

Multimodal Evaluation of *Oureta lanata* (L.) Kuntze against Scopolamine-induced Alzheimer's-like Neurocognitive dysfunction in Wistar Albino Rats

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder associated with cognitive decline, cholinergic dysfunction, oxidative stress, and neuronal damage. The present study evaluated the neuroprotective and anti-amnesic potential of *Oureta lanata* (L.) Kuntze stem extracts against scopolamine hydrobromide-induced AD-like neurocognitive dysfunction in Wistar albino rats. Petroleum ether, chloroform, ethanolic, aqueous, and total aqueous extracts were prepared and subjected to physicochemical, phytochemical, TLC, FTIR, and LC-MS analyses. In vitro acetylcholinesterase (AChE) inhibitory and DPPH radical-scavenging activities were assessed. Acute oral toxicity of the total aqueous extract was evaluated according to OECD 423. Cognitive and behavioral functions were assessed using the Morris water maze, T-maze, Y-maze, novel object recognition, open-field, and locomotor activity tests. Brain AChE, lipid peroxidation, superoxide dismutase, and reduced glutathione were estimated, followed by hippocampal histopathological examination. The total aqueous extract showed the highest extraction yield (3.48%), while LC-MS analysis identified several bioactive constituents in the ethanolic extract, including naringenin, catechin, acacetin, an isorhamnetin derivative, a kaempferol-derived conjugate, and a rutin-type glycoside. The ethanolic extract exhibited strong AChE inhibition (87% at 400 µg/mL; IC₅₀ 33.04 µg/mL), while ethanolic, aqueous, and total aqueous extracts showed antioxidant activity. Treatment improved cognitive and behavioral performance and attenuated scopolamine-induced alterations in AChE, lipid peroxidation, SOD, and GSH levels. Histopathology further supported neuronal preservation. Overall, *Oureta lanata* (L.) Kuntze stem extracts, particularly the ethanolic and total aqueous extracts, demonstrated promising anti-amnesic, antioxidant, and neuroprotective effects.

Keywords: *Oureta lanata* (L.) Kuntze; Acetylcholinesterase; Antioxidant activity; Scopolamine; Neuroprotection. 1.

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INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by deterioration of memory, learning, cognitive abilities and functional independence [1]. The disease involves complex pathological changes including amyloid-β accumulation, tau hyperphosphorylation, synaptic dysfunction and neuronal loss [2]. Cholinergic dysfunction, neuroinflammation and oxidative stress further contribute to neuronal and cognitive impairment [3].

Acetylcholine is an important neurotransmitter involved in learning, memory and attention [4]. Acetylcholinesterase (AChE) hydrolyses acetylcholine at cholinergic synapses, and inhibition of this enzyme enhances cholinergic neurotransmission and stays an established approach for the symptomatic management of cognitive impairment in AD [5]. However, currently available pharmacological treatments provide mainly symptomatic benefits, and the multifactorial nature of AD has encouraged the investigation of therapeutic strategies capable of targeting multiple pathological mechanisms [6].

Oxidative stress is an important factor in AD-associated neuronal damage. Excessive production of reactive oxygen and nitrogen species can overwhelm endogenous antioxidant defense systems, resulting in lipid peroxidation, mitochondrial dysfunction, protein damage and neuronal injury [7]. The interaction between oxidative stress, neuroinflammation and other pathological processes may further contribute to progressive cognitive decline. Therefore, therapeutic agents that have antioxidant and neuroprotective properties may provide complementary benefits in the management of neurodegenerative disorders.

Medicinal plants and their bioactive phytochemicals have gained considerable attention as potential sources of neuroprotective agents. Phenolic compounds, flavonoids and other secondary metabolites have been associated with antioxidant, anti-

inflammatory and anticholinesterase activities [8]. Recent studies have highlighted the potential of medicinal plants to influence multiple mechanisms involved in AD, supporting their investigation as multitarget therapeutic candidates [9].

Oureta lanata (L.) Kuntze, belonging to the family Amaranthaceae, is a medicinal plant traditionally used for various ailments. Phytochemical investigations have reported the presence of diverse secondary metabolites, particularly phenolic and flavonoid constituents, in different parts of the plant [10]. These constituents may contribute to the antioxidant and other pharmacological activities reported for *Oureta lanata* (L.) Kuntze extracts [11]. Recent analytical studies have further supported the presence of bioactive constituents in the plant [12]. However, the neuroprotective potential of *Oureta lanata* (L.) Kuntze, particularly its stem extracts, against Alzheimer's disease-like cognitive dysfunction has not been comprehensively investigated.

Scopolamine-induced cognitive impairment is a widely used pharmacological model for evaluating learning and memory deficits associated with cholinergic dysfunction [13]. Scopolamine produces reproducible cognitive impairment through blockade of central muscarinic cholinergic signaling and is therefore useful for screening potential cognition-enhancing and neuroprotective agents [14]. Recent experimental studies have continued to employ this model for evaluating behavioral, biochemical and neuroprotective responses [15]. Although the model does not reproduce the complete pathological spectrum of human AD, it provides a useful experimental approach for studying cholinergic-related cognitive dysfunction.

Considering the multifactorial nature of AD and the potential benefits of agents with complementary pharmacological activities, the present study was designed to characterize the stem extracts of *Oureta lanata* (L.) Kuntze and evaluate their neuroprotective potential using complementary in-vitro and in-vivo approaches [16].

The study included phytochemical and analytical characterization, in-vitro AChE inhibitory and antioxidant assays, and multimodal in-vivo evaluation using scopolamine hydrobromide-induced Alzheimer's disease-like neurocognitive dysfunction in albino rats [17]. Behavioral, biochemical and hippocampal histopathological assessments were performed to provide an integrated evaluation of the neuroprotective potential of the stem extracts [18].

Neuropathology of Alzheimer's Disease (AD)

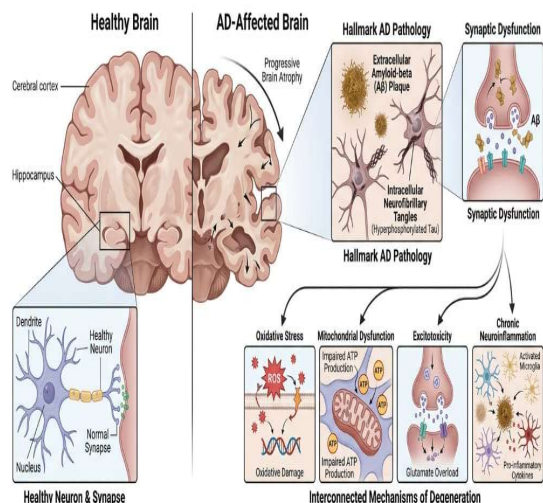


Figure 1. Conceptual neuropathology of Alzheimer's disease reproduced from the thesis.

2. MATERIALS AND METHODS

2.1 Plant Material and Authentication:

The stems of *Oureit lanata* (L.) Kuntze were collected from the Western Ghats region of Tamil Nadu, India, on [28-12-2026]. The stems were washed, shade-dried at room temperature (25–30°C) for 7–10 days and powdered. The plant material was authenticated by Dr. M. Murugan, Department of Botany, Ayyanadar Janakiammal College, Tamil Nadu, based on standard morphological and taxonomical characteristics [19].



Figure 2. Whole plant of *Oureit lanata* (L.) Kuntze (L.) Kuntze

2.2 Pharmacognostic Evaluation:

Fresh stems and powdered material were evaluated for organoleptic, macroscopic and microscopic characteristics. Transverse sections were stained with safranin, mounted in glycerine and examined microscopically. Powder microscopy was performed to find diagnostic structures such as epidermal cells, parenchyma, fibres and xylem vessels. Physicochemical parameters, including loss on drying, total ash, acid-insoluble ash and water-soluble ash, were decided using standard pharmacognostic procedures [20].



Figure 3. Stem part of *Oureit lanata* (L.) Kuntze

2.3 Extraction and Percentage Yield:

The dried powdered stem was subjected to successive cold maceration with petroleum ether, chloroform, ethanol and distilled water. Approximately 250 g of powdered material was macerated with each solvent for 72 h with occasional stirring. A separate 250 g part was directly extracted with distilled water to obtain the total aqueous extract. The extracts were filtered, concentrated using a rotary evaporator at controlled temperature and dried. The dried extracts were stored in airtight containers at 4°C until further use [21,22].

$$\text{Percentage yield (\%)} = \left(\frac{\text{Weight of dried extract}}{\text{Weight of crude drug}} \right) \times 100$$

2.4 Preliminary Phytochemical Screening:

The petroleum ether, chloroform, ethanolic, aqueous and total aqueous extracts were subjected to preliminary phytochemical screening using standard qualitative chemical tests [23]. The extracts were examined for alkaloids, carbohydrates, flavonoids, phenolic compounds, tannins, saponins, glycosides, steroids, terpenoids, fixed oils and fats, and gums and mucilage [24].

2.5 TLC Profiling:

TLC profiling of ethanolic, aqueous and total aqueous extracts was performed on pre-coated silica gel 60 F254 plates using ethyl acetate: methanol: water (5:4:1, v/v/v) as the mobile phase. The developed plates were examined under UV light at 254 and 366 nm, and Rf values were calculated [25].

$$R_f = \frac{\text{Distance travelled by the compounds}}{\text{Distance travelled by the solvent front}}$$

2.6 FTIR Analysis:

FTIR analysis of the ethanolic, aqueous and total aqueous extracts was performed using KBr pellet method. Approximately 1 mg of each extract was mixed with 100 mg spectroscopic-grade KBr, compressed into pellets and scanned over 4000–400 cm⁻¹ at a resolution of 4 cm⁻¹ with 16 scans. Characteristic absorption bands were assigned to the corresponding functional groups based on standard spectral data [26].

2.7 LC-MS Analysis:

The dried ethanolic stem extract of *Oureit lanata* (L.) Kuntze was dissolved in HPLC-grade methanol (1 mg/mL), sonicated for 10 min and filtered through a 0.22 μm membrane filter. Chromatographic separation was performed using a reverse-phase C18 column (250 × 4.6 mm, 5 μm) with water having 0.1% formic acid (mobile phase A) and acetonitrile having 0.1% formic acid (mobile phase B) under gradient elution. The flow rate was 0.5 mL/min, the injection volume was 10 μL and the column temperature was maintained at 30°C. Mass spectrometric detection was performed using electrospray ionization in positive and negative modes over an m/z range of 100–1000, with a capillary voltage of 3.5 kV and a drying gas temperature of 350°C. Putative phytoconstituents were characterized based on retention times, molecular ion peaks and fragmentation patterns, with reference to published literature and spectral databases [27].

2.8 In-Vitro Studies:

AChE Inhibitory Assay:

AChE inhibitory activity was decided using a modified Ellman colorimetric method [28]. Ethanolic, aqueous and total aqueous extracts were tested at 25, 50, 100, 200 and 400 μg/mL, with donepezil as the reference standard. The reaction mixture had

acetylthiocholine iodide (15 mM), DTNB (3 mM), Tris-HBr buffer (50 mM, pH 8.0) having 0.1% BSA, AChE and test sample. After 5 min incubation at room temperature, absorbance was measured at 415 nm. Percentage inhibition and IC₅₀ values were decided from the concentration-response relationship [28].

$$\% \text{ Inhibition} = \frac{\text{Change in OD per min of control} - \text{Change in OD per min of test samples}}{\text{Change in OD per min of control}} \times 100$$

DPPH Radical Scavenging Assay:

DPPH radical scavenging activity was evaluated for the ethanolic, aqueous and total aqueous extracts using ascorbic acid as the standard [29]. Extracts were tested at 20, 40, 60, 80 and 100 µg/mL. Equal volumes of sample and DPPH solution were mixed and incubated in the dark for 30 min at room temperature, followed by absorbance measurement at 517 nm [30].

$$\text{DPPH Scavenging activity (\%)} = \frac{[(A \text{ control} - A \text{ sample}) / A \text{ control}] \times 100}{}$$

2.9 In- Vivo Studies:

Experimental Animals and Design:

Healthy adult Wistar albino rats (180–220 g) were maintained under standard laboratory conditions (22 ± 2°C, 50–60% relative humidity, 12 h light/dark cycle) with food and water ad libitum and acclimatized for one week. The study was approved by the Institutional Animal Ethics Committee (Registration No: AKCP/IAEC/05/2025-2026). (Sanction No. 30 Animals, dated [27-12-2026]) [31]. Twenty-four rats were randomly divided into six groups (n = 4) and treated for 14 days. Scopolamine hydrobromide (1 mg/kg) was used to induce cognitive dysfunction, while donepezil (1 mg/kg) served as the standard. The groups comprised normal control (Saline), Disease control (scopolamine HBr 1 mg/kg), Standard (donepezil 1 mg/kg) + scopolamine HBr (1 mg/kg), and ethanolic extract of *Ourel lanata* (L.) Kuntze (400 mg/kg) + scopolamine HBr (1 mg/kg), aqueous extract *Ourel lanata* (L.) Kuntze (400 mg/kg) + scopolamine HBr (1 mg/kg) and total aqueous extract *Ourel lanata* (L.) Kuntze (400 mg/kg) + scopolamine HBr (1 mg/kg) [32].

Acute Oral Toxicity:

Acute oral toxicity of the total aqueous extract was evaluated according to OECD Guideline 423 [33]. Female Wistar rats received a single oral dose of 2000 mg/kg by gavage and were seen for toxic signs and mortality for 14 days. Behaviour, food and water intake, and body weight were also checked. As no mortality or treatment-related toxicity was seen, the oral LD₅₀ was >2000 mg/kg [33].

Morris Water Maze:

Spatial learning and memory were assessed using the Morris Water Maze. Animals received three days of acquisition training with four trials per day, and escape latency was recorded. On day 4, a 60-s probe trial was conducted, and time spent in the target quadrant was recorded as an index of spatial memory retention [34,35].

$$\text{Escape Latency (Acquisition):} = \frac{\text{Time from trial start to platform reach (in seconds)}}{}$$

$$\text{Target Quadrant Preference (Probe Trial):} = \frac{\text{Target Preference \%}}{\text{Time spent in target quadrant / Total trial time} \times 100}$$

T-Maze:

Working memory was evaluated using a T-maze. Animals were acclimatized to the apparatus for 30 min and allowed to explore the maze according to the experimental protocol. Correct arm choices and correct trials were recorded as measures of working memory [36].

$$\text{Correct Response \%} = \frac{\text{Number of correct choices}}{\text{Total number of trials}} \times 100$$

Y-Maze Test:

A Y-maze with three identical arms (40 × 10 × 15 cm) arranged at 120°, 70% ethanol and a stopwatch were used. Animals were acclimatized for 30 min and allowed to explore the maze freely for 5 min. The sequence and number of arm entries were recorded, and percentage spontaneous alternation was calculated. The maze was cleaned with 70% ethanol between animals [37].

$$\text{Spontaneous alternation (\%)} = \frac{[\text{Number of actual alternations} / (\text{Total arm entries} - 2)] \times 100}{}$$

Spontaneous Locomotor Activity Test:

A digital Actophotometer, 70% ethanol and a stopwatch were used. Animals were acclimatized to the experimental room for 30 min and individually placed in the Actophotometer chamber for 5 min. Locomotor activity was automatically recorded as infrared beam interruption counts. The chamber was cleaned with 70% ethanol between animals, and total activity counts were recorded [38].

$$\text{Total activity} = \text{Total number of beam interruptions during 5 min}$$

Open Field Test:

An open field apparatus, 70% ethanol and a stopwatch were used. Animals were acclimatized for 30 min and individually allowed to explore the arena for 5 min. The number of squares crossed (ambulation), rearing, grooming and time spent in the centre were recorded. The apparatus was cleaned with 70% ethanol between animals [38].

$$\text{Ambulation} = \text{Total number of squares crossed during 5 min}$$

Novel Object Recognition:

Recognition memory was assessed using the Novel Object Recognition test. Animals were familiarized with the arena and two identical objects, followed by exposure to one familiar and one novel object after the retention interval. Exploration time was recorded, and the Recognition Index was calculated [39].

$$\text{DI} = \frac{\text{Time novel} - \text{Time familiar}}{\text{Time novel} + \text{Time familiar}}$$

2.10 Biochemical Estimations:

After behavioral assessment, animals were sacrificed under appropriate anesthesia, and brains were exercised, washed with ice-cold saline, and homogenized in 0.1 M phosphate buffer (pH 7.4) to obtain a 10% w/v homogenate. The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C, and the supernatant was used for biochemical analysis. Brain AChE activity was estimated by the Ellman method at 412 nm [40]. Lipid peroxidation was assessed by TBARS/MDA estimation at 532 nm [41]. SOD activity was determined based on inhibition of nitro blue tetrazolium reduction and expressed as unit's/min/mg protein [42]. Reduced glutathione was estimated using DTNB and expressed as µg/mg [43].

2.11 Histopathological Evaluation:

Hippocampal tissues were fixed in 10% neutral buffered formalin, processed, paraffin-embedded and sectioned at 4–5 µm. Sections were stained with haematoxylin and eosin and examined microscopically for neuronal degeneration and architectural alterations [44]. Changes were graded as 0 = none, 1 = minimal, 2 = mild and 3 = moderate multifocal neuronal degeneration [45].

2.12 Statistical Analysis:

In-vitro data were expressed as Mean ± SD (n = 3) and analysed descriptively. *In-vivo* data were expressed as Mean ± SEM (n = 4). Statistical analysis was performed using GraphPad Prism, with one-way ANOVA followed by Tukey's multiple comparisons test. A p value < 0.05 was considered statistically significant [46].

3. RESULT

3.1 Pharmacognostic Evaluation of the Stem of *Ourel lanata* (L.) Kuntze:

The stem of *Ourel lanata* (L.) Kuntze was greenish brown in color, with a characteristic odour, slightly bitter taste and coarse texture. Macroscopic evaluation revealed an herbaceous, cylindrical stem with a slightly rough surface, green to brown color, fibrous fracture, and hard texture. Transverse section microscopy showed a distinct epidermal layer with parenchymatous cells and well-defined vascular tissues. Powder microscopy revealed characteristic epidermal cells, parenchymatous tissues, fibers and xylem vessels. Physicochemical evaluation of the stem powder showed a loss on drying of 7.24 % w/w, total ash value of 8.36 % w/w, acid-insoluble ash value of 1.42 % w/w and water-soluble ash value of 2.68 % w/w.

3.2 Extraction and Percentage Yield:

The powdered stems of *Ourel lanata* (L.) Kuntze were subjected to cold maceration using petroleum ether, chloroform, ethanol and water. For each extraction, 250 g of stem powder was used. The respective extracts were concentrated and dried, and the percentage

yield was calculated based on the weight of the dried extract obtained compared to the first weight of the plant material. The percentage yield obtained from the different extracts was 0.88% for petroleum ether, 0.92% for chloroform, 1.00% for ethanol, 1.64% for aqueous extract and 3.48% for total aqueous extract. Among the extracts, the total aqueous extract showed the highest yield (3.48%), while the petroleum ether extract showed the lowest yield (0.88%).

3.3 Preliminary Phytochemical Screening:

Preliminary phytochemical screening was performed on the petroleum ether, chloroform, ethanolic, aqueous and total aqueous extracts of the stem of *Ourel lanata* (L.) Kuntze. The screening revealed the presence of different classes of phytoconstituents, including alkaloids, flavonoids, tannins, glycosides, carbohydrates, saponins, steroids, terpenoids, phenolic compounds, and gums and mucilage. The ethanolic and aqueous extracts showed (8nos) phytoconstituents each, while the total aqueous extract showed the highest number of phytoconstituents (9nos). The chloroform and petroleum ether extracts showed (4nos) and (2nos) phytoconstituents, respectively. The detailed results are presented in (Table 1).

Table 1. Preliminary phytochemical screening of different stem extracts of *Ourel lanata* (L.) Kuntze:

Constituent class	Petroleum ether	Chloroform	Ethanol	Aqueous	Total aqueous
Alkaloids	–	–	+	+	+
Flavonoids	–	–	+	+	+
Tannins	–	–	+	+	+
Glycosides	+	+	+	+	+
Carbohydrates	–	–	+	+	+
Saponins	+	–	+	+	+
Steroids	–	+	–	+	–
Terpenoids	–	+	+	–	+
Phenolics	–	–	+	+	+
Gums/mucilage	–	+	–	–	+
Number of phytoconstituents detected	2	4	8	8	9

Note: + Present; – Absent.

3.4 Thin Layer Chromatography (TLC) and Fluorescence Analysis:

TLC analysis was performed to obtain the chromatographic profile of the ethanolic, aqueous and total aqueous extracts of the stem of *Ourel lanata* (L.) Kuntze. A single fluorescent spot was seen for each extract under UV light. The ethanolic extract showed a bright yellow, fluorescent spot with an R_f value of 0.78, while the aqueous extract showed a light green, fluorescent spot with an R_f value of 0.63. The total aqueous extract showed a yellowish-green, fluorescent spot with an R_f value of 0.69.

3.5 Fourier Transform Infrared Spectroscopy (FTIR) Analysis:

FTIR analysis was carried out in the range of $4000\text{--}400\text{ cm}^{-1}$ to characterize the major functional groups present in the extracts. The ethanolic extract exhibited absorption peaks at 3317.56 , 1641.42 , 509.21 and 433.98 cm^{-1} , corresponding to O–H, C=C, C–Cl and C–Br functional groups, respectively. The aqueous extract showed peaks at 3402.43 , 3197.98 , 2341.58 , 1639.49 and 503.42 cm^{-1} , corresponding to O–H, O=C=O, C=C and C–Cl functional groups. The total aqueous extract showed peaks at 3402.43 , 3201.83 , 2351.23 , 1639.49 and 754.17 cm^{-1} , corresponding to O–H, O=C=O, C=C and C–H functional groups. The FTIR spectra of the three extracts are presented in (Figure 1).

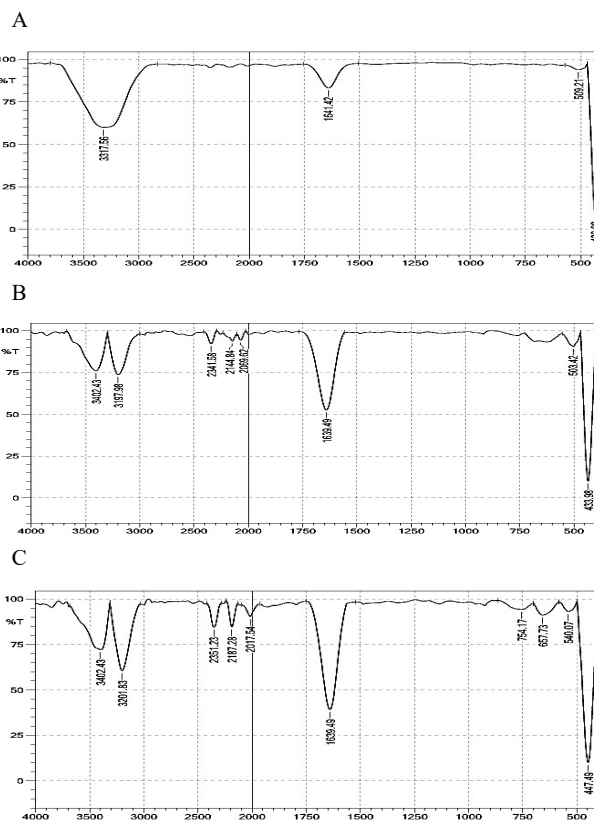


Figure 4. (A) Ethanolic, (B) Aqueous, (C) Total Aqueous in FTIR spectra

3.6 LC-MS Analysis:

LC-MS profiling of the ethanolic stem extract of *Ourel lanata* (L.) Kuntze revealed several characteristic peaks with distinct retention times and m/z values (Table 2; Figure 5). The detected constituents included naringenin, catechin, acacetin, an isorhamnetin derivative, a kaempferol-derived conjugate and a rutin-type glycoside. The identified compounds were predominantly flavonoids and flavonoid glycosides, showing the presence of diverse phenolic constituents in the ethanolic extract.

Table 2. LC-MS Analysis of the Ethanolic Extract of the Stem of *Ourel lanata* (L.) Kuntze.

S. No	Retention Time (min)	m/z Value	Tentatively Identified Compound	Class of Compound
1	0.989	273	Naringenin	Flavonoid
2	21.893	511	Kaempferol-derived conjugate	Flavonoid conjugate
3	13.338	292	Catechin	Flavanol
4	20.878	312	Isorhamnetin derivative	Flavonoid
5	23.510	283	Acacetin	Flavonoid
6	11.236	612	Rutin-type glycoside	Flavonoid glycoside

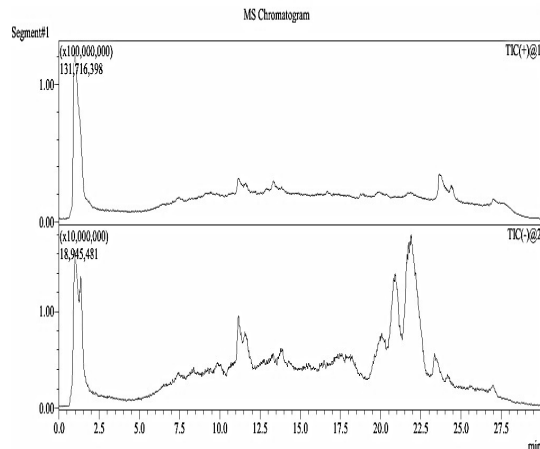


Figure 5: LC-MS chromatogram of ethanolic extract

3.7 In-Vitro Studies:

Acetylcholinesterase (AChE) Inhibitory Activity:

The ethanolic, aqueous and total aqueous stem extracts of *Oureta lanata* (L.) Kuntze exhibited concentration-dependent AChE inhibitory activity over the tested concentration range. Among the extracts, the ethanolic extract showed the strongest inhibitory response and showed a lower IC_{50} value than the reference standard, donepezil. The aqueous and total aqueous extracts showed comparatively weaker inhibitory activity. The AChE inhibitory activity and IC_{50} values are presented in (Table 2).

DPPH Radical Scavenging Activity:

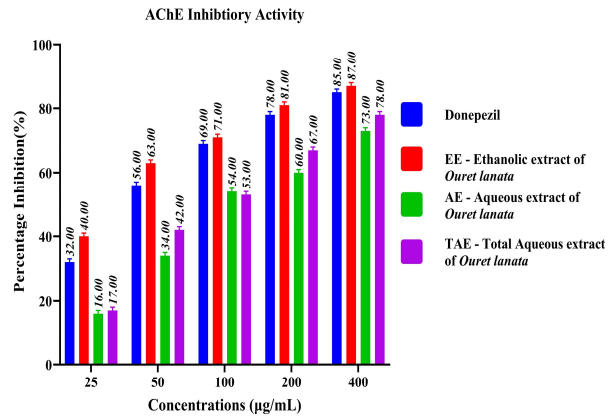
All the tested stem extracts proved concentration-dependent DPPH radical scavenging activity. The ethanolic and aqueous extracts showed comparatively stronger antioxidant activity, with IC_{50} values close to that of the reference standard, ascorbic acid. The total aqueous extract showed a comparatively higher IC_{50} value among the tested extracts. The DPPH radical scavenging activity and IC_{50} values are presented (Table 2) and illustrated in (Figure 6).

Table 2. In-vitro AChE inhibitory and DPPH radical scavenging activities.

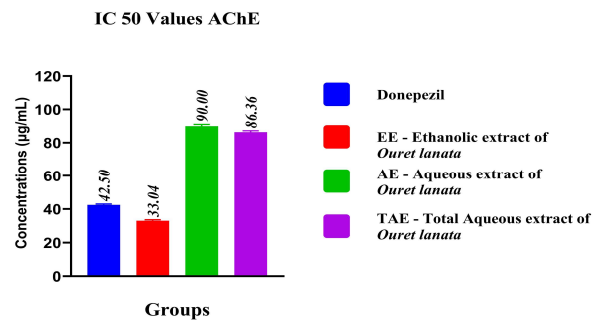
Assay	Concentration (µg/mL)	Standard (%)	Ethanolic extract (%)	Aqueous extract (%)	Total aqueous extract (%)
AChE inhibition	25	32 ± 0.42	40 ± 0.58	16 ± 0.47	17 ± 0.36
	50	56 ± 0.63	63 ± 0.72	34 ± 0.55	42 ± 0.51
	100	69 ± 0.48	71 ± 0.45	54 ± 0.61	53 ± 0.67
	200	78 ± 0.54	81 ± 0.52	60 ± 0.68	67 ± 0.59
	400	85 ± 0.39	87 ± 0.43	73 ± 0.56	78 ± 0.47
	IC_{50}	42.50 ± 0.50	33.04 ± 0.61	90.00 ± 1.00	86.36 ± 0.84
DPPH scavenging	20	11.10 ± 0.17	18.05 ± 0.23	15.27 ± 0.31	16.66 ± 0.34
	40	25.60 ± 0.26	29.16 ± 0.29	23.61 ± 0.32	26.38 ± 0.37
	60	40.60 ± 0.36	38.88 ± 0.70	38.88 ± 0.37	41.66 ± 0.44
	80	53.90 ± 0.30	55.55 ± 0.33	56.94 ± 0.46	51.38 ± 0.37
	100	72.30 ± 0.30	70.83 ± 0.35	69.44 ± 0.36	70.83 ± 0.35
	IC_{50}	74.14 ± 0.31	73.34 ± 0.36	72.31 ± 0.32	77.16 ± 0.34

Values are expressed as Mean ± SD (n = 3). Donepezil was used as the standard for AChE inhibition and ascorbic acid was used as the standard for DPPH radical scavenging activity.

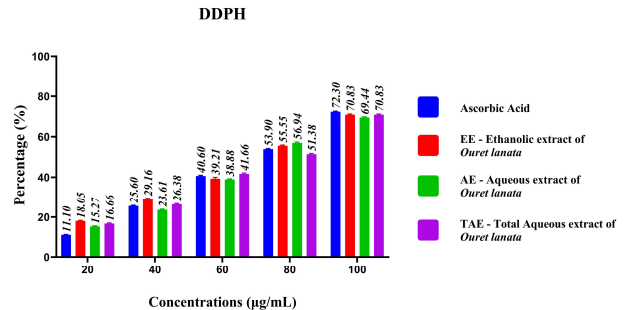
A



B



C



D

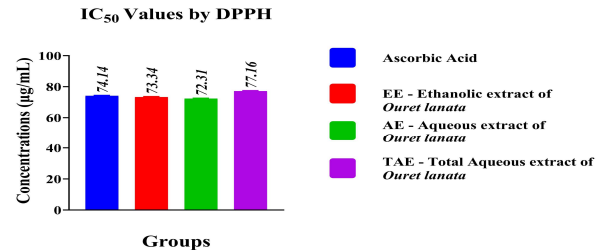


Figure 6. (A) AChE inhibitory Activity, (B) AChE in IC_{50} value, (C) DPPH radical scavenging Activity, (D) DPPH in IC_{50} value.

3.8. In-Vivo Studies:

Acute Oral Toxicity Study:

The acute oral toxicity of the total aqueous stem extract of *Oureta lanata* (L.) Kuntze was evaluated according to OECD Guideline 423 at a single oral dose of 2000 mg/kg in female Wistar albino rats. No mortality or treatment-related clinical signs of toxicity were seen.

during the 14-day observation period. Food and water intake remained normal, and the animals showed a gradual increase in body weight during the observation period. Based on these observations, the oral LD₅₀ of the total aqueous extract was greater than 2000 mg/kg body weight.

Morris Water Maze (MWM) Test:

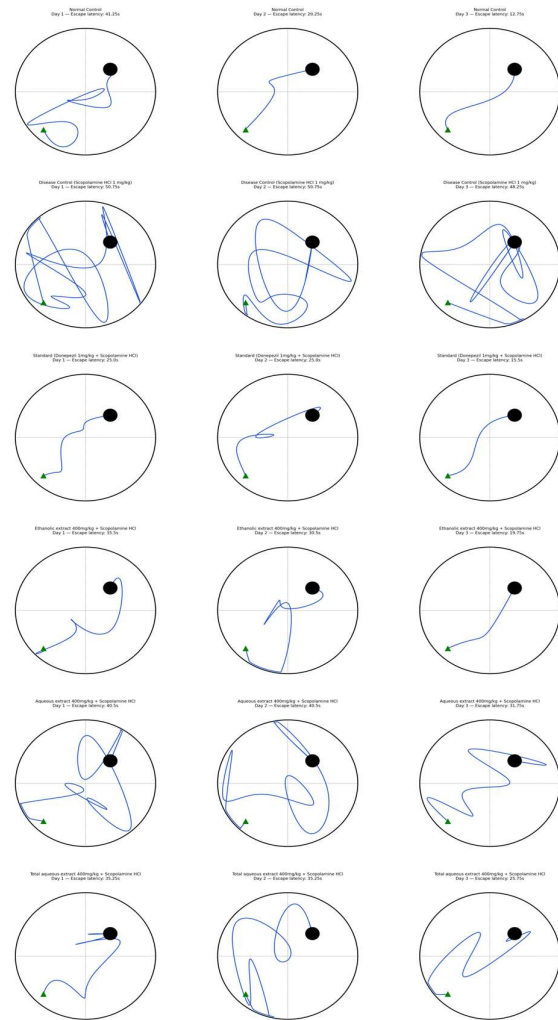
The disease control group showed impaired spatial learning and memory, with increased escape latency during the acquisition trial and reduced time spent in the target quadrant during the probe trial. Donepezil and the stem extract improved MWM performance compared with the disease control group. Among the extracts, the ethanolic extract showed the most pronounced improvement, followed by the total aqueous and aqueous extracts. The results are presented in (Table 3), Tracking method in animal reading was (Figure 7) and illustrated in (Figure 8).

Table 3. Morris Water Maze (MWM) performance

Groups	Day 1 (sec)	Day 2 (sec)	Day 3 (sec)	Target Quadrant (sec)
Normal Control	41.25 ± 1.75*	20.25 ± 1.38****	12.75 ± 1.11****	27.50 ± 1.56****
Disease Control (Scopolamine HBr 1 mg/kg)	50.75 ± 2.14	50.75 ± 1.75	48.25 ± 1.75	11.25 ± 1.11
Standard (Donepezil 1 mg/kg) + Scop. HBr (1 mg/kg)	25.00 ± 1.58****	25.00 ± 1.96****	15.50 ± 1.32****	22.75 ± 1.38****
Ethanolic extract (400 mg/kg) + Scop. HBr (1 mg/kg)	35.50 ± 1.94***	30.50 ± 1.56****	19.75 ± 1.11****	21.00 ± 1.29***
Aqueous extract (400 mg/kg) + Scop. HBr (1 mg/kg)	40.50 ± 1.94*	40.50 ± 1.94**	31.75 ± 1.25****	14.25 ± 1.49ns
Total aqueous extract (400 mg/kg) + Scop. HBr (1 mg/kg)	35.25 ± 1.75***	35.25 ± 1.75****	25.75 ± 1.38****	19.00 ± 1.29**

Values are expressed as Mean ± SEM (n = 4). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test. ns = not significant; *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001 compared with the disease control group.

A



B

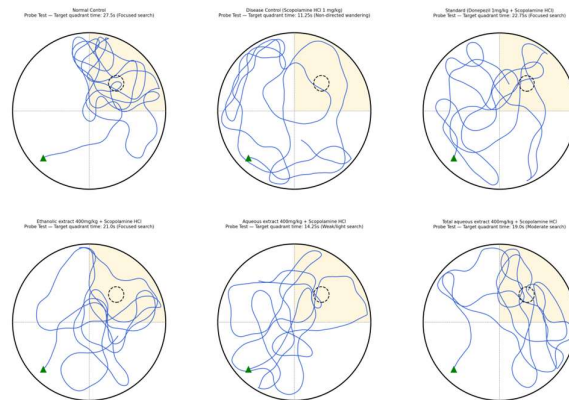
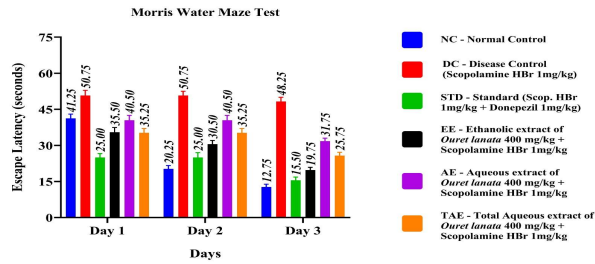


Figure 7: Tracking-assisted Morris water maze test showing (A) escape latency and (B) target quadrant time.

A



B

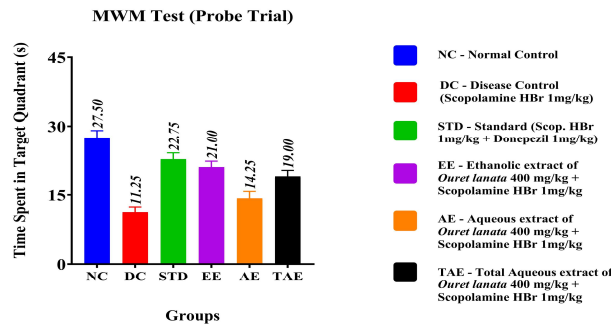


Figure 8. (A) Morris Water Maze escape latency during the acquisition trial and (B) target quadrant during the probe trial

Spontaneous Locomotor Activity:

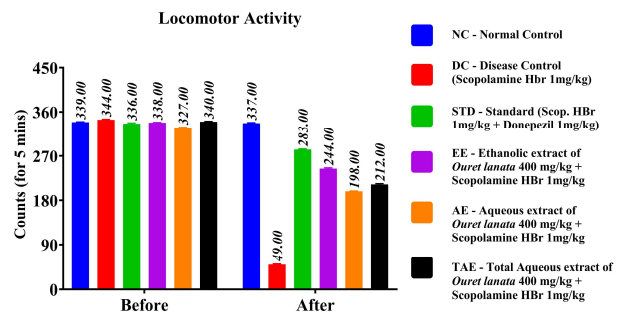
Scopolamine hydrobromide markedly reduced locomotor activity compared with the normal control group. Treatment with donepezil and *Oureta lanata* (L.) Kuntze stem extracts significantly restored locomotor activity compared with the disease control group. Among the extracts, the ethanolic extract showed the greatest improvement, followed by the total aqueous and aqueous extracts. The findings show that the ethanolic extract produced the most pronounced protective effect on scopolamine-induced locomotor impairment. The results are presented in (Table 4) and illustrated in (Figure 9).

Table 4. Spontaneous Locomotor Activity

Groups	Locomotor Activity Score (Counts/5 min)		
	Before	After	% Change In Activity
Normal Control	339 ± 0.91	337 ± 1.29****	00.59 ± 0.12
Disease Control (Scopolamine HBr 1 mg/kg)	344 ± 1.23	49 ± 1.23	85.76 ± 0.42
Standard (Donepezil 1 mg/kg) + Scop. HBr (1 mg/kg)	336 ± 1.68	283 ± 1.29****	15.77 ± 0.25
Ethanolic extract (400 mg/kg) + Scop. HBr (1 mg/kg)	338 ± 0.58	244 ± 1.68****	27.81 ± 0.31
Aqueous extract (400 mg/kg) + Scop. HBr (1 mg/kg)	327 ± 1.68	198 ± 0.91****	39.45 ± 0.28
Total aqueous extract (400 mg/kg) + Scop. HBr (1 mg/kg)	340 ± 0.91	212 ± 1.68	37.65 ± 0.30

Values are expressed as Mean ± SEM (n = 4). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test. ns = not significant; *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001 compared with the disease control group.

A



B

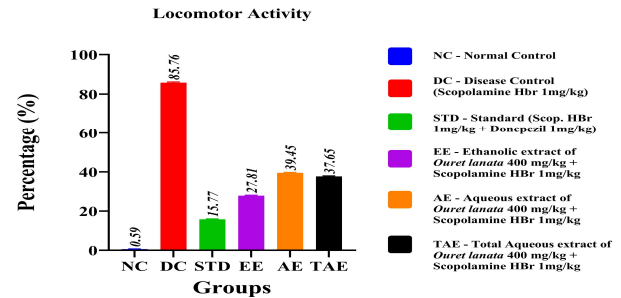


Figure 9. (A) Locomotor Activity in Before-After value and (B) Locomotor % Change in Activity

Open Field Test:

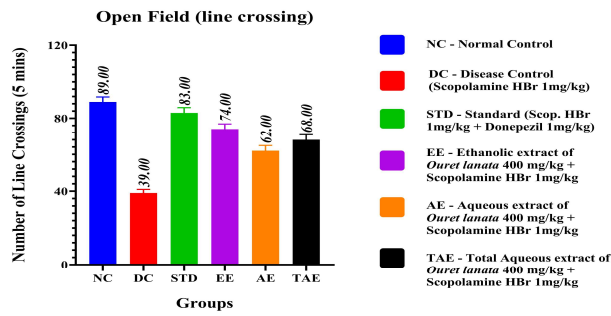
Scopolamine hydrobromide markedly reduced line crossing, rearing and grooming activities compared with the normal control group. Treatment with donepezil and *Oureta lanata* (L.) Kuntze stem extracts increased line crossing activity compared with the disease control group, with the ethanolic extract showing the greatest improvement, followed by the total aqueous and aqueous extracts. Rearing and grooming activities also showed improvement following treatment, although the changes were not significant in several extract-treated groups. Overall, the ethanolic extract showed the most consistent improvement in open-field behavioural parameters. The results are presented in (Table 5) and illustrated in (Figure 10).

Table 5. Open Field Test

Groups	Line Crossing (5min)	Rearing (5min)	Grooming (5min)
Normal Control	89 ± 2.74****	27 ± 2.97*	16 ± 2.20**
Disease Control (Scopolamine HBr 1 mg/kg)	39 ± 1.96	13 ± 2.35	5 ± 1.29
Standard (Donepezil 1 mg/kg) + Scop. HBr (1 mg/kg)	83 ± 3.34****	25 ± 2.86 ^{ns}	14 ± 2.20*
Ethanolic extract (400 mg/kg) + Scop. HBr (1 mg/kg)	74 ± 3.13****	22 ± 2.97 ^{ns}	11 ± 1.83 ^{ns}
Aqueous extract (400 mg/kg) + Scop. HBr (1 mg/kg)	62 ± 2.68***	17 ± 3.24 ^{ns}	8 ± 1.83 ^{ns}
Total aqueous extract (400 mg/kg) + Scop. HBr (1 mg/kg)	68 ± 2.86****	20 ± 3.24 ^{ns}	9 ± 1.96 ^{ns}

Values are expressed as Mean ± SEM (n = 4). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test. ns = not significant; *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001 compared with the disease control group.

A



B

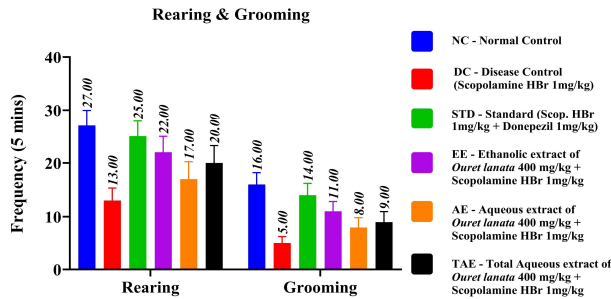


Figure 10. Open Field Test in (A) Line Crossing and (B) Rearing & Grooming

T-maze Test:

The T-maze test showed impaired spatial learning and working memory in the scopolamine hydrobromide-treated disease control group, as reflected by reduced correct alternation and correct trial performance. Treatment with donepezil and the different stem extracts improved T-maze performance compared with the disease control group. Among the extract-treated groups, the ethanol extract produced the most pronounced improvement, followed by the total aqueous and aqueous extracts.

Y-Maze Test

Scopolamine hydrobromide significantly reduced spontaneous alternation and total arm entries compared with the normal control group, showing impaired spatial working memory and exploratory activity. Treatment with donepezil and *Oureit lanata* (L.) Kuntze stem extracts significantly improved spontaneous alternation compared with the disease control group. The ethanol extract showed the greatest improvement, followed by the total aqueous and aqueous extracts. Total arm entries were also increased following treatment, with the ethanol and total aqueous extracts showing significant improvement, while the aqueous extract showed a non-significant change. The T – Maze & Y – Maze results are presented in (Table 6) and illustrated in (Figure 11).

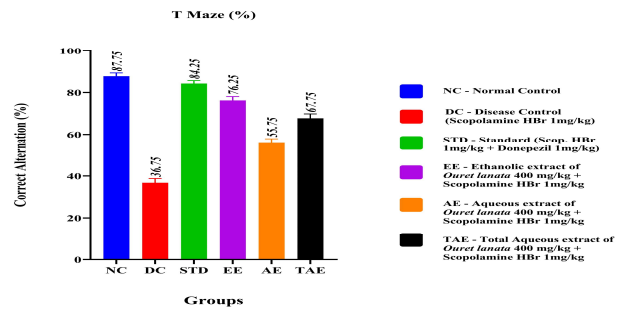
Table 6. T - Maze & Y – maze.

Group	T-Maze: Correct Alternation (%)	T-Maze: Correct Trials	Y-Maze: Spontaneous Alternation (%)	Y-Maze: Total Arm Entries
Normal Control	87.75 ± 1.56****	4.50 ± 0.29**	95.50 ± 1.43****	30.50 ± 1.04****
Disease Control (Scopolamine HBr 1 mg/kg)	36.75 ± 2.01	1.75 ± 0.48	43.50 ± 2.46	20.25 ± 1.25
Standard (Donepezil 1mg/kg) + Scop. HBr (1mg/kg)	84.25 ± 1.42****	4.25 ± 0.25**	87.25 ± 1.36****	28.75 ± 1.25***
Ethanol extract (400 mg/kg) + Scop. HBr (1 mg/kg)	76.25 ± 1.81****	3.75 ± 1.10*	78.75 ± 1.69****	27.25 ± 1.11**
Aqueous extract (400 mg/kg) + Scop. HBr (1 mg/kg)	55.75 ± 2.14****	3.00 ± 0.41ns	59.25 ± 1.75****	23.50 ± 1.32ns
Total aqueous extract (400 mg/kg) + Scop.	67.75 ± 2.07****	3.50 ± 0.50ns	71.50 ± 1.62****	25.75 ± 1.44*

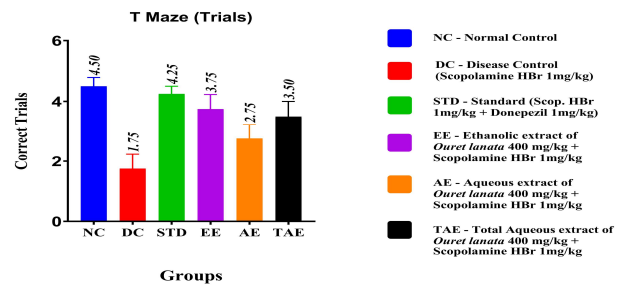
HBr (1 mg/kg)

Values are expressed as Mean ± SEM (n = 4). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test. ns = not significant; *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001 compared with the disease control group.

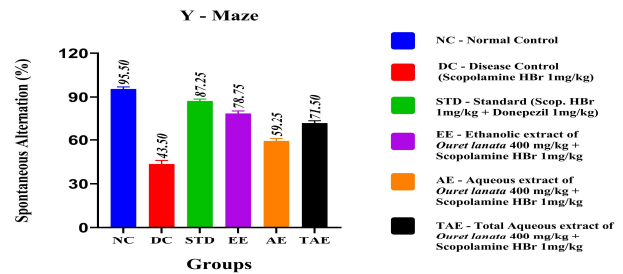
A



B



C



D

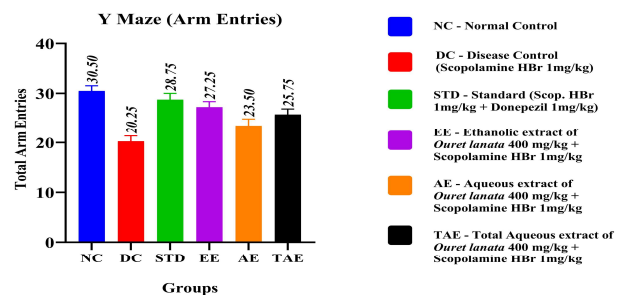


Figure 11. T - Maze & Y – maze Test in (A) Correct Alternation (%), (B) Correct Trials, (C) Spontaneous Alternation (%), (D) Total Arm Entries

Novel Object Recognition (NOR) Test:

In the Novel Object Recognition (NOR) test, the disease control group showed reduced exploration of the novel object and a lower Recognition Index, showing impaired recognition memory. Treatment with donepezil and the stem extracts improved novel object exploration and Recognition Index compared with the disease

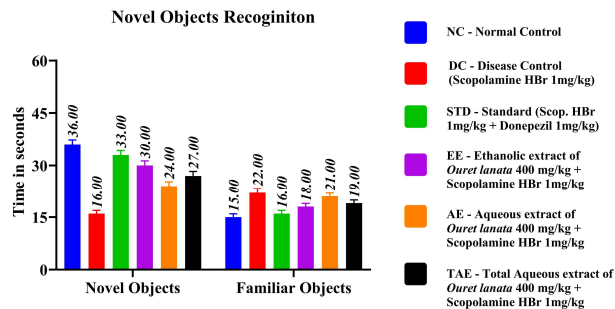
control group. Among the extracts, the ethanolic extract showed the most pronounced improvement, followed by the total aqueous and aqueous extracts. The results of the T-maze and NOR tests are presented in (Table 7), and the corresponding behavioral outcomes are illustrated in (Figure 12).

Table 7. Novel Object Recognition (NOR)

Groups	Familiar Object (sec)	Novel Object (sec)	Recognition Index (%)
Normal Control	15 ± 0.91**	36 ± 1.29****	70.66 ± 0.54****
Disease Control (Scopolamine HBr 1 mg/kg)	22 ± 1.29	16 ± 0.91	42.11 ± 0.21
Standard (Donepezil 1mg/kg) + Scop. HBr (1mg/kg)	15.25 ± 1.70**	33 ± 1.47****	67.40 ± 0.52****
Ethanolic extract (400 mg/kg) + Scop. HBr (1 mg/kg)	18 ± 1.79 ^{ns}	30 ± 1.29****	62.52 ± 0.24****
Aqueous extract (400 mg/kg) + Scop. HBr (1 mg/kg)	21 ± 1.08 ^{ns}	24 ± 1.87**	53.30 ± 0.31****
Total aqueous extract (400 mg/kg) + Scop. HBr (1 mg/kg)	19 ± 1.47 ^{ns}	27.25 ± 1.25****	58.70 ± 0.17****

Values are expressed as Mean ± SEM (n = 4). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test. ns = not significant; *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001 compared with the disease control group.

A



B

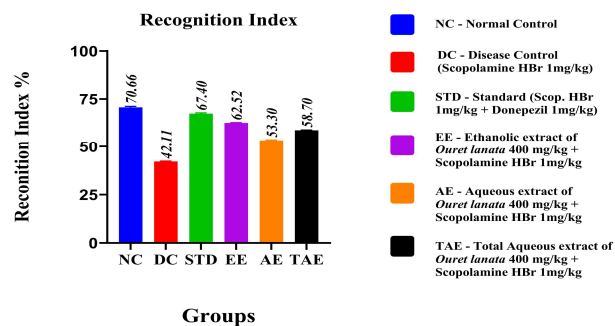


Figure 12. T - Maze & Y – maze Test in (A) Familiar Object (sec) & Novel Object (sec) and (B) Recognition Index (%)

3.9 Biochemical Parameters:

Acetylcholinesterase (AChE) Activity:

The disease control group showed increased brain AChE activity compared with the normal control group. Treatment with donepezil and the stem extracts reduced AChE activity, with the ethanolic

extract showing the greatest reduction, followed by the total aqueous and aqueous extracts.

Lipid Peroxidation (LPO) Levels:

Brain LPO levels were markedly increased in the disease control group compared with the normal control group. Treatment with donepezil and the stem extracts reduced LPO levels, with the ethanolic extract producing the most pronounced reduction, followed by the total aqueous and aqueous extracts.

Superoxide Dismutase (SOD) Activity:

The disease control group showed reduced brain SOD activity compared with the normal control group. Donepezil and the stem extract increased SOD activity, with the ethanolic extract showing the greatest improvement, followed by the total aqueous and aqueous extracts.

Reduced Glutathione (GSH) Levels:

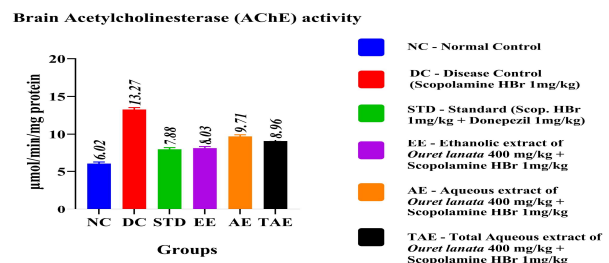
Brain GSH levels decreased in the disease control group compared with the normal control group. Treatment with donepezil and the stem extracts increased GSH levels, with the ethanolic extract showing the most pronounced improvement, followed by the total aqueous and aqueous extracts. The combined biochemical findings are presented in (Table 8) and illustrated in (Figure 13).

Table 8. Biochemical parameters.

Groups	AChE (µg/min/g protein)	LPO (nmol MDA/mg protein)	SOD (unit's/min/mg protein)	GSH (µg/mg protein)
Normal Control	6.02 ± 0.20****	1.03 ± 0.05****	9.11 ± 0.19****	30.19 ± 0.31****
Disease Control (Scopolamine HBr 1 mg/kg)	13.27 ± 0.25	7.49 ± 0.15	4.04 ± 0.12	18.64 ± 0.26
Standard (Donepezil 1 mg/kg) + Scop. HBr (1 mg/kg)	7.88 ± 0.22****	2.73 ± 0.10****	8.77 ± 0.17****	32.66 ± 0.37****
Ethanolic extract (400 mg/kg) + Scop. HBr (1 mg/kg)	8.03 ± 0.21****	3.17 ± 0.10****	8.03 ± 0.16****	29.51 ± 0.51****
Aqueous extract (400 mg/kg) + Scop. HBr (1 mg/kg)	9.71 ± 0.21****	4.61 ± 0.12****	6.19 ± 0.17****	25.70 ± 0.48****
Total aqueous extract (400 mg/kg) + Scop. HBr (1 mg/kg)	8.96 ± 0.19****	3.83 ± 0.14****	7.27 ± 0.16****	27.96 ± 0.35****

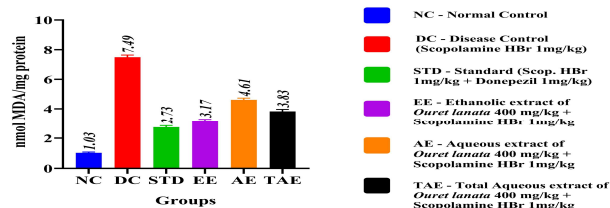
Values are expressed as Mean ± SEM (n = 4). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test. ns = not significant; *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001 compared with the disease control group.

A



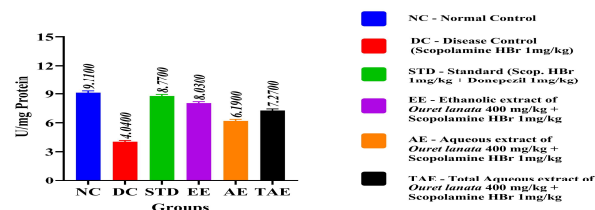
B

Lipid Peroxidation (LPO) Levels



C

Brain Superoxide Dismutase (SOD) Activity



D

Brain Reduced Glutathione (GSH) Levels

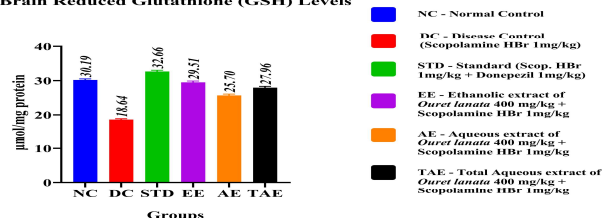


Figure 13. biochemical parameters in (A) AChE, (B) LPO, (C) SOD and (D) GSH

3.10 Histopathological Evaluation:

Histopathological examination of the hippocampal tissue revealed distinct differences among the experimental groups. (A) The normal control group showed preserved hippocampal architecture with no clear neuronal degeneration. In contrast, (B) the disease control group treated with scopolamine hydrobromide exhibited moderate multifocal neuronal degeneration. (C) Treatment with donepezil resulted in marked preservation of hippocampal architecture with only minimal neuronal degeneration. Among the extract-treated groups, (D) the ethanolic and (E) total aqueous extracts showed minimal neuronal degeneration, while (F) the aqueous extract showed mild neuronal degeneration. Overall, the ethanolic and total aqueous extracts showed better preservation of hippocampal neuronal architecture compared with the aqueous extract. Representative histopathological sections of the hippocampus are shown (Figure 14).

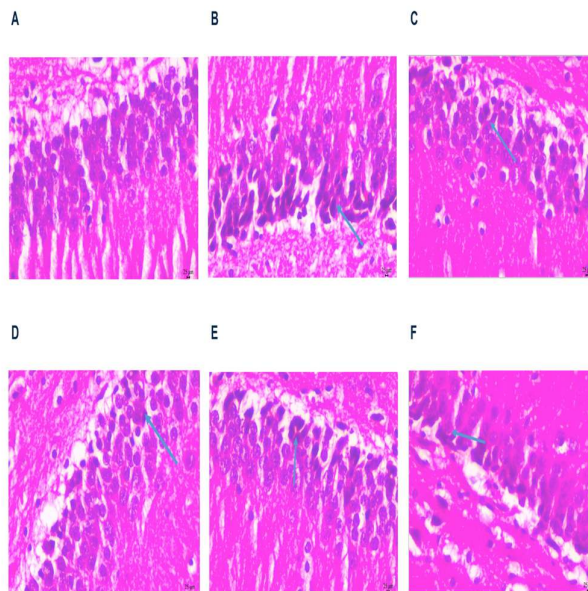


Figure 14. Histopathological evaluation of hippocampal tissue of experimental rats following treatment with different stem extracts of *Oureta lanata* (L.) Kuntze.

4. DISCUSSION

The present study evaluated the protective effects of *Oureta lanata* (L.) Kuntze stem extracts against scopolamine hydrobromide-induced Alzheimer's disease-like neurocognitive dysfunction using in-vitro and in-vivo approaches. The extracts showed a broad phytochemical profile, with phenolic and flavonoid constituents being prominent in the active extracts. Recent investigations have reported diverse phytoconstituents and antioxidant properties in *Oureta lanata* (L.) Kunze extracts, supporting the phytochemical findings of the present study [47,48].

The extracts proved concentration-dependent AChE inhibitory and DPPH radical scavenging activities, with the ethanolic extract showing the strongest AChE inhibition. Medicinal plants that have bioactive phenolic and flavonoid constituents have been reported to have anticholinesterase activity, supporting the potential relevance of the present in-vitro findings [49]. The observed antioxidant activity may also contribute to neuroprotection by reducing oxidative damage associated with cognitive dysfunction [50].

Scopolamine administration produced cognitive impairment in the MWM, T-maze and NOR tests, while treatment with donepezil and the extracts improved behavioral performance. Similar improvements in spatial learning and memory have been proved in scopolamine-treated rats following treatment with neuroprotective agents [51,52]. The behavioral improvement seen with the ethanolic extract was consistent with its stronger in-vitro activity.

The biochemical findings showed increased AChE and LPO levels with reduced SOD and GSH levels following scopolamine administration. Comparable alterations in cholinergic function and oxidative stress markers have been reported in scopolamine-induced cognitive dysfunction [53]. Natural compounds have also been shown to improve scopolamine-induced cognitive impairment through modulation of AChE activity and antioxidant defense mechanisms [54]. In the present study, the ethanolic extract produced the most pronounced overall biochemical response.

Histopathological examination further supported the neuroprotective effects of the extracts. Moderate multifocal neuronal degeneration seen in the disease control group was reduced following treatment, with minimal degeneration in the ethanolic and total aqueous extract groups. Earlier studies have proved that attenuation of scopolamine-induced cognitive impairment is accompanied by preservation of hippocampal neuronal architecture and reduction of oxidative damage [55,56].

Overall, *Ourea lanata* (L.) Kunze stem extracts showed multimodal protective effects against scopolamine-induced neurocognitive dysfunction. The ethanolic extract proved the most consistent activity across the in-vitro, behavioral, biochemical and histopathological assessments, suggesting that modulation of cholinergic dysfunction and oxidative stress may contribute to its neuroprotective effects.

5. CONCLUSION

The present study proved the protective potential of *Ourea lanata* (L.) Kuntze stem extracts against scopolamine hydrobromide-induced Alzheimer's disease-like neurocognitive dysfunction in albino rats. The extracts showed concentration-dependent acetylcholinesterase inhibitory and DPPH radical scavenging activities in vitro, while in vivo treatment improved cognitive performance, attenuated alterations in brain AChE and lipid peroxidation, and enhanced antioxidant defense. Histopathological findings further supported the protective effects of the extracts on hippocampal neuronal architecture. Among the tested extracts, the ethanolic extract showed the most consistent overall activity across the in vitro, behavioral and biochemical assessments. These findings suggest that *Ourea lanata* (L.) Kunze stem extracts, particularly the ethanolic extract, may have promising cholinergic and antioxidant-mediated neuroprotective potential and call for further investigation to set up their active constituents and underlying mechanisms.

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