



Cognition-Enhancing Effect of *Artemisia Pallens* Leaf Extract Against Scopolamine-Induced Amnesia in Rats

Rishabh Tripathi, Urvesh Singh Narwaria

Abstract: *Background:* Cognitive impairment is a major feature of neurodegenerative disorders, with scopolamine widely used to induce experimental amnesia. Plant-derived phytochemicals are increasingly explored as safer alternatives to synthetic nootropics. *Objective:* To evaluate the cognition-enhancing potential of the methanolic leaf extract of *Artemisia pallens* against scopolamine-induced amnesia in Wistar rats. *Methods:* *A. pallens* leaves were extracted using a Soxhlet apparatus with 80% methanol. Phytochemical screening, total phenolic content (Folin-Ciocalteu method), and total flavonoid content (AICls method) were determined. Rats were divided into five groups: vehicle, scopolamine (2 mg/kg, i.p.), *A. pallens* extract (100 and 200 mg/kg, p.o.) + scopolamine, and piracetam (20 mg/kg, p.o.) + scopolamine. Cognitive performance was assessed using the Elevated Plus Maze paradigm, with transfer latency as the behavioural index. *Results:* Phytochemical analysis confirmed the presence of alkaloids, phenolics, tannins, proteins, and flavonoids. The extract showed high phenolic (71.53 ± 1.51 mg GAE/g) and flavonoid (59.36 ± 0.64 mg QE/g) content. Scopolamine significantly impaired cognition, while *A. pallens* extract produced dose-dependent reductions in transfer latency (-41.7% and -50.8% at 24 h; -47.0% and -56.2% at 48 h for 100 and 200 mg/kg, respectively). Piracetam showed the strongest effect (-55.1% at 24 h; -59.9% at 48 h). *Conclusion:* *Artemisia pallens* leaf extract exhibits significant cognition-enhancing activity, likely mediated by its phenolic and flavonoid constituents. It may represent a potential phytotherapeutic agent for managing memory deficits.

Keywords: *Artemisia Pallens*; Scopolamine-Induced Amnesia; Cognitive Enhancement; Elevated Plus Maze; Piracetam

Nomenclature:

GAE: Gallic Acid Equivalent

TL: Transfer Latency

APE: *A. Pallens* Extract

I. INTRODUCTION

Cognitive impairment and memory deficits are hallmark features of several neurodegenerative disorders, including Alzheimer's disease, and represent a major global health challenge (Hoffman & Monroe, 2001) [1]. Among the pharmacological models used to study amnesia,

Scopolamine—a muscarinic acetylcholine receptor antagonist—is widely used because it induces reversible memory impairment by disrupting cholinergic neurotransmission (Levi, 2016) [2]. Current therapeutic agents, such as piracetam and other nootropics, provide symptomatic relief but are often limited by side effects and variable efficacy (Rates, 2001) [3]. This has prompted increasing interest in plant-derived bioactive compounds as safer alternatives for cognitive enhancement.

Medicinal plants are rich sources of phytochemicals, including alkaloids, flavonoids, and phenolic compounds, which have been reported to exert neuroprotective effects through antioxidant activity, modulation of neurotransmitter systems, and enhancement of synaptic plasticity (Singh et al., 2015) [6]; Baraiya & Chauhan, 2021) [7]. *Artemisia* species, belonging to the family Asteraceae, are traditionally used in ethnomedicine for their diverse pharmacological properties, including antimicrobial, anti-inflammatory, and neuroprotective activities (Kirtikar & Basu, 1999) [4]; Nadkarni, 2009) [5]. *Artemisia pallens*, commonly known as “Davana,” is an aromatic herb cultivated in India and valued for its essential oils and phytoconstituents. Preliminary phytochemical investigations have revealed flavonoids, phenolics, and terpenoids in *A. pallens*, suggesting its potential role in modulating cognitive functions (Arora, 2019) [8].

Given the established role of oxidative stress and cholinergic dysfunction in memory impairment, evaluating *A. pallens* extract against scopolamine-induced amnesia provides a rational approach to identify novel cognition-enhancing agents. The present study was therefore designed to investigate the anti-amnesic potential of methanolic leaf extract of *A. pallens* in Wistar rats using the Elevated Plus Maze paradigm, with piracetam serving as the standard reference drug.

II. MATERIAL AND METHODS

A. Preparation of Plant Material for Extraction

The leaves of *Artemisia pallens* were collected from local plants around Gwalior city and were authenticated by a botanist at RB Science, Bhopal. The dried leaves were powdered using a blender at low speed for extraction.

i. Extraction of Leaves (Arora, 2019) [9]

The powdered leaves were extracted using the hot continuous extraction method with a Soxhlet apparatus. 87.5 g of leaf powder was evenly placed in the extractor, and 350 mL of 80% methanol was

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poured over it and allowed to collect in the attached flask. Extraction was achieved by heating the solvent at 75°C for 7 h, until a colourless solution was collected from the siphon tube. The extract was filtered through a Whatman filter and concentrated using a rotary vacuum evaporator. The resinous extract was collected and stored in a desiccator to remove excess moisture. The dried extract was stored in a desiccator for further processing.

ii. *Preliminary Phytochemical Screening (Shabir et al., 2011)[10]*

All extracts were evaluated by qualitative phytochemical screening to identify the types of plant secondary metabolites present. The screening was performed for triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic acids. Colour intensity or precipitate formation was used as the analytical response for these tests.

iii. *Total Phenolic Content (Ansari et al., 2013)[11]*

The extract was dissolved in methanol to obtain a 50 mg/mL solution. The solution was stored at 4°C in an amber bottle and served as the stock solution for subsequent analyses.

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% Na₂CO₃ 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. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.

iv. *Total Flavonoid Content (Tiwari et al., 2017) [12]*

Determination of total flavonoid content was based on the 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 the volume was made up to 100 mL. One mL (1mg/ml) of this extract was used for flavonoid estimation. Add 1 mL of 10% sodium nitrite solution was added to 1 mL of extract or quercetin solution and allowed to stand for 10 min. Then, 1 mL of 2% AlCl₃ methanolic solution was added and allowed to stand for 60 min at room temperature; absorbance was measured at 420 nm.

B. Evaluation of Anti-Amnesic Potential

i. *Grouping of Animals used for Study*

Healthy Wistar rats of either sex, weighing 180-250 g, were used for the study. The animals were housed in cages during the experimental period, maintained on a 12-hour day/night cycle, and kept at 17-26°C. The animals were fed standard rodent pellet feed and water *ad libitum*. The animals were fasted

for 12 hours before the experiment, with free access to water only. The animals were divided into 5 groups of 6 animals each. The grouping and treatment per group are presented below.

- **Group 1:** control vehicle (0.9% w/v NaCl); Group 2 was injected with scopolamine (SCOP) 2mg/kg intraperitoneally for 21 days; Group 3 was administered with extract of *Artemisia pallens* (APE) at a dose of 100 mg/kg/p.o and injected with SCOP (2 mg/kg) for 21 days; **Group 4** was administered with extract of *Artemisia pallens* (APE) at a dose of 200 mg/kg/p.o and injected with SCOP (2 mg/kg) for 21 days; **Group 5** was administered with piracetam at a dose of 20 mg/kg/p.o and injected with SCOP (2 mg/kg) for 21 days.
- **Elevated Plus Maze Test** (Schneider et al., 2011) [13]; Itoh et al., 1990) [14] The elevated plus maze comprised two open (50 cm × 10 cm) and two enclosed (50 cm × 10 cm × 40 cm) arms radiating from a central platform (10 cm × 10 cm) to form a plus sign. The maze was constructed of black acrylic sheet. The plus maze was elevated to 50 cm above floor level by a single central support. The trial was started by placing an animal on the central platform of the maze facing an open arm. During the 90-second experiment, the rat's behaviour was recorded as the time required for the rat to enter the closed arm with all four paws in. The rat was considered to have entered an arm when all four paws were on the arm. The apparatus was cleaned thoroughly between trials with damp and dry towels. All behavioural recordings were carried out with the observer unaware of the treatment the rat had received. The change in transfer latency was calculated using the formula:

$$\text{Change (\%)} = \frac{TL_t - TL_{\text{training}}}{TL_{\text{training}}} \times 100$$

C. Statistical Analysis

All analyses were performed using GraphPad Prism 5 for Windows. All statistical analyses were expressed as mean ± standard error of the mean (SEM). Two-way ANOVA was used to analyse the data.

III. RESULTS AND DISCUSSION

The extraction yield of *Artemisia pallens* leaves in 80% methanol by hot continuous extraction was 12.25% w/w. The extract was resinous and black. Phytochemical analysis suggests the presence of alkaloids, sterols, phenolics and tannins, proteins, and flavonoids in the 80% methanolic leaf extract.

A. Total Phenolic Content

The total phenolic content of the 80% methanolic extract of *Artemisia pallens* leaves was quantified. A standard curve of gallic acid was plotted in distilled water.





The total phenolic content of the extract was examined using the Folin-Ciocalteu method. The total phenolic content of the 80% methanolic extract of *Artemisia pallens* leaves was found to be 71.53 ± 1.514 GAE mg/g.

B. Total Flavonoid Content

The total flavonoid content of the 80% methanolic extract of *Artemisia pallens* leaves was quantified. A quercetin standard curve was plotted. The total flavonoid content of the extract was examined using the aluminium chloride

method. The total flavonoid content of the 80% methanolic extract of *Artemisia pallens* leaves was found to be 59.36 ± 0.641 QE mg/g.

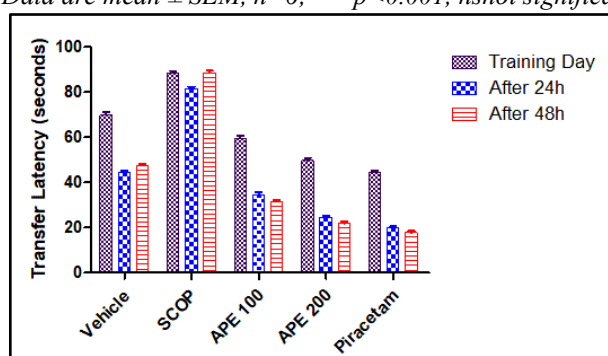
C. Determination of Cognition Enhancement Potential

The rats were induced to amnesia by administration of scopolamine for 21 days. The effect of co-administration of the extract of *Artemisia pallens* (APE) or piracetam with scopolamine for 21 days and further 48 h was observed (Table I).

Table I: Transfer Latency from Open to Closed Arm

Treatment	Dose (mg/kg)	Transfer Latency (Seconds)		
		Training Day	After 24 h	After 48 h
Vehicle	0.5 ml/kg, i.p.	69.83 ± 2.83	$44.5 \pm 1.54^{***}$	$47.5 \pm 1.54^{***}$
SCOP	2 mg/kg, i.p.	88.5 ± 2.00	$81.5 \pm 2.00^{***}$	88.67 ± 1.78^{ns}
APE	100 mg/kg, p.o.	59.5 ± 2.38	$34.67 \pm 1.78^{***}$	$31.5 \pm 1.54^{***}$
APE	200 mg/kg, p.o.	49.83 ± 1.91	$24.5 \pm 1.54^{***}$	$21.83 \pm 1.76^{***}$
Piracetam	20 mg/kg, p.o.	44.5 ± 1.54	$20.0 \pm 1.67^{***}$	$17.83 \pm 1.42^{***}$

Data are mean $\pm$ SEM, $n=6$; $^{***}p<0.001$, ns not significant

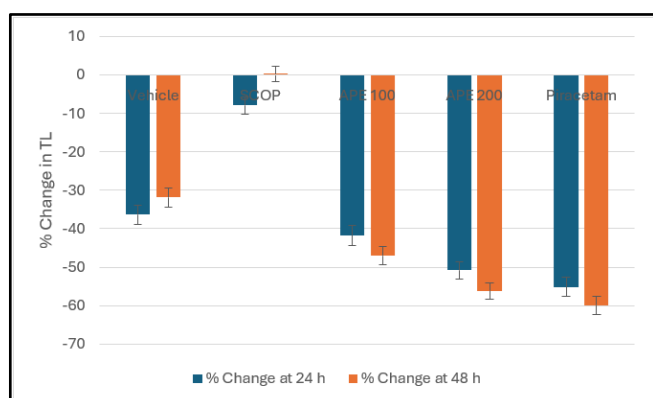


[Fig.1: Graph Showing Transfer Latency Across Various Treatment Groups on Different Days]

The change in transfer latency was calculated from the transfer latency data (Table 2).

Table II: Per Cent Change in Transfer Latency

Treatment	% Change at 24 h (Mean $\pm$ SEM)	% Change at 48 h (Mean $\pm$ SEM)
Vehicle	$-36.3\% \pm 2.5$	$-31.9\% \pm 2.4$
SCOP	$-7.9\% \pm 2.2$	$+0.2\% \pm 2.0$
APE 100	$-41.7\% \pm 2.6$	$-47.0\% \pm 2.4$
APE 200	$-50.8\% \pm 2.3$	$-56.2\% \pm 2.5$
Piracetam	$-55.1\% \pm 2.4$	$-59.9\% \pm 2.3$



[Fig.2: Bar Graph Showing Per Cent Change in TL Compared to Training Day]

In the present study, the cognition-enhancing potential of *Artemisia pallens* leaf extract was evaluated using the Elevated Plus Maze paradigm. Transfer latency (TL) served as the behavioural index of learning and memory. The

results demonstrated that scopolamine administration produced a marked impairment in cognition, as reflected by persistently high TL values and negligible per cent change at 24 h and 48 h. In contrast, co-administration of *A. pallens* extract (APE) produced a significant and dose-dependent reduction in TL, indicating improved acquisition and retention of memory. At 100 mg/kg, APE reduced TL by approximately 41.7% at 24 h and 47.0% at 48 h, while the higher dose of 200 mg/kg produced even greater reductions of 50.8% and 56.2%, respectively. Piracetam, used as a standard nootropic, showed the strongest effect with TL reductions of 55.1% at 24 h and 59.9% at 48 h, thereby validating the experimental model.

The per cent change analysis further highlights APE's dose-dependent efficacy. Vehicle-treated animals showed natural memory retention with TL reductions of 36.3% and 31.9% at 24 h and 48 h, respectively. Scopolamine-treated animals, however, exhibited only a minor reduction at 24 h (-7.9%) and virtually no change at 48 h (+0.2%), confirming its amnesic effect. In contrast, APE treatment produced consistent improvements, with the higher dose approaching piracetam's efficacy. These findings strongly suggest that *A. pallens* extract possesses cognition-enhancing properties capable of counteracting scopolamine-induced deficits.

The behavioural outcomes correlate well with the extract's phytochemical profile. Phytochemical screening revealed alkaloids, phenolics, tannins, proteins, and flavonoids. Quantitative estimation showed high levels of total phenolic content (71.53 ± 1.51 mg GAE/g) and total flavonoid content (59.36 ± 0.64 mg QE/g). Both phenolics and flavonoids are known to exert neuroprotective effects through antioxidant activity, modulation of cholinergic neurotransmission, and enhancement of synaptic plasticity. Their abundance in the extract provides a mechanistic basis for the observed cognitive benefits. The strong correlation between high TPC/TFC values and significant reductions in TL supports the conclusion that these phytochemicals are the primary contributors to the memory-enhancing activity of *A. pallens*.



IV. CONCLUSION

The present study demonstrated that the methanolic leaf extract of *Artemisia pallens* possesses significant cognition-enhancing activity against scopolamine-induced amnesia in Wistar rats. The extract produced a dose-dependent reduction in transfer latency in the Elevated Plus Maze paradigm, indicating improved learning and memory acquisition. At 200 mg/kg, the extract approached the efficacy of piracetam, a standard nootropic agent. Phytochemical analysis revealed high levels of phenolics and flavonoids, which are known to exert neuroprotective effects through antioxidant mechanisms and modulation of cholinergic neurotransmission. These findings suggest that *A. pallens* may serve as a promising natural source for developing phytotherapeutic agents to counteract memory deficits associated with neurodegenerative disorders such as Alzheimer's disease. Further studies involving isolation of active constituents and mechanistic investigations are warranted to validate its therapeutic potential.

DECLARATION STATEMENT

Some of the cited references are older and are noted explicitly as [1], [3], [4], [5], [6], [10], [11], [13] and [14]. However, these works remain significant for the current study, as they are pioneering in their fields.

After aggregating input from all authors, I must verify the accuracy of the following information as the article's author.

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- **Funding Support:** This article has not been funded by any organisations or agencies. This independence ensures the research is conducted objectively and without external influence.
- **Ethical Approval and Consent to Participate:** The content of this article does not necessitate ethical approval or consent to participate, with supporting documentation.
- **Data Access Statement and Material Availability:** The adequate resources of this article are publicly accessible.
- **Authors' Contributions:** The authorship of this article is contributed equally to all participating individuals.

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Certificate

This is to certify that the project proposal no RBS/AE/26/001 entitled "Exploring the Cognition Enhancing Effect of *Artemisia Pallens* Leaf Extract in Rodent" submitted by Mr. Richabh Tripathi, has been approved/recommended by the IAEC of Richa Brijesh Science LLP, Bhopal (Registration Number - 2356/PO/Re/S/2025/CCSEA) in its meeting held on 14/03/2026 and 30 Wistar rats have been sanctioned under this proposal for a duration of 3 months.

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