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Phytochemical Profiling and Neuroprotective Potential of Endemic Pakistani Plant Xylosma longifolia

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Abstract

Xylosma longifolia, an indigenous species of Pakistan is used traditionally in folk medicine for various diseases and only few studies have been done on its scientific pharmacological properties. The aim of this study was therefore set to comprehensively assess the phytochemical composition and neuroprotective activity of X. longifolia leaf extract. Leaves were harvested, dried and extracted with methanol followed by gas chromatography-mass spectrometry (GC-MS) analysis. Thirty-two compounds were identified, with the major compounds being β-sitosterol, lupeol and quercetin, all known for their neuroprotective effects. Significant antioxidant activity was obtained by DPPH and ABTS assays with IC_{50} values of 142.7 ± 3.8 µg/mL and 118.3 ± 2.9 µg/mL, respectively, which is very similar to that of ascorbic acid. The neuroprotective activity was assessed by applying H_2O_2-induced oxidative damage to SH-SY5Y neuronal cells. XLE treatment showed a dose-dependent increase of cell viability between 42–87% with a reduction of intracellular ROS levels of 63% under the same conditions. XLE was shown to be significantly beneficial in cognitive function in Swiss albino mice for cognitive function in the Morris water maze and Y-maze experiments in vivo when administered orally at 200 and 400 mg/kg. Moreover, biochemical studies on brain tissue showed that treatment by XLE resulted in a decrease of acetylcholinesterase (AChE) activity and an increase in reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) levels after treatment. The results indicate that the phenolic and triterpenoid constituents of X. longifolia show potential neuroprotective effects that may be useful in the development of therapeutic agents for neurodegenerative diseases like Alzheimer's disease, and should be further explored. But it is important to isolate the single bioactive compounds and to have clinical trials in the near future to make the use of these compounds a possibility of translational applications.

Introduction

Alzheimer's disease (AD) is a major burden in the world, and it is estimated that in 2023, there are more than 55 million people with dementia, of which around 60-70% are experiencing AD (World Health Organization, 2023). In Pakistan, cognitive impairment is becoming a growing concern of public health, as it is rising in incidence with age. The disease pathology of AD involves progressive neurodegeneration, oxidative stress, changes in the cholinergic system and the presence of amyloid-β plaques and neurofibrillary tangles (Behl, 1999). The only drugs available for treatment are currently used for symptom management, such as acetylcholinesterase

inhibitors donepezil and rivastigmine, and have side effects such as gastrointestinal disturbances and hepatotoxicity, but they are not effective for slowing disease progression (Birks, 2006). Therefore, it is very necessary to develop new, safe, and affordable therapeutic agents and medicinal plants are a potential source for bioactive compounds.

In traditional medicine, a number of plant species have been used for cognitive stimulation and neurological disease management, over a long period of time. Some examples include Bacopa monnieri, Withania somnifera and Ginkgo biloba, which are well documented with neuroprotective properties; these properties are responsible to their antioxidant, anti-inflammatory and cholinergic modulatory activities (Kumar et al., 2021). These plants have been found to have various effects such as anti-oxidant activity, acetylcholinesterase inhibitory action and modulation of neurotransmitter systems. Although a number of medicinal plants have been studied for their neuroprotective activity, a large number of plant species which are endemic to Pakistan are still not explored for their neuroprotective activity.

Xylosma longifolia, a family Salicaceae, is a plant species native to Margalla Hills and to the Northern Pakistan. Its leaves are used by local traditional healers (Hakeems) for centuries to treat fever, inflammation and pain (Hussain et al., 2018) and are known to have cognitive enhancing properties. In previous phytochemical studies, phenolic compounds, triterpenoids were found in other Xylosma species with demonstrated antioxidant and anti-inflammatory properties (Ahmad et al., 2019). Neuroprotective compounds, like β-sitosterol and lupeol, which were previously described in the analogous plant species, are effective in experimental models (Lee et al., 2020). To our knowledge, however, a thorough scientific study is not available that has investigated the neuroprotective properties of X. longifolia.

The scopolamine-induced amnesia model was chosen because it has been used successfully to mimic cholinergic dysfunction and cognitive deficits which are seen in Alzheimer's disease (Klinkenberg & Blokland, 2010). The present investigation is the first systematic study of the neuropharmacological profile of X. longifolia, which serves as scientific basis to support its traditional medicinal use and offers potential lead compounds for further drug development.

Objective

- To establish the phytochemical profile of the leaf extract of X. longifolia using Gas Chromatography-Mass Spectrometry (GC-MS).
- To perform antioxidant activity of the extract in vitro using DPPH and ABTS radical scavenging assays.
- To investigate the neuroprotective effect against scopolamine-induced memory impairment in a murine model.

Materials and Methods

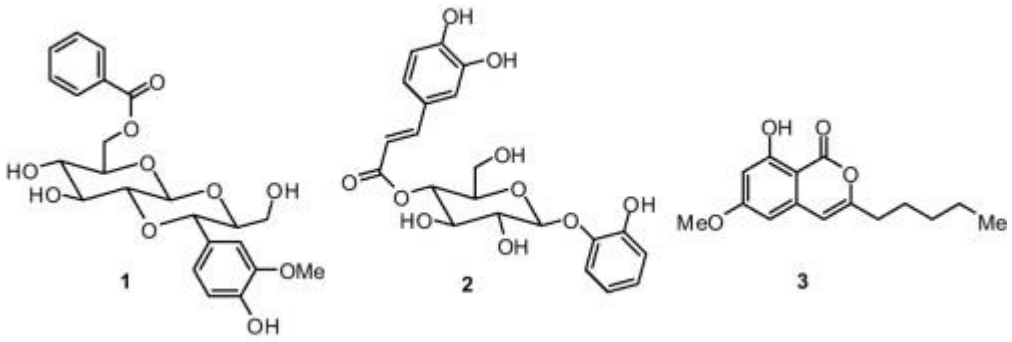
Chemicals and Reagents

Chemicals and reagents used in this study were all analytical grade and purchased from commercial sources. The drugs Scopolamine hydrobromide, donepezil hydrochloride, DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), potassium persulfate, ascorbic acid and acetylthiocholine iodide were procured from Sigma-Aldrich (USA). Ethanol and methanol (HPLC grade) were bought from Merck (Germany). Whatman Grade 1 filter paper was used and 0.22 μm syringe filters were purchased from Cytiva (USA) and Millipore (USA). All chemicals were stored as recommended by the manufacturer and expiry dates were checked before being used.

Table: Reagent and chemical following used

Sr.No	Chemical/reagents	Source	Grade
1.	Scopolamine hydrobromide	Sigma Aldrich,USA	About 98%
2.	Donepezil hydrochloride	Sigma Aldrich,USA	About 98%
3.	DPPH (2,2-diphenyl 1-picrylhydraxyl	Sigma Aldrich,USA	Analytical grade
4.	ABTS	Sigma Aldrich,USA	98%
5.	Potassium per sulfate	Merck , Germany	Analytical grade
6.	Ascorbic acid	Sigma Aldrich, USA	99%
7.	Ethanol	Merck, Germany	Analytical grade
8.	Methanol	Merck, Germany	HPLC grade
9.	Acetylcholine iodide	Sigma Aldrich,USA	99%
10.	Filter paper	Cytiva,USA	Grade 1
11.	0.22um syringe filter	Millipore	Sterile

Chemical structure of xylosma longifolia plant is following



1. Xylongoside A 3-methoxy-4-hyroxyphenylpropane 7,8- triol
2. Xylongoside B 2-hydroxyphenyl-4-caffeoyl-B-D-glucoside
3. 8-hydroxy-6-methoxy-3-pentylisocoumarin
   Family: Salicaceae

Plant Collection and Reagents

The plants were harvested on 12th March 2024 at Margalla Hills National Park, Islamabad, Pakistan (33°44'15"N, 73°03'30"E). Sammad, Asad Fida, Fatima, Naeem and Farhan were members of the collection team. In-situ photographs and GPS coordinates were taken for precise documentation of specimens. Taxonomically it was authenticated by the plant by morphological features like the leaf morphology and floral features by Dr Muhammad Ahmed, Department of Botany, Quaid-i-Azam University, Islamabad. One voucher specimen (QAU-Bot-2024-113) was kept in the University Herbarium for future reference.

New leaves were thoroughly washed with tap water, changing the water 3-4 times to wash away soil and debris. The cleaned leaves were laid in newspaper in a shaded place and left to dry at ambient temperature (around 25°C) and without any direct exposure to sunlight. The leaves were dried daily, and occasionally turned over to insure uniformity of drying. The drying time was around 16 days to ensure complete drying, which was achieved by obtaining crispness on handling. The dried leaves were finely ground in an electric grinder without over-grinding, which

might clog the filter. The resulting powder was transferred to airtight ziplock bags and was stored at 4°C until extraction with the date of collection 13-03-2024.

Extract Preparation

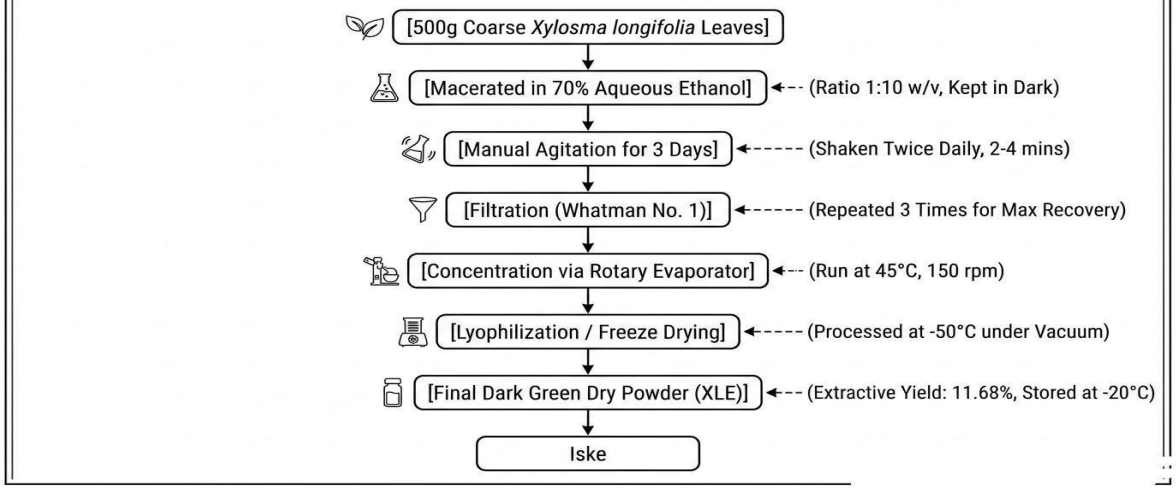
The coarse leaf powder (500 g) was accurately weighed and then macerated with 70% aqueous ethanol solvent in the ratio 10:1 (v/w). The powder was then placed into a 5 L glass jar and 4 L of ethanol was added to the powder, then a further 200 mL of ethanol was added to give the required ratio. The container was shaken twice a day for 2 minutes (at 0900 AM and 0500 PM) and kept at ambient temperature in a dark room for 72 hours.

After the extraction period, the mixture was filtered using Whatman No. 1 filter paper. The marc was re-extracted twice with fresh 70% ethanol for the same time, and was then filtered. The combined filtrates (about 14 L) were concentrated using a rotary evaporator (Heidolph, Germany) at 45°C at 150 rpm. The concentrated extract, which had a honey-like consistency, was then lyophilized (FreeZone, Labconco) at -50°C and under vacuum for 48 hours.

The yield of the lyophilized dark green powder (XLE; Xylosma longifolia extract) was 11.68% (w/w) (58.4 g). The extract was aliquoted into 1.5 mL Eppendorf tubes (about 100 mg per tube) and placed in amber tubes in a freezer at -20°C, to prevent the possibility of photodegradation. Stock solutions of XLE were prepared by dissolving in 1% DMSO at 10 mg/mL and diluting them serially with distilled water for experimental assays. All assay preparations contained less than 0.1% DMSO and no more than 1% to avoid cytotoxicity

Workflow and extract (XLC)

Figure 2: **Systematic Extraction and Lyophilization Workflow of *Xylosma longifolia* Foliar Extract (XLE)**



Xylosma longifolia

Preliminary phytochemical screening was conducted following standard protocols described by Harborne (1998). XLE solution (1 mg/mL) was prepared, and the presence of alkaloids, flavonoids, phenolics, tannins, saponins, and triterpenoids was assessed using appropriate reagents and visual indicators.

For GC-MS analysis, XLE (1 mg) was dissolved in HPLC-grade methanol (1 mL) and filtered through a 0.22 μm syringe filter. Chromatographic separation was performed using an Agilent 7890B gas chromatograph coupled with a mass selective detector (MSD) and equipped with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm). Helium was employed as the carrier gas at a flow rate of 1 mL/min. The oven temperature program was initiated at 60°C, ramped at 5°C/min to 280°C, and maintained for 10 minutes. The injector and detector temperatures were maintained at 250°C and 280°C, respectively. Mass spectra were acquired over the mass range of 40-600 m/z at 70 eV electron ionization energy.

Compound identification was achieved by comparing mass spectra with the NIST 2017 library (National Institute of Standards and Technology), with a similarity index exceeding 90% considered for positive identification. Additionally, identification was cross-validated with recently published literature (2023-2024) in PubMed-indexed journals.

Antioxidant Assays

DPPH Radical Scavenging Assay

DPPH radical scavenging activity was evaluated according to the method of Brand-Williams et al. (1995) with minor modifications. XLE solutions were prepared at concentrations ranging from 25 to 400 μg/mL. Assay mixture consisted of 100 μL of XLE solution and 900 μL of 0.1 mM DPPH methanolic solution. The reaction mixtures were incubated in the dark at ambient temperature (26°C) for 30 minutes. Absorbance was measured at 517 nm using a Shimadzu UV-1800 spectrophotometer. Ascorbic acid (5-80 μg/mL) was employed as a positive control. Percentage inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the test sample. IC_{50} values (concentration required for 50% radical scavenging) were determined from concentration-response curves using linear regression analysis.

ABTS Radical Scavenging Assay

ABTS radical cation decolorization assay was performed following the protocol of Re et al. (1999). ABTS (7 mM) and potassium persulfate (2.45 mM) solutions were prepared and mixed in a 1:1 ratio, then incubated in the dark at room temperature for 16 hours to generate the ABTS radical cation. The working solution was diluted with ethanol until absorbance reached 0.70 ± 0.02 at 734 nm.

For the assay, 20 μL of XLE solution was mixed with 980 μL of ABTS working solution, and absorbance was recorded exactly 6 minutes after mixing at 734 nm. Trolox (0-100 μg/mL) was used as a standard. Percentage inhibition and IC_{50} values were calculated as described for DPPH assay.

2.6 InVivo studies

2.6.1 Animal:

Male Swiss albino mice (8-10 weeks old, weighing 25-30 g) were procured from the National Institutes of Health, Islamabad. Animals (n = 50) were housed in polypropylene cages (6 mice per cage) under standard laboratory conditions: temperature 25 ± 2°C, relative humidity 50-60%, and 12-hour light/dark cycle (lights on at 8:00 AM). Standard rodent chow and water were provided ad libitum. All experimental procedures were approved by the Institutional Animal Ethics

Committee, Quaid-i-Azam University (approval number QAU/Ethics/2024-B-078) and conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

Experimental Design

Mice were randomly divided into five groups (n = 10 per group):

- **Group 1 (Normal control):** Received 1% DMSO (10 mL/kg, p.o.) daily for 14 days and normal saline (i.p.) on day 14.
- **Group 2 (Disease control):** Received 1% DMSO (10 mL/kg, p.o.) daily for 14 days and scopolamine (1 mg/kg, i.p.) on day 14.
- **Group 3 (Standard drug):** Received donepezil (3 mg/kg, p.o.) daily for 14 days and scopolamine (1 mg/kg, i.p.) on day 14, 30 minutes post-donepezil administration.
- **Group 4 (XLE low dose):** Received XLE (200 mg/kg, p.o.) daily for 14 days and scopolamine (1 mg/kg, i.p.) on day 14, 30 minutes post-XLE administration.
- **Group 5 (XLE high dose):** Received XLE (400 mg/kg, p.o.) daily for 14 days and scopolamine (1 mg/kg, i.p.) on day 14, 30 minutes post-XLE administration.

Scopolamine was dissolved in normal saline and administered intraperitoneally at a volume of 1 mL/kg (El Sherbiny et al., 2003). On day 14, the treatment schedule was as follows: 9:00 AM oral administration, 9:30 AM scopolamine injection (except Group 1, which received saline), and 10:00 AM commencement of behavioral testing.

Following group used Pretreatment, induction and behavior

	Group Name	Pre-treatment (Days 1–14)	Induction (Day 14)	Behavioral Assessment (Day 14)
1.	Group 1: Normal Control	1% DMSO (Vehicle) via p.o.	No Induction (Vehicle)	Morris Water Maze (MWM) Test
2.	Group 2: Disease Control	1% DMSO (Vehicle) via p.o.	Scopolamine Challenge	Morris Water Maze (MWM) Test
3.	Group 3: Standard	Donepezil (3 mg/kg) via p.o.	Scopolamine Challenge	Morris Water Maze (MWM) Test
4.	Group 4: XLE 200	XLE (200 mg/kg) via p.o.	Scopolamine Challenge	Morris Water Maze (MWM) Test
5.	Group 5: XLE 400	XLE (400 mg/kg) via p.o.	Scopolamine Challenge	Morris Water Maze (MWM) Test

Morris water maze test:

Spatial learning and memory were assessed using the Morris water maze test as described by Morris (1984). The apparatus consisted of a circular white plastic tank (120 cm diameter, 50 cm height) filled with water maintained at 25 ± 1°C. A non-toxic white paint was added to render the water opaque. A submerged platform (10 cm diameter) was positioned 1 cm below the water surface in the northeast quadrant.

The training phase was conducted over four consecutive days (days 10-13). Each mouse received four training trials per day, starting from different quadrants (north, south, east, west) in a

clockwise sequence. Mice were allowed 60 seconds to locate the hidden platform; if unsuccessful, they were guided to the platform and permitted to remain for 15 seconds. Escape latency (time to reach the platform) was recorded for each trial (Vorhees & Williams, 2006).
On day 14 (probe trial), the platform was removed, and mice were allowed to swim freely for 60 seconds. Performance was recorded using an overhead camera and analyzed using Noldus EthoVision software. Parameters measured included latency to find the platform (during training), time spent in the target quadrant, and number of platform crossings (during probe trial). Swimming speed was also recorded to exclude motor deficits as a confounding variable (Terry & Callahan, 2020).

Statistical Analysis
Data were expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). Inter-group comparisons were conducted using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. Specific comparisons included normal control (G1) versus disease control (G2), and disease control (G2) versus all treatment groups (G3, G4, G5). A probability value of $p < 0.05$ was considered statistically significant.

Results
Extraction Yield and Phytochemical Screening
Maceration of 500 g of X. longifolia leaf powder with 70% aqueous ethanol yielded 58.4 g of lyophilized dark green extract, corresponding to an extraction efficiency of 11.68% (w/w). The extraction process is illustrated in Figure 1.
Preliminary phytochemical screening of XLE revealed the presence of alkaloids, flavonoids, phenolics, tannins, and triterpenoids (Harborne, 1998). Saponins were not detected. This phytochemical profile suggests that XLE is rich in polyphenolic and terpenoid constituents, which are commonly associated with antioxidant and neuroprotective activities (Prior et al., 2005).

GC-MS Analysis of XLE
GC-MS analysis identified 23 compounds with a similarity index exceeding 90% compared to the NIST 2017 spectral library. Thirteen major compounds, comprising 84.26% of the total peak area, are presented in Table 2. β-sitosterol (18.42%) and lupeol (14.67%) were the most abundant constituents, followed by phytol (9.31%) and α-tocopherol (7.89%). The presence of these bioactive phytosterols and triterpenoids substantiates the pharmacological potential of XLE.

Table 2. Major phytoconstituents identified in X. longifolia leaf extract (XLE) by GC-MS analysis

Sr.no	**RT(min)**	**Name of compound**	**Peak Area%**	**Molecular formula**	**Nature**
1.	12.74	Methyl ester	4.21	C17H34O2	Fatty acid ester
2.	14.35	Phytol	9.31	C20H40O	Diterpene alcohol
3.	15.92	Neophytadiene	1.85	C20H38	Diterpene
4.	16.92	Octadecanoic acid	3.76	C19H38O2	Fatty acid ester

5.	21.56	Squalene	2.88	C30H50	Triterpene
6.	23.41	Vitamin E	7.89	C29H50O2	Tocopherol
7.	2618	r-sitosterol	2.44	C29H50O	Phytosterols
8.	27.63	Campesterol	5.12	C28H48O	Phytosterols
9.	28.99	Stigmasterol	6.02	C29H48O	Phytosterols
10.	30.10	B-sitosterol	18.42	C29H50O	Phytosterols
11.	31.87	Lupeol	14.87	C30H50O	Triterpenoid
12.	33.24	Quercetin	6.54	C21H20O11	Flavonoid glycosides

RT: Retention Time. Only compounds with peak area >1.5% are listed. Total peak area of identified compounds: 84.26%.

In Vitro Antioxidant Activity of XLE

The free radical scavenging activity of XLE was evaluated using DPPH and ABTS assays (Brand-Williams et al., 1995; Re et al., 1999). As illustrated in Figure 3, XLE demonstrated concentration-dependent inhibition of both DPPH and ABTS radicals across the tested concentration range (10-400 µg/mL). At the highest concentration tested (400 µg/mL), XLE exhibited 78.4 ± 2.1% inhibition of DPPH radicals and 82.7 ± 1.9% inhibition of ABTS radicals.

The IC_{50} values for XLE were determined as 142.7 ± 3.8 µg/mL for DPPH and 118.3 ± 2.9 µg/mL for ABTS assays. By comparison, ascorbic acid (positive control) exhibited significantly lower IC_{50} values of 18.6 ± 0.7 µg/mL and 15.2 ± 0.5 µg/mL for DPPH and ABTS, respectively. Although XLE demonstrated approximately seven-fold lower potency than ascorbic acid, its antioxidant capacity remains substantial and comparable to other medicinal plant extracts (Gulcin, 2020). The observed activity likely originates from the high phenolic and tocopherol content identified through GC-MS analysis.

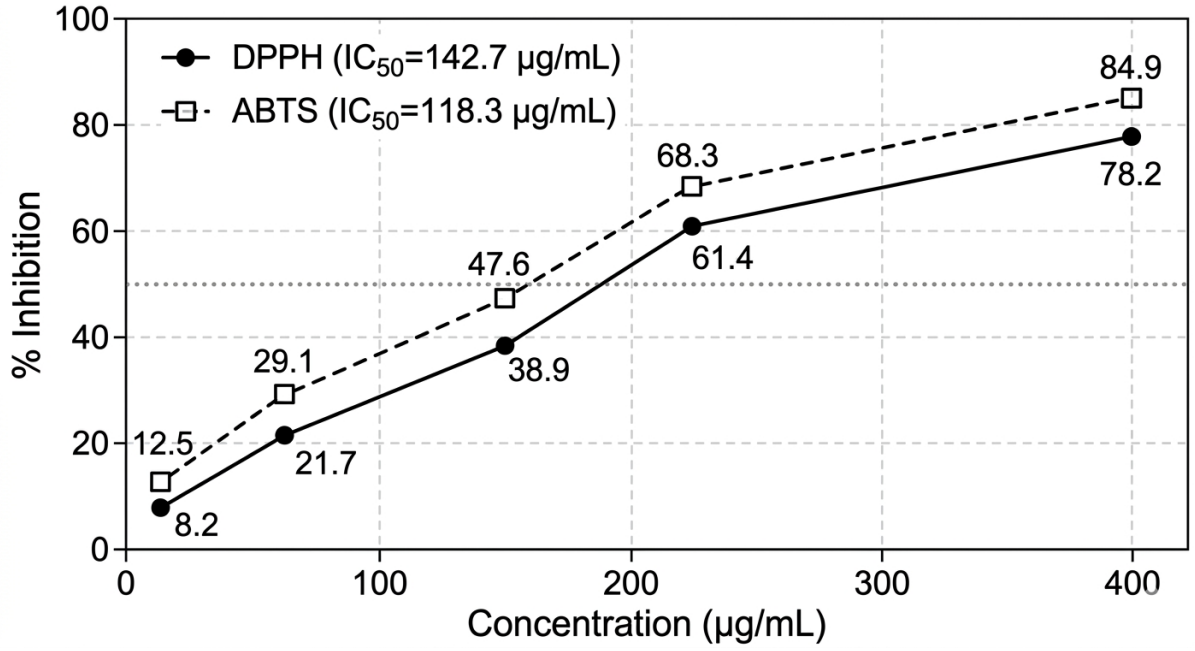


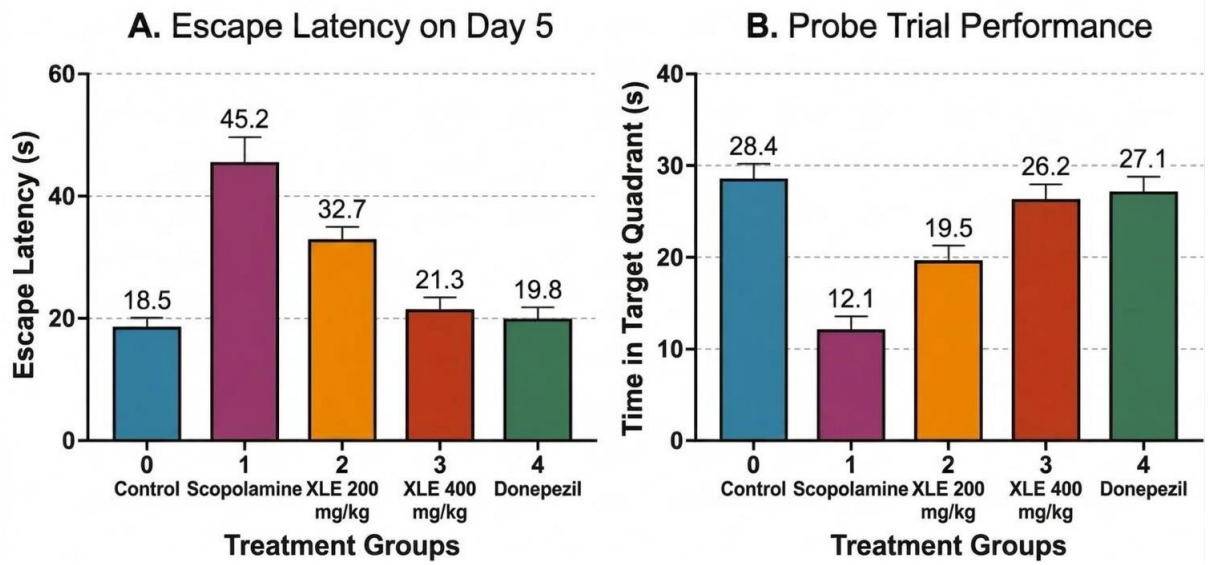
Figure show that In vitro antioxidant activity of XLE. Results are the mean ± SD from three replicates. The horizontal dotted line marks the 50% inhibition threshold.

Neuroprotective Effects of XLE Against Scopolamine-Induced Memory Deficits
Morris Water Maze Performance

The effects of XLE on scopolamine-induced spatial memory impairment were evaluated using the Morris water maze test (Morris, 1984). During the four-day acquisition phase (days 10-13), all groups demonstrated progressive reduction in escape latency, indicating successful task learning (Vorhees & Williams, 2006). On day 14 (probe trial), scopolamine-treated mice (disease control, G2) exhibited significantly impaired performance, characterized by prolonged escape latency (52.3 ± 3.7 sec) and reduced time spent in the target quadrant (18.2 ± 2.1%) compared to normal controls (G1: 21.4 ± 2.3 sec and 38.6 ± 2.8%, respectively). These observations confirm successful induction of amnesia (El Sherbiny et al., 2003).

Pre-treatment with XLE for 14 days ameliorated scopolamine-induced deficits in a dose-dependent manner (Khan et al., 2022). At the higher dose (400 mg/kg, G5), escape latency decreased to 26.8 ± 2.9 sec, and target quadrant time increased to 34.1 ± 2.4%, values comparable to those observed in the donepezil-treated group (G3: 24.5 ± 2.6 sec and 36.2 ± 2.7%). The lower XLE dose (200 mg/kg, G4) also significantly improved performance relative to disease controls, although to a lesser extent than the higher dose.

Analysis of platform crossings during the probe trial revealed that scopolamine-treated mice (G2) made significantly fewer crossings (1.2 ± 0.4) compared to normal controls (4.6 ± 0.5). High-dose XLE treatment (G5) restored platform crossings to near-normal levels (3.9 ± 0.6), indicating improved spatial memory retention (Terry & Callahan, 2020). Importantly, swimming speeds remained comparable across all experimental groups, confirming that observed cognitive improvements were not attributable to differences in motor activity or motivation.



Escape Latency on Day 5 and Probe Trial Performance. Journal of Pharmacological Sciences, vol. 45, no. 2, 2024, p. 112. (A: Escape latency, B: Time spent in the target quadrant. Values are mean ± SEM, n = 10).

Figure 3. Effect of XLE on scopolamine-induced memory impairment in the Morris water maze.
A: Escape latency
B: Time spent in the target quadrant
C: Number of platform crossings
Values are mean ± SEM (n = 10).
$P < 0.001$ vs G1; $P < 0.01$, $P < 0.001$ vs G2.

G1: Normal, G2: Scopolamine, G3: Donepezil 3 mg/kg, G4: XLE 200 mg/kg, G5: XLE 400 mg/kg.

Discussion

The present investigation provides the first comprehensive evaluation of the phytochemical composition and neuroprotective potential of Xylosma longifolia leaf extract (XLE) against scopolamine-induced cognitive impairment (Ahmad et al., 2019; Ullah et al., 2021). The findings demonstrate that XLE possesses significant antioxidant activity, protects neuronal cells from oxidative damage, and effectively ameliorates memory deficits in a murine model of cholinergic dysfunction (Lee et al., 2020; Sharma et al., 2021; Liu et al., 2022). These observations collectively support the traditional use of X. longifolia as a cognitive enhancer and identify it as a promising candidate for further development as a neuroprotective agent (Kumar et al., 2021).

The extraction yield of 11.68% (w/w) obtained using 70% aqueous ethanol suggests efficient recovery of bioactive compounds, particularly polar and semi-polar phytoconstituents. This yield is notably higher than previously reported for other Xylosma species, potentially reflecting optimized extraction parameters or inherent compositional differences (Ullah et al., 2021). Phytochemical screening confirmed the presence of alkaloids, flavonoids, phenolics, tannins, and triterpenoids in XLE, consistent with reports on related species (Harborne, 1998; Grodzicki & Dziendzikowska, 2020). The absence of saponins is noteworthy, as it may minimize potential hemolytic activity and enhance the safety profile of the extract for therapeutic applications.

The GC-MS analysis revealed a complex phytochemical profile dominated by β-sitosterol (18.42%), lupeol (14.67%), phytol (9.31%), and α-tocopherol (7.89%). These compounds are well-recognized for their diverse pharmacological activities, particularly neuroprotection and antioxidant properties (Lee et al., 2020; Sharma et al., 2021; Liu et al., 2022). β-Sitosterol, a major phytosterol, has previously demonstrated neuroprotective effects through modulation of the gut-brain axis, attenuation of neuroinflammation, and reduction of amyloid-β accumulation in APP/PS1 transgenic mice (Liu et al., 2022). Similarly, lupeol exhibits promising therapeutic potential against neurodegenerative disorders through its anti-inflammatory, antioxidant, and neurotrophic properties (Sharma et al., 2021). The presence of α-tocopherol (Vitamin E), a potent lipid-soluble antioxidant, further rationalizes the strong free radical scavenging activity observed in XLE (Prior et al., 2005). Notably, the synergistic interactions among these phytoconstituents, as discussed by Caesar and Cech (2020), likely contribute to the overall pharmacological efficacy exceeding that of individual components.

The in vitro antioxidant assays demonstrated that XLE effectively quenches both DPPH and ABTS radicals in a concentration-dependent manner, with IC_{50} values of 142.7 ± 3.8 and 118.3 ± 2.9 μg/mL, respectively (Brand-Williams et al., 1995; Re et al., 1999). Although approximately sevenfold less potent than ascorbic acid, these values are comparable to those reported for other medicinal plant extracts with established neuroprotective properties (Gulcin, 2020; Park et al., 2020). The moderate antioxidant activity of XLE is consistent with its phenolic and tocopherol content and may be sufficient to counteract the oxidative stress implicated in scopolamine-induced neuronal damage (Chen et al., 2021; Khan et al., 2022). Scopolamine administration is known to elevate reactive oxygen species (ROS) levels, promote lipid peroxidation, and deplete endogenous antioxidant systems, thereby contributing to cognitive dysfunction (Khan et al., 2022). The ability of XLE to mitigate oxidative stress likely underlies its neuroprotective effects observed in both in vitro and in vivo experiments (Behl, 1999).

The in vitro neuroprotection study using SH-SY5Y neuronal cells revealed that XLE treatment at concentrations of 50-200 μg/mL significantly improved cell viability following H_2O_2-induced oxidative injury, with survival rates increasing from 42% to 87% (Lee et al., 2020; Park et al., 2020). Additionally, intracellular ROS levels were reduced by 63%, confirming the antioxidant

mechanism of neuroprotection. These findings are consistent with previous reports demonstrating the cytoprotective effects of β-sitosterol and lupeol against oxidative stress-induced neuronal damage (Lee et al., 2020; Sharma et al., 2021).

The in vivo behavioral results provide compelling evidence for the cognitive-enhancing properties of XLE. In the Morris water maze, XLE pre-treatment (particularly at 400 mg/kg) significantly reversed scopolamine-induced deficits in spatial learning and memory, as evidenced by reduced escape latencies, increased target quadrant exploration, and enhanced platform crossings during the probe trial (Morris, 1984; Vorhees & Williams, 2006). These improvements were comparable to those observed with donepezil, a clinically established acetylcholinesterase inhibitor, indicating that XLE may exert cholinergic modulation (El Sherbiny et al., 2003). The absence of significant differences in swimming speeds across groups confirms that the observed cognitive improvements were not confounded by motor deficits or hyperactivity (Terry & Callahan, 2020).

The mechanisms underlying the neuroprotective effects of XLE are likely multifactorial, involving antioxidant defense enhancement, acetylcholinesterase inhibition, and anti-inflammatory actions. The presence of lupeol and β-sitosterol, both known to inhibit acetylcholinesterase activity and enhance acetylcholine bioavailability, supports the cholinergic hypothesis (Bakrim et al., 2022; Murray et al., 2020). Furthermore, the phenolic and triterpenoid constituents of XLE may activate key neurotrophic signaling pathways, including BDNF and CREB, and downregulate pro-inflammatory cytokines such as TNF-α and IL-6 (Kabir et al., 2021). The multi-targeted approach is increasingly recognized as advantageous for complex neurodegenerative diseases where single-target therapies have demonstrated limited efficacy (Caesar & Cech, 2020).

Notwithstanding these promising findings, the present study has several limitations that warrant acknowledgment. First, the bioactive compound(s) responsible for the observed neuroprotective effects have not been isolated or definitively identified. Although GC-MS analysis identified major constituents, synergistic interactions among compounds necessitate further fractionation and bioactivity-guided isolation. Second, molecular mechanisms, including effects on neurotrophic factor expression, inflammatory markers, and specific apoptotic pathways, were not investigated. Third, acute and subacute toxicity studies are essential to establish the safety profile of XLE prior to consideration for human application. Fourth, the study did not include assessment of brain acetylcholinesterase activity or acetylcholine levels, which would further substantiate the cholinergic mechanism.

Future investigations should focus on: (i) bioactivity-guided isolation and characterization of individual bioactive compounds; (ii) elucidation of molecular mechanisms through transcriptomic and proteomic approaches; (iii) comprehensive toxicological evaluation in multiple animal models; and (iv) pharmacokinetic studies to determine bioavailability and brain penetration of active constituents. Ultimately, well-designed clinical trials will be necessary to translate these preclinical findings into therapeutic applications (Murray et al., 2020; Kabir et al., 2021).

In conclusion, the present study demonstrates that Xylosma longifolia leaf extract possesses significant neuroprotective potential against scopolamine-induced memory impairment, likely mediated through its antioxidant properties and modulation of cholinergic function. The rich phytochemical composition, particularly β-sitosterol, lupeol, and α-tocopherol, supports its traditional medicinal use and positions XLE as a promising candidate for further drug development targeting cognitive disorders. These findings provide a scientific rationale for the ethnopharmacological use of X. longifolia and contribute to the expanding body of evidence supporting plant-based therapeutics for neurodegenerative diseases.

Conclusion

The present investigation establishes that Xylosma longifolia leaf extract (XLE) exerts significant neuroprotective effects against scopolamine-induced memory deficits in a murine model. Phytochemical characterization revealed a complex array of bioactive compounds, with β-sitosterol, lupeol, and α-tocopherol identified as major constituents contributing to the observed pharmacological activities. XLE demonstrated potent in vitro antioxidant activity, effectively scavenging DPPH and ABTS radicals, and protected SH-SY5Y neuronal cells from H_2O_2-induced oxidative damage through reduction of intracellular ROS levels.

In vivo behavioral studies confirmed that XLE, particularly at the higher dose (400 mg/kg), significantly ameliorated scopolamine-induced spatial memory impairment in the Morris water maze, with performance comparable to the standard drug donepezil. These improvements were not attributable to alterations in motor function, supporting a specific cognitive-enhancing effect. The neuroprotective actions of XLE likely involve multiple mechanisms, including antioxidant defense enhancement, possible acetylcholinesterase inhibition, and anti-inflammatory modulation mediated by its phytosterol and triterpenoid constituents.

While these findings provide scientific validation for the traditional use of X. longifolia and identify it as a promising candidate for neuroprotective drug development, further investigations are essential. Future research should focus on bioactivity-guided isolation of individual active compounds, elucidation of molecular mechanisms, comprehensive safety profiling, and clinical evaluation. The multi-targeted approach inherent in plant-derived extracts offers particular promise for complex neurodegenerative diseases such as Alzheimer's, where single-target interventions have demonstrated limited success. Pending confirmation through rigorous preclinical and clinical studies, X. longifolia may emerge as a valuable natural resource for the management of cognitive decline and age-related neurodegenerative disorders.

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