International Journal of Agricultural Technology 2024 Vol. 20(5):2035-2054
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

In vivo analgesic, antipyretic and anti-inflammatory potential of ethanol extract from *Plukenetia volubilis* Linneo leaves in mice

Nhung, T. T. P. and Quoc, L. P. T.*

Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Ho Chi Minh City, Vietnam.

Nhung, T. T. P. and Quoc, L. P. T. (2024). *In vivo* analgesic, antipyretic and anti-inflammatory potential of ethanol extract from *Plukenetia volubilis* Linneo leaves in Swiss albino mice. International Journal of Agricultural Technology 20(5):2035-2054.

Abstract *Plukenetia volubilis* L. is traditionally utilized for managing joint pain, muscle discomfort, wound healing, and infection prevention. Despite this extensive use, the specific analgesic, anti-inflammatory, and antipyretic activities of the ethanol extract from *P. volubilis* leaves (PVEE) remain underexplored, warranting further investigation. The study results revealed that PVEE possesses notable analgesic effects, as evidenced by a significant increase in tail-flick latency ($p < 0.05$), inhibition of pain responses ($p < 0.05$), a reduction in writhing frequency ($p < 0.05$), and a decrease in paw swelling ($p < 0.05$) in inflammatory models. Additionally, PVEE demonstrated antipyretic properties by significantly reducing rectal temperature ($p < 0.05$) in a yeast-induced fever model. Furthermore, the extract exhibited strong anti-inflammatory effects, as it significantly reduced paw edema ($p < 0.05$) and inhibited the release of pro-inflammatory cytokines, such as IL-6, IL-1β, and TNF-α ($p < 0.05$). These results highlight the potential of PVEE as a promising therapeutic option for pain relief, fever reduction, and inflammation management.

Keywords: Analgesic, Antipyretic, Anti-inflammatory, Biological effects, Pharmacological potential

Introduction

Throughout history, human societies have accumulated extensive knowledge of medicinal plants and their applications for health improvement. The therapeutic benefits of these plants are largely due to their production of secondary metabolites. These compounds serve as natural antibiotics, providing plants with a defense mechanism against a range of threats, including fungi, bacteria, herbivores, and competing vegetation. Secondary metabolites, such as polyphenols, alkaloids, and flavonoids, are known for their antioxidant and antimicrobial activities, which help inhibit the growth and spread of harmful microorganisms. These properties play a crucial role in enhancing the survival and adaptability of plants in their natural habitats. As a result, medicinal plants

*Corresponding Author: Quoc, L. P. T.; Email: lephamtanquoc@iuh.edu.vn

have been extensively used in both traditional and modern medical practices, particularly for managing conditions related to inflammation and pain (Ammam *et al*., 2023).

Inflammation is a generalized immune reaction to damaging agents, for example chemicals, pathogens, or injury, and plays a crucial role in many pathological conditions. In the acute phase, it serves as a defense mechanism by releasing mediators like TNF-α, IL-1β, IL-6, and nitric oxide at the site of damage to limit damage. However, excessive mediator release can worsen inflammation, causing tissue damage, organ failure, and death (Nhung and Quoc, 2023a). These mediators also activate pain receptors, leading to pain, insomnia, and increased sensitivity at the injury site (Nhung and Quoc, 2023b). Inflammation can trigger the arachidonic acid pathway, producing prostaglandin E2 (PGE2), which binds to EP3 receptors in the hypothalamus, causing fever (Nhung and Quoc, 2024a). Synthetic drugs like corticosteroids and NSAIDs are commonly used to manage inflammation, pain, and fever. NSAIDs inhibit cyclooxygenase (COX), an enzyme involved in prostaglandin synthesis (Aslam *et al*., 2023). However, long-term use of NSAIDs can lead to liver and kidney damage, gastric ulcers, and immune suppression. Their toxicity and recurrence of symptoms upon cessation highlight the need for safer alternatives (Mustafa *et al*., 2023). Natural compounds, particularly from medicinal plants, have attracted attention for their anti-inflammatory, analgesic, and antipyretic properties, thanks to their secondary metabolites (Radha *et al*., 2020).

Plukenetia volubilis L., also known as Sacha inchi, belongs to the Euphorbiaceae family, which includes approximately 300 genera and 7,500 species. Indigenous to the rainforests of Peru and northwestern Brazil, *P. volubilis* is well-adapted to the tropical forests of the Americas, particularly thriving at elevations between 200 and 1500 meters. Its remarkable nutritional value has led to its commercial cultivation in several Asian countries and Central and South America regions. Traditionally, *P. volubilis* has been used to treat joint issues and muscle pain, and it provides skincare benefits including moisturizing, wound healing, insect bite treatment, and infection prevention (Rahman *et al*., 2023). Various parts of *P. volubilis* contain bioactive substances that enhance its therapeutic and dietary advantages. The seeds, in particular, are highly valued for their rich content of essential fatty acids, especially omega-3, omega-6, and omega-9 fatty acids. The oil extracted from *P. volubilis* seeds is especially noted for its high concentration of unsaturated fatty acids, making it a nutritionally important resource. Additionally, the presence of tocopherols, which are antioxidants that protect the oil from oxidation, makes *P. volubilis* seed oil a valuable addition to a healthy diet

(Goyal *et al*., 2022). The leaves of *Plukenetia volubilis* are rich in bioactive compounds such as phenolics, flavonoids, and terpenoids, which are responsible for their antioxidant and anti-inflammatory effects. Additionally, leaf extracts from *Plukenetia volubilis* have shown therapeutic potential in managing low-grade joint inflammation induced by Complete Freund's Adjuvant (CFA) in mice (Tran *et al*., 2023). The extracts are non-toxic and exhibit detoxifying properties (Tran and Tran, 2021). *P. volubilis* provides a variety of nutrients and active compounds that may confer multiple health benefits, such as reducing risk factors for cardiovascular disease and alleviating joint inflammation (Rahman *et al*., 2023). Despite its promising pharmacological effects, the specific mechanisms and efficacy of *P. volubilis* in pain relief, anti-inflammatory, and antipyretic activities remain incompletely understood. This research aimed to investigate the analgesic, antipyretic, and anti-inflammatory properties of ethanol leaf extract from *P. volubilis* in a mouse model, and to evaluate its capability as an adjunctive treatment for inflammation, pain, and fever.

Materials and methods

Chemicals and reagents

The carrageenan used for experimental purposes was procured from Sigma-Aldrich (USA). Acetic acid, formaldehyde, tramadol, and yeast were obtained from Toan Thang Co., Ltd. (Vietnam). Paracetamol, meloxicam, and aspirin were sourced from a pharmacy in Saigon, Vietnam. All reagents and chemicals used in the experiments were of analytical grade and exhibited high purity.

Collection of material and preparation of the extract

Plukenetia volubilis leaves were harvested in October 2023 from the farm of Eastern Agriculture Company in Daklak province, Vietnam. The collected leaves underwent a stringent selection process to eliminate any damaged or pest-infested material. The selected leaves were thoroughly washed and sun-dried for three days, followed by additional drying in a UN55 dryer (Memmert, Germany) until the weight remained constant. The dried leaves were pulverized using a DP1215 herbal grinder (Đuc Phat, Vietnam). The resulting leaf powder was stored in bags at room temperature for use in the next experiments.

Five hundred grams of powdered material were subjected to cold maceration in 2.5 liters of ethanol for 72 hours, with intermittent agitation to

enhance extraction efficiency. Post-maceration, the solution was decanted to separate the solid residues, followed by filtration to purify the extract. The filtrate was subsequently concentrated using a rotary evaporator (RV 10 Digital V-C, IKA, Germany) at 40 °C until a viscous extract was achieved. This concentrated extract, designated as PVEE, was kept in moisture-proof containers at 4 °C, for future use in analysis.

Phytochemical screening and quantitative analysis in the extract

Phytochemical analysis was conducted by screening the phytochemicals in the extract of leaves (Joshi *et al*., 2013; Nhung and Quoc, 2024b).

*Quantification of total polyphenol, terpenoid, and flavonoid content***:** These values was quantified according to the procedure outlined by Nhung and Quoc (2024b).

Animal housing and conditions

Swiss albino mice had averaged weight of 30 ± 2 grams, and they were individually housed in glass cages at 25 ± 2 °C and a relative humidity of 55-60%. A 12-hour light/12-hour dark cycle was implemented throughout both the acclimation and experimental phases. The mice were provided with ad libitum access to standard laboratory chow and water throughout the study. Their diet was not restricted in any way during the experiment. All animal handling and experimental procedures strictly adhered to ethical guidelines for animal research (Alison, 2010).

Experimental protocol

In this study, the effects of different doses of ethanol extract from *P. volubilis* leaves (PVEE) administered orally (100, 200, and 300 mg/kg) were compared with reference drugs: aspirin (ASA, 10 mg/kg, op), tramadol (TRM, 5 mg/kg, ip), indomethacin (IND, 20 mg/kg, op), and paracetamol (PCM, 20 mg/kg, op). These treatments were evaluated against control groups receiving saline (5 mg/kg, op) in various models of pain, inflammation, and fever. Specifically, the analgesic effects were assessed using pain perception tests, the anti-inflammatory effects were evaluated using carrageenan-induced paw edema tests, and the antipyretic effects were measured using yeast-induced pyrexia tests. All treatments were examined 30 minutes post-administration (Román-Vargas *et al*., 2023).

Evaluation of the analgesic activity of the extract

Design of analgesic testing: Healthy Swiss albino mice (30 ± 2 g) of the same age were utilized for the study and were divided into five groups, each comprising five mice. The negative control group (saline group) was administered saline (5 mg/kg, op). The positive control groups were administered either aspirin (ASA, 10 mg/kg, op) for the peripheral analgesic model or tramadol (TRM, 5 mg/kg, ip) for the central analgesic model. The experimental groups (PVEE100, PVEE200, and PVEE300 groups) were treated with PVEE at doses of 100-300 mg/kg orally.

Tail-flick test: The central analgesic activity of PVEE was assessed using the tail-flick test, as described by Román-Vargas *et al.* (2023) with minor modifications. This method involved five groups (n = 5): a saline group (5 mg/kg, orally), three PVEE groups at different doses (100, 200, and 300 mg/kg, orally), and a tramadol group (TRM, 5 mg/kg, intraperitoneally). Radiant heat was applied to the base of the tail using a tail-flick apparatus (Ugo-Basile, Italy), and the latency to remove the tail from the stimulus was recorded. The intensity of the thermal stimulus was set to induce a tail-flick response with a maximum duration of twenty seconds to avoid harming the tissue. Tail withdrawal latency was measured at 0, 30, 60, 90, and 120 minutes after administration of the extract and the standard drug. The results were expressed as a percentage of the maximum possible effect (MPE):

$$\text{MPE (\%)} = \frac{\text{Test latency} - \text{Control latency}}{\text{Test latency}} \times 100$$

Haffner tail clip test: Metal artery clips were applied to the base of the mice's tails to induce pain. The Haffner tail-clip test was conducted according to the protocol described by Nhung and Quoc (2023b), with minor modifications. Before the test, sensitivity assessments were performed to choose appropriate mice; those that did not react and remove the clip from the tail in ten seconds were excluded from the study. A total of 25 mice were randomly assigned to various treatment groups. Tail clips were applied thirty minutes following the delivery of PVEE (100, 200, and 300 mg/kg, orally), tramadol (TRM, 5 mg/kg, intraperitoneally), or saline (5 mg/kg, orally). Reaction times were monitored and recorded at intervals of 0, 5, 10, and 15 minutes post-application. An analgesic effect was deemed present if no escape attempt was observed within 10 seconds. The percentage of pain inhibition from the tail clip (PIC) was determined using the following formula:

$$\text{PIC (\%)} = \frac{\text{Latency (test)} - \text{Latency (control)}}{\text{Cut} - \text{off time (10s)} - \text{Latency (test)}} \times 100$$

Acetic acid-induced writhing test: The writhing model is a chemical nociception test that simulates peritonitis in animals by injecting acetic acid into the peritoneal cavity (Nhung and Quoc, 2024a). After administering PVEE (100, 200, and 300 mg/kg, op), saline (5 mg/kg, op), or aspirin (ASA, 10 mg/kg, op) for 30 minutes, mice were injected intraperitoneally with 0.1 mL of 1% acetic acid solution. The mice were then individually placed in glass cages and allowed to acclimate for five minutes. Subsequently, each mouse was observed for ten minutes, and the writhing frequency was documented. Writhes were defined as abdominal tightening accompanied by the extension of at least one hind limb. The percentage inhibition of writhing (PIW) was calculated using the following formula:

$$\text{PIW (\%)} = \frac{\text{No. of writhes in control group} - \text{No. of writhes in test group}}{\text{No. of writhes in control group}} \times 100$$

Evaluation of the antipyretic activity of the extract

Study design for antipyretic testing: Thirty mice were assigned to six distinct treatment groups, with five mice per group. The normal control group was given an oral saline solution (5 mg/kg) without fever induction. The negative control group (Yeast group) was induced with fever through an intraperitoneal injection of 20% yeast (10 mL/kg) and received an oral saline solution (5 mg/kg). The positive control group (Yeast+PCM group) was similarly induced with fever by a twenty percent yeast injection (10 mL/kg) and treated with the antipyretic agent paracetamol (150 mg/kg). The test groups (Yeast+PVEE100, Yeast+PVEE200, and Yeast+PVEE300) were subjected to fever induction using the 20% yeast injection (10 mL/kg) and subsequently treated with PVEE at oral doses of 100-300 mg/kg, respectively.

Evaluation of fever-reducing effects: The fever-lowering effect was assessed using a mouse model of fever induced by yeast, according to the methodology described by Nhung and Quoc (2024a). Before the experiment, the mice were fasted overnight but had free access to water. Rectal temperatures were measured with a TESTO 106 digital thermometer (Germany). At an interval of 18 hours after subdermal yeast administration, mice that exhibited a rise with a rectal temperature increase of approximately 0.3-0.5 °C were chosen for fever-reducing evaluation. Rectal temperatures were recorded at 1-, 2-, and 3-hours post-yeast injection. The proportion of fever reduction (PFR) was determined using the formula below:

$$\text{PER (\%)} = \frac{\text{Post} - \text{fever temperature} - \text{Temperature after 1, 2, and 3 hours}}{\text{Post} - \text{fever temperature} - \text{Normal body temperature}} \times 100$$

Assessment of anti-inflammatory activity

Anti-inflammatory test design: 30 healthy mice (30 ± 2 g) of similar age were assigned to six groups, with each group comprising five mice. The normal control group (Control group) received physiological saline (5 mg/kg, orally). The negative control group (CAR group) was injected with 50 µL of carrageenan (CAR) and received saline (5 mg/kg, orally). The positive control group (CAR+IND group) was injected with 50 µL of CAR and received indomethacin (IND, 20 mg/kg, orally). The experimental groups (CAR+PVEE100, CAR+PVEE200, and CAR+PVEE300) were also injected with 50 µL of CAR and treated with PVEE at doses of 100- 300 mg/kg orally, respectively.

Evaluation of anti-inflammatory activity: The anti-inflammatory effect of PVEE was investigated using the carrageenan (CAR)-induced paw edema model in mice, as detailed by Nhung and Quoc (2023a) with slight modifications. Mice were administered PVEE (100, 200, and 300 mg/kg), saline (5 mg/kg), or indomethacin (IND, 20 mg/kg), and 30 minutes later, 50 µL of a 1% CAR solution in physiological saline was administered into the subplantar area of the right hind paw. Paw edema, representing the increase in paw diameter, was measured in millimeters using a Mitutoyo digital caliper (Japan) at 1, 2, 3, 4, and 5 hours after CAR injection. The percentage of edema inhibition (PEI) was calculated using the following formula:

$$\text{PEI (\%)} = \frac{\text{Swollen leg diameter} - \text{Leg diameter 1,2, 3, 4, and 5 hours}}{\text{Swollen leg diameter} - \text{Normal leg diameter}} \times 100$$

Quantification of TNF- α, IL-1β, and IL-6: The levels of IL-6, TNF-α, and IL-1β were quantified using enzyme-linked immunosorbent assay (ELISA) combined with an immunoadsorption technique, following the protocol described by Nhung and Quoc (2023a). In this test, capture antibodies targeting IL-1β were fixed onto the wells of a 96-well plate and left to bind overnight. Following incubation with tissue samples or standard antigens, a biotin-conjugated detection antibody was added. The reaction was then developed with streptavidin, resulting in a color shift from purple to yellow. The absorbance of the solution was measured at 450 nm and the concentrations of TNF-α, IL-6, and IL-1β in each sample were reported as mg/g of protein.

Statistical analysis

Data were analyzed using Statgraphics Centurion XVI software (USA). Results are expressed as mean ± standard deviation (SD). Statistical significance between control and treatment groups was assessed using one-way ANOVA followed by Tukey's post hoc test, with a significance level set at $p < 0.05$.

Results

Phytochemical assessment and quantitative evaluation of the extract

Phytochemical analysis of the ethanol extract of *P. volubilis* leaves (PVEE) revealed the presence of tannins, flavonoids, terpenoids, saponins, steroids, alkaloids, and polyphenols, with no detectable cardiac glycosides (Table 1). The analgesic and antipyretic effects of plant extracts are largely attributed to the phytochemicals present within them. Quantitative analysis of PVEE showed flavonoid content at 43.48 ± 1.31 mg QE/g, terpenoid content at 69.93 ± 1.75 mg TAE/g, and polyphenol content at 68.54 ± 1.76 mg GAE/g (Table 1).

Table 1. Qualitative analysis of phytochemicals and quantitative determination of flavonoids, polyphenols, and terpenoids in the extract of *P. volubilis* leaves

Phytoconstituents	Present in PVEE	Quantification of phytochemicals
Tannins	+	NT
Flavonoids	+	43.48 ± 1.31 (mg QE/g)
Terpenoids	+	69.93 ± 1.75 (mg TAE/g)
Saponins	+	NT
Steroids	+	NT
Cardiac glycosides	-	NT
Alkaloids	+	NT
Polyphenol	+	68.54 ± 1.76 (mg GAE/g)

Presence of phytochemicals in PVEE: (+) present and (-) absent; NT: not tested.

Evaluation of the analgesic activity of the extract

Tail-flick test

Baseline latency was comparable across all groups in the tail-flick analgesia model in mice (Table 2). The analgesic effects of PVEE at doses of

100, 200, and 300 mg/kg were significant from 30 to 120 minutes, with statistically significant differences compared to the saline group ($p < 0.05$). The effect peaked at 90 minutes, showing the highest efficacy among the time points ($p < 0.05$) and maintaining statistical significance when compared to the tramadol group ($p < 0.05$). At 60, 90, and 120 minutes, the maximum possible effect (MPE) (% maximum analgesia) of the PVEE100-300 groups was statistically significant compared to both the saline group ($p < 0.05$) and the tramadol (TRM) group ($p < 0.05$) (Figure 1).

Table 2. The analgesic effects of ethanol extract of *P. volubilis* leaves in the tail-flick test

Time (min)	Saline group	TRM group	PVEE100 group	PVEE200 group	PVEE300 group
0	$21,92 \pm 0,17^{a}$	$22,05 \pm 0,14^{a}$	$21,98 \pm 0,16^{a}$	$21,89 \pm 0,14^{a}$	$22,03 \pm 0,13^{a}$
30	$14,61 \pm 0,16^{a}$	$33,08 \pm 0,19^{e}$	$17,58 \pm 0,12^{b}$	$22,98 \pm 0,15^{c}$	$30,84 \pm 0,21^{d}$
60	$12,18 \pm 0,13^{a}$	$39,69 \pm 0,24^{e}$	$24,18 \pm 0,18^{b}$	$28,46 \pm 0,18^{c}$	$37,45 \pm 0,26^{d}$
90	$7,31 \pm 0,09^{a}$	$66,15 \pm 0,39^{e}$	$48,36 \pm 0,26^{b}$	$54,73 \pm 0,25^{c}$	$63,89 \pm 0,35^{d}$
120	$6,85 \pm 0,06^{a}$	$55,13 \pm 0,28^{e}$	$37,37 \pm 0,24^{b}$	$43,78 \pm 0,20^{c}$	$52,87 \pm 0,28^{d}$

Different letters (a-e) denote statistically significant differences between groups ($p < 0.05$).

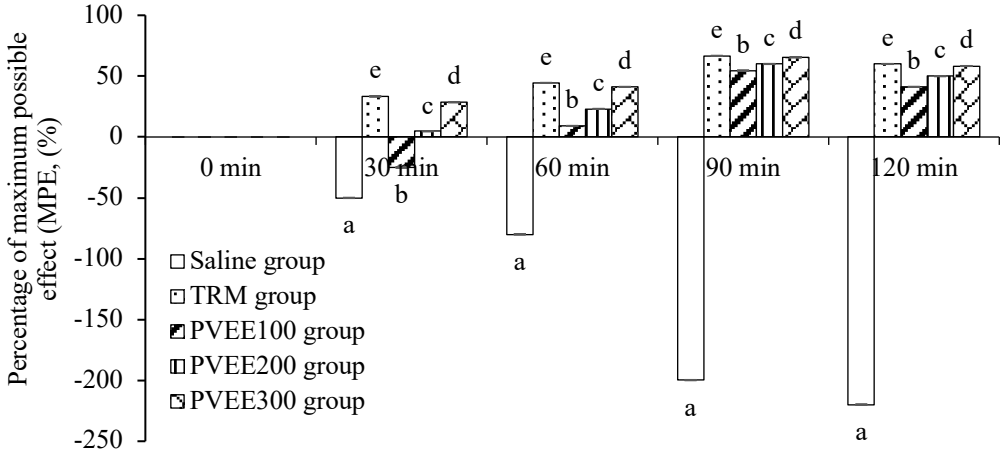

Figure 1. Maximum analgesic efficacy of ethanol extract of *P. volubilis* leaves in the tail-flick test, Distinct letters (a-e) signify statistically significant differences among groups ($p < 0.05$)

Haffner tail clip test

The analgesic properties of PVEE are illustrated in Table 3 and Figure 2. Haffner's tail-clip test demonstrated dose- and time-dependent antinociceptive effects, indicated by increased tail-flick latency and percentage inhibition of pain (PIC) in mice. The analgesic effects of PVEE at doses of 100-300 mg/kg,

as well as TRM (5 mg/kg), were significant from 5 to 15 minutes, with statistically significant differences compared to the saline group ($p < 0.05$). Notably, administration of PVEE at doses of 100-300 mg/kg resulted in mean tail-flick latencies of 3.41, 4.37, and 5.59 seconds, respectively, corresponding to average PIC values of 45.54%, 56.08%, and 66.43% at the 10-minute survey time, which were significantly higher compared to the 5 and 15-minute survey time ($p < 0.05$).

Table 3. The analgesic effects of extract of *P. volubilis* leaves in the Haffner tail clip test

Time (min)	Saline group	TRM group	PVEE100 group	PVEE200 group	PVEE300 group
0	1.91 ± 0.05^{a}	1.93 ± 0.04^{a}	1.86 ± 0.03^{a}	1.92 ± 0.04^{a}	1.88 ± 0.04^{a}
5	2.22 ± 0.04^{a}	5.81 ± 0.13^{e}	3.25 ± 0.07^{b}	4.18 ± 0.08^{c}	5.34 ± 0.15^{d}
10	2.28 ± 0.06^{a}	6.08 ± 0.11^{e}	3.41 ± 0.08^{b}	4.37 ± 0.12^{c}	5.59 ± 0.13^{d}
15	2.25 ± 0.05^{a}	5.63 ± 0.14^{e}	3.15 ± 0.06^{b}	4.05 ± 0.09^{c}	5.18 ± 0.12^{d}

Different letters (a-e) denote statistically significant differences between groups ($p < 0.05$)

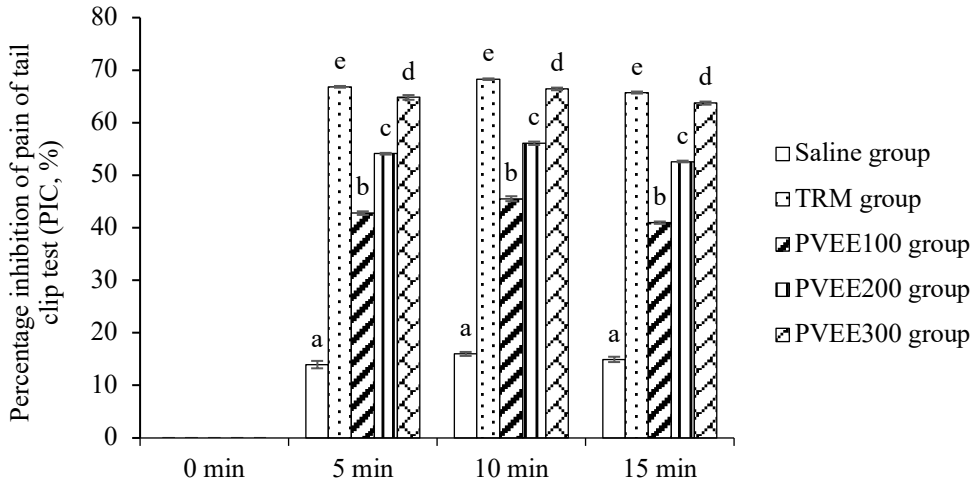

Figure 2. Percentage inhibition of pain of ethanol extract of *P. volubilis* leaves of tail clip test, Distinct letters (a-e) signify statistically significant differences among groups ($p < 0.05$)

Acetic acid-induced writhing test

The data on the effects of PVEE on acetic acid-induced writhing in mice are presented in Table 4. PVEE at doses of 100-300 mg/kg significantly reduced the number of times writhes compared to the saline group ($p < 0.01$). Notably, PVEE at 300 mg/kg exhibited a significantly greater reduction in

writhing compared to the 100 and 200 mg/kg doses ($p < 0.05$). The reference drug ASA (10 mg/kg) also significantly inhibited the number of writhes ($p < 0.05$) compared to the saline group. The percent pain inhibition effect highest was observed in the ASA and PVEE300 groups (58.97% and 56.18%, respectively) ($p < 0.05$), followed by the PVEE200 group (46.81%) ($p < 0.05$), and the lowest in the PVEE100 group (30.14%) ($p < 0.05$) (Table 4).

Table 4. The analgesic effects of extract of *P. volubilis* leaves in the acetic acid-induced writhing pain

Parameters	Saline group	ASA group	PVEE100 group	PVEE200 group	PVEE300 group
No. of times writhing (times)	27.80 ± 1.92^{d}	11.40 ± 1.14^{a}	19.40 ± 1.14^{c}	14.80 ± 1.30^{b}	12.20 ± 1.30^{a}
% pain inhibition of PVEE (%)	0.00 ± 0.00^{a}	58.97 ± 3.28^{d}	30.14 ± 2.53^{b}	46.81 ± 1.35^{c}	56.18 ± 2.21^{d}

Different letters (a-e) denote statistically significant differences between groups ($p < 0.05$)

Evaluation of the antipyretic activity of the extract

The fever-reducing effectiveness of PVEE was assessed by observing the progressive decrease in rectal temperature across all treated mice (Table 5 and Figure 3). Rectal temperature in the yeast-induced fever group increased significantly compared to the control group ($p < 0.05$). The standard drug (paracetamol, PCM) and PVEE at doses of 100-300 mg/kg demonstrated a significant reduction in temperature from the first to the third hour ($p < 0.05$) compared to the yeast group. The percentage fever reduction (PER) also increased significantly in the PCM (85.69%) and PVEE groups (66.65%, 71.59%, and 76.96%, respectively) ($p < 0.05$).

Assessment of anti-inflammatory activity

Evaluation of the anti-inflammatory effects of PVEE

The baseline mean paw diameters were comparable across all groups in the carrageenan (CAR)-induced paw edema model in mice (Table 6). Two hours post-CAR injection, the paw diameter of mice in the CAR group increased significantly compared to the control group ($p < 0.05$). However, PVEE at various doses (100-300 mg/kg) exhibited anti-inflammatory effects against CAR-induced inflammation. At 1- and 2-hours post-injection, all PVEE-treated groups showed a reduction in paw diameter compared to the CAR group ($p < 0.05$). From 3 to 5 hours post-injection, a statistically

significant reduction in paw diameter was observed in all treated groups compared to the CAR group ($p < 0.05$). The percentage inhibition of edema (PEI) over the 1 to 5-hour period was calculated (Figure 4). After five hours, the PEI for the IND group (87.42%) and the PVEE300 group (84.50%) was significantly higher than that of the other groups ($p < 0.05$). At a dose of 300 mg/kg, the PEI of PVEE was nearly equivalent to that of IND ($p > 0.05$).

Table 5. The antipyretic effects of ethanol extract of *P. volubilis* leaves in yeast-induced fever

Experimental group	Initial (ºC)	Fever (ºC)	1 h (ºC)	2 h (ºC)	3 h (ºC)
Control group	36.39 ± 0.03[a]	36.43 ± 0.04[a]	36.51 ± 0.02[a]	36.48 ± 0.03[a]	36.47 ± 0.02[a]
Yeast group	36.42 ± 0.04[a]	40.79 ± 0.05[b]	41.88 ± 0.06[f]	42.25 ± 0.04[f]	42.61 ± 0.05[f]
Yeast+PCM group	36.51 ± 0.03[a]	40.89 ± 0.04[c]	39.43 ± 0.02[b]	38.29 ± 0.03[b]	37.14 ± 0.02[b]
Yeast+PVEE100 group	36.49 ± 0.05[a]	41.96 ± 0.02[f]	40.51 ± 0.03[c]	39.41 ± 0.03[c]	38.31 ± 0.04[c]
Yeast+PVEE200 group	36.44 ± 0.03[a]	41.96 ± 0.04[e]	40.08 ± 0.02[d]	38.99 ± 0.04[d]	37.89 ± 0.03[d]
Yeast+PVEE300 group	36.46 ± 0.04[a]	41.54 ± 0.03[d]	39.74 ± 0.05[e]	38.65 ± 0.03[e]	37.55 ± 0.04[e]

Different letters (a-e) denote statistically significant differences between groups ($p < 0.05$)

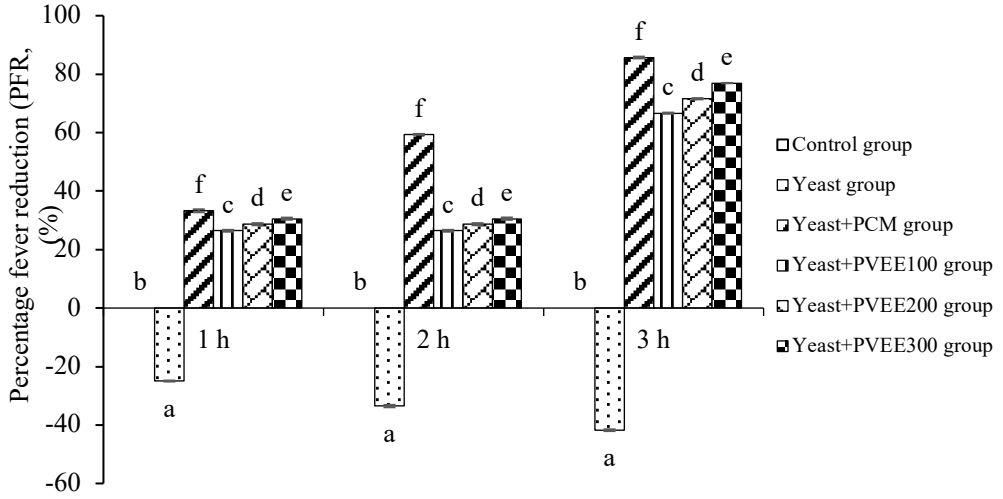

Figure 3. Percentage fever reduction of ethanol extract of *P. volubilis* leaves in the yeast-induced fever, Distinct letters (a-e) signify statistically significant differences among groups ($p < 0.05$)

Table 6. The anti-inflammatory effects *of* ethanol extract of *P. volubilis* leaves in the carrageenan-induced paw edema model in mice

Experimental group	Paw diameter (mm)						
	0 h	Foot swelling	1 h	2 h	3 h	4 h	5 h
Control group	0.61 ± 0.01^{a}	0.62 ± 0.01^{a}	0.63 ± 0.03^{a}	0.62 ± 0.02^{a}	0.61 ± 0.01^{a}	0.63 ± 0.02^{a}	0.62 ± 0.03^{a}
CAR group	0.63 ± 0.01^{b}	0.72 ± 0.01^{b}	0.73 ± 0.01^{d}	0.75 ± 0.01^{d}	0.77 ± 0.01^{d}	0.78 ± 0.01^{d}	0.79 ± 0.01^{d}
CAR + IND group	0.63 ± 0.01^{a}	0.71 ± 0.01^{b}	0.68 ± 0.01^{b}	0.67 ± 0.01^{b}	0.66 ± 0.01^{b}	0.65 ± 0.01^{b}	0.64 ± 0.01^{b}
CAR + PVEE100 group	0.62 ± 0.01^{c}	0.73 ± 0.01^{c}	0.71 ± 0.01^{c}	0.69 ± 0.01^{c}	0.68 ± 0.01^{c}	0.68 ± 0.01^{c}	0.66 ± 0.01^{c}
CAR + PVEE200 group	0.62 ± 0.01^{b}	0.72 ± 0.01^{bc}	0.71 ± 0.01^{c}	0.70 ± 0.01^{c}	0.68 ± 0.01^{c}	0.67 ± 0.01^{bc}	0.65 ± 0.01^{bc}
CAR + PVEE300 group	0.63 ± 0.01^{c}	0.71 ± 0.01^{b}	0.68 ± 0.01^{b}	0.67 ± 0.01^{b}	0.66 ± 0.01^{b}	0.65 ± 0.01^{b}	0.64 ± 0.01^{c}

Different letters (a-e) denote statistically significant differences between groups ($p < 0.05$)

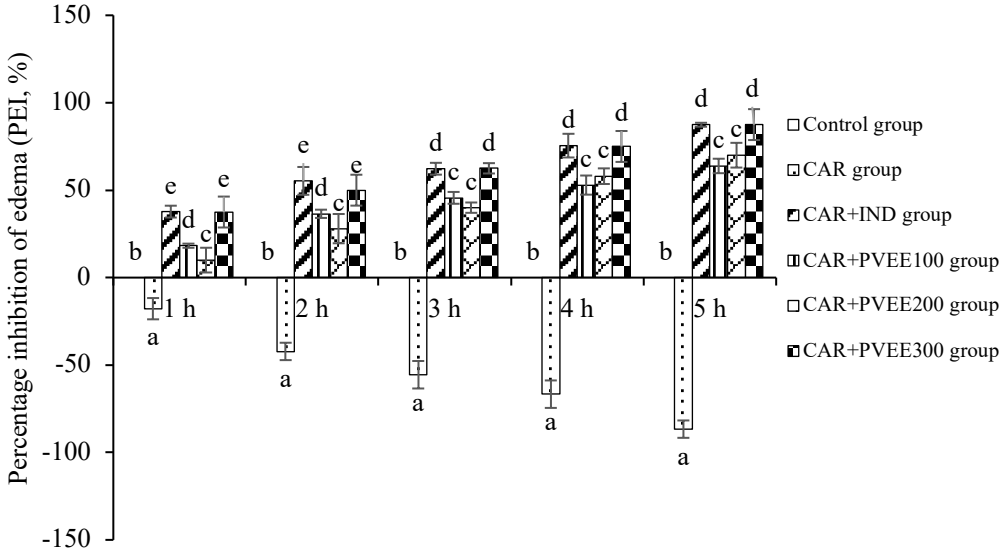

Figure 4. Percentage inhibition of edema of ethanol extract of *P. volubilis* leaves in the carrageenan-induced paw edema model in mice, Distinct letters (a-e) signify statistically significant differences among groups ($p < 0.05$)

Quantification of TNF-α, IL-1β, and IL-6

After treatment with CAR, the serum levels of IL-6, IL-1*β*, and TNF-*α* was significantly increased in the treated groups compared to the control group ($p < 0.05$) as shown in Figure 5. Evaluation of cytokine concentrations demonstrated to decrease ($p < 0.05$) in the released of IL-6, IL-1*β*, and TNF-*α* in the serum of mice treated with PVEE at all three doses (100, 200, and 300 mg/kg) or indomethacin (IND), compared to the negative control (CAR group).

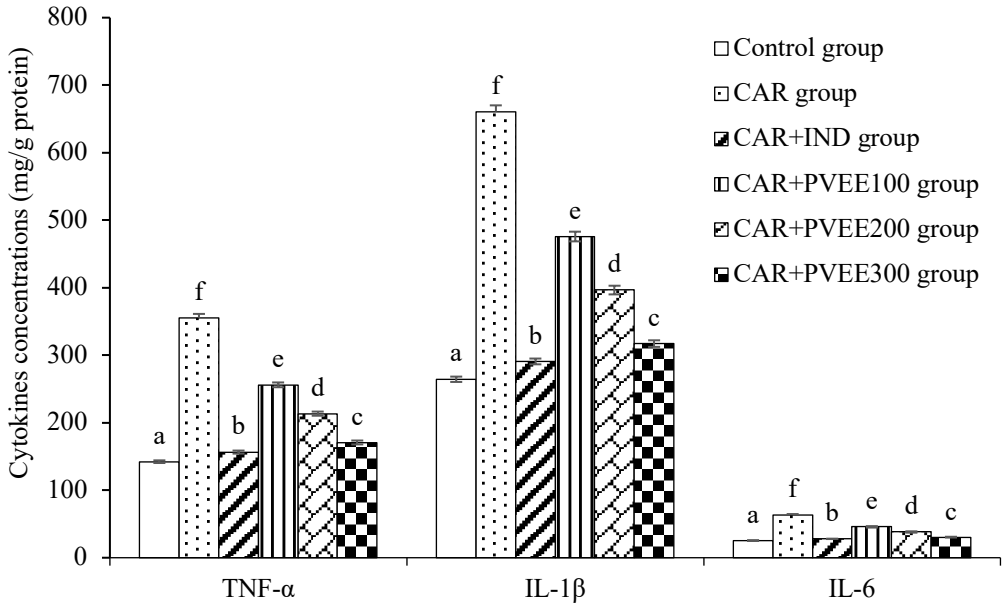

Figure 5. Cytokine concentrations in the carrageenan-induced paw edema model in mice, Distinct letters (a-e) signify statistically significant differences among groups ($p < 0.05$)

Discussion

Medicinal plants and herbs, enriched with essential phytochemicals, encompass a wide range of primary and secondary plant metabolites responsible for antibacterial, anti-inflammatory, analgesic, antipyretic, and other known biological activities (Singh *et al*., 2022). Preliminary phytochemical profiling indicates that the ethanol extract of *P. volubilis* leaves (PVEE) is enriched with various essential phytochemicals, including tannins, flavonoids, terpenoids, saponins, steroids, alkaloids, and polyphenols. Establishing a robust scientific foundation for using PVEE necessitates identifying the efficacy of its major phytochemicals-flavonoids, terpenoids, and

polyphenols-which possess antioxidant, analgesic, and antipyretic properties. Flavonoids, abundant in plant extracts, inhibit enzymes involved in inflammation and modulate pain perception by interacting with the central nervous system. Terpenoids have been demonstrated to alleviate pain and reduce fever by inhibiting the synthesis of inflammatory mediators. Polyphenols are recognized for their anti-inflammatory properties, contributing to pain relief and fever reduction (Sherif *et al*., 2023). The flavonoid content at 43.48 ± 1.31 mg QE/g, terpenoid content at 69.93 ± 1.75 mg TAE/g, and polyphenol content at 68.54 ± 1.76 mg GAE/g in PVEE indicate its significant potential for analgesic, antipyretic, and anti-inflammatory effects. The levels of flavonoids suggest strong anti-inflammatory and pain-relieving properties, as flavonoids are known to inhibit inflammatory enzymes and modulate pain perception through interaction with the central nervous system. The substantial terpenoid content supports the extract's ability to alleviate pain and reduce fever by inhibiting the synthesis of pro-inflammatory mediators. Additionally, the polyphenol content in the extract underscores the extract's potent anti-inflammatory effects, which contribute further to pain relief and fever reduction. Together, these phytochemicals in PVEE highlight its efficacy in managing inflammation, pain, and fever.

The tail-flick analgesia method is highly effective for evaluating the efficacy and effects of centrally acting analgesic drugs. The tail-flick response, a spinal reflex to thermal stimuli, measures the transient model of acute pain. When the tail is exposed to noxious heat stimuli, pain receptors such as TRPV1 are activated, leading to an influx of Na^+ and Ca^{2+} ions into the nociceptors. This generates action potentials that travel along the sensory neuron axons to the dorsal horn of the spinal cord. Here, excitatory neurotransmitters like glutamate and substance P are released into the synaptic cleft between the nociceptor and second-order neurons in the spinal cord. The second-order neurons transmit the pain signal via the spinothalamic tract to the thalamus for sensory relay, which then forwards the signal to the somatosensory cortex, where the perception of pain occurs (Román-Vargas *et al*., 2023). In our study, oral administration of the ethanol extract of *P. volubilis* leaves (PVEE) moderately altered the thermal pain threshold, reflecting changes in the behavioral response to pain. The analgesic mechanism of PVEE in the tail-flick test involves multiple pathways and biochemical processes. Flavonoids in PVEE interact with various receptors in the central and peripheral nervous systems, enhancing GABAergic neurotransmission to increase the inhibition of pain signals. Terpenoids act on opioid receptors and inhibit the transmission of pain signals within the nervous system. PVEE also affects serotonin and dopamine release, which slows neurotransmission in the central nervous

system. Additionally, PVEE modulates ion channels (such as TRPV1 and voltage-gated sodium channels), reducing the excitability of pain receptors. Some phytochemicals in PVEE exhibit opioid-like activity, binding to opioid receptors and mimicking the effects of endogenous opioids, thereby modulating and reducing pain perception (Lalan *et al*., 2020).

The investigation continued utilizing the Haffner tail-clip test, a mechanical nociceptive model employed to assess the central analgesic efficacy of PVEE. In the Haffner tail-clip experiment, mechanical force is applied to the tail of the experimental animal. This mechanical stimulation activates specialized sensory nerve endings located within the tail tissues, generating action potentials along sensory nerve fibers, which then propagate to the central nervous system. Upon reaching the dorsal horn of the spinal cord, the action potentials trigger the release of neurotransmitters, thereby activating second-order nerve cells, and transmitting pain signals to brain centers including the brainstem and cortex. These pain signals are processed and interpreted in various brain regions associated with pain perception, resulting in conscious pain sensation (Yam *et al*., 2020). In this study, the Haffner tail-clip test method was employed to assess the anti-inflammatory and analgesic effects of the PVEE. Compounds present in PVEE inhibit the activation of pain receptors in response to mechanical stimuli, thereby reducing the excitability of sensory nerve cells. PVEE modulates the release of neurotransmitters at the dorsal horn of the spinal cord and influences central pain processing, thereby activating descending pain inhibitory pathways and modulating neurotransmitter systems (endogenous opioids) involved in pain modulation.

Peripheral analgesic effects were assessed using acetic acid-induced pain in mice, a visceral pain model commonly employed for pain relief drug screening. This model utilizes the noxious chemical stimulus of acetic acid to induce pain responses. Acetic acid causes irritation and inflammation in the peritoneal cavity, activating peripheral nociceptive receptors capable of detecting damage. The stimulation by acetic acid triggers the release of pain-reducing mediators, including prostaglandins, bradykinin, histamine, and serotonin, from injured tissues and immune cells, sensitizing and eliciting rapid responses from peripheral nerve fibers, amplifying pain signal transmission through sensory nerve cells to the spinal cord's dorsal horn. Pain signals are further processed and transmitted to higher brain centers associated with pain perception. Activation of pain pathways in the central nervous system leads to the manifestation of pain behaviors, such as abdominal writhing, stretching, and contraction of abdominal muscles in experimental animals (Ullah *et al*., 2023). Peripheral analgesic effects of PVEE were assessed using this model, which significantly reduced the number of writhes at 100, 200, and 300 mg/kg b.w

doses. The reference drug ASA also helped alleviate visceral pain in this model. PVEE modulates ion channels or signaling pathways related to the sensitivity of pain receptors, thereby reducing the sensitivity of peripheral pain receptors and their responsiveness to chemical stimuli. Active components in PVEE regulate pain transmission in the spinal cord by intervening in releasing neurotransmitters or signaling pathways involved in pain perception processing, thereby reducing pain signal transmission from the periphery to the central nervous system and diminishing overall pain responses. PVEE also activates descending pain inhibition pathways originating from the brainstem and descending to the spinal cord, releasing endogenous opioids and other neurotransmitters that block pain signal transmission at the spinal level, thus reducing pain perception.

Yeast induces fever through the production and accumulation of prostaglandins in the hypothalamus, leading to an increase in rectal temperature. The injection of yeast triggers white blood cells (macrophages and monocytes) to produce and release pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α). These cytokines travel to the hypothalamus in the brain, which regulates body temperature, and stimulates the production of prostaglandin E2 (PGE2) via the cyclooxygenase-2 (COX-2) pathway. PGE2 acts on EP3 receptors in the hypothalamus, altering the body's set point temperature. This causes the body to perceive its current temperature as lower than it is, thereby initiating heat-producing mechanisms such as vasoconstriction, muscle shivering, vasodilation, and increased sweating. These responses lead to an elevation in body temperature, resulting in fever (Sherif *et al*., 2023). The ethanol extract of *P. volubilis* leaves (PVEE) exhibits antipyretic effects through multiple mechanisms, including the reduction of pro-inflammatory cytokine production, inhibition of PGE2 synthesis, enhancement of anti-inflammatory activity, regulation of ion channels and thermoreceptors, and activation of pain inhibitory pathways. PVEE may contain bioactive compounds such as flavonoids, terpenoids, and polyphenols that inhibit the production and release of pro-inflammatory cytokines, thereby reducing inflammatory signaling to the hypothalamus, which regulates body temperature. Additionally, these compounds inhibit the activity of the cyclooxygenase-2 (COX-2) enzyme, leading to decreased PGE2 production and preventing alterations in the hypothalamic set point temperature. PVEE may also modulate the activity of ion channels and thermoreceptors (such as TRPV1), and activate descending pain inhibitory pathways from the brainstem to the spinal cord, releasing neurotransmitters like endogenous opioids, serotonin, and norepinephrine. These actions inhibit pain and inflammation signaling at the

spinal and brain levels, reducing the sensitivity of the nervous system to thermal and inflammatory stimuli.

Inflammation is triggered by living tissues damaged due to infection by bacteria, viruses, and various other factors. The primary function of inflammation is to prevent and eliminate infectious agents and harmful substances from the tissues. An additional function of inflammation is to create favorable conditions for the repair of damaged tissues, organs, or systems by removing injured tissue components. Injection of carrageenan (CAR) into the paw leads to the release of histamine and serotonin from mast cells, increasing the permeability of blood vessels, serum leakage, and initial swelling. During this stage, bradykinin and kinins are produced, further increasing vascular permeability and causing pain and swelling. Subsequently, the synthesis and release of prostaglandins (especially PGE2) and nitric oxide continue to dilate vessels and increase permeability. Neutrophils and other leukocytes infiltrate the inflammation site to release additional inflammatory mediators such as cytokines (TNF-α, IL-1β, IL-6) and reactive oxygen species (ROS), prolonging the inflammatory response (Xiang *et al*, 2023). The ethanol extract of *P. volubilis* leaves (PVEE) modulates several biochemical pathways and inflammatory responses. PVEE inhibits the cyclooxygenase-2 (COX-2) enzyme, which in turn suppresses the synthesis of prostaglandin E2 (PGE2), leading to reduced inflammation and associated pain. The flavonoids, terpenoids, and polyphenols present in PVEE possess strong antioxidant properties, enabling them to scavenge reactive oxygen species (ROS), thereby reducing oxidative stress and limiting tissue damage. PVEE stabilizes lysosomal membranes in leukocytes, preventing the release of lysosomal enzymes. It also interferes with chemotactic signals that attract neutrophils and other leukocytes to the inflammation site, thus reducing the release of inflammatory mediators. Additionally, PVEE downregulates the production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, resulting in decreased leukocyte recruitment and activation. PVEE can inhibit inducible nitric oxide synthase (iNOS), leading to reduced nitric oxide (NO) production. It also modulates the activity of transient receptor potential vanilloid 1 (TRPV1) channels and other ion channels involved in pain and inflammation, thereby decreasing the sensitivity of sensory neurons to inflammatory stimuli. The significant reduction in paw diameter and the percentage inhibition of edema observed in the current study highlight the potential of PVEE as an effective anti-inflammatory agent.

In this study, the ethanol extract from *Plukenetia volubilis* leaves (PVEE) demonstrated significant analgesic, antipyretic, and anti-inflammatory effects in mice. PVEE exhibited dose-dependent analgesic properties in both the tail-flick

analgesia model and Haffner's tail-clip test, significantly reducing pain responses. Moreover, PVEE effectively attenuated acetic acid-induced writhing, highlighting its potential as an analgesic agent. Additionally, PVEE demonstrated remarkable antipyretic activity by significantly reducing yeast-induced fever in mice. Furthermore, PVEE exhibited potent anti-inflammatory effects against carrageenan-induced paw edema, as evidenced by a significant reduction in paw diameter and inhibition of pro-inflammatory cytokine release. These findings underscore the therapeutic potential of PVEE as a natural remedy for pain, fever, and inflammation.

References

Alison, A. (2010). Basel declaration defends animal research. Nature, 468:742.

Ammam, A., Zemour, H., Kaid, M., Villemin, D., Soufan, W., and Belhouadjeb, F. A. (2023). Assessment of the anti-inflammatory and analgesic effects of *Opuntia ficus* indica L. Cladodes extract. Libyan Journal of Medicine, 18:2275417.

Aslam, B., Hussain, A., Bari, M. U., Faisal, M. N., Sindhu, Z. U. D., Alonaizan, R., Al-Akeel, R. K., Naz, S. and Khan, R. U. (2023). Anti-pyretic, analgesic, and anti-inflammatory activities of meloxicam and curcumin co-encapsulated PLGA nanoparticles in acute experimental models. Metabolites, 13:935.

Goyal, A., Tanwar, B., Sihag, M. K. and Sharma, V. (2022). Sacha inchi (*Plukenetia volubilis* L.): An emerging source of nutrients, omega-3 fatty acids, and phytochemicals. Food Chemistry, 373:131459.

Joshi, A., Bhobe, M. and Saatarkar, A. (2013). Phytochemical investigation of the roots of *Grewia microcos* Linn. Journal of Chemical and Pharmaceutical Research, 5:80-87.

Lalan, B. K., Hiray, R. S. and Ghongane, B. B. (2020). Evaluation of analgesic and anti-inflammatory activity of extract of *Holoptelea Integrifolia* and *Argyreia Speciosa* in animal models. Journal of Clinical and Diagnostic Research, 9:1-4.

Mustafa, A., Indiran, M. A., Shanmugham, R. and Ramalingam, K. (2023). Anti-inflammatory activity of lauric acid, thiocolchicoside, and thiocolchicoside-lauric acid formulation. Bioinformation, 19:1075-1080.

Nhung, T. T. P. and Quoc, L. P. T. (2023a). Investigation of the inflammatory, antipyretic, and analgesic potential of ethanol extract from *Hedyotis capitellata* Wall. ex G. Don leaves in mice. Tropical Journal of Natural Product Research, 7:5501-5508.

Nhung, T. T. P. and Quoc, L. P. T. (2023b). Analgesic and antipyretic activities of ethanol extract of *Gardenia jasminoides* Ellis fruits in mice. Tropical Journal of Natural Product Research, 7:4902-4907.

Nhung, T. T. P. and Quoc, L. P. T. (2024a). Efficacy of black shallot extract in analgesic and antipyretic activities in experimental mice. Tropical Journal of Natural Product Research, 8:6609-6616.

Nhung, T. T. P. and Quoc, L. P. T. (2024b). Ethanol extract of *Caryota urens* Lour fruits alleviates oxidative stress in a murine model of rheumatoid arthritis induced by Freund's complete adjuvant. Tropical Journal of Natural Product Research, 8:6948-6956.

Radha, M. J. and Mahaboob-Basha P. (2020). Hepatotoxic evaluation of Di-n-butyl phthalate in Wistar rats upon sub-chronic exposure: A multigenerational assessment. Toxicology Reports, 7:772-778.

Rahman, I. Z. A., Hisam, N. S. N., Aminuddin, A., Hamid, A. A., Kumar, J. and Ugusman, A. (2023). Evaluating the potential of *Plukenetia volubilis* Linneo (Sacha Inchi) in alleviating cardiovascular disease risk factors: A mini review. Pharmaceuticals, 16:1588.

Román-Vargas, Y., Porras-Arguello, J. D., Blandón-Naranjo, L., Pérez-Pérez, L. D. and Benjumea, D. M. (2023). Evaluation of the analgesic effect of high-cannabidiol-content cannabis extracts in different pain models by using polymeric micelles as vehicles. Molecules, 28:4299.

Sherif, A. E., Sajid-ur-Rehman, M., Asif, M., Qadeer, I. and Khan, K. U. R. (2023). The anti-inflammatory, analgesic, and antipyretic potential of *Oxystelma esculentum* (L. f.) Sm. using in vitro, in vivo, and in silico studies. Frontiers in Pharmacology, 14:1326968.

Singh, P. K., Singh, J., Medhi, T. and Kuma, A. (2022). Phytochemical screening, quantification, FT-IR analysis, and *in silico* characterization of potential bio-active compounds identified in HR-LC/MS analysis of the polyherbal formulation from Northeast India. ACS Omega, 7:33067-33078.

Tran, T. P. N. and Tran, T. T. N. (2021). Evaluation of acute and subchronic toxicity induced by the crude ethanol extract of *Plukenetia volubilis* Linneo leaves in swiss albino mice. BioMed Research International, 2021:6524658.

Tran, T. P. N., Nguyen, T. T. and Gia-Buu Tran. (2023). Anti-arthritis effect of ethanol extract of Sacha inchi (*Plukenetia volubilis* L.) leaves against complete Freund's adjuvant-induced arthritis model in mice. Tropical Life Sciences Research, 34:237-257.

Ullah, Q., Ali, Z., Rashid, U., Ali, G., Ahmad, N., Khan, R., Ullah, S., Ayaz, M. and Murthy, H. C. A. (2023). Involvement of the opioidergic mechanism in the analgesic potential of a novel indazolone derivative: efficacy in the management of pain, neuropathy, and inflammation using *in vivo* and *silico* approaches. ACS Omega, 8:22809-22819.

Xiang, L., Huang, Q., Chen, T., He, Q., Yao, H. and Gao, Y. (2023). Ethanol extract of *Paridis rhizoma* attenuates carrageenan-induced paw swelling in rats by inhibiting the production of inflammatory factors. BMC Complementary Medicine and Therapies, 23:437.

Yam, M. F., Loh, Y. C., Oo, C. W. and Basir, R. (2020). Overview of neurological mechanism of pain profile used for animal "pain-like" behavioral study with proposed analgesic pathways. International Journal of Molecular Sciences, 21:4355.

(Received: 3 June 2024, Revised: 5 September 2024, Accepted: 7 September 2024)