RESEARCH PAPER

Analgesic and anti-inflammatory effects of *Artemisia pallens* leaf extract in rodent

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Abstract
Pain and inflammation are major global health concerns, typically managed with synthetic drugs such as NSAIDs and opioids. Despite their efficacy, these agents are associated with adverse effects and dependency risks, highlighting the need for safer, plant-based alternatives. The present study investigates the analgesic and anti-inflammatory potential of *Artemisia pallens* leaf extract in rodent models. Phytochemical screening of the methanolic extract revealed the presence of flavonoids, phenolics, alkaloids, proteins, and sterols. Quantitative analysis showed high phenolic (71.39 ± 0.611 mg GAE/g) and flavonoid (61.10 ± 0.468 mg QE/g) content. Anti-inflammatory activity was assessed using carrageenan-induced paw edema, where the extract at 200 mg/kg inhibited edema by 58.24% at 6 h, compared to 79.72% inhibition by ibuprofen. Analgesic activity was evaluated using the tail-flick method, with the extract significantly prolonging response time in a dose-dependent manner. These findings suggest that *A. pallens* possesses moderate but significant analgesic and anti-inflammatory properties, likely mediated through cyclo-oxygenase inhibition and central pain modulation. The study validates traditional claims and supports the potential of *A. pallens* as a natural therapeutic agent for managing pain and inflammation.

Keywords
Artemesia pallens, anti-inflammatory, analgesic, flavonoids, natural therapeutics
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Introduction

Pain and inflammation are among the most common health challenges worldwide, often managed with synthetic drugs such as NSAIDs and opioids [1]. While effective, these treatments carry risks of side effects, dependency, and long-term complications. There is a pressing need for safer, plant-based alternatives that combine efficacy with sustainability [2].

Artemisia pallens plant contains flavonoids, alkaloids, glycosides, phenols, saponins, triterpenes, and steroids, all of which are known to influence nociception and inflammatory pathways and oxidative stress [3]. In Ayurveda and folk medicine, *Artemisia pallens* has been valued for its analgesic and anti-inflammatory properties. Related species in the *Artemisia* family have shown anti-inflammatory, anagesic, and antioxidant activities, indicating that *A. pallens* may share similar bioactivity [4-7].

Limited systematic studies exist on *Artemisia pallens* for pain and inflammation, present a knowledge gap for the present study. Also, the plant's phytochemicals may act on key pathways (e.g., prostaglandin synthesis, cytokine modulation), offering novel mechanisms for relief.

The study is expected to bridge traditional wisdom with modern science, validating an underexplored plant for inflammatory conditions. It is also aimed to open pathways for novel product development, from supplements to therapeutic formulations. The study will contribute to expanding the arsenal of natural therapeutics for pain and inflammation.

Material and Methods

The leaves of *Artemisia pallens* were collected from local plants around Bhopal and was authenticated by botanist at RB Science, Bhopal. The dried leaves powdered using a blender at low speed. The powdered leaves were stored in air tight container until taken for further processing.

Extraction and phytochemical Screening

The powdered leaves were used for the extraction process by hot continuous extraction method using soxhlet apparatus. 87.5 g of leaf powder was evenly placed in the extractor of the apparatus and 350 mL of 80% methanol was poured over it and was allowed to collect in the attached flask. Extraction was achieved by heating the solvent at 75°C for 7 h till a colorless solution was collected from the siphon tube of the apparatus. The extract was filtered through Whatman filter and concentrated using rotary vacuum evaporator [8]. The resinous extract was collected and stored in desiccator to remove the excessive moisture**.** The dried extract was stored in desiccator for further processing.

All the extracts were evaluated by qualitative phytochemical screening in order to identify the type of plant secondary metabolites present in them. The screening was performed for triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic acids. The color intensity or the precipitate formation was used as analytical responses to these tests [9].

Total Phenolic Content

For total phenolic content determination, 200 µL of sample was mixed with 1.4 mL purified water and 100 µL of Folin-Ciocalteu reagent. After at least 30 s (but not exceeding 8 min), 300 µL of 20% Na_2CO_3 aqueous solution was added and the mixture allowed to stand for 2 h. The absorbance was measured at 765 nm with a UV-Vis spectrophotometer. Standard solutions of gallic acid (10-100 ppm) were similarly treated to plot the analytical curve. The control solution contained 200 µL of methanol and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples [10]. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.

Total Flavonoid Content

Determination of total flavonoids content was based on aluminium chloride method. 50 mg quercetin was dissolved in 50 ml methanol, and various aliquots of 25- 150µg/ml were prepared in methanol. 0.1 g of dried extract was extracted with 10 ml methanol, filtered, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was for the estimation of flavonoid. 1 mL of 10% sodium nitrite solution was added to 1 mL of extract or standard solution and allowed to stand for 10 min. To this was added 1 ml of 2% $AlCl_3$ methanolic solution nd allowed to stand for 60 min at room temperature; absorbance was measured at 420 nm [11].

Animals

Healthy Wistar rats of either sex, weighing 180-250g were used for the study. The animals were housed in cages during the course of experimental period and maintained at 12 day and night schedule with a temperature [17-26°C] maintained at standard experimental condition. The animals were fed with standard rodent pellet feed and water *ad libitum*. The animals were fasted 12 hours before the experiment with free access to only water. The experiments were approved by the institutional animal ethical committee.

Experimental grouping of animals

Animals were divided into 4 groups (n = 6) as follows:

Group I (Control) - treated with vehicle (normal saline); Group II (Standard) – Ibuprofen treated; Group III– *Artemisia pallens* extract (APE) administered in dose of 100 mg/kg; Group IV– *Artemisia pallens* extract (APE) administered in dose of 200 mg/kg.

Carrageenan induced rat paw edema method for anti-inflammatory action

The carrageenan induced rat paw edema method was used for evaluating the anti-inflammatory activity of *Artemisia pallens* extract [12]. Paw oedema was induced by subcutaneous injection of 0.1mL (1% solution) of Carrageenan into the plantar surface of the right hind paw of the rat. The test sample was administered in dose of 10 mg/kg in different groups of animals, 30 min prior to carrageenan injection. Ibuprofen (10 mg/kg i.p.) was used as a standard anti-inflammatory drug which was administered 30 min prior to carrageenan injection.

Paw diameters were measured immediately before the administration of the Carrageenan and thereafter at 1, 2, 4 and 6 h using vernier caliper. The results obtained were compared with control group. The percentage inhibition of paw inflammation exhibited by each group was calculated by using following formula:

% inhibition = C-T/ C x 100

C= Paw diameter (mm) in vehicle treated group (control); T= Paw diameter (mm) in drug treated group

Tail flick method for analgesic action

The analgesic activity was evaluated using tail flick method [13]. About 5 cm from the distal end, tail of each rat was immersed in warm water maintained at 50°C. The reaction time (in seconds) was the time taken by the rat to flick its tail due to pain. The first reading was omitted and reaction time was taken as the average of the next two readings. The reaction time was recorded before (0 min) and at 15, 30, 45, and 60 min after the administration of the treatments. The maximum reaction time was fixed at 15 sec to prevent any tail tissue injury. If the reading exceeds 15 sec, it would be considered as maximum analgesia. The maximum possible analgesia (MPA) was calculated as follows:

$$MPA = \frac{\text{Reaction time for treatment} - \text{reaction time for saline}}{15\text{ sec} - \text{reaction time for saline}} X\ 100$$

Results and Discussion

Extraction Yield and phytochemical screening

The extraction yield of the flower of *Artemisia pallens* leaves in 80% methanol by hot continuous extraction was found to be 12.91% w/w. The extract was resinous and black in color. The observation of the phytochemical analysis suggest the presence of alkaloids, sterols, phenolics and tannins, proteins and flavonoids in the 80% methanolic extract of the leaves (Table 1).

Table 1. Phytochemical screening of *Artemisia pallens* leaves extract

Chemical Tests	Observation	Inference

Alkaloids		
Mayer's reagent	cream colour precipitate	+
Hager's reagent	yellow colour precipitate	-
Wagner's reagent	reddish brown precipitate	-
Dragendorff's reagent	reddish brown precipitate	+
Glycosides		
Froth test	Frothing is seen	-
Kedde's Test	No color	-
Bontrager's Test	Rose pink or red color in the ammonical layer not found	-
Keller-Kiliani	No color in acetic acid layer	-
Phenols/Tannins		
Ferric chloride	Blue green color	+
Gelatin Solution	White precipitate	+
Alkaline reagent test	Yellow to red precipitate	+
Vanillin HCl test	Purplish red color	+
Flavonoids		
Shinoda test	red color	+
Alkaline reagent test	Yellow color that turns red on acidification	+
Zinc HCl reductino test	red color	+
Proteins		
Millon's Test	white precipitate, turns red on heating	+
Ninhydrin Test	Voilet color	+
Sterols/triterpenoids		
Liberman-Burchard Test	Brown ring at junction Upper layer turns green	-
Salkowski Test	Yellow color in lower layer	+

*+ positive; - negative

Phenolic and flavonoid content

The 80% methanolic extract of *Artemisia pallens* leaves was evaluated for quantifying the total phenolic content. Standard curve of gallic acid was plotted in distilled water. The result of the total phenolic content of the extract examined using Folin-Ciocalteu method. The total phenolic content of 80% methanolic extract of *Artemisia pallens* leaves was found to be 71.39 ± 0.611 GAE mg/g. The result of the total flavonoid content of the extract examined using Aluminium chloride method revealed that the 80% methanolic extract of *Artemisia pallens* leaves contained 61.10 ± 0.468 QE mg/g of flavonoids.

Evaluation of analgesic and anti-inflammatory action

Table 2 shows the effect of *Artemisia pallens* leaves extract and standard drug as compared to the normal saline control at different hours in carrageenan-induced rat paw edema model using vernier caliper. Ibuprofen at dose of 10 mg/Kg inhibited 79.73% edema after 6h of administration while the APE 100 and APE 200 were able to inhibit 40.75% and 58.24% edema formation respectively (Table 3). This suggests comparatively significant anti-inflammatory potential in the 80% methanolic extract of *Artemisia pallens* leaves extract at both low and high doses.

Table 2. Effect of *Artemisia pallens* extract on rat paw edema

Group	Change in Paw thickness (mm)			
	1h	2h	4h	6h
Normal Saline	1.544 ± 0.018	3.488 ± 0.057	3.732 ± 0.051	3.956 ± 0.017
Ibuprofen	0.384 ± 0.018	0.792 ± 0.023	0.862 ± 0.041	0.802 ± 0.008
APE 100	1.336 ± 0.042	2.128 ± 0.033	3.012 ± 0.010	2.344 ± 0.018
APE 200	1.044 ± 0.021	1.572 ± 0.019	2.134 ± 0.018	1.652 ± 0.023

Values are mean ± SD (n = 6)

Table 3. % inhibition of edema by *Artemisia pallens* extract

Group	[% inhibition of edema]			
	1h	2h	4h	6h
Ibuprofen	75.13	77.29	76.9	79.72
APE 100	13.47	38.99	19.29	40.75
APE 200	32.38	54.93	42.82	58.24

Carrageenan-induced acute inflammation is one of the most suitable test procedures to screen anti-inflammatory agents. As shown in the table, *Artemisia pallens* leaves extract was not able to inhibit edema significantly in the early hours but was able to inhibit edema considerably at 6h. The anti-inflammatory effect of *Artemisia pallens* leaves was less as compared to Ibuprofen (Figure 1).

Carrageenan-induced paw edema model in rats is known to be sensitive to cyclo-oxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents, which primarily inhibit the cyclo-oxygenase involved in prostaglandin synthesis [14]. Therefore, it can be inferred that the inhibitory effect of *Artemisia pallens* leaves on carrageenan-induced inflammation may be due to inhibition of the enzyme cyclo-oxygenase leading to inhibition of prostaglandin synthesis.

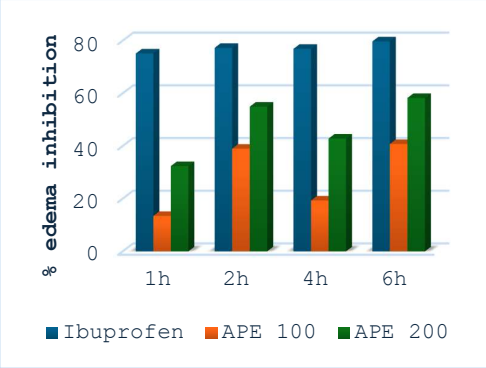

Figure 1. Comparison of anti-inflammatory effect of ibuprofen and *Artemisia pallens*

The results of analgesic activity of *Artemisia pallens* leaves extract by tail flick method are shown in Table 4. Rats treated with normal saline (vehicle control) did not exhibit any significant difference in the response time on tail-flick throughout the 60 min duration of observation.

The duration of response time in Ibuprofen and *Artemisia pallens* leaves was significantly higher as compared to the saline treated animals. The reaction time for APE 100 and APE 200 was 6.072 ± 0.086 sec and 6.656 ± 0.250 sec respectively at 60 min post administration while it was 3.416 ± 0.047 sec and 8.070 ± 0.139 sec for vehicle treated group and Ibuprofen respectively at the same time duration.

Table 4. Effect of *Artemisia pallens* extract on tail flick response

Group	Response Time in seconds				
	0 min	15 min	30 min	45 min	60 min
Vehicle Control	3.096 ± 0.044	3.312 ± 0.043	3.534 ± 0.033	3.454 ± 0.056	3.416 ± 0.047
Ibuprofen	4.250 ± 0.096	5.698 ± 0.284	6.274 ± 0.140	6.860 ± 0.089	8.070 ± 0.139
APE 100	3.582 ± 0.169	4.550 ± 0.369	5.844 ± 0.091	6.010 ± 0.129	6.072 ± 0.086
APE 200	3.672 ± 0.145	4.854 ± 0.142	6.050 ± 0.145	6.282 ± 0.256	6.656 ± 0.250

Values are mean ± SD (n = 6)

Analgesics are drugs that act on peripheral or central nervous system to selectively relieve pain without significantly altering consciousness [15]. The animal model used for screening of analgesic activity in this study is pain-state model using thermal stimuli which includes tail-flick method. The tail-flick method is known to mediate a spinal reflex to a nociceptive stimulus [16]. Ibuprofen was used as the reference drug, which is considered as mild analgesic.

The tail-flick method is based on the observation that morphine-like compounds are selectively able to prolong the response time of typical tail-withdrawal in rats in response to pain stimulus. Analgesic drugs which are centrally acting elevate pain threshold of animals towards heat and pressure [17]. Therefore, the analgesic effect of *Artemisia pallens* leaves on this pain-state model indicates that it might be centrally acting.

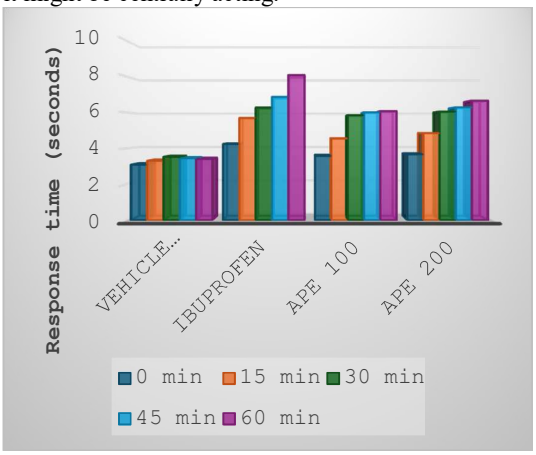

Figure 2. Comparison of analgesic effect of ibuprofen and *Artemisia pallens*

Conclusion

The study establishes that the 80% methanolic extract of *Artemisia pallens* leaves possesses moderate but significant anti-inflammatory and analgesic properties. The pharmacological effects are likely attributable to the presence of flavonoids and phenolic compounds, which may act via inhibition of cyclo-oxygenase and modulation of pain pathways. Although the activity was lower than ibuprofen, the extract exhibited clear dose-dependent efficacy, supporting its potential as a natural therapeutic agent.

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