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PHARMACOLOGICAL EVALUATION OF OXYSTELMA ESCULENTUM EXTRACT ON ALZHEIMERS DISEASE IN MICE

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Abstract:

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by memory impairment and cognitive decline. In this study, the anti-Alzheimer's potential of Oxystelma esculentum R.Br. was investigated using in vitro and in vivo models. Sequential extraction of the aerial parts of the plant was performed using hexane, chloroform, ethyl acetate, and ethanol. Phytochemical analysis revealed that the ethanolic extract contained glycosides, flavonoids, phytosterols, alkaloids, terpenoids, and phenolic compounds, indicating its rich bioactive profile.

The acetylcholinesterase inhibition assay demonstrated significant in vitro activity, with the ethanolic extract showing the lowest IC50 value (38.54 µg/mL) compared to other extracts, suggesting potential to enhance cholinergic neurotransmission. Acute toxicity studies in Swiss albino mice indicated the ethanolic extract was safe up to 2000 mg/kg body weight, with no mortality or significant behavioral changes observed.

In vivo evaluation using scopolamine-induced amnesia in mice demonstrated that oral administration of ethanolic extract (200 and 400 mg/kg) significantly improved memory and learning, as evidenced by Y-maze, open-field, and traction tests. Biochemical assessments revealed a reduction in malondialdehyde (MDA) levels and enhancement of antioxidant enzymes such as catalase and glutathione, indicating a protective effect against oxidative stress.

The findings suggest that the ethanolic extract of O. esculentum exhibits neuroprotective activity and may ameliorate cognitive deficits, potentially through cholinesterase inhibition and antioxidant mechanisms. These results support the traditional use of the plant in managing memory disorders and highlight its potential as a source of therapeutic agents for Alzheimer's disease.

Keywords

Oxystelma esculentum, Alzheimer's disease, Acetylcholinesterase inhibition, Phytochemicals, Ethanol extract, Neuroprotection, Oxidative stress, Scopolamine-induced amnesia, Memory enhancement, Antioxidant enzymes

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INTRODUCTION:

Alzheimer's Disease

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that leads to the loss of brain cells, resulting in memory impairment and cognitive decline. Symptoms often begin mildly and worsen over time. The condition was first described by German physician Alois Alzheimer in 1906. According to the National Institute on Aging, AD is the sixth leading cause of death in the United States, primarily affecting older adults.

Pathophysiology

The key pathological features of AD include the formation of amyloid plaques and neurofibrillary tangles, accompanied by the loss of neuronal connections. The human brain contains approximately 100 billion neurons, responsible for functions such as learning, memory, hearing, and sensory processing. In AD, neuronal damage disrupts these networks, leading to cell death.

Plaques

Amyloid plaques are formed by the aggregation of beta-amyloid fragments derived from amyloid precursor protein (APP) via β-secretase activity. These plaques are toxic to neurons and impair cell-to-cell communication, inducing oxidative stress and neuronal death.

Tangles

Tau proteins, which support intracellular transport, form neurofibrillary tangles in AD. These tangles disrupt the transport of nutrients and essential molecules within neurons, causing further neurotoxicity.

Neurotransmitter Dysfunction

Cholinergic and glutamatergic systems are primarily affected. Memory loss is linked to reduced acetylcholine levels, treatable with acetylcholinesterase inhibitors. Glutamatergic dysregulation via NMDA receptor overactivation also contributes to cognitive decline.

Global Burden

AD affects approximately 36.5 million people worldwide, with prevalence higher in high-income countries. Women are more frequently affected than men. In the U.S., Alzheimer's-related deaths rose 16% during the COVID-19 pandemic.

Risk Factors

AD results from a combination of genetic, environmental, and lifestyle factors, including aging, family history, cardiovascular diseases, diabetes, poor diet, sedentary lifestyle, head injury, smoking, alcohol use, and low mental activity.

Symptoms

AD progresses slowly, with varying manifestations:

- **Early:** Memory lapses, difficulty in decision-making, confusion.
- **Middle:** Disorientation, impulsivity, mood swings.
- **Late:** Hallucinations, delusions, loss of speech, incontinence, severe memory deficits.

Diagnosis

Diagnosis relies on medical and family history, behavioral observation, and neurological assessments. Advanced diagnostic tools include blood biomarkers, MRI/CT, PET, SPECT scans, lumbar puncture, genotyping, and EEG.

Management

Treatment aims to maintain cognitive function, manage behavioral symptoms, improve quality of life, and reduce morbidity. Cholinesterase inhibitors and NMDA receptor modulators are commonly used. Recent therapies target disease mechanisms, though AD's exact cause remains unclear.

Herbal and Alternative Therapies

Herbal medicines from ancient traditions in India, China, and Egypt have shown promise in AD management. Plants such as Bacopa monnieri, Centella asiatica, Valeriana officinalis, and Myristica fragrans exhibit neuroprotective, antioxidant, anti-inflammatory, anticholinesterase, and memory-enhancing effects. Numerous other plants worldwide have demonstrated similar potential, highlighting the growing interest in natural therapies for cognitive support.

Materials and Methods

Plant Collection

Leaves of *Oxystelma esculentum* were collected from Koyalaguem village, Yadadri Bhuvanagiri District, Telangana, India. The plant material was poisoned, mounted on herbarium sheets, and taxonomically identified by the Botanical Survey of India (BSI), Deccan Regional Centre, Hyderabad. The identified specimens were deposited in the Herbarium Hyderabadense, Department of Botany, Osmania University, Hyderabad.

Preparation of Extracts

The collected leaves were shade-dried, powdered, and sequentially extracted using a Soxhlet apparatus with hexane, chloroform, ethyl acetate, and ethanol. The extracts were concentrated using a rotary vacuum evaporator and stored in a desiccator until further use.

Preliminary Phytochemical Screening
All extracts were screened for the presence of secondary metabolites, including alkaloids, carbohydrates, glycosides, phytosterols, flavonoids, tannins, phenolics, proteins, amino acids, saponins, and fixed oils, using standard chemical tests such as Dragendorff's, Wagner's, Molisch, Fehling, Benedict, Legal's, Liebermann-Burchard, Shinoda, Biuret, and Spot tests.

In Vitro Anti-Alzheimer Activity
Acetylcholinesterase Inhibition Assay
The anti-Alzheimer potential of hexane, chloroform, ethyl acetate, and ethanol extracts was evaluated using a spectrophotometric acetylcholinesterase (AChE) inhibition assay based on Ellman's method. Different concentrations (25–800 µg/mL) of each extract were incubated with AChE and DTNB. Formation of the yellow-colored thiocholine-DTNB complex was monitored at 405 nm for 5 minutes. The extract showing the lowest IC50 was selected for further isolation of active compounds using column chromatography.

Acute Toxicity Study
Acute oral toxicity of the active ethanolic extract was performed in Swiss albino mice (25–30 g) according to OECD guideline 423. Mice were acclimatized for 7 days, and the extract was administered orally at 2000 mg/kg as a 1% carboxymethylcellulose suspension. Observations for signs of toxicity, mortality, behavior, and physiological changes were recorded for 14 days. Based on the results, one-fifth and one-tenth of the safe dose were used for in vivo studies.

In Vivo Anti-Alzheimer Activity
Experimental Design
Thirty male Swiss mice (6–7 weeks old, 20–30 g) were randomly divided into five groups (n=6):

Group	Treatment	Drug/Dose
I	Control	Normal saline
II	Disease control	Scopolamine (1 mg/kg, i.p.)
III	Test dose 1	Scopolamine + low-dose extract
IV	Test dose 2	Scopolamine + high-dose extract
V	Standard	Scopolamine + Donepezil (5 mg/kg)

Extract and Donepezil were administered orally for 15 days. Scopolamine was administered from day 8 to 14 to induce amnesia. Behavioral tests were performed 1 hour after treatment on the 15th day.

Behavioral Studies
- **Y-Maze Test:** Assessed spatial working memory. Mice were placed in a three-arm maze, and spontaneous alternation behavior was recorded for 5 minutes. Percent alternation was calculated as (number of alternations ÷ possible alternations) × 100.
- **Open-Field Test:** Measured locomotor activity and exploration in a 60 × 60 × 25 cm arena divided into squares. Parameters recorded included line crossings, rearing, and time spent in the center.
- **Traction Test:** Evaluated motor coordination using a 12 mm horizontal bar. The time mice held onto the bar (grip index) was recorded.

Biochemical Analysis
After behavioral studies, mice were sacrificed, and brains were homogenized in PBS (pH 7.4) to obtain 10% tissue homogenates for biochemical assays.

- **Malondialdehyde (MDA):** Lipid peroxidation was measured using TBA assay, absorbance at 530 nm.
- **Catalase Activity:** Measured via H2O2 decomposition, absorbance at 240 nm.
- **Reduced Glutathione (GSH):** Determined using Ellman's reagent, absorbance at 412 nm.

Statistical Analysis
Data are expressed as mean ± SEM. Statistical comparisons were performed using one-way ANOVA, followed by Duncan's multiple range test ($P < 0.05$).

RESULTS AND DISCUSSION:

Percentage Yield of Extracts
The percentage yields and appearances of the extracts obtained from *Oxystelma esculentum* R.Br. are summarized in Table 1.

Table 1. Percentage Yield and Appearance of Extracts

Extract	Appearance	Yield (% w/w)
Hexane	Green	11.2
Chloroform	Dark green	9.2
Ethyl acetate	Yellowish green	7.3
Ethanol	Reddish brown	12.5

The highest yield was observed with ethanol (12.5%), followed by hexane, chloroform, and ethyl acetate.

Phytochemical Screening

Preliminary phytochemical analysis revealed the presence of various primary and secondary metabolites in the extracts (Table 2).

Table 2. Phytochemical Screening of Extracts

Compounds	Hexane	Chloroform	Ethyl Acetate	Ethanol
Alkaloids	-	+	+	+
Reducing Sugar	-	+	+	+
Saponins	+	+	-	-
Phytosterol	-	+	+	+
Tannins	-	+	-	+
Flavonoids	-	+	+	+
Protein & Amino Acid	-	-	-	+
Terpenoids	+	+	+	+
Glycosides	-	+	+	+
Fixed Oils & Fats	+	+	-	-

Results Interpretation:

- Hexane extract: terpenoids, saponins, oils/fats
- Chloroform extract: alkaloids, carbohydrates, phytosterols, tannins, glycosides, oils/fats
- Ethyl acetate extract: alkaloids, carbohydrates, phytosterols, flavonoids, terpenoids, glycosides
- Ethanol extract: alkaloids, glycosides, flavonoids, terpenoids, phytosterols, phenolics

Discussion:

Ethanolic extract exhibited the richest phytochemical profile, containing glycosides, flavonoids, phytosterols, alkaloids, terpenoids, and phenolic compounds. These bioactive constituents are known for diverse pharmacological activities including anti-inflammatory, antimicrobial, diuretic, and neuroprotective effects.

In Vitro Anti-Alzheimer Activity
Acetylcholinesterase (AChE) Inhibition:

All extracts were tested for AChE inhibition, and IC50 values are shown in Table 3.

Table 3. AChE Inhibition by Extracts

Extract	IC50 (µg/mL)
Galantamine (Standard)	14.22
Hexane	726.07
Chloroform	171.41
Ethyl Acetate	72.01
Ethanol	38.54

Results:
The ethanol extract showed the strongest AChE inhibition with an IC50 of 38.54 µg/mL, indicating significant anti-Alzheimer potential compared to other extracts.

Discussion:
Acetylcholinesterase (AChE) catalyzes the breakdown of acetylcholine, and its inhibition improves cholinergic neurotransmission, enhancing memory and cognitive function. The ethanol extract, rich in flavonoids and polyphenols, demonstrated promising AChE inhibition, suggesting its potential as a natural cognitive enhancer comparable to standard drugs like galantamine.

Acute Toxicity Study

Table 4. Acute Toxicity of Ethanol Extract (2000 mg/kg)

Observation	30 min	4 h	24 h	14th day
Body weight	-	-	-	-
Preterminal death	-	-	-	-
Convulsions	-	-	-	-
Righting reflex	+	+	+	+
Lacrimation	-	-	-	-
Salivation	-	-	-	-
Respiration	+	+	+	+
Diarrhea	-	-	-	-
Sedation	-	-	-	-
Excitation	+	+	-	+
Aggression	+	+	+	+

Results:
No mortality or severe toxic effects were observed, indicating that the ethanol extract is safe up to 2000 mg/kg. Low (200 mg/kg) and high (400 mg/kg) doses were selected for in vivo studies.

In Vivo Anti-Alzheimer Activity

Y-Maze Test

Table 5. Percentage Alternation in Y-Maze

Group	% Alternation (Mean ± SEM)
Control	60.33 ± 2.50
Scopolamine	16.54 ± 1.50***
Scopolamine + 200 mg/kg	65.45 ± 6.55***
Scopolamine + 400 mg/kg	76.50 ± 5.45***
Donepezil (5 mg/kg)	72.34 ± 4.56***

Results:

Scopolamine significantly reduced spontaneous alternation. Both doses of ethanol extract significantly improved memory performance (**p < 0.001).

Open-Field Test

Table 6. Locomotor and Exploratory Behavior

Parameter	Control	Scopolamine	Low Dose	High Dose	Donepezil
Crossing	75 ± 2.8	45 ± 3.1***	60 ± 3.4***	71 ± 4.7***	75 ± 1.8***
Rearing	9 ± 1.9	5 ± 0.8***	6 ± 1.5	8 ± 1.1	10 ± 1.1**
Time in center (min)	4 ± 1.1	2.5 ± 0.8***	3 ± 1.0	4.5 ± 0.8**	5 ± 2.0***

RESULTS & DISCUSSION:

The ethanol extract enhanced exploratory behavior and reduced scopolamine-induced memory deficits. The high dose (400 mg/kg) showed superior improvement in locomotor activity, rearing, and time spent in the center, suggesting cognitive and anxiolytic benefits.

Traction Test

Table 7. Retention Time on Horizontal Bar (seconds)

Group	Retention Time (s)
Control	14.00 ± 1.10
Scopolamine	4.50 ± 1.50**
Scopolamine + 200 mg/kg	9.50 ± 2.55*
Scopolamine + 400 mg/kg	28.57 ± 2.00***
Donepezil	26.34 ± 4.56***

Results:

The high-dose ethanol extract (400 mg/kg) significantly improved motor coordination and balance (**p < 0.001), indicating neuroprotective effects.

Biochemical Estimation

Table 8. MDA, Glutathione, and Catalase Levels

Group	MDA (mmol/g)	Glutathione (mmol/g)	Catalase (mmol/g)
Control	59.30 ± 3.54	5.56 ± 0.36	287.56 ± 10.34
Scopolamine	97.32 ± 3.14**	1.57 ± 0.10**	107.32 ± 4.65***
200 mg/kg + Scopolamine	70.87 ± 3.87*	3.72 ± 0.16*	250.44 ± 8.78**
400 mg/kg + Scopolamine	64.80 ± 4.47***	4.36 ± 0.09**	315.72 ± 18.89***
Donepezil	59.30 ± 6.89***	4.98 ± 0.12**	387.60 ± 10.14***

Discussion:

Scopolamine induced oxidative stress, evidenced by elevated MDA and decreased catalase and glutathione levels. Treatment with ethanol extract, particularly at 400 mg/kg, significantly reduced MDA and increased antioxidant enzyme levels, demonstrating neuroprotective and antioxidant potential.

Overall Discussion

The study demonstrated that ethanol extract of *Oxystelma esculentum* R.Br. is rich in bioactive phytochemicals, exhibits in vitro acetylcholinesterase inhibition, and improves cognitive function in scopolamine-induced amnesic mice. Behavioral tests (Y-maze, open-field, traction) and biochemical analyses confirmed that the extract enhances memory, learning, motor coordination, and antioxidant defense. Polyphenols, flavonoids, terpenoids, and glycosides are likely responsible for these neuroprotective effects, suggesting the extract's potential for managing Alzheimer's disease and related neurodegenerative disorders.

CONCLUSION:

The ethanolic extract of *Oxystelma esculentum* demonstrated significant anti-Alzheimer's activity in a scopolamine-induced amnesia model. Its rich phytochemical composition, including flavonoids, glycosides, and terpenoids, likely contributed to the observed neuroprotective effects and cognitive enhancement in mice. Additionally, Kanokoside D, an iridoid glycoside isolated from the plant, was identified as a key compound capable of mitigating neurotoxicity and improving memory function. These findings suggest that *O. esculentum* holds potential as a natural therapeutic agent for managing Alzheimer's disease and related neurodegenerative disorders.

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