J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 50(2): 371-388, 2026
Doi: 10.33483/jfpau.1691113
Research Article / Araştırma Makalesi

ANTI-AMNESIC AND NEUROPROTECTIVE EFFECTS OF *JASMINUM SAMBAC* LEAF EXTRACT IN SCOPOLAMINE-INDUCED AMNESIC RATS

SKOPOLAMİN İLE İNDÜKLENMİŞ AMNEZİK SIÇANLARDA JASMİNUM SAMBAC YAPRAK EKSTRESİNİN ANTİ-AMNEZİK VE NÖROKORUYUCU ETKİLERİ

Kailas MALI[1]*, Nitin SALUNKHE[1], Kajal HOWAL[1], Niranjan MAHAJAN[1], Remeth DIAS[2]

[1]Adarsh College of Pharmacy, Department of Pharmacology, Vita, Sangli, Maharashtra, India
[2]Government College of Pharmacy, Department of Pharmacy, Karad, Satara, Maharashtra, India

ABSTRACT

Objective: *This study investigated the anti-amnesic effects of Jasminum sambac ethanolic leaf extract in scopolamine-induced amnesic rats, using Donepezil (5 mg/kg) as the reference standard.*
Material and Method: *The ethanolic extract was prepared by maceration and characterized through qualitative and quantitative phytochemical analyses. GC–MS analysis was performed to identify bioactive constituents. Cognitive function was evaluated using the elevated plus maze (EPM), hole board test, Morris water maze (MWM), and actophotometer. Biochemical parameters, including acetylcholinesterase (AChE) activity and antioxidant status, were also assessed.*
Result and Discussion: *Phytochemical screening confirmed the presence of flavonoids, phenols, anthraquinones, saponins, and coumarins, with total phenolic (0.29 mg GAE/g leaf powder) and flavonoid (0.39 mg QE/g leaf powder) contents. The DPPH assay showed 62.55% inhibition at 200 µg/ml (IC_{50}: 109.38 µg/ml). GC–MS identified 29 compounds, including six with antioxidant potential. Behavioral studies demonstrated significant memory enhancement ($p < 0.05$), evidenced by reduced escape latency (MWM), decreased transfer latency (EPM), increased exploratory behavior, and improved locomotor activity. The findings suggest neuroprotective effects mediated via AChE inhibition and attenuation of oxidative stress, supporting further investigation for cognitive impairment and Alzheimer's disease management.*
Keywords: *Alzheimer's disease, amnesia, anti-amnesic activity, dementia, Jasminum sambac*

ÖZ

Amaç: *Bu çalışma, Jasminum sambac'ın etanollü yaprak ekstraktının skopolamin ile indüklenmiş amnezik sıçanlarda anti-amnezik etkilerini, referans standart olarak Donepezil (5 mg/kg) kullanarak araştırmayı amaçlamıştır.*
Gereç ve Yöntem: *Etanollü ekstrakt maserasyon yöntemi ile hazırlanmış ve kalitatif ile kantitatif fitokimyasal analizlerle karakterize edilmiştir. Biyoaktif bileşenlerin tanımlanması için GC-MS analizi yapılmıştır. Bilişsel fonksiyonlar; yükseltilmiş artı labirent (EPM), delik tahta testi, Morris su labirenti (MWM) ve aktophotometre kullanılarak değerlendirilmiştir. Ayrıca asetilkolinesteraz*

* **Corresponding author:** Kailas Mali
E-mail: malikailas@gmail.com
Submitted date: 04.05.2025 **Accepted date:** 07.04.2026 **Published date:** 19.05.2026
Citation: Mali, K., Salunkhe, N., Howal, K., Mahajan, N., Dias, R. (2026). Anti-amnesic and neuroprotective effects of *Jasminum sambac* leaf extract in scopolamine-induced amnesic rats. Journal of Faculty of Pharmacy of Ankara University, 50(2), 371-388.

(AChE) aktivitesi ve antioksidan durum belirlenmiştir.
Sonuç ve Tartışma: *Fitokimyasal analizler flavonoidler, fenoller, antrakinonlar, saponinler ve kumarinlerin varlığını doğrulamıştır. Toplam fenolik madde miktarı 0.29 mg GAE/g yaprak tozu, toplam flavonoid miktarı ise 0.39 mg QE/g yaprak tozu olarak bulunmuştur. DPPH yöntemiyle 200 µg/ml'de %62.55 inhibisyon (IC_{50}: 109.38 µg/ml) saptanmıştır. GC-MS analizinde antioksidan potansiyele sahip altı bileşen dâhil olmak üzere toplam 29 bileşik belirlenmiştir. Davranışsal testler, kaçış latansında azalma (MWM), transfer latansında düşüş (EPM), keşif davranışında ve lokomotor aktivitede artış ile anlamlı hafıza iyileşmesi ($p < 0.05$) göstermiştir. Bulgular, AChE inhibisyonu ve oksidatif stresin azaltılması yoluyla nöroprotektif etkiyi desteklemekte olup, Alzheimer hastalığı ve bilişsel bozuklukların yönetiminde ileri araştırmalar gerektirmektedir.*
Anahtar Kelimeler: *Alzheimer hastalığı, amnezi, anti-amnezik aktivite, bunama, Jasminum sambac*

INTRODUCTION

Alzheimer's disease (AD) was first described in 1906 by Alois Alzheimer, a German neuropathologist and psychiatrist. Today, an estimated 24 million people worldwide suffer from dementia, with AD being the most common form. The disease is characterized by oxidative stress damage to brain proteins, nucleic acids, and mitochondria, leading to cognitive decline and neurodegeneration. Memory impairment is a hallmark symptom [1]. Dementia encompasses multiple disorders with distinct symptoms and causes. AD, the most prevalent form, is primarily driven by β-amyloid plaque accumulation in the brain, resulting in severe cognitive deterioration [2]. Amnesia, a specific type of memory loss, impairs the ability to form new memories. Key symptoms include significant memory gaps or an inability to retain new information [3].

In 2022, global spending on AD-related healthcare reached $321 billion, while in the US alone, AD caused 121,499 deaths in 2019. By 2020, approximately 491,000 Americans aged 65 and older developed AD [4]. Worldwide, an estimated 50 million people live with AD, a number projected to double every five years, reaching 152 million by 2050 [5]. In Mumbai, India, a study of 24,488 individuals identified 105 AD and amnesia cases, with prevalence rates of 0.43% (age 40+), 2.44% (age 65), and 1.81% (age 65+). For AD alone, prevalence was 0.25% overall and 1.5% in those aged 65 and above [6].

Currently, there is no definitive cure for Alzheimer's disease (AD), dementia, or amnesia. Existing treatments only provide symptomatic relief, primarily targeting cognitive decline. Only two classes of drugs are approved for AD management: N-methyl-D-aspartate (NMDA) antagonists and cholinesterase inhibitors. Tacrine, the first FDA-approved cholinesterase inhibitor, works by boosting acetylcholine levels in muscarinic neurons [5].

For centuries, herbal remedies have been employed in traditional medicine to manage AD, dementia, and amnesia [1]. Among these, *Jasminum sambac (J. sambac)* (L.) Aiton has shown efficacy in enhancing cognitive function. Various parts of this plant have been used to treat diverse ailments, including paralysis, diabetes, and rheumatism [7]. Modern research has validated its broad-spectrum pharmacological properties, such as anticancer, antifungal, anti-inflammatory, antioxidant, antimicrobial, antidiabetic, analgesic, antipyretic, gastroprotective, cardiovascular disease and antiparkinson's activity [6-14]. Additionally, it exhibits stress-relieving and mood-enhancing properties [15]. *J. sambac* contains a rich profile of bioactive compounds, including phenols, terpenes, flavonoids (quercitrin, isoquercitrin, and rutin), coumarins, alkaloids, tannins, saponins, essential oils, and anthraquinones. Its leaf extract, in particular, demonstrates significant antioxidant, anti-inflammatory, analgesic, antipyretic, wound-healing, and neuroprotective activities. Flavonoids, the predominant phytochemicals in the extract, are believed to play a key role in improving cognitive function by mitigating oxidative stress and inflammation [6]. These findings highlight *J. sambac* as a promising candidate for developing therapeutic interventions against cognitive disorders.

The leaf extract of *J. sambac* contains bioactive flavonoids that demonstrate potent antioxidant properties. These compounds show therapeutic potential against AD through multiple mechanisms such as reducing oxidative stress, improving mitochondrial dysfunction, decreasing insulin resistance, and alleviating memory impairment [16]. Although the flower extract has been documented to possess memory-enhancing effect, to date, no studies have investigated the anti-amnesic properties of the leaf

extract [17,18]. This represents a significant gap in current research, particularly given the leaf's established neuroprotective flavonoid content and its demonstrated efficacy in addressing key pathological features of AD.

Considering the above facts, the study aimed to investigate the anti-amnesic effect of *J. sambac* leaves extract against scopolamine-induced amnesia in rats using different screening models.

MATERIAL AND METHOD

Plant Material

The leaves of *J. sambac* plant were collected from Kundal, Sangli, Maharashtra, India. The plant material was submitted to the Department of Botany, Balwant College, Vita, 415311, Sangli, Maharashtra, India, for identification. The voucher number for the specimen is KPH001.

Preparation of Extract

The leaves were collected, washed with water, and shade-dried. The leaves were crushed in an electrical grinder and sieved to obtain fine powder. For the extract preparation, the maceration method was used. In brief, 100 g of powder material was soaked in 500 ml of ethanol in a beaker and kept for three days wrapped with aluminum foil, with periodic stirring. After three days, the mixture was filtered using a fine muslin cloth, followed by filtration using Whatman filter paper. The filtrate was evaporated by using a rotary vacuum evaporator (Equitron, India) and stored in a desiccator until further use [7]. The dry extract was suspended in carboxymethyl cellulose (CMC; 0.5% w/v) and administered orally to rats according to their body weight [19].

Qualitative Phytochemical Analysis

Ethanolic extract of *J. sambac* was screened for the phytoconstituents such as flavonoids, tannin, alkaloids, phenols, anthraquinones, saponin and coumarins. Furthermore, the amounts of specific phytoconstituents present in the extract were determined using appropriate quantitative methods. A one percent solution of extract was prepared in ethanol and used for further phytochemical tests [20,21].

Estimation of Phenolic Content

A test sample was prepared by dissolving 100 mg of *J. sambac* ethanolic extract in 100 ml of distilled water. For the reference standard, a gallic acid solution (100 µg/ml) was prepared in distilled water. A series of gallic acid dilutions was made, ranging from 10 to 100 µg/ml. For the assay, 1 ml of the gallic acid solution was combined with 1 ml of Folin-Ciocalteu reagent. After 5 minutes, 10 ml of a 7% sodium carbonate solution was added. The mixture was then diluted to 25 ml with 13 ml of distilled water and incubated in the dark for 90 minutes. The same procedure was followed for the test sample. The absorbance of both the test sample and standard solutions was measured at 760 nm using a UV spectrophotometer (Shimadzu 1800, Japan), with a blank solution prepared without gallic acid. The total phenolic content was calculated and expressed as gallic acid equivalents [22,23].

Estimation of Flavonoid Content

The test sample was prepared according to the procedure described for determining phenolic content. For the standard, a stock solution of quercetin (100 mg in 100 ml methanol) was prepared, and 1 ml of this solution was diluted to 10 ml with methanol to obtain a working concentration of 100 µg/ml. Aliquots of the 100 µg/ml quercetin solution were taken and mixed with 4 ml of distilled water, followed by the addition of 0.3 ml of 5% sodium nitrite. After 5 minutes, 0.3 ml of 10% aluminum chloride was added. At the 6-minute mark, 2 ml of 1 M sodium hydroxide was added, and the final volume was adjusted to 10 ml with distilled water to achieve concentrations ranging from 20 to 150 µg/ml. The same procedure was applied to one ml of the test sample (100 µg/ml). The absorbance of the solutions was measured at 510 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan) against a blank. The flavonoid content was determined and expressed as quercetin equivalents [23,24].

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

The chemical composition of *J. sambac* ethanolic extract was analyzed using gas chromatography-mass spectrometry (GC-MS; Shimadzu TQ 8050 Plus, Japan) equipped with an HS 20 autosampler. Before analysis, the AOC-20i+s autosampler performed a cleaning cycle involving two pre-solvent and two post-solvent rinses (8 µl wash volume). The injection port operated at 260°C with a 0.3-second well time and 5 pumping cycles. Chromatographic separation was achieved at a constant column flow rate of 1.01 ml/min under a pressure of 54.4 kPa, with the oven temperature maintained at 50°C for 2 minutes. The system employed direct injection at 250°C with a linear speed of 36.5 cm/sec. Data acquisition and instrument control were performed using Shimadzu GC-MS Solution software. Compound identification was accomplished by comparing mass spectra against the NIST14.lib reference database (National Institute of Standards and Technology) [25].

Antioxidant Assay

The antioxidant potential of the plant extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. The test was performed by mixing 3 ml of 0.1 mM DPPH solution with 100 mg/ml extract solution, and then diluting to a final volume of 5 ml with methanol. A control containing only DPPH solution and a standard containing butylated hydroxytoluene (BHT, 100 mg/ml) were prepared in parallel. After thorough mixing, all samples were incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm against a methanol blank using a UV-Visible spectrophotometer (UV-1800 Shimadzu, Japan). The percentage of free radical scavenging activity was calculated using the following equation [26,27]:

$$\%\ inhibition = \frac{(A_{con} - A_{test})}{A_{con}} x\ 100 \quad (1)$$

Where, A_{con} is the absorbance of the control, and A_{test} is the absorbance of the test.

Acute Toxicity Test

The acute toxicity profile of *J. sambac* extract was evaluated according to OECD Guideline 423 using female Wistar rats (180-250 g). Animals were maintained under controlled environmental conditions (25±2°C, 12-hour light/dark cycle) in standard polypropylene cages with free access to *ad libitum* and rodent diet, following CPCSEA guidelines. A single high dose of 2000 mg/kg body weight, suspended in 0.5% CMC, was administered via oral gavage. Post-administration, the animals were systematically observed for toxicological endpoints at specified intervals (30 min, 4 h, 24 h, 48 h, and 72 h), followed by daily monitoring for 14 days. The observational protocol included assessment of neurological status (reactivity, motor coordination), autonomic functions (secretions, thermoregulation), and general behaviour (locomotor activity, feeding patterns). Mortality, if any, was recorded throughout the study period to determine the safety profile of the extract at the tested dose level [28].

Drugs and Chemicals

Scopolamine hydrobromide and Donepezil were procured from Tokyo Chemical Industry Pvt. Ltd., India. Normal saline was obtained from the local vendor. All other chemicals used were of analytical grade and supplied by Loba Chemie, Mumbai.

Behavioural Study (Training period)

All rats underwent a 7-day behavioural training period before experimental interventions. During this acclimatization phase, animals received no pharmacological treatments. Only subjects demonstrating complete task acquisition were selected for subsequent experimental groups.

Experimental Design

The study employed a standardized protocol to evaluate potential therapeutic interventions for memory impairment. Rats (n=30) were randomly allocated into five experimental groups (n=6 per group) as detailed in Table 1. Following a 14-day pretreatment period with daily oral administration of test extracts, animals received scopolamine hydrobromide (1 mg/kg, *i.p.*) to induce memory impairment during the final 7 days of the study. A reference standard drug was administered orally concurrently

with scopolamine treatment. All test compounds were administered 30 minutes before scopolamine injection to allow for proper absorption and distribution. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee of Biocyte Institute of Research and Development, Sangli, Maharashtra (Approval No: BIRD/05/04/24-25), ensuring compliance with ethical guidelines for animal research. This comprehensive design enabled simultaneous evaluation of both behavioural parameters and biochemical markers associated with memory function.

Table 1. Experimental design

No	Group	Dose	Days
1	Normal control (Normal saline)	10 ml/kg, *p.o.*	14 days
2	Disease control (Scopolamine)	1 mg/kg, *i.p.*	7 days
3	Standard (Donepezil) + Scopolamine	5 mg/kg, *p.o.*+ 1 mg/kg, *i.p.*	7 days
4	Low extract *(J. sambac)* + Scopolamine	200 mg/kg, *p.o.* + 1 mg/kg, *i.p.*	14 days
5	High extract *(J. sambac)* + Scopolamine	400 mg/kg, *p.o.* +1 mg/kg, *i.p.*	14 days

Elevated Plus Maze

The elevated plus maze consisted of two open arms (50 × 10 cm) and two closed arms (50 × 10 × 40 cm) positioned opposite each other, with a central square measuring 10 × 10 cm. The maze was raised to 50 cm above the ground. In this model, each animal was placed individually at the end of an open arm, and the transfer latency (time taken to move from the open arm to a closed arm) was recorded. If an animal failed to enter a closed arm within 90 seconds, it was gently guided into the closed arm. Following the training period and 14 days of dosing, transfer latency was assessed on the 15th acquisition day (learning) during treatment and on the 16th retention day (memory) in the absence of dosing [29].

Hole Board Test

The rat hole board measured 40 × 40 cm and consisted of two acrylic floors. The walls of the board were 10 cm high and 1.8 cm thick. The apparatus was positioned 5 cm above the ground, with 16 uniformly spaced holes, each 3 cm in diameter, on the first floor. The ground floor was used to place scented and attractive food for the rats in designated patterns. Each animal was placed alone in the center of the first floor with its back to the observer for the first trial, and their movements were recorded for 5 minutes. After the training period and the 14-days dosing, for the behavioural study, the number of nose poking (nose poking inside the hole) was recorded on the 15th acquisition (learning) day with dosing and on the 16th retention (memory) day without dosing [30].

Morris Water Maze

The Morris water maze, consisting of a circular pool measuring 100 cm in diameter and 35 cm in height, was filled with water up to a depth of 25 cm. A transparent platform measuring 10 × 10 cm was placed 1 cm below the water. The circular pool was divided into four equal quadrants. The platform location remained constant throughout the behavioural investigation. Each animal was conducted four consecutive trials each day. The rat was carefully dropped into the water with a different drop location for every trial and given 120 seconds to find the platform. If they failed to find it, then guided them and allowed them to remain on the platform for 20 s. After the training period and the 14-days dosing, for the behavioural study, the escape latency of the animals was recorded (moving outside the water). The time taken by an animal to move from the starting quadrant to the hidden platform is referred to as the escape latency. Escape latency was recorded on the 15th, 16th, 17th and 18th day. On day 19, a probe trial was performed without administering treatments, during which the platform was removed. The rat was placed in a maze and allowed to explore the hidden platform for 300 seconds. The probe trial means time spent in the target quadrant [31].

Actophotometer

The actophotometer was designed to measure the locomotor activity of animals. The 16 light beams were used to record the activity count of animals. When the animal interrupted the light beam, the activity score was recorded by a digital counter. Rats with body weights ranging from 180 to 250 g were assigned identification numbers. Following the training period and 14 days of dosing in the behavioural study, animals were administered their assigned treatments on the 15th day. The animals were placed individually inside the actophotometer for 10 minutes, and the locomotor activity score was measured [32].

Tissue Preparation

Following behavioural testing, the animals were euthanized under deep anesthesia induced by intraperitoneal injection of thiopental sodium (40 mg/kg). Cervical decapitation was then performed to ensure death. The brains were promptly extracted from the skull and weighed. For homogenate preparation, each fresh brain was placed in a chilled glass homogeniser with 10 volumes of ice-cold phosphate buffer (0.1 M, pH 8.0) and thoroughly homogenised on ice. The homogenate was centrifuged at 3000 rpm for 10 minutes at 4°C in a refrigerated centrifuge. The resulting supernatant, which appeared slightly cloudy, was collected for subsequent analysis of acetylcholinesterase activity [29,31].

Brain Acetylcholinesterase Activity

A 0.4 ml aliquot of the brain homogenate was transferred to a cuvette containing 2.6 ml of phosphate buffer (0.1 M, pH 8.0) and 100 µl of DTNB (5,5′-dithiobis-2-nitrobenzoic acid). The mixture was homogenised by gentle air bubbling, and the initial absorbance was measured at 412 nm using a spectrophotometer. After the absorbance stabilized, a baseline reading was recorded. Subsequently, 20 µl of the substrate (acetylcholine) was added to the cuvette, and the change in absorbance was monitored to assess acetylcholinesterase activity [33]. The enzyme activity is calculated using the following formula:

$$Acetylcholinesterase\ activity\ \left(\frac{M}{mL}\right) = \frac{(\mathrm{A/min} \times \mathrm{V_t})}{[\varepsilon \times b \times \mathrm{V_s}]} \qquad (2)$$

Where,
A/min is the change in the absorbance per minute
$\mathcal{E} = 1.361 \times 10^4\ M^{-1}cm^{-1}$
b = Pathlength (1 cm)
V_t = total volume (3.1 ml)
V_s = sample volume (0.4 ml)
The final reading of enzyme activity was expressed as µ moles/ml sample.

Statistical Analysis

The experimental results are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism (version 10.1.0). Intergroup comparisons were conducted using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. A *p*-value of < 0.05 was considered statistically significant.

RESULT AND DISCUSSION

Phytochemical Analysis

A detailed summary of the phytochemical screening of *J. sambac* is presented in Table 2. The phytochemical analysis revealed that the leaf extract of *J. sambac* was enriched with flavonoids, phenols, anthraquinones, saponins, and coumarins. The *J. sambac* extract showed positive results for flavonoids, phenols, tannins, anthraquinones, triterpenoids, coumarins, carbohydrates, glycosides, and saponins. The test for alkaloid was found to be negative.

Table 2. Results of phytochemical analysis

No	Name	Test	Result
1	Alkaloid	Wagner's test	Absent
2	Saponin	Frothing test	Present
3	Phenol/Tannin	Ferric chloride test	Present
4	Steroid	Salkowski's test	Present
5	Flavonoid	Shinoda test	Present
6	Anthraquinone	Brontrager's test	Present
7	Triterpenoid	Liebermann's Burchard test	Present
8	Coumarins	10% NaOH Test	Present
9	Glycoside	Bromine water	Present
10	Carbohydrate	Benedict test	Present

Estimation of Phenolic Content

Calibration curve of gallic acid was found to be linear in the range of 10 to 100 µg/ml with a regression coefficient of 0.999. Concentration of phenols in the ethanolic extract of *J. sambac* was estimated by using the regression equation Y=0.0042X+0.0404 equivalent to gallic acid. The phenolic content present in *J. sambac* was found to be 0.29 mg GAE/g of leaf powder.

Estimation of Flavonoid Content

Calibration curve of gallic acid was found to be linear in the range of 20 to 150 µg/ml with a regression coefficient of 0.999. Concentration of flavonoids in the ethanolic extract of *J. sambac* was estimated by using the regression equation Y=0.0019X+0.0406, equivalent to quercetin. The flavonoid content present in *J. sambac* was found to be 0.39 mg QE/g of leaf powder.

Gas Chromatography Mass Spectroscopy Analysis

The results of the GC-MS analysis of the ethanolic extract of *J. sambac* are presented in Figure 1. A total of 29 compounds were identified from the chromatogram using the NIST14 library (Table 3). Among these, six compounds exhibit antioxidant activity, which may help reduce free radicals, mitigate oxidative stress, and potentially prevent conditions such as Alzheimer's disease, dementia, and amnesia.

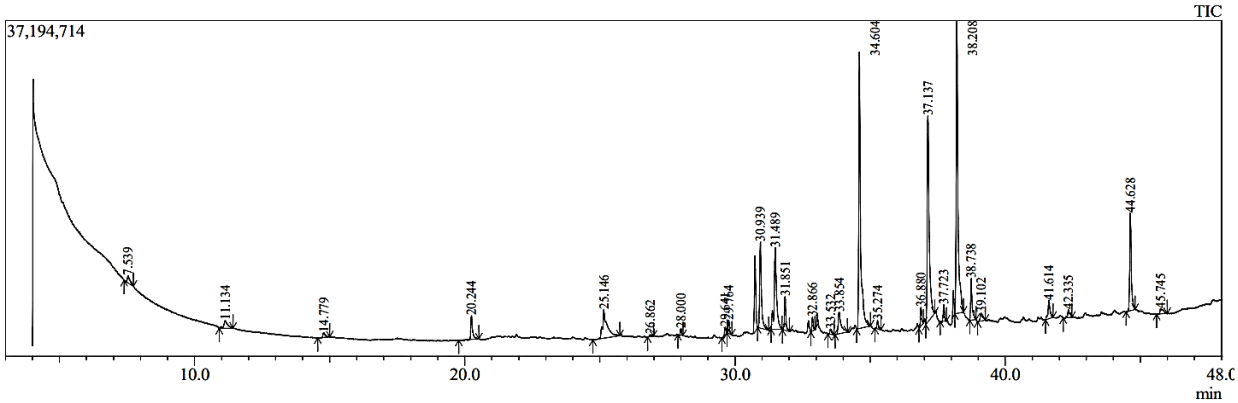

Figure 1. GC-MS Chromatogram of ethanolic extract of *J. sambac*

Table 3. Compound identified in the ethanolic extract of *J. sambac*

Peak No	RT	Compound	Class	Activity	Reference
1	7.539	Butane, 1,1-diethoxy-3-methyl-	Aliphatic acetal	No activity reported	-
2	11.134	Propane, 1,1,3-triethoxy-	Aliphatic acetal	No activity reported	-
3	14.779	Dodecane	Alkane	No activity reported	-
4	20.244	Tetradecane	Alkane	No activity reported	-
5	25.146	Hexadecane	Alkane	No activity reported	-
6	26.862	Undecanal	Saturated fatty aldehyde	No activity reported	-
7	28.000	2-Hexyldecyl acetate	Fatty acid ester	No activity reported	-
8	29.641	Tetradecanoic acid, ethyl ester	Fatty acid esters	No activity reported	-
9	29.764	Heneicosane	Alkane	Antimicrobial, antioxidant	[34]
10	30.939	2-Pentadecanone, 6,10,14-trimethyl-	Aliphatic ketone	Hypocholesterolemic, anti-inflammatory, antibacterial, anti-nociceptive, antioxidant, and lubricating	[35]
11	31.489	1,2-Benzenedicarboxylic acid, bis (2- methylpro*pyl*) ester	Aromatic diester	Anticancer	[36]
12	31.851	Neophytadiene	Acyclic diterpene	Analgesic, antioxidant, antimicrobial, anti-cancer, antimalarial, and neuroprotective	[37]
13	32.866	2- Piperidinone, N-[4-bromo-n-butyl]-	δ-lactam	Antimicrobial	[38]
14	33.532	Isophytol	Terpenoid	Antibacterial / antifungal	[39]
15	33.854	Dibutyl phthalate	Phthalic acid diester	No activity reported	-
16	34.604	Hexadecanoic acid, ethyl ester	Fatty acid ester	Anticancer, antimicrobial	[40,41]
17	35.274	Z, Z-6,28-Heptatriactontadien-2-one	Aliphatic methyl ketone	No activity reported	-
18	36.880	6-Octadecenoic acid, methyl ester, (Z)-	Fatty acid methyl ester	No activity reported	-
19	37.137	Phytol	Acyclic diterpenoid	Antinociceptive, antioxidant, anticancer, antiinflammatory, antimicrobial, diuretic, chemopreventive properties	[42]
20	37.723	Cyclopentanone, 2-(5-oxohexyl)-	Bicyclic diketone	No activity reported	-
21	38.208	Ethyl oleate	Fatty acid ethyl ester	No activity reported	-
22	38.738	Octadecanoic acid, ethyl ester	Fatty acid ethyl ester	Antioxidant, anti-inflammatory	[42]
23	39.102	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Phytane diterpenoid	Antiasthmatic, antimicrobial, cancer preventive, Anti-inflammatory	[43]
24	41.614	4,8,12,16-Tetramethylheptadecan-4-olide	Macrocyclic lactone	Anticancer	[44]
25	42.335	Eicosanoic acid, ethyl ester	Fatty acid ethyl ester	No activity reported	-
26	44.628	Bis(2-ethylhexyl) phthalate	Phthalate ester	Antibacterial	[45]
27	45.745	Docosanoic acid, ethyl ester	Fatty acid ethyl ester	Anticancer	[40]
28	48.574	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	Phthalate-diester	No activity reported	-
29	49.906	Squalene	Triterpene	Antioxidant, antibacterial, anticancer	[42,46]

Antioxidant Activity

The free radicals were the major cause of various diseases such as Alzheimer's, amnesia, dementia, cancer, etc. The antioxidants play a major role in scavenging the free radicals. The DPPH method is widely used to evaluate antioxidant activity. DPPH, which is purple in color, changes to yellow upon reacting with the plant extract [47]. It indicates the presence of free radical scavenging activity to plant. The result of maximum % inhibition of the ethanolic extract of *J. sambac* leaves and standard BHT was 62.55 and 71.33 % at a concentration of 200 µg/ml. The IC_{50} value of the ethanolic extract of *J. sambac* leaves was found to be 109.38 µg/ml (Figure 2). The ethanolic extract of *J. sambac* showed less antioxidant activity than the standard (BHT).

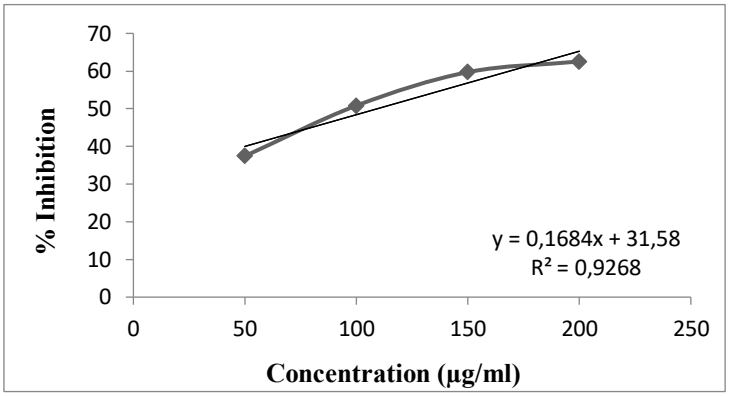

Figure 2. Percent inhibition of the ethanolic extract of *J. sambac* leaves

Acute Toxicity Study

The acute toxicity assessment demonstrated that oral administration of *J. sambac* ethanolic extract at the maximum tested dose (2000 mg/kg) did not produce any mortality in the experimental animals. Throughout the observation period, no significant alterations were noted in body weight, neurological parameters, or behavioural patterns. These findings establish the safety profile of *J. sambac* extract, with 2000 mg/kg being confirmed as the no-observed-adverse-effect level (NOAEL). Based on this safety evaluation, two working doses were selected for subsequent studies: 200 mg/kg (10% of the NOAEL) as the therapeutic dose and 400 mg/kg as the higher dose for dose-response assessment [28,48].

Table 4. Effect of treatment on transfer latency

Group	Drug and Dose	Transfer Latency (Sec)	
		Acquisition Day (15^{th} day)	Retention Day (16^{th} day)
Normal Control	Normal saline (*p.o.*)	42 ± 0.61 $^{\#c}$	39 ± 0.54 $^{\#c}$
Disease Control	Scopolamine (1 mg/kg, *i.p.*)	57 ± 0.40 $^{\$c}$	56 ± 0.61$^{\$c}$
Standard Drug	Donepezil 5 mg/kg (*p.o.*) + Scopolamine (1 mg/kg, *i.p.*)	19 ± 0.17 $^{\#c,\$c}$	18 ± 0.27 $^{\#c,\$c}$
Low-dose Extract	*J. sambac* 200 mg (*p.o.*) + Scopolamine (1 mg/kg, *i.p.*)	30 ± 1.07$^{\#c,\$c,+c}$	30 ± 0.49 $^{\#c,\$c,+c}$
High-dose Extract	*J. sambac* 400 mg (*p.o.*) + Scopolamine (1 mg/kg, *i.p.*)	22 ± 0.49 $^{\#c,\$c,+c,\ *c}$	21 ± 0.33 $^{\#c,\$c,+c,\ *c}$

All data are presented as mean ± SEM (n=6 animals per group). Statistical comparisons were performed using one-way ANOVA with Tukey's post hoc test for multiple comparisons. a: $p < 0.05$, b: $p < 0$ .01, c: $p < 0.001$, #: Data compared with disease control, $: Data compared with control, +: Data compared with standard, *: Data compared with low-dose group, ** Data compared with high dose group

Elevated Plus Maze

The elevated plus maze was performed to determine the transfer latency of *J. sambac* leaf extract

in rats. The scopolamine hydrobromide (1mg/kg, *i.p.*) injected for the last 7 days to the disease control group caused impairment of learning and memory. The effect of treatment on transfer latency is given in Table 4 and Figure 3.

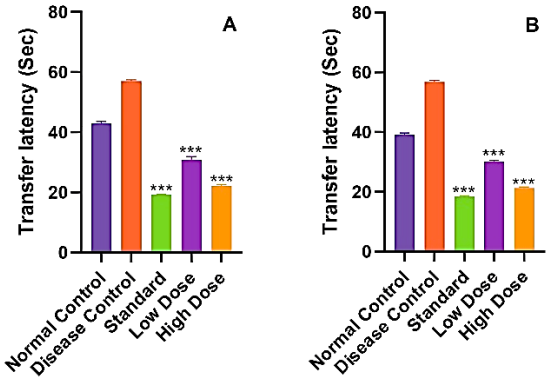

Figure 3. Effect of *J. sambac* extract on transfer latency (in seconds) on the Elevated plus maze, 15th day (A) and 16th day (B) (Data compared with disease control; *** $p < 0.001$)

The normal control, standard, low-dose, and high-dose extract groups showed a significant decrease ($p < 0.05$) in transfer latency on the 15th acquisition (learning) day and 16th retention (memory) day compared with the scopolamine-treated group. Transfer latency was significantly higher ($p < 0.05$) in the disease control group compared with the normal control group. Similarly, transfer latency on the 15th and 16th days was significantly decreased ($p < 0.05$) in the standard, low-dose, and high-dose groups compared with the normal control group. The treatment group (200 and 400 mg/kg) showed significantly ($p < 0.05$) higher transfer latency on the 15th and 16th day compared with the standard group (donepezil). Treatment with the high-dose extract (400 mg/kg) resulted in a significant reduction ($p < 0.05$) in transfer latency compared with the low-dose extract. Decrease in transfer latency on the 15th day and 16th day, indicating significant improvement in cognitive function in the brain. Elevated plus maze results demonstrated that oral administration of both low and high doses of *J. sambac* extract for 14 days effectively protected rats from scopolamine-induced deficits in learning and memory.

Table 5. Effect of treatment on the nose poking entries

Group	Drug and Dose	Nose Poking Entries	
		Acquisition Day (15th day)	Retention Day (16th day)
Normal Control	Normal saline (*p.o.*)	59 ± 1.54 $^{\#c}$	62 ± 1.49 $^{\#c}$
Disease Control	Scopolamine (1 mg/kg, *i.p.*)	20 ± 0.36 $^{\$c}$	22 ± 0.60 $^{\$c}$
Standard Drug	Donepezil 5 mg/kg (*p.o.*) + Scopolamine (1 mg/kg, *i.p.*)	68 ± 1.22 $^{\#c}$	71 ± 1.09 $^{\#c}$
Low-dose Extract	*J. sambac* 200 mg (*p.o.*) + Scopolamine (1 mg/kg, *i.p.*)	51 ± 1.52 $^{\#c,+c}$	54 ± 1.10 $^{\#c,+c}$
High-dose Extract	*J. sambac* 400 mg (*p.o.*) + Scopolamine (1 mg/kg, *i.p.*)	60 ± 1.25 $^{\#c,+c,*c}$	63 ± 1.52 $^{\#c,+c,*c}$

All data are presented as mean ± SEM (n=6 animals per group). Statistical comparisons were performed using one-way ANOVA with Tukey's post hoc test for multiple comparisons. a: $p < 0.05$, b: $p < 0.01$, c: $p < 0.001$, #: Data compared with disease control, $: Data compared with control, +: Data compared with standard, *: Data compared with low-dose group, ** Data compared with high dose group

Hole Board Test

The hole board test was performed to determine the effect of nose pocking entries of *J. sambac*

leaf extract in rats. Administering scopolamine hydrobromide (1 mg/kg, *i.p.*) for 7 days to the disease control group resulted in low nose-poking entries as compared to the normal control group. The results of the hole board test are presented in Table 5 and Figure 4.

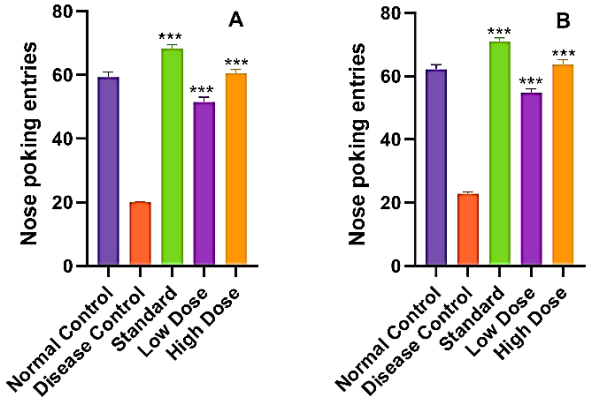

Figure 4. Effect of *J. sambac* extract on nose poking entries (in seconds) on the hole board test, 15th day (A) and 16th day (B) (Make change as poking in figure too) (Data compared with disease control; *** $p < 0.001$)

The normal control group, standard group, low-dose and high-dose extract groups showed a significant increase ($p < 0.05$) in nose poking entries on the 15th acquisition (learning) and 16th retention (memory) days as compared with the disease control group. The disease control group showed a significant decrease ($p < 0.05$) in nose poking entries as compared with the normal control group. The ethanolic extract of *J. sambac* at high dose (400 mg/kg) showed a non-significant ($p > 0.05$) increase in nose poking entries as compared with the normal control group. Both the low-dose and high dose treatment groups exhibited significantly lower ($p < 0.05$) nose poking entries on the 15th and 16th days compared with the standard group. The high-dose group (400 mg/kg) had significantly higher ($p < 0.05$) nose poking entries compared with low-dose group. The ethanolic extract of *J. sambac* at high and low dose extract groups showed significant improvement in nose poking entries, indicating a rise in memory on the 15th acquisition day and 16th retention day.

Morris Water Maze

The Morris water maze was performed to determine the escape Latency (EL) of *J. sambac* leaf extract in rats. Results of the Morris water maze are shown in Table 6 and Figure 5.

Table 6. Effect of treatment on the escape latency

Group	Drug and Dose	Escape Latency on 18th Day (Sec)
Normal Control	Normal saline (*p.o.*)	$33\pm0.67^{\#c}$
Disease Control	Scopolamine (1 mg/kg, *i.p.*)	$38\pm0.79^{\$c}$
Standard Drug	Donepezil 5 mg/kg (*p.o.*) + Scopolamine (1 mg/kg, *i.p.*)	$22\pm0.43^{\#c,**c}$
Low-dose Extract	*J. sambac* 200 mg (*p.o.*) + Scopolamine (1 mg/kg, *i.p.*)	$30\pm0.479^{\#c,+c}$
High-dose Extract	*J. sambac* 400 mg (*p.o.*) + Scopolamine (1 mg/kg, *i.p.*)	$22\pm 0.45^{\#c,+c,*c}$

All data are presented as mean ± SEM (n=6 animals per group). Statistical comparisons were performed using one-way ANOVA with Tukey's post hoc test for multiple comparisons. a: $p < 0.05$, b: $p < 0.01$, c: $p < 0.001$, #: Data compared with disease control, $: Data compared with control, +: Data compared with standard, *: Data compared with low-dose group, ** Data compared with high dose group

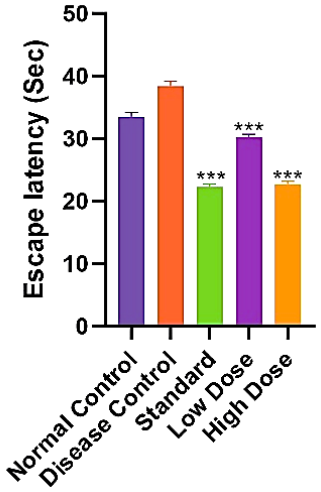

Figure 5. Effect of *J. sambac* extract on escape latency (in seconds) on the Morris water maze on 18th day. (Data compared with disease control; *** $p < 0.001$)

The disease control group exhibited markedly higher escape latency compared with the other groups. On the 15th acquisition (learning) and 16th retention (memory) days, the normal control, standard, and both low- and high-dose extract groups exhibited a significant reduction ($p < 0.05$) in escape latency relative to the disease control group. Escape latency was significantly higher ($p < 0.05$) in the disease control group relative to the normal control group. From the 18th day onwards, both the low-dose and high-dose treatment groups exhibited a significant increase ($p < 0.05$) in escape latency relative to the standard donepezil group. The group that received a high dose (400 mg/kg) showed a significant decrease ($p < 0.05$) in escape latency compared with the group received a low-dose. Escape latency in the standard donepezil group did not differ significantly ($p > 0.05$) from that in the high-dose extract group. The treatment group reduces the time of escape latency in the Morris water maze, indicating improvement in the cognitive function of the brain.

Actophotometer

The actophotometer was used to determine the locomotor activity of *J. sambac* leaf extract in rats. Results of locomotor activity are given in Table 7 and Figure 6.

Table 7. Effect of treatment on the locomotor activity

Group	Drug and Dose	Locomotor Activity Score
Normal Control	Normal saline (*p.o.*)	$498 \pm 3.06^{\#c}$
Disease Control	Scopolamine (1 mg/kg, *i.p.*)	$214 \pm 7.01^{\$c}$
Standard Drug	Donepezil 5 mg/kg (*p.o.*) + Scopolamine (1 mg/kg, i.p.)	$502 \pm 4.47^{\#c}$
Low-dose Extract	*J. sambac* 200 mg (*p.o.*) + Scopolamine (1 mg/kg, *i.p.*)	$308 \pm 3.26^{\#c,+c}$
High-dose Extract	*J. sambac* 400 mg (*p.o.*) + Scopolamine (1 mg/kg, *i.p.*)	$411 \pm 2.95^{\#c,+c,*c}$

All data are presented as mean ± SEM (n=6 animals per group). Statistical comparisons were performed using one-way ANOVA with Tukey's post hoc test for multiple comparisons. a: $p < 0.05$, b: $p < 0.01$, c: $p < 0.001$, #: Data compared with disease control, $: Data compared with control, +: Data compared with standard, *: Data compared with low-dose group, ** Data compared with high dose group

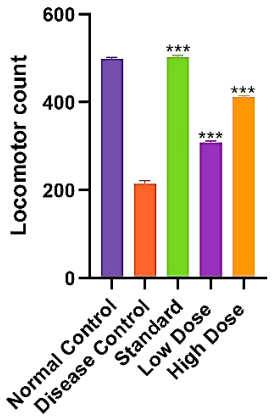

Figure 6. Effect of *J. sambac* extract on locomotor activity (in seconds) on the actophotometer. (Data compared with disease control; *** $p < 0.001$)

Treatment of the disease control group with scopolamine hydrobromide (1 mg/kg, *i.p.*) for 7 days induced learning and memory deficits, as evidenced by decreased locomotor counts. The normal control, standard, low-dose, and high-dose extract groups showed significant increases ($p < 0.05$) in locomotor activity compared with the disease control group. The standard drug donepezil showed no significant difference in locomotor activity ($p > 0.05$) compared with the normal control group. On the 15th and 16th days, both the low-dose and high-dose treatment groups showed a significant decrease ($p < 0.05$) in locomotor activity relative to the standard donepezil group. Locomotor activity was significantly reduced ($p < 0.05$) in the disease control group relative to the normal control group. The high-dose (400 mg/kg) group showed a significantly higher locomotor count ($p < 0.05$) compared with the low-dose group. The observed increase in locomotor activity in the extract-treated group suggests a potential enhancement of memory function in rats.

Brain Acetylcholinesterase Activity

The acetylcholinesterase activity was performed to determine the acetylcholinesterase level in the brain. Result of acetylcholinesterase activity is given in Table 8. The normal control, standard, low-dose, and high-dose extract groups exhibited lower AChE levels in the rat brain. The disease control group showed the highest acetylcholinesterase level. The acetylcholinesterase level decreased in both extract groups (200 and 400 mg/kg), indicating improvement of memory in rats.

Table 8. Effect of treatment on Acetylcholinesterase activity

Group	Drug and Dose	Acetylcholinesterase Activity (µ moles/ml)
Normal Control	Normal saline (*p.o.*)	20.5
Disease Control	Scopolamine (1 mg/kg, *i.p.*)	22.78
Standard Drug	Donepezil 5 mg/kg (p.o.) + Scopolamine (1 mg/kg, *i.p.*)	18.22
Low-dose Extract	*J. sambac* 200 mg (p.o.) + Scopolamine (1 mg/kg, *i.p.*)	19.93
High-dose Extract	*J. sambac* 400 mg (*p.o.*) + Scopolamine (1 mg/kg, *i.p.*)	16.52

The present study aimed to evaluate the anti-amnesic activity of *J. sambac* in rats. Previous studies have demonstrated that medicinal plants contribute significantly to the enhancement of cognitive functions in experimental animal models. This study explored the effect of the ethanolic extract of *J.*

sambac leaves on amnesic rats. Both *in vitro* and *in vivo* methods were employed to evaluate and confirm the anti-amnesic effects of *J. sambac* in rats.

The ethanolic extract was prepared by the maceration method. The practical yield of the extract was ~4.9 % w/w. The phytochemical investigation of the ethanolic extract of *J. sambac* confirms the presence of flavonoids, tannin, phenols, anthraquinones, saponin and coumarins except alkaloid [6,20]. Quantitative estimation of ethanolic extract indicated the presence of 0.29 mg GAE/g of leaf powder as phenols and 0.39 mg QE/g of leaf powder flavonoids. Observed phenolic content is less than the literature value reported for flower extract. Leaf extract contains a higher percentage of flavonoids than the flower extract [26].

GC-MS analysis identified 29 bioactive compounds in the extract, six of which have antioxidant activity. The flower extract contains bioactive compounds such as phytol and squalene, which are also present in *J. sambac* leaf extract. The antioxidant activity of leaf bioactive compounds may play a role in attenuating memory loss. The observed antioxidant activity of *J. sambac* leaf extract may be attributed to the combined and synergistic actions of multiple bioactive compounds [34-47] identified by GC-MS analysis [17,49]. A recent GC–MS study by Vishnupandi and Ganga (2025) on colchicine-induced *J. sambac* floral variants reported terpenoids, fatty acid esters, and long-chain hydrocarbons as major volatile constituents. In agreement with these findings, GC–MS analysis of the *J. sambac* leaf extract in the present study also revealed fatty acid ethyl esters (e.g., hexadecanoic acid ethyl ester, octadecanoic acid ethyl ester, ethyl oleate), terpenoid-related compounds (phytol, isophytol, neophytadiene, squalene), and long-chain hydrocarbons. The observed differences in compound abundance are likely attributable to variations in plant organ (leaf vs flower), genetic background (native vs colchicine-induced variants), and extraction methodology. Nevertheless, the presence of similar chemical classes across studies supports the consistent phytochemical profile of *J. sambac* and its potential contribution to antioxidant and neuroprotective activities [50].

The maximum percentage inhibition of the ethanolic extract and standard BHT was found to be 62.55% and 71.33%, respectively, at a concentration of 200 µg/ml. The IC_{50} value of the ethanolic leaf extract of *J. sambac* was 109.38 µg/ml. The reported IC_{50} value for *J. sambac* flower extract (208 µg/ml) is more than that of the leaf extract. Hence, one can replace flower extract with leaf extract when antioxidant activity is required [26].

OECD guideline 423 was used to perform the acute oral toxicity of the ethanolic extract. Acute toxicity evaluation demonstrated that a single oral dose of 2000 mg/kg of the ethanolic leaf extract of *J. sambac* did not induce any observable signs of toxicity in rats. Hence, ethanolic extract of *J. sambac* leaf is safe for further use in animal studies [51].

The effects of *J. sambac* on memory function in amnesic rats were assessed using multiple *in-vivo* behavioral paradigms, including the Morris water maze, elevated plus maze, hole board test, and actophotometer [52].

The scopolamine was used as a disease inducer. It blocks cholinergic signalling, thereby reducing levels of ACh, which causes memory loss [53]. Donepezil was used as a disease controller. It inhibits the acetylcholine hydrolysis, thereby increasing acetylcholine availability at the synapses and enhancing cholinergic transmission [54,55].

The transfer latency of rats was increased after administration of scopolamine in the elevated plus maze model. This effect may be associated with scopolamine-induced alterations in acetylcholinesterase activity in the cortical and hippocampal regions [56]. Treatment with both low and high doses of the extract resulted in a significant reduction in transfer latency. The significant reduction in transfer latency time indicates increased levels of ACh in the brain, which may be responsible for the anti-amnesic effect. The *J. sambac* ethanolic extract may act against scopolamine and reflect the good functioning of the memory [57].

The hole board test result showed that the scopolamine-treated group decreased the number of nose poking entries by forming senile plaque and neurofibrillary tangles in the brain [58]. The ethanolic extract of *J. sambac* significantly increases the number of nose poking entries, indicating enhancement of the cognitive functions in the rat brain [30].

The escape latency in rats was increased after administration of scopolamine in the Morris water maze model. The *J. sambac* ethanolic extract may act against scopolamine and decrease the escape

latency time. The standard drug donepezil binds reversibly to acetylcholinesterase, thereby enhancing acetylcholine levels in the brain and producing a significant reduction in escape latency time [31].

Actophotometer assessment revealed a significant decrease in locomotor activity in rats after scopolamine administration. It causes atrophy in the hippocampus and cortical region in the brain [59]. The ethanolic extract of *J. sambac* increased locomotor activity in rats. This enhancement in locomotor activity after treatment may reflect improved brain function [32].

Rats treated with low- and high- doses of *J. sambac* leaf extract exhibited lower AChE activity than the control group [60]. The study revealed that the memory-enhancing effect of *J. sambac* leaf extract may be associated with inhibition of AChE activity, leading to increased acetylcholine levels in the brain and attenuation of excessive free radical generation. It inhibits the formation of senile plaques and neurofibrillary tangles in the rat brain, thereby reducing the effects of cognitive decline [33].

The findings suggest that *J. sambac* possesses significant neuroprotective potential. Thus, it seems that *J. sambac* may prove to be useful in the treatment of AD, dementia and amnesia [48].

The ethanolic extract of *J. sambac* leaves demonstrated significant neuroprotective and anti-amnesic effects in scopolamine-induced amnesic rats, supporting its potential therapeutic role in AD and related cognitive disorders. Notably, the extract was safe even at a high dose of 2000 mg/kg, with no observed mortality.

Phytochemical analysis confirmed the presence of flavonoids, phenols, anthraquinones, saponins, and coumarins, while alkaloids were absent. The high flavonoid and phenolic content correlated with potent antioxidant activity, as evidenced by DPPH radical scavenging, which may contribute to reducing oxidative stress- a key factor in neurodegeneration. Behavioural assessments revealed improved cognitive function, including reduced transfer and escape latencies, increased exploratory behaviour (nose poking), and enhanced locomotor activity, suggesting memory enhancement.

Mechanistically, the extract exhibited acetylcholinesterase inhibitory activity, increasing acetylcholine levels in the brain, which is crucial for memory retention. Additionally, its antioxidant properties help mitigate free radical-induced damage, potentially preventing the formation of amyloid plaques and neurofibrillary tangles-hallmarks of AD. GC-MS analysis further identified bioactive compounds with known neuroprotective and antioxidant effects.

In conclusion, *J. sambac* leaf extract shows promise as a natural therapeutic agent for memory disorders, including AD and dementia, through multimodal mechanisms involving cholinergic enhancement and oxidative stress reduction. Further clinical studies are warranted to validate its efficacy in humans.

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to Adv. Vaibhav (Dada) Patil, President of the Loknete Hon. Hanmantrao Patil Charitable Trust, Vita, for providing the necessary facilities to conduct this research. The authors also acknowledge Dr. Sandip Patil from Biocyte, Sangli, for his valuable assistance with the *in vivo* studies.

AUTHOR CONTRIBUTIONS

Concept: K.M., K.H.; Design: K.M., K.H.; Control: N.S., N.M.; Sources: R.D., N.S.; Materials: K.M., K.H.; Data Collection and/or Processing: K.M., K.H., N.S.; Analysis and/or Interpretation: K.M., R.D.; Literature Review: K.H., N.S., N.M.; Manuscript Writing: K.H., K.M.; Critical Review: R.D., N.M.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

Before the execution of experimentation animal study protocol was approved by Biocyte Institute of Research and Development, Sangli, Maharashtra, India 416416 with project proposal number

IAEC/BiRD/Sangli/2024-24-04.

REFERENCES

1. Akram, M., Nawaz, A. (2017). Effects of medicinal plants on Alzheimer's disease and memory deficits. Neural Regeneration Research, 12(4), 660. [CrossRef]
2. Gale, S. A., Acar, D., Daffner, K.R. (2018). Dementia. The American Journal of Medicine, 131(10), 1161-1169. [CrossRef]
3. Hall, J. (1887). Amnesia. Science, ns-10(250), 250-251. [CrossRef]
4. Alzheimer's Association Report (2020). 2020 Alzheimer's disease facts and figures. Alzheimer's & Dementia, 16(3), 391-460. [CrossRef]
5. Breijyeh, Z., Karaman, R. (2020). Comprehensive review on Alzheimer's disease: Causes and treatment. Molecules, 25(24), 5789. [CrossRef]
6. Khan, I.A., Hussain, M., Munawar, S.H., et al. (2021). *Jasminum sambac*: A potential candidate for drug development to cure cardiovascular ailments. Molecules, 26(18), 5664. [CrossRef]
7. AlRashdi, A.S., Salama, S.M., Alkiyumi, S.S., et al. (2012). Mechanisms of gastroprotective effects of ethanolic leaf extract of *Jasminum sambac* against HCl/ethanol-induced gastric mucosal injury in rats. Evidence-Based Complementary and Alternative Medicine, 2012, 1-15. [CrossRef]
8. Kalaiselvi, M., Narmadha, R., Ragavendran, P., et al. (2013). Chemopreventive effect and HPTLC fingerprinting analysis of *Jasminum sambac* (L.) Ait. Extract against DLA-induced lymphoma in experimental animals. Applied Biochemistry and Biotechnology, 169(4), 1098-1108. [CrossRef]
9. Santhanam, J., Abd Ghani, F.N., Basri, D.F. (2014). Antifungal activity of *Jasminum sambac* against *Malassezia* sp. and non- *Malassezia* sp. isolated from human skin samples. Journal of Mycology, 2014, 1-7. [CrossRef]
10. Sengar, N., Joshi, A., Prasad, S.K., et al. (2015). Anti-inflammatory, analgesic and anti-pyretic activities of standardized root extract of *Jasminum sambac*. Journal of Ethnopharmacology, 160, 140-148. [CrossRef]
11. El-Hawary, S.S., EL-Hefnawy, H.M., Osman, S.M., et al. (2021). Phenolic profiling of different *Jasminum* species cultivated in Egypt and their antioxidant activity. Natural Product Research, 35(22), 4663-4668. [CrossRef]
12. Redfern, J., Kinninmonth, M., Burdass, D., et al. (2014). Using Soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. Journal of Microbiology & Biology Education, 15(1), 45-46. [CrossRef]
13. Umar, U., Ahmed, S., Iftikhar, A., et al. (2023). Phenolics extracted from *Jasminum sambac* mitigates diabetic cardiomyopathy by modulating oxidative stress, apoptotic mediators and the Nfr-2/HO-1 pathway in alloxan-induced diabetic rats. Molecules, 28(14), 5453. [CrossRef]
14. Beena, V., Richa, M. (2023). Evaluation of *in-vivo* antioxidant of ethanolic extract of *Jasminum sambac* flower. International Journal of Pharmaceutical and Biological Science Archive, 11(5), 60-67.
15. Ceccacci, S., Lucia, A. De, Tortora, A., et al. (2022). *Jasminum sambac* cell extract as antioxidant booster against skin aging. Antioxidants, 11(12), 2409. [CrossRef]
16. Minocha, T., Birla, H., Obaid, A.A., et al. (2022). Flavonoids as promising neuroprotectants and their therapeutic potential against Alzheimer's disease. Oxidative Medicine and Cellular Longevity, 2022, 1-13. [CrossRef]
17. Ayaz, M., Sadiq, A., Junaid, M., et al. (2017). Neuroprotective and anti-aging potentials of essential oils from aromatic and medicinal plants. Frontiers in Aging Neuroscience, 9, 168. [CrossRef]
18. Gupta, J., Kulshreshtha, M. (2018). Pharmacological investigation and unraveling mechanism of action of jasminum sambac flowers for predicated treatment of Alzheimer's disease. Current Nutrition & Food Science, 14(2), 128-135. [CrossRef]
19. Dhote, V., Dangi, U., Mandloi, A.S., et al. (2021). Preferential cyclooxygenase inhibition by Jasminum sambac: A possible relationship with potent anti-arthritic activity. Journal of Traditional and Complementary Medicine, 11(3), 217-227. [CrossRef]
20. Dubale, S., Kebebe, D., Zeynudin, A., et al. (2023). Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia. Journal of Experimental Pharmacology, 15, 51-62. [CrossRef]
21. Gul, R., Jan, S.U., Faridullah, S., et al. (2017). Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from ephedra intermedia indigenous to Balochistan. The Scientific World Journal, 2017(1), 5873648. [CrossRef]
22. Mali, K.K., Salunkhe, N.H., Jadhav, B.D., et al. (2025). *In vivo* evaluation of analgesic activity of *Indigofera cordifolia* B. Heyne Ex. Roth in mice compared to diclofenac, tramadol, and indomethacin.

Bulletin of Pharmaceutical Sciences Assiut University, 48(2), 1599-1612. [CrossRef]
23. Phuyal, N., Jha, P.K., Raturi, P.P., et al. (2020). Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of *Zanthoxylum armatum* DC. The Scientific World Journal, 2020, 1-7. [CrossRef]
24. Anima, P., Arun, M., Satish, S. (2019). Scientific validation of wound healing potential of *Jasminum sambac* Ait. South African Journal of Botany, 121, 584-589. [CrossRef]
25. Willie, P., Uyoh, E.A., Aikpokpodion, P.O. (2020). Gas Chromatography-Mass Spectrometry (GC-MS) assay of bio-active compounds and phytochemical analyses in three species of Apocynaceae. Pharmacognosy Journal, 13(2), 383-392. [CrossRef]
26. Wu, L.C., Lin, C.L., Peng, C.C., et al. (2021). Development from *Jasminum sambac* flower extracts of products with floral fragrance and multiple physiological activities. Evidence Based Complementary and Alternative Medicine, 2021, 1-12. [CrossRef]
27. Mali, K.K., Kokate, M.D., Ghorpade, V.S., et al. (2025). Exploring the antiarthritic potential of *Caralluma fimbriata*: Phytochemical screening and preliminary observations in a rat model. Journal of Ethnopharmacology, 353, 120417. [CrossRef]
28. OECD website. (2002). Test No. 423: Acute Oral toxicity - Acute Toxic Class Method. Oecd Guideline for Testing of Chemicals Section 4. https://www.oecd.org/en/publications/test-no-423-acute-oral-toxicity-acute-toxic-class-method_9789264071001-en.html/. Accessed date: 04.01.2025.
29. Vasudevan, M., Parle, M. (2007). Memory enhancing activity of Anwala churna (*Emblica officinalis* Gaertn.): An ayurvedic preparation. Physiology & Behavior, 91(1), 46-54. [CrossRef]
30. Apu, A., Hossain, F., Bhuyan, S., et al. (2013). Study of pharmacological activities of methanol extract of *Jatropha gossypifolia* fruits. Journal of Basic and Clinical Pharmacy, 4(1), 20-24. [CrossRef]
31. Dhingra, D., Kumar, V. (2012). Memory-enhancing activity of palmatine in mice using Elevated Plus Maze and Morris Water Maze. Advances in Pharmacological Sciences, 2012, 1-7. [CrossRef]
32. Goverdhan, P., Sravanthi, A., Mamatha, T. (2012). Neuroprotective effects of meloxicam and selegiline in scopolamine-induced cognitive impairment and oxidative stress. International Journal of Alzheimer's Disease, 2012, 1-8. [CrossRef]
33. Bhattacharjee, A., Shashidhara, S.C., Saha, S. (2015). Nootropic activity of *Crataeva nurvala* Buch-Ham against scopolamine induced cognitive impairment. EXCLI journal, 14, 335-45.
34. Vanitha, V., Vijayakumar, S., Nilavukkarasi, M., et al. (2020) Heneicosane-A novel microbicidal bioactive alkane identified from *Plumbago zeylanica* L. Industrial Crops and Products, 154, 112748. [CrossRef]
35. Hasan M.R., Haque, M.M., Hoque, M.A., et al. (2024). Antioxidant activity study and GC-MS profiling of *Camellia sinensis* Linn. Heliyon, 10(1), e23514. [CrossRef]
36. Kumar, A., Kaur, S., Dhiman, S., et al. (2022). Targeting Akt/NF-κB/p53 pathway and apoptosis inducing potential of 1,2-benzenedicarboxylic acid, bis(2-methylpropyl)ester isolated from *Onosma bracteata* Wall. against human osteosarcoma (MG-63) cells. Molecules, 27(11), 3478. [CrossRef]
37. Rajeswaran, S., Rajan, D.K. (2025). Neophytadiene: Biological activities and drug development prospects. Phytomedicine, 143, 156872. [CrossRef]
38. Al-Bahadily, D., Shari, F., Najm, M., et al. (2019). Antimicrobial activity of the compound 2-piperidinone, *N*-[4-bromo-*n*-butyl]-extracted from Pomegranate peels. Asian Journal of Pharmaceutics, 13(1), 46-53.
39. Tao, R., Wang, C.Z., Kong, Z.W. (2013). Antibacterial/antifungal activity and synergistic interactions between polyprenols and other lipids isolated from *Ginkgo biloba* L. Leaves. Molecules, 18(2), 2166-82. [CrossRef]
40. Nisa, S., Bibi, Y., Masood, S., et al. (2022) Isolation, characterization and anticancer activity of two bioactive compounds from *Arisaema flavum* (Forssk.) Schott. Molecules, 27(22), 7932. [CrossRef]
41. Shaaban, M.T., Ghaly, M.F., Fahmi, S.M. (2021). Antibacterial activities of hexadecanoic acid methyl ester and green-synthesized silver nanoparticles against multidrug-resistant bacteria. Journal of Basic Microbiology, 61, 557-568. [CrossRef]
42. Ganesh, M., Mohankumar, M. (2017). Extraction and identification of bioactive components in *Sida cordata* (Burm.f.) using gas chromatography-mass spectrometry. Jornal of Food Science and Technology, 54, 3082–3091. [CrossRef]
43. Padmashree, M.S., Ashwathanarayana, R., Naika, R., et al. (2018) Antioxidant, cytotoxic and nutritive properties of *Ipomoea staphylina* Roem & Schult. plant extracts with preliminary phytochemical and GCMS analysis. Asian Journal of Pharmacy and Pharmacology, 4(4), 473-492. [CrossRef]
44. Swantara, M.D., Rita, W.S., Suartha, N., et al. (2019). Anticancer activities of toxic isolate of *Xestospongia testudinaria* sponge. Veterinary World, 12(9), 1434-1440. [CrossRef]
45. Javed, M.R., Salman, M., Tariq, A., et al. (2022). The antibacterial and larvicidal potential of bis-(2-ethylhexyl)phthalate from *Lactiplantibacillus plantarum*. Molecules, 27(21), 7220. [CrossRef]

46. Popa, I., Babeanu, N., Nita, S., et al. (2014). Squalene-natural resources and applications. Farmacia, 62(5), 840-862. [CrossRef]
47. Liu, X.L., Sato, S., Dai, W., et al. (2001). The protective effect of hepatocyte growth-promoting factor (pHGF) against hydrogen peroxide-induced acute lung injury in rats. Medical Electron Microscopy, 34(2), 92-102. [CrossRef]
48. Mali, K.K., Sutar, G.V, Dias, R.J., et al. (2021). Evaluation of nootropic activity of *Limonia acidissima* against scopolamine-induced amnesia in rats. Turkish Journal of Pharmaceutical Sciences, 18(1), 3-9. [CrossRef]
49. Pham-Huy, L.A., He, H., Pham-Huyc, C. (2008). Free radicals, antioxidants in disease and health. International Journal of Biomedical Science, 4(2), 89-96. [CrossRef]
50. Vishnupandi, S., Ganga, M., Rajamani, K., et al. (2025) Floral volatile composition of *Jasminum sambac* variants developed through colchicine. Natural Product Research, 39(9), 2444-2452. [CrossRef]
51. Kunhachan, P., Banchonglikitkul, C., Kajsongkram, T., et al. (2012). Chemical composition, toxicity and vasodilatation effect of the flowers extract of *Jasminum sambac* (L.) Ait. "G. Duke of Tuscany." Evidence Based Complementary and Alternative Medicine, 2012, 1-7. [CrossRef]
52. Parle, M., Bansal, N. (2011). Antiamnesic activity of an ayurvedic formulation chyawanprash in mice. Evidence Based Complementary and Alternative Medicine, 2011(1), 1-10. [CrossRef]
53. Thongrong, S., Promsrisuk, T., Sriraksa, N., et al. (2024). Alleviative effect of scopolamine-induced memory deficit via enhancing antioxidant and cholinergic function in rats by pinostrobin from *Boesenbergia rotunda* (L.). Biomedical Reports, 21(3), 130. [CrossRef]
54. Rahimzadegan, M., Soodi, M. (2018). Comparison of memory ımpairment and oxidative stress following single or repeated doses administration of scopolamine in rat hippocampus. Basic and Clinical Neuroscience Journal, 9(1), 5-14. [CrossRef]
55. Asiri, Y.A., Mostafa, G.A.E. (2010). Donepezil. Profiles of Drug Substances, Excipients and Related Methodology, 35,117-150. [CrossRef]
56. Otari, K.V., Bichewar, O.G., Shete, R.V., et al. (2012). Effect of hydroalcoholic extract of *Vitex negundo* Linn. leaves on learning and memory in normal and cognitive deficit mice. Asian Pacific Journal of Tropical Biomedicine, 2(1), S104-S111. [CrossRef]
57. Li, R., Chan, W., Mat, W., et al. (2017). Antiaging and anxiolytic effects of combinatory formulas based on four medicinal herbs. Evidence Based Complementary and Alternative Medicine, 2017(1), 4624069. [CrossRef]
58. Casarrubea, M., Di Giovanni, G., Aiello, S., et al. (2023). The hole-board apparatus in the study of anxiety. Physiology & Behavior, 271, 114346. [CrossRef]
59. George, S.D., Archana, R., Parasuraman, S. (2022). Caloric vestibular stimulation induced enhancement of behavior and neurotrophic factors in chronic mild stress induced rats. Frontiers in Pharmacology, 13, 1-9. [CrossRef]
60. Yadang, F.S.A., Nguezeye, Y., Kom, C.W., et al. (2020). Scopolamine-induced memory impairment in mice: Neuroprotective effects of *Carissa edulis* (Forssk.) Valh (Apocynaceae) aqueous extract. International Journal of Alzheimer's Disease, 2020, 1-10. [CrossRef]