Phytochemical and Neuroprotective activity of *Coccinia grandis* leaves using hydroxydopamine (6-OHDA) Model

[*]Vishal Kumar, Saurabh Sharma, Sandeep Kumar, Rizwan Ahmad and Darakhshan Gazala Bari

School of Pharmacy, Vivek University, Bijnor, Uttar Pradesh

[*]Email- vishal.thakur229@gmail.com

DOI 10.51129/ujpah-2026-40-1(4)

Received –May 15, 2026
Revised – May 17, 2026
Accepted –May 21, 2026
Published – June 20, 2026

Abstract- Cognitive impairment in neurodegenerative disorders is closely associated with oxidative stress, neuro-inflammation, and cholinergic dysfunction. *Coccinia grandis*, a medicinal plant rich in bioactive constituents, has been explored for its potential neuroprotective effects. The present study aimed to evaluate the neuroprotective activity of hydroalcoholic fruit extract of *Coccinia grandis* and its fractions against scopolamine induced memory impairment in rats. The extract was subjected to phytochemical screening, thin-layer chromatography (TLC), and column chromatography for characterization. Neurobehavioral assessments were conducted using the Morris Water Maze (MWM) and Elevated Plus Maze (EPM) models. Biochemical parameters including acetylcholinesterase (AChE), interleukin-1β (IL-1β), malondialdehyde (MDA), superoxide dismutase (SOD), and catalase were evaluated. Scopolamine administration significantly impaired cognitive function, as evidenced by increased escape and transfer latency and reduced retention time. Treatment with *Coccinia grandis* extract (200mg/kg) significantly improved behavioral performance, comparable to the standard drug rivastigmine. Biochemical findings showed reduced AChE, IL-1β, and MDA levels, along with enhanced antioxidant enzyme activity (SOD and catalase). Acute toxicity studies confirmed safety up to 1000 mg/kg. Overall, the findings suggest that *Coccinia grandis* exhibits significant neuroprotective activity via antioxidant, anti-inflammatory, and cholinergic modulation mechanisms.

Introduction

The disturbance of the normal anatomical and functional integrity of the skin or underlying tissues due to physical, chemical, or biological forces is referred to as a wound. Any damage to the skin triggers a complicated series of physiological processes intended to restore tissue integrity since the skin serves as the body's main defense against environmental dangers and microbial invasion[1]. Trauma, burns, surgery, infections, and chronic illnesses like diabetes can all cause wounds that seriously hinder the healing process[2]. Wounds are categorized as either acute or chronic based on their ability to heal; chronic wounds provide significant treatment problems because of prolonged inflammation and delayed repair[3]. Hemostasis, inflammation, proliferation, and remodeling are the four overlapping stages of the tightly controlled process of wound healing. Coordinated

interactions between immune cells, fibroblasts, keratin-ocytes, cytokines, and growth factors are necessary for these stages[4]. Any interference with these processes brought on by oxidative stress, infection, or systemic diseases might cause problems and postpone healing. Clinically, wounds are a major worldwide burden that frequently results in morbidity, higher medical expenses, and a lower standard of living[5].

The epidermis, dermis, and hypodermis make up the skin, which is essential for immunological defense, thermoregulation, and protection. When this structure is damaged, the repair process is initiated and its barrier function is compromised[6]. Due to their interference with collagen synthesis, angiogenesis, and cellular regeneration, infection, oxidative stress, nutritional deficiencies, aging, and systemic disorders like diabetes are among the many factors that affect wound healing.

In wound healing, oxidative stress has two functions. Excessive reactive oxygen species (ROS) can harm proteins, lipids, and DNA, which eventually delays tissue repair, while moderate ROS levels support cellular signaling and microbial defense[7]. Antioxidants are therefore crucial for preserving redox equilibrium and encouraging repair.

Antiseptics, antibiotics, anti-inflammatory medications, and synthetic dressings are examples of conventional wound treatments. Despite their effectiveness, these methods have drawbacks such cytotoxicity, antimicrobial resistance, high cost, and insufficient healing in long-term circumstances[1-8]. Alternative remedies are becoming more popular as a result of these difficulties.

The abundance of bioactive substances found in medicinal plants, such as flavonoids, tannins, and phenolics, which have antibacterial, anti-inflammatory, and antioxidant properties, has drawn attention[9]. These characteristics make plant-based treatments viable substitutes for wound care because they promote increased collagen synthesis, angiogenesis, and tissue regeneration[4,9].

Because they offer structurally varied molecules with substantial pharmacological potential, natural products have historically been essential to drug discovery. The relevance of plants in therapeutic development is highlighted by the fact that many contemporary medications are derived from them[10]. Since ancient systems like Ayurveda and Unani have traditionally used plant-based formulations to treat skin injuries, ethnomedical knowledge further supports the use of plants in wound healing[11].

Chronic wounds continue to be a major problem despite developments in contemporary medicine, requiring the creation of safer and more potent treatments. *Mimosa tenuiflora* has bioactive chemicals with possible therapeutic properties and is traditionally used to heal burns, wounds, and infections. Its ability to cure wounds, however, has to be systematically validated by science using experimental models.

Coccinia grandis

The perennial climbing vine *Coccinia grandis* (L.) Voigt (Cucurbitaceae) is found throughout tropical regions, including Southeast Asia, India, and Sri Lanka. It thrives in open fields and hedges and is sometimes referred to as ivy gourd (Tindora, Kovai). The plant produces white

dioecious flowers and simple, alternating leaves with three to five lobes. Fruits are cylindrical, length (2–6 cm), green when immature and scarlet when ripe, and packed with seeds. Fruits, leaves, and roots have historically been used to treat inflammation, fever, and diabetes. The fruits are eaten as vegetables as well. Along with nutritional elements like fiber and vitamin C, phytochemical ingredients include flavonoids (quercetin, kaempferol), triterpenoids (cucurbitacins), and other substances like alkaloids, glycosides, and phenolics.

Pharmacologically, the herb has antipyretic, anti-inflammatory, anti-diabetic, and antioxidant properties. Fruits are picked when they are ripe for therapeutic uses and when they are green for consumption. Formulations typically use powdered or fresh fruits (10–20 g/day)[12].

Material and Methods

Plant Material Standardization- Ash levels and moisture content were among the pharmacopoeial characteristics used to standardize the authorized plant material. To assess purity and inorganic content, total ash, water-soluble ash, and acid-insoluble ash were measured. Loss on drying using an oven technique was used to determine the moisture content. To guarantee the purity and identification of the crude medication, these procedures were performed in accordance with conventional standards[13].

Table-1 List of Chemicals

Sr .no	Chemicals	Supplied by
1	Ethanol	Solanki enterprise pune
2	H2so4	Solanki enterprise pune
3	Ferric chloride	Solanki enterprise pune
4	Iodine	Solanki enterprise pune
5	Ethyl acetate	Solanki enterprise pune
6	Chloroform	Solanki enterprise pune
7	Petroleum ether	Solanki enterprise pune

Extraction of *Coccinia grandis* - Soxhlet extraction was performed on 150 g of dried *Coccinia grandis* leaf powder using a hydroalcoholic solvent (ethanol:water, 80:20). The extraction was done for 16 hours at 30 to 40°C. A semisolid extract (~100 ml) was produced by concentrating the extracted material (~500 ml) through evaporation[14].

Phytochemicals Screening - To find the main bioactive components, the ethanolic extract was put through a qualitative phytochemical screening. Based on distinctive color changes and precipitate formation, standard tests verified the presence of carbohydrates (Molisch test), alkaloids (Hager's test), saponins (foam test), tannins (ferric chloride test), terpenoids (Salkowski test), glycosides (Keller–Killiani test), steroids, phenols, amino acids (ninhydrin test), flavonoids, and anthraquinones[15].

Thin-layer chromatography (TLC) - Using silica gel-coated plates as the stationary phase and suitable solvent systems as the mobile phase, TLC was used to separate and identify phytoconstituent. Plates were produced in a saturated chamber after samples were applied as spots. Following development, spots were

seen using detecting reagents and UV light, and Rf values were computed to describe the constituents[16].

Column Chromatography - Column chromatography packed with silica gel (60–120 mesh) was used to separate bioactive substances. After the extract was injected into the column and adsorbed onto silica, it was eluted using more polar solvents (ethyl acetate, petroleum ether, and chloroform). Fractions were gathered, condensed, and then examined further[17].

Phytochemical Analysis of Fractions- Standard qualitative assays were used to screen collected fractions for phyto-constituents. Based on distinctive color responses, the presence of carbohydrates, alkaloids, saponins, tannins, terpenoids, glycosides, steroids, phenols, amino acids, flavonoids, and anthraquinones was confirmed.

Bioactive Fraction Characterization by TLC - With an observed Rf value of 0.81, TLC profiling of bioactive fractions employing methanol:water (8:2) as the mobile phase revealed the presence of phenolic chemicals, validating successful separation and identification[18].

In-vivo Studies

Institutional Animal Ethics Committee (IAEC) Approval- All experimental procedures involving animals were conducted in accordance with the ethical guidelines for the care and use of laboratory animals as prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used in the study.

Experimental Animals - A certified and registered laboratory animal supplier provided healthy adult Wistar albino rats of any sex, weighing between 180 and 220 g. The animals were kept in the institutional animal facility under conventional environmental conditions, which included a 12-hour light-dark cycle, a controlled temperature of 22 ± 2°C, and a relative humidity of 50–60%. To maintain hygienic conditions, rats were housed in sterile polypropylene cages with sterile rice husk bedding that was changed on a regular basis. For the duration of the study, the animals were given a regular pellet meal and unlimited access to water. To reduce stress and guarantee physiological stability, all animals were acclimated to laboratory conditions for at least seven days prior to the start of the experiment. Throughout the course of the trial, animals were handled with care and kept in uniform conditions.

Experimental Design - The experimental study was carried out using Wistar albino rats (180–220 g), randomly divided into **six groups (n=6 per group)**. Cognitive impairment was induced using **scopolamine (1 mg/kg, i.p.)**, and treatment was continued for **28 days**.

Table-2 Grouping and Treatment

Group	Treatment	Dose / Details
Group I	Normal Control	Normal saline (p.o.)
Group II	Negative Control	Scopolamine (1 mg/kg, i.p.)
Group III	Standard	Rivastigmine (0.5 mg/kg, p.o.) + Scopolamine
Group IV	Test I (CGEE)	*Coccinia grandis* extract (200 mg/kg, p.o.) + Scopolamine
Group V	Test II (PEF)	Petroleum ether fraction (200 mg/kg, p.o.) + Scopolamine
Group VI	Test III (EAF)	Ethyl acetate fraction (200 mg/kg, p.o.) + Scopolamine

Oral Acute Toxicity - According to OECD 423, the acute toxicity of *Coccinia grandis* ethanolic extract (CGEE) and its fractions was evaluated. To determine a safe dose range, animals were given single oral doses (100–2000 mg/kg) and monitored for behavioral, neurological, physiological, and mortality changes over a 14-day period.

Behavioral Evaluation (Memory and Learning)- The Morris Water Maze (MWM) and Elevated Plus Maze (EPM) were used to assess cognitive function:
• **Elevated Plus Maze (EPM):** The main measure of memory and learning was transfer latency (TL). Increased open-arm entries and time spent demonstrated decreased anxiety and higher cognitive ability, while a decrease in TL on successive trials suggested improved memory retention.

• **Morris Water Maze (MWM):** Time spent in the target quadrant during the probe trial and escape latency (EL) during training trials were used to measure spatial learning and memory. While longer retention times in the target quadrant showed memory consolidation, lower EL across sessions suggested learning acquisition.
To determine the effectiveness of neuro-protective therapy, behavioral assessments were carried out both before and after 28 days of treatment.

Cognitive Impairment Induction- Scopo-lamine and chronic restraint stress (6 hours per day for 28 days) were used to generate memory impairment, which resulted in neuroinflammation and cognitive deficits.
Estimation of Biochemistry - Brain homogenates were examined for:
• Activity of acetylcholinesterase (AChE) (412 nm)

• ELISA measurement of interleukin-1β (IL-1β) levels

Neuroinflammation and cholinergic function were evaluated using these markers.

Model 6-OHDA - 6-OHDA was used to promote neurodegeneration. CGEE (200 and 400 mg/kg) and rivastigmine were given for 28 days. To ascertain neuroprotective effects, behavioral and physiological markers were assessed.

Statistical Analysis- One-way ANOVA and Dunnett's test were used to evaluate the data, which were presented as Mean ± SEM (n = 6). Significance was defined as $P < 0.05$.

Results

Gathering, Drying, and Verification- Fruits of *Coccinia grandis* were collected, cleaned, and washed to remove impurities. The material was shade dried or oven dried (40–50°C), powdered, and stored in airtight containers. The plant was authenticated based on morphological characteristics by a qualified authority.

Table- 3 Physicochemical Evaluation of *Coccinia grandis* Extract

Sr. No.	Evaluation parameters	Values *Coccinia grandis* extract
1	Total ash value	4.6 %
2	Acid insoluble ash	1.4 %
3	Water soluble ash	3%
4	Moisture content	7 %

Conclusion

The *Coccinia grandis* extract's physico-chemical parameters—total ash (4.6%), acid-insoluble ash (1.4%), water-soluble ash (3%), and moisture content (7%)—were all within allowable bounds, suggesting minimal impurity levels and strong ability. These results validate the extract's purity and applicability for additional pharmaco-logical and neuroprotective research.

Extraction- The *Coccinia grandis*, extracted with various 80% ethanol and 20% water.

Table-4 Yield of various extracts obtained from the fruit

Sr. No.	Evaluation Parameters	Color	Nature	Percentage Yield (% W/W)
1.	Ethanol (80%) and water (20%)	Bright red	Smooth and soft	10

Phytochemical Screening- The extraction procedure is as described previously, using ethanol (800 mL) as the solvent and keeping the solid-to-solvent ratio at about 1:8 (w/v) for 24 hours at 90°C.

Table-5 Ethanolic extraction of *Coccinia grandis*

Sr no	Test name	Procedure	Observation	Result
1.	Alkaloid Test: B) Wagner's test:	2ml extract + 1-2 drops of wagner's reagent	No reddish brown precipitate	Alkaloid absent
2.	Glycosides Test: A) Keller Killani test:	1 ml extract + 1.5 ml glacial acetic acid + 1 drop of 5 % ferric chloride + conc.H_2SO_4	A blue coloured solution (in the layer)	Glycosides present
3.	Flavonoid Test: A) Ferric chloride test:	Extract solution + few drops 10 % ferric chloride solution	Green precipitate	Flavonoid present
4.	Tannins Test: A) Dil. HNO_3:	Few ml extract + dil. HNO_3	Yellow precipitate	Tannis absent
5.	Phenol Test: A) Ferric Chloride Test:	Extract + ferric chloride solution	dark blue or greenish black colour appeared	Phenol absent

Conclusion

Phytochemical screening revealed the absence of alkaloids but the presence of glycosides, flavonoids, tannins, and phenolic substances. These constituents indicate the presence of bioactive compounds that may contribute to the pharmacological potential of the extract, including antidiabetic activity.

Table-6 TLC for Phytoconstituent

Type of Constituent	Mobile phase	Observation	RF value found
Flavonoids	n-Hexane: Ethanol 6:4	Light green	0.67
Glycosides	Chloroform : Methanol 6:4	Light green	0.81

Conclusion

Using the proper solvent systems, thin layer chromatographic profiling of *Coccinia grandis* extract showed discrete spots corresponding to flavonoids (Rf 0.67) and glycosides (Rf 0.81). These findings indicate the extract's potential neuroprotective action and its appropriateness for additional pharmacological testing by confirming the presence of antioxidant rich phyto-constituents.

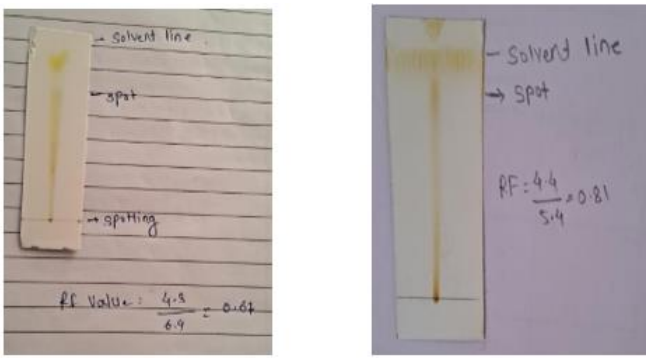


Figure-1 TLC for Flavonoids and Glycosides

Column Chromatography- The hydro-alcoholic extract's initial phytochemical examination revealed the existence of phenolic compounds, which was corroborated by TLC profiling that revealed several phytoconstituents. In light of these results, column chromatography was used to separate the extract's bioactive constituents.

Table-7 Phytoconstituents Tests of Bioactive Fractions

Sr no.	Phytoconstituent	Test performed and reagents	Ethanolic fraction	Pet ether fraction	Ethyl acetate fraction
1	Alkaloids	i)Mayer's test ii)Dragondroff's test iii)Hager's test	+ + +	+ + +	+ + +
2	Carbohydrates	i) Benidict's test ii) Fehling's test iii) Molisch's test	+ + +	+ + +	+ + +
3	Glycosides	• Keller-killani test	+	+	+
4	Tannins	i) Gelatin test • Lead acetate test	+ +	+ +	+ +
5	Saponins	• Foam test	+	+	+
6	Flavonoids	i) Ferric chloride test ii) Alkaline • reagent test	+ +	+ +	+ +
7	Steroids	**1.** Liebermann-Burchard' test	+	+	+
8	Terpenoids	**2.** Salkowski's test	+	+	+
9	Amino acid	**3.** Ninhydrin Test	_	_	_
10	Anthraquinones	**4.** Borntranger's test	_	_	_

(+ Present , - Absent)

Conclusion

While amino acids and anthraquinones were not present, phytochemical screening of ethanolic, petroleum ether, and ethyl acetate fractions revealed the presence of alkaloids, carbohydrates, glycosides, tannins, saponins, flavonoids, steroids, and terpenoids. These findings show that the plant has a variety of bioactive components that contribute to its medicinal potential.

In-vivo Study

Oral Acute Toxicity- Mice were used to test the acute oral toxicity of *Coccinia grandis* fractions (PEF, CF, and EAF) over a 14-day period. Up to 1000 mg/kg, no

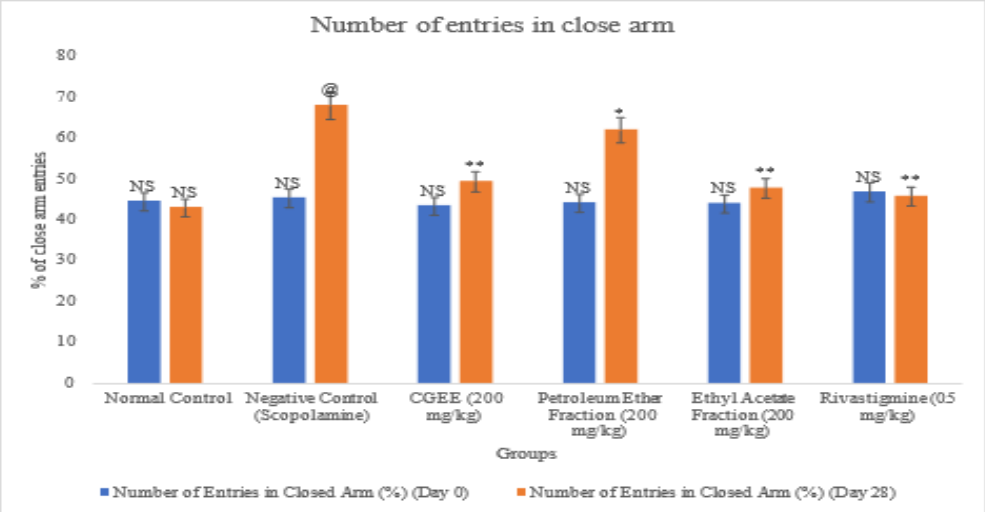
mortality or notable behavioral changes were seen, and normal body weight, eating, and movement were maintained. Mild and temporary lethargy was observed at 2000 mg/kg without significant toxicity. These results show that all fractions are safe and have high tolerance. LD50 was predicted to be greater than 1000 mg/kg, and all fractions were safe up to 1000 mg/kg. For additional neuroprotective testing, a dosage of 200 mg/kg was chosen.

I. Estimation of Behavioural Study

A. Elevated Plus Maze (EPM) Test

Table-8 Effect of *Coccinia grandis* fraction on Number of Entries in Closed Arm in Elevated Plus Maze

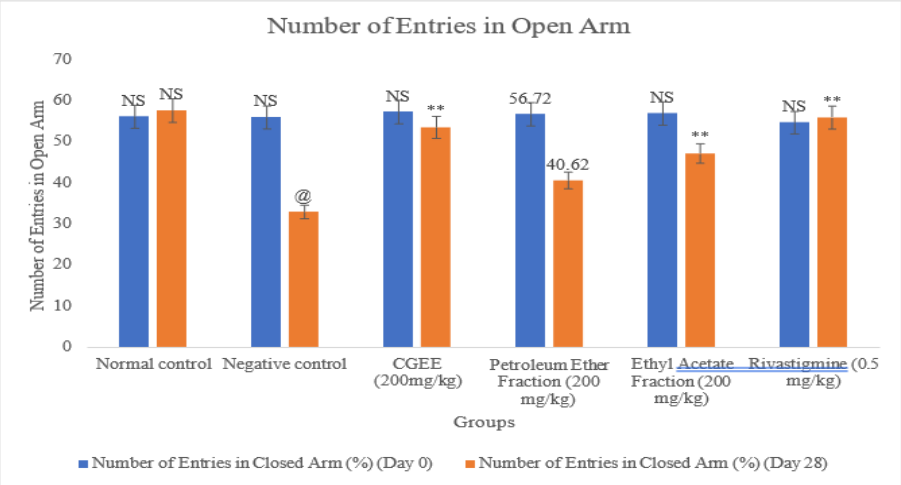
Sr. No.	Groups	Number of Entries in Closed Arm (%) (Day 0)	Number of Entries in Closed Arm (%) (Day 28)
1	Normal Control	44.62 ± 0.47	43.12 ± 0.68
2	Negative Control (Scopolamine)	45.45 ± 0.59ns	68.20 ± 1.61@
3	CGEE (200 mg/kg)	44.12 ± 0.52ns	49.84 ± 0.61**
4	Petroleum Ether Fraction (200 mg/kg)	44.25 ± 0.58ns	62.15 ± 0.56**
5	Ethyl Acetate Fraction (200 mg/kg)	44.08 ± 0.47ns	47.92 ± 0.49**
6	Rivastigmine (0.5 mg/kg)	46.92 ± 0.22ns	45.92 ± 0.50**



Graph-1 Effect of *Coccinia grandis* fraction on number of entries in close arm of rats in EPM apparatus

Table-9 Effect of *Coccinia grandis* fraction on number of entries in open arm of rats in EPM apparatus

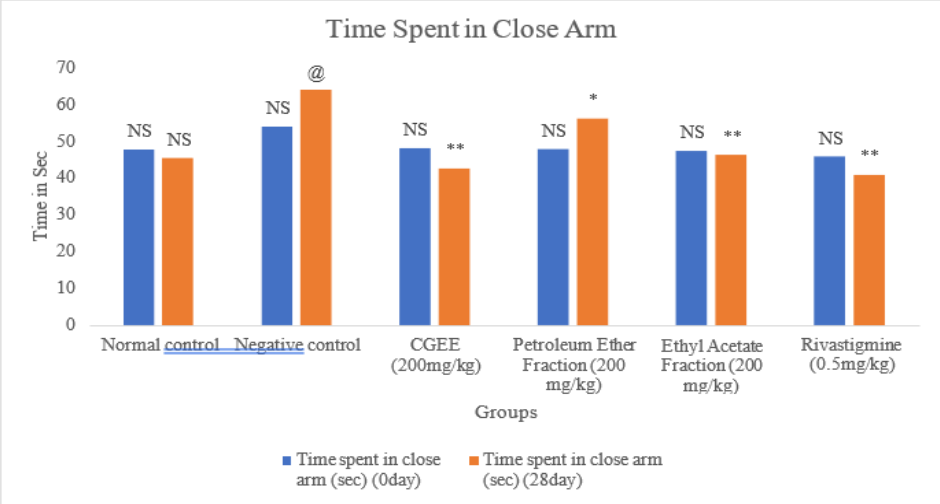
Sr. No.	Groups	Number of Entries in Open Arm (%) (Day 0)	Number of Entries in Open Arm (%) (Day 28)
1	Normal Control	56.12 ± 0.77	57.62 ± 0.51
2	Negative Control (Scopolamine)	55.95 ± 0.58ns	32.95 ± 0.66@
3	CGEE (200 mg/kg)	56.84 ± 0.42ns	53.15 ± 0.73**
4	Petroleum Ether Fraction (200 mg/kg)	56.72 ± 0.39ns	40.62 ± 0.68**
5	Ethyl Acetate Fraction (200 mg/kg)	56.91 ± 0.47ns	47.18 ± 0.55**
6	Rivastigmine (0.5 mg/kg)	54.67 ± 1.68ns	55.92 ± 0.50**



Graph-2 Effect of *Coccinia grandis* fraction on number of entries in open arm of rats in EPM apparatus

Table-10 Effect of *Coccinia grandis* fraction on time spend in close arm by rats in EPM apparatus

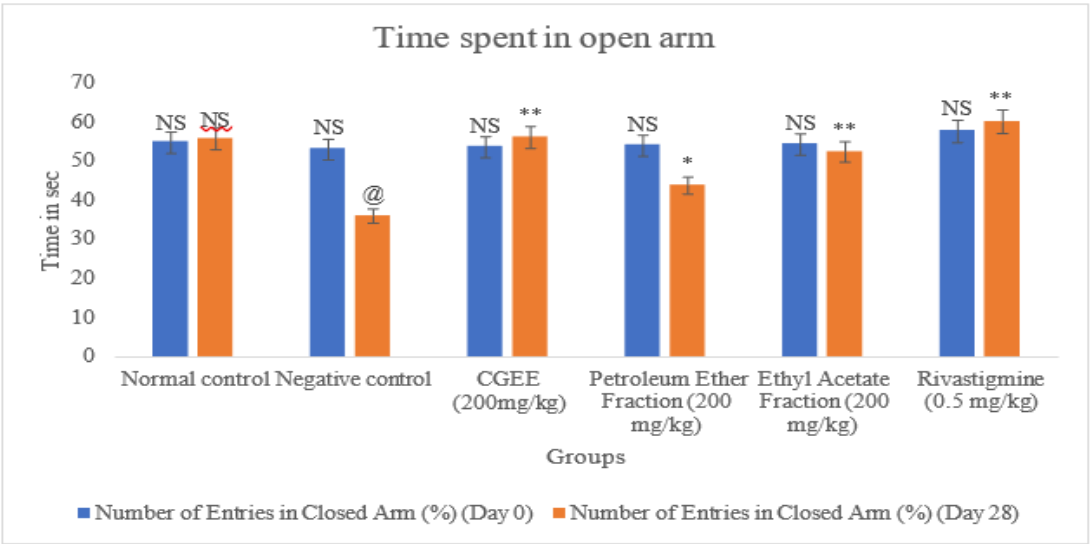
Sr. No.	Groups	Time Spent in Closed Arm (sec) (Day 0)	Time Spent in Closed Arm (sec) (Day 28)
1	Normal Control	47.75 ± 0.85	45.50 ± 0.24
2	Negative Control (Scopolamine)	53.96 ± 1.23ns	63.96 ± 1.23@
3	CGEE (200 mg/kg)	48.10 ± 1.37ns	42.32 ± 0.42**
4	Petroleum Ether Fraction (200 mg/kg)	47.88 ± 1.12ns	56.14 ± 0.51*
5	Ethyl Acetate Fraction (200 mg/kg)	47.35 ± 1.05ns	46.28 ± 0.44**
6	Rivastigmine (0.5 mg/kg)	45.85 ± 3.2	40.86 ± 0.51**



Graph-3 Effect of *Coccinia grandis* fraction on time spend in close arm by rats in EPM apparatus

Table-11 Effect of *Coccinia grandis* fraction on time spend in open arm by rats in EPM apparatus

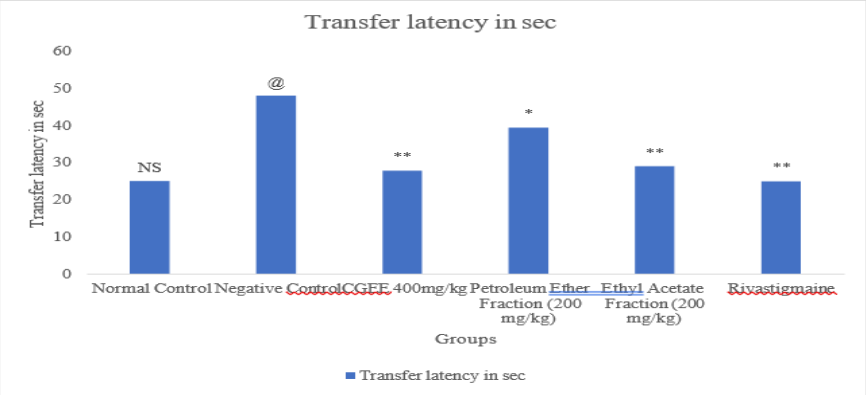
Sr. No.	Groups	Time Spent in Open Arm (sec) (Day 0)	Time Spent in Open Arm (sec) (Day 28)
1	Normal Control	54.82 ± 0.43	55.82 ± 0.66
2	Negative Control (Scopolamine)	53.07 ± 1.15ns	36.07 ± 1.83@
3	CGEE (200 mg/kg)	54.18 ± 0.61ns	56.92 ± 1.74**
4	Petroleum Ether Fraction (200 mg/kg)	54.05 ± 0.58ns	43.86 ± 1.62**
5	Ethyl Acetate Fraction (200 mg/kg)	54.36 ± 0.47ns	52.48 ± 1.51**
6	Rivastigmine (0.5 mg/kg)	57.70 ± 0.44ns	60.20 ± 2.18**



Graph-4 Effect of *Coccinia grandis* fraction on time spend in open arm by rats in EPM apparatus

Table-12 Effect of *Coccinia grandis* fraction on transfer latency of rats in EPM apparatus.

Sr. No.	Groups	Transfer Latency (sec)
1	Normal Control	25.01 ± 1.15
2	Negative Control (Scopolamine)	48.03 ± 1.16@
3	CGEE (200 mg/kg)	36.97 ± 1.13**
4	Petroleum Ether Fraction (200 mg/kg)	39.42 ± 1.08**
5	Ethyl Acetate Fraction (200 mg/kg)	28.94 ± 0.85**
6	Rivastigmine (0.5 mg/kg)	24.94 ± 1.16**

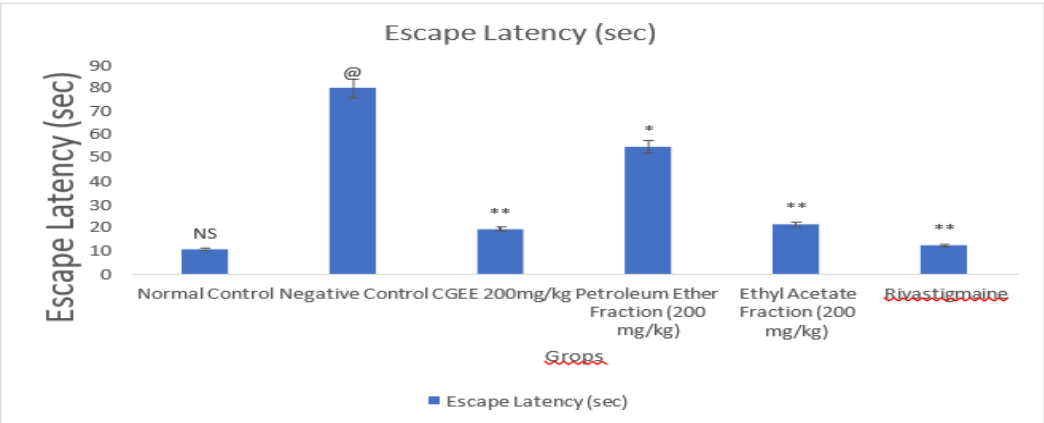


Graph-5 Effect of *Coccinia grandis* fraction on transfer latency of rats in EPM apparatus.

B. Morris Water Maze Apparatus

Table - 13 Effect of *Coccinia grandis* fraction on escape latency of rats in MWM apparatus.

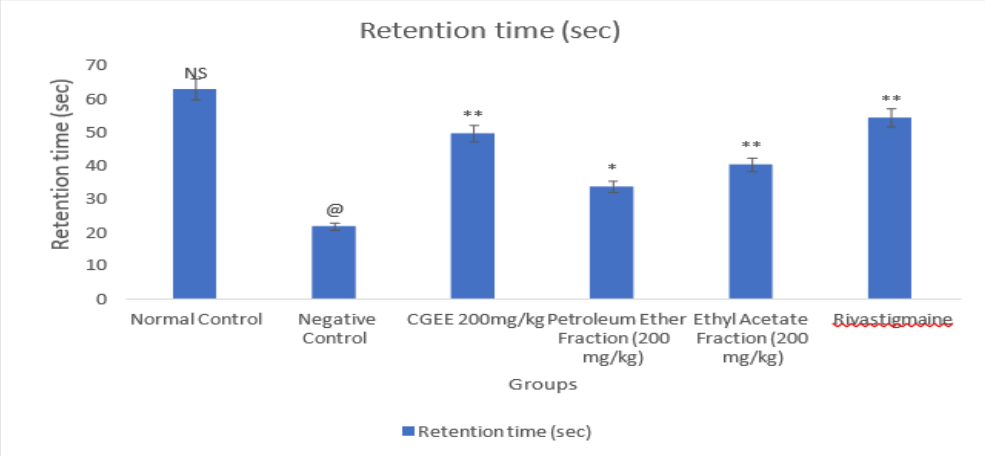
Sr. No.	Groups	Escape Latency (sec)
1	Normal Control	10.50 ± 1.29
2	Negative Control (Scopolamine)	80.25 ± 1.72@
3	CGEE (200 mg/kg)	19.14 ± 1.65**
4	Petroleum Ether Fraction (200 mg/kg)	54.76 ± 1.88**
5	Ethyl Acetate Fraction (200 mg/kg)	21.35 ± 1.54**
6	Rivastigmine (0.5 mg/kg)	12.25 ± 1.78**



Graph-6 Effect of *Coccinia grandis* fraction on escape latency of rats in MWM apparatus

Table-14 Effect of *Coccinia grandis* fraction on retention time of rats in MWM apparatus.

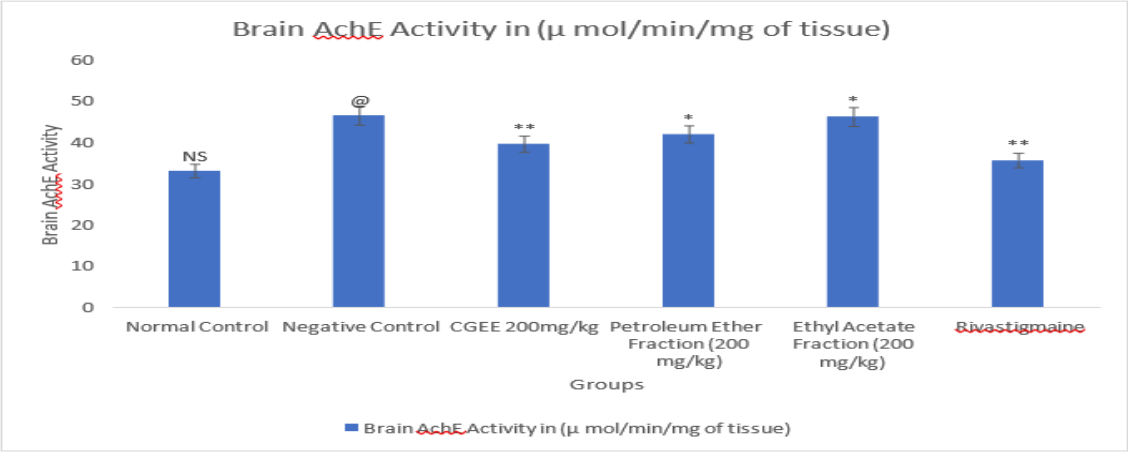
Sr. No.	Groups	Retention Time (sec)
1	Normal Control	62.75 ± 1.78
2	Negative Control (Scopolamine)	21.69 ± 1.82@
3	CGEE (200 mg/kg)	49.5 ± 2.11**
4	Petroleum Ether Fraction (200 mg/kg)	33.62 ± 1.94**
5	Ethyl Acetate Fraction (200 mg/kg)	40.18 ± 1.63**
6	Rivastigmine (0.5 mg/kg)	54.25 ± 1.71**



Graph -7 Effect of *Coccinia grandis* fraction on escape latency of rats in MWM apparatus.

II. Estimation of Biochemical Parameter

Effect of *Coccinia grandis fraction* and Rivastigmine on Brain Acetyl Cholinesterase (AChE) Activity in rats



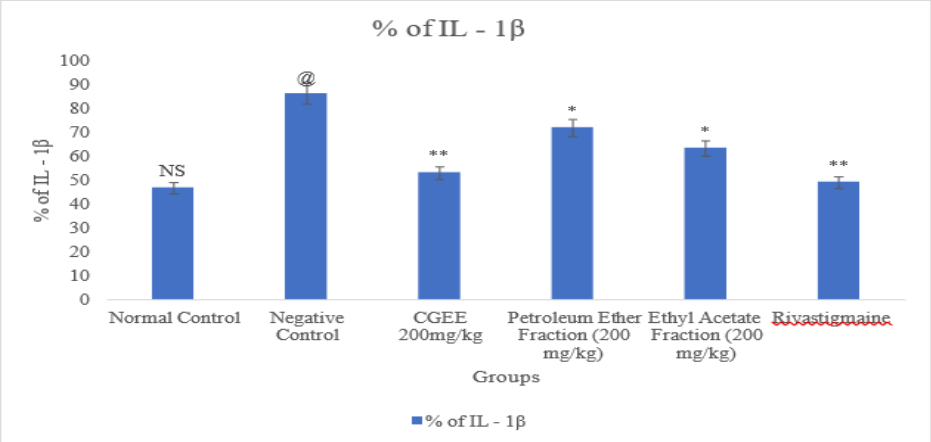
Graph-8 Effect of *Coccinia grandis* fraction on brain ache activity in rats.

A. Estimation of Interleukin -1β level.

Table-15 Effect of *Coccinia grandis* fraction on Iinterleukin -1β level in rats.

Sr. No.	Groups	IL-1β (%)
1	Normal Control	47.02 ± 0.99
2	Negative Control (Scopolamine)	86.52 ± 0.61@

3	CGEE (200 mg/kg)	53.45 ± 0.92**
4	Petroleum Ether Fraction (200 mg/kg)	72.18 ± 0.88**
5	Ethyl Acetate Fraction (200 mg/kg)	63.64 ± 0.76**
6	Rivastigmine (0.5 mg/kg)	49.32 ± 0.87**

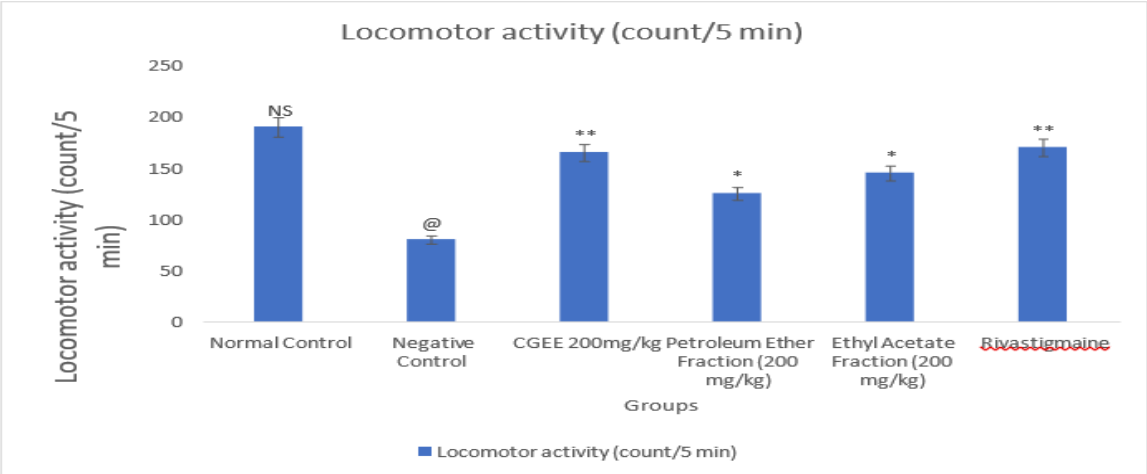


Graph-9 Effect of *Coccinia grandis* fraction on Iinterleukin -1β level in rats.

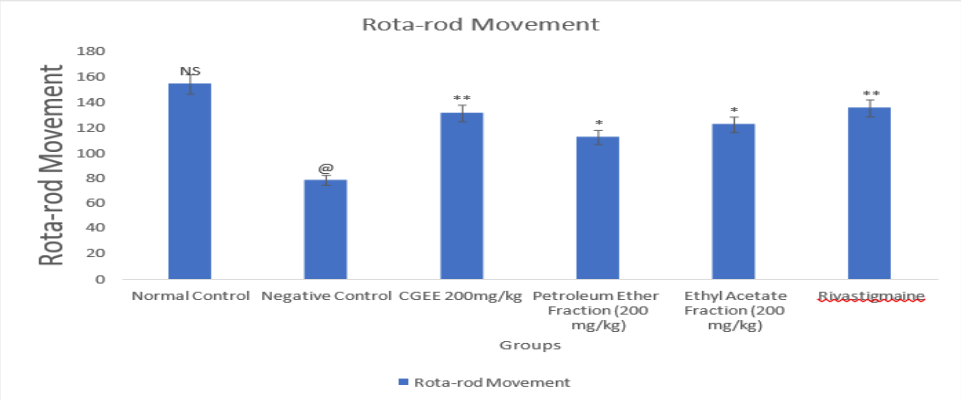
B. 6 - hydroxydopamine (6-OHDA) Model

Table-17 Effect of *Coccinia grandis* fraction on Locomotor Activity (Actophotometer)

Sr. No.	Group Name	Locomotor Activity (count/5 min)
1	Normal Control	190 ± 8
2	Negative Control (Scopolamine)	80 ± 6
3	CGEE (200 mg/kg)	165 ± 7
4	Petroleum Ether Fraction (200 mg/kg)	125 ± 6
5	Ethyl Acetate Fraction (200 mg/kg)	145 ± 6
6	Rivastigmine (0.5 mg/kg)	170 ± 5



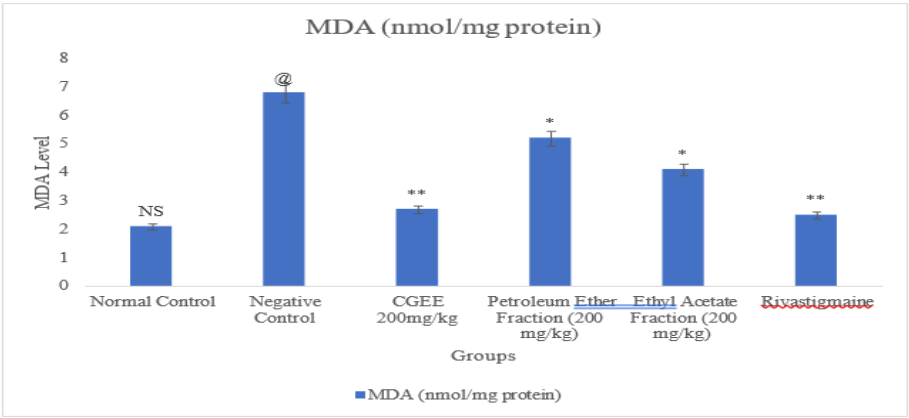
Graph-10 Effect of *Coccinia grandis* fraction – Locomotor activity (Actophotometer)



Graph-11 Effect of *Coccinia grandis* fraction on rota-rod apparatus

Table-18 Effect of *Coccinia grandis* fraction on MDA levels

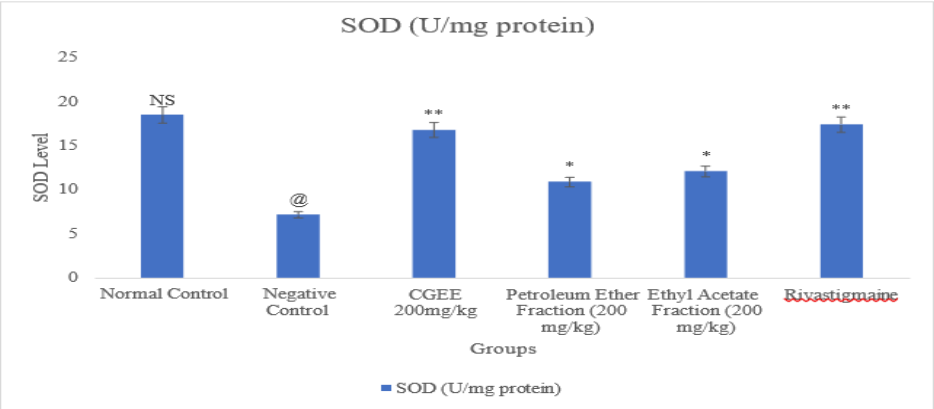
Sr. No.	Group Name	MDA (nmol/mg protein)
1	Normal Control	2.1 ± 0.2
2	Negative Control (Scopolamine)	6.8 ± 0.3
3	CGEE (200 mg/kg)	2.7 ± 0.3
4	Petroleum Ether Fraction (200 mg/kg)	5.2 ± 0.3
5	Ethyl Acetate Fraction (200 mg/kg)	4.1 ± 0.2
6	Rivastigmine (0.5 mg/kg)	2.5 ± 0.2



Graph-12 Effect of *Coccinia grandis* fraction on MDA levels

Table-19 Effect of *Coccinia grandis* fraction on SOD (Superoxide Dismutase)

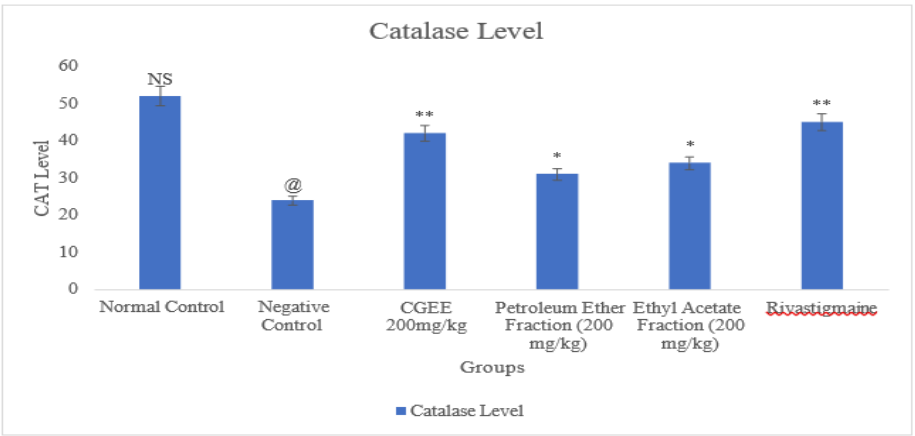
Sr. No.	Group Name	SOD (U/mg protein)
1	Normal Control	18.5 ± 0.6
2	Negative Control (Scopolamine)	7.2 ± 0.4
3	CGEE (200 mg/kg)	16.8 ± 0.5
4	Petroleum Ether Fraction (200 mg/kg)	10.9 ± 0.5
5	Ethyl Acetate Fraction (200 mg/kg)	12.1 ± 0.6
6	Rivastigmine (0.5 mg/kg)	17.4 ± 0.5



Graph-13 Effect of *Coccinia grandis* fraction on SOD (Superoxide Dismutase)

Table-20 Effect of *Coccinia grandis* fraction on Catalase Activity

Sr. No.	Group Name	Catalase Level (U/mg protein)
1	Normal Control	52 ± 0.6
2	Negative Control (Scopolamine)	24 ± 0.4
3	CGEE (200 mg/kg)	42 ± 0.5
4	Petroleum Ether Fraction (200 mg/kg)	31 ± 0.5
5	Ethyl Acetate Fraction (200 mg/kg)	34 ± 0.6
6	Rivastigmine (0.5 mg/kg)	45 ± 0.5



Conclusion

In scopolamine induced cognitive impairment, learning and memory were considerably enhanced by *Coccinia grandis* extract and its fractions. Behavioral outcomes (EPM, Morris Water Maze) showed marked improvement, with CGEE exhibiting effects comparable to rivastigmine. Biochemical findings (↓AChE, IL-1β, MDA; ↑SOD, catalase) further support its neuroprotective and antioxidant potential.

Discussion

The current study used scopolamine-induced cognitive impairment in rats to assess the neuroprotective potential of *Coccinia grandis* extract and its fractions. It

is well known that scopolamine disrupts cholinergic transmission, resulting in memory impairments akin to those seen in neurodegenerative diseases like Alzheimer's.

Scopolamine significantly increased transfer latency and decreased open arm exploration, indicating impaired learning and memory, according to behavioral tests using the Elevated Plus Maze (EPM). These effects were considerably reversed by treatment with *Coccinia grandis* extract (CGEE) and its fractions, as seen by enhanced open arm entries and time spent and reduced transfer delay. The most noticeable effect among the fractions was CGEE, which was comparable to the activity of the common medication rivastigmine (Tables 8–11).

Similarly, scopolamine-treated rats had a significant decrease in retention time and an increase in escape latency in the Morris Water Maze (MWM) test, indicating compromised spatial learning and memory. Improved memory acquisition and retention were indicated by treatment with CGEE and its fractions, which dramatically decreased escape latency and increased time spent in the target quadrant. Once more, CGEE outperformed other fractions in terms of efficacy (Tables 12–13). The behavioral results were further corroborated by biochemical studies. Acetylcholinesterase (AChE) activity was markedly boosted by scopolamine treatment, which resulted in decreased acetylcholine levels and higher oxidative stress (MDA) and inflammatory (IL-1β) indicators. AChE activity and IL-1β levels were dramatically reduced after treatment with *Coccinia grandis* extract, indicating a reduction in neuroinflammation and a restoration of cholinergic function. Furthermore, a notable decrease in MDA levels and a rise in the activity of antioxidant enzymes (SOD and catalase) were noted, suggesting that oxidative stress had been attenuated.

Coccinia grandis's rich phytochemical makeup, especially flavonoids and phenolic chemicals, which are recognized for their anti-inflammatory and antioxidant qualities, may be responsible for the plant's demonstrated neuroprotective effects. By scavenging free radicals, lowering lipid peroxidation, and adjusting neuro-transmitter levels, these substances probably help protect neuronal cells.

Overall, the results indicate that *Coccinia grandis* has strong neuroprotective action, which may be mediated by anti-inflammatory, antioxidant, and cholinergic pathways. The findings also show that the crude extract (CGEE) is more effective than specific fractions, perhaps as a result of several phytoconstituents working in concert.

Conclusion

In behavioral animals, *Coccinia grandis* extract showed notable neuroprotective efficacy against scopolamine-induced memory impairment, enhancing learning, memory, and spatial ability. Reduced oxidative stress and restoration of cholinergic function are indicated by biochemical data (MDA, AChE, SOD, catalase). Flavonoids and phenolic chemicals are probably responsible for the action, indicating a multi-targeted mechanism. All things considered, *Coccinia grandis* exhibits potential as a natural treatment for cognitive conditions like Alzheimer's.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

References

1. Guyon, I. and Elisseeff, A. An introduction to feature extraction. In Feature extraction: foundations and applications. *Berlin, Heidelberg: Springer Berlin Heidelberg*; 2006; $p_{p.}$ 1-25.
2. Heneka, M.T. Neuro-inflammation in Alzheimer's disease Lancet neurology. 2015; 14: 388–405 doi: 10.1016. S1474-4422 (15):70016-5.
3. Xia, M.; Yang, L.; Sun, G. and Qi S, Li B. Mechanism of depression as a risk factor in the development of Alzheimer's disease: the function of AQP4 and the glymphatic system. Psychopharmacology. 2017; Feb; 234(3):365-79.
4. Abdul Manap, A.S.; Vijayabalan, S.; Madhavan, P.; Chia, Y.Y.; Arya, A.; Wong, E.H.; Rizwan, F.; Bindal, U. and Koshy, S. Bacopa monnieri, a neuroprotective lead in Alzheimer disease: a review on its properties, mechanisms of action, and preclinical and clinical studies. *Drug target insights*; 2019; Jul;13: 1177392819866412.
5. Bradley-Whitman, M.A. and Lovell, MA. Biomarkers of lipid peroxidation in Alzheimer disease (AD): an update. *Archives of toxicology*. 2015; Jul; 89(7):1035-44.
6. Armstrong, R.A. The molecular biology of senile plaques and neurofibrillary tangles in Alzheimer's disease. *Folia neuropathologica.;* 2009; Jan. 1; 47(4):289-99.
7. Sultana, R. and Butterfield, DA. Role of oxidative stress in the progression of Alzheimer's disease. *Journal of Alzheimer disease;* 2010; Jan. 6;1 9(1):341-53.
8. Sharifi-Rad, J.; Salehi, B.; Varoni, E.M.; Sharopov, F.; Yousaf, Z.; Ayatollahi, S.A.; Kobarfard, F.; Sharifi-Rad, M.; Afdjei, M.H.; Sharifi-Rad, M. and Iriti, M. Plants of the Melaleuca genus as antimicrobial agents: From farm to pharmacy. *Phytotherapy research*; 2017; Oct. 31; (10):1475-94.
9. Billowria, K.; Ali, R.; Rangra, N.K.; Kumar, R. and Chawla, P.A. Bioactive flavonoids: a comprehensive review on pharmacokinetics and analytical aspects. *Critical Reviews in Analytical Chemistry*; 2024; Jul. 3; 54(5):1002-16.
10. Albensi, B.; Cadonic, C.; Thompson, E. and Chowdhury, S.K.; Snow, W. and Ferny-hough, P. Mitochondrial Dysfunction in Alzheimer's Disease. *In Mitochondrial Medicine;* 2014.
11. Durazzo, A. and Lucarini, M. A current shot and re-thinking of antioxidant research strategy. *Bra. J. Anal. Chem.;* 2018; 5:9-11.
12. Yashashwini, Y.C. Evaluation of learning and memory enhancing activity of *Coccinia grandis* fruits in rats. *J. of Drug Delivery & Therapeutics*; 2018; Sep. 1; 8 (5).
13. Joshi, V.K.; Joshi, A. and Dhiman, K.S. The Ayurvedic Pharmacopoeia of India, development and perspectives. *J. of ethnopharmacology*. 2017; Feb. 2; 197:32-8.
14. Kshirsagar, P.R.; Mohite, A.; Suryawanshi, S.; Chavan, J.J.; Gaikwad, N.B. and Bapat, V.A. Plant regeneration through direct and indirect organogenesis, phyto-molecular profiles, antioxidant properties and swertiamarin production in elicitated cell suspension cultures of Swertia minor (Griseb.) Knobl. *Plant Cell, Tissue and*

Organ Culture (PCTOC); 2021; Feb.; 144 (2):383-96.

15. Shrestha, D.; Magar, A.B.; Pakka, S. and Sharma, K.R. Phytochemical analysis, antioxidant, antimicrobial, and toxicity studies of Schima wallichii growing in Nepal. *International Journal of Food Properties*; 2024; Dec. 31; 27(1):273-85.
16. Harborne, A.J. Phytochemical methods a guide to modern techniques of plant analysis. *Springer science & business media*; 1998; Apr. 30.
17. Sharma, O.P.; Kumar, N; Singh, B. and Bhat, T.K. An improved method for thin layer chromatographic analysis of saponins. *Food Chemistry*; 2012; May 1; 132(1):671-4.
18. Wagner, H. and Bladt, S. Plant Drug Analysis. *A Thin Layer Chromatography Atlas*.