < 0.05 compare to positive control group (dic. sod.); (c) *p* value < 0.05 compared to PLE-20 (d) *p* value < 0.05 compared to PLE-40; (e) *p* value < 0.05 compared to PLE-80. PLE: *Passiflora edulis* leaves extract

Assessment of the effectiveness in reducing oedema and evaluating the anti-inflammatory effect of PLE, can also be seen from the calculation of AUC on the graph obtained in the test conducted for 5 hours. The larger AUC value will indicate the smaller the effectiveness of an anti-inflammatory drug. In this study, the average AUC value of the negative control (10.98 ± 0.14) was higher than the Dic. sod (6.52 ± 0.36), PLE-20 (7.80 ± 0.27), PLE-40 (7.64 ± 0.35) and PLE-80 (7.23 ± 0.18). This indicates that carrageenan is able to induce oedema formation in rat feet. The AUC values of Dic. sod, PLE-20, PLE-40, and PLE-80 groups were lower than the AUC value of the negative control group. This indicates that all groups of Dic. sod, PLE-20, PLE-40 and PLE-80 have anti-inflammatory effects. Of the three doses of PLE, it was found that PLE-80 was better at inhibiting oedema formation in rat paws, as indicated by the lowest mean AUC value (Figure 2). There is a decreased value of AUC in the anti-inflammatory test on rats (volume of oedema) after treatment with dic. sod. and PLE-20, PLE-40, PLE-80.

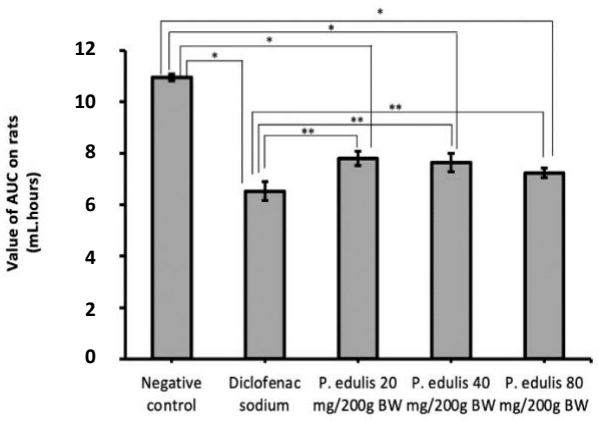

Figure 2: Comparison of AUC values. (*) *p* value < 0.05 compared to the negative control group; (**) *p* value < 0.05 compared to the positive control group (dic. sod.); PLE: *Passiflora* edulis leaves extract

Discussion

The development of new drugs to manage pain and inflammatory symptoms is very important to continue to be developed. Preclinical research can scientifically prove the efficacy of natural ingredients that have been used empirically and the active compounds that can have an effect. Some herbal and phytotherapeutic plants have demonstrated their effectiveness in treating pain and inflammation. In terms of science, using medicinal plants is crucial for creating new goods as well as making a significant impact on the fundamental healthcare system (Junior *et al.*, 2020). *Passiflora edulis* is among the widely used traditional medicinal plants for treating pain, and inflammation. Therefore, to support its claimed traditional use, it may be worthwhile to do scientific research on the antinociceptive and anti-inflammatory properties of *P. edulis* leaf extract in vivo.

In this study, PLE showed peripheral pain relief activity by reducing the number of writhes in comparison to the negative control. These findings confirmed that the peripheral antinociceptive activity of the PLE. This was obtained due to an increase in the concentration of phytoconstituents possessing antinociceptive activity at increasing doses of PLE. Two mechanisms that suppress pain are inhibition of the production and release of various endogenous pro-inflammatory mediators and suppression of the sensitivity of peripheral nociceptors to pain (Chy *et al.*, 2021). The proposed mechanism for how the PLE's antinociceptive effects work by activating the released endogenous peptides (i.e. endorphin or enkephalin). Endorphin or enkephalin acts as an inhibitor of pain impulse transmission and, through the inhibition of pro-inflammatory mediators such as prostaglandins, leukotrienes, and other endogenous substances, plays a role in central pain transmission (Sangeetha *et al.*, 2021). In this study, the phytochemical screening showed that P. *edulis* leaves contain polyphenols, flavonoids, alkaloids, tannins, saponins, and

triterpenoids. Therefore, it can be stated that these phytochemical contents provide the antinociceptive effect of PLE. This mechanism is consistent with reports that claim certain phytoconstituents, such as flavonoids, alkaloids, tannins, and steroids extracted from herbs, have been shown to have strong antinociceptive properties (Chhetri *et al.*, 2020).

PLE-20, PLE-40, and PLE-80 significantly ($p < 0.05$) reduced oedema formation starting from the first phase of inflammation i.e. 1 hour after carrageenan induction and the effect lasted until the 5th hour of observation (second phase of inflammation). This finding suggests that the phytoconstituents in PLE can inhibit the release of pro-inflammatory mediators, thereby suppressing both phases of acute inflammation. Thus, the proposed mechanism is that PLE has anti-inflammatory effects on various endogenous inflammatory mediators involved in the early and late phases of inflammation, such as prostaglandins, bradykinin, cyclooxygenase, and leukotrienes and may have antioxidant activity (Kaur & Singh, 2022).

Previous reports from scientific journals claim plants that mainly contain phenolic elements, flavonoids, saponins, alkaloids, and triterpenoids show anti-inflammatory effects that potentially have anti-inflammatory action. Therefore, the anti-inflammatory effect of PLE in this study is due to the presence of these phytoconstituents. Findings in previous studies also showed that antinociceptive and anti-inflammatory effects depend on the amount of phytoconstituents of the plant, so the higher the dose, the stronger the activity and this is consistent with the current study. Alkaloids work by suppressing prostaglandin production through inhibition of the cyclooxygenase enzyme thereby reducing the formation of proinflammatory cytokines such as Interleukin-1, Interleukin-6, and Tumor Necrosis Factor (Harikrishnan *et al.*, 2018), terpenoids are thought to inhibit cyclooxygenase-2 activity, PLA2, Tumor Necrosis Factor, iNOS expression, and NF-κB activation, while saponins are thought to inhibit prostaglandin production through inhibition of Cox-2 and iNOS expression thereby obtaining anti-inflammatory effects (Wang *et al.*, 2022). Polyphenols work to reduce inflammation by inhibiting the formation of pro-inflammatory chemokines and cytokines, suppressing cyclooxygenase and iNOS activity, and reducing the amount of free radicals (Tasneem *et al.*, 2019).

In general, it can be concluded that the anti-inflammatory activity of PLE may be due to the cumulative effect of the active phytoconstituents in reducing the synthesis, release and action of various pro-inflammatory mediators that play a role in the development of inflammation. The authors realise that this study also has some limitations, namely, the mechanism of action of PLE in inhibiting pain and oedema has not yet been tested by measuring inflammatory mediators such as prostaglandins, IL-6, TNF alpha, nitric oxide, etc.

Conclusion

In conclusion, this is the first novel study to clearly prove the antinociceptive and anti-inflammatory properties of *P. edulis* leaf extract. This study suggests to determine the exact mechanism of action of the extract and its active compounds, more thorough follow-up studies are required, including measuring the expression of pro-inflammatory mediators.

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