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Original Article

Evaluation of Anti-Alzheimer's Activity of Spirulina Platensis and *Clerodendreme inerme* Plant Extracts

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ABSTRACT

Background: Alzheimer's Disease (AD) is the most prevalent cause of dementia globally and its frequency increases as the world's population ages. AD is one of the largest healthcare issues of the 21st century as the primary cause of dementia and neurodegenerative illnesses. An acquired loss of cognitive function across many cognitive areas is referred to as dementia. The animal studies conducted in this research article to determine whether chemical compounds cause AD may be useful in understanding the disease's mechanism and treatment options. **Objectives:** The current study assessed the effectiveness of C-Phycocyanin (C-PC) from *Spirulina platensis* and ethanolic extract of *Clerodendreme inerme* against scopolamine-induced Alzheimer's in mice. Since *Clerodendreme inerme* and C-PC are rich in active chemical constituents, they function as beneficial antioxidants. These constituents include saponins, oil, fat, phenolic compounds, tannins, alkaloids, flavonoids, glycosides, terpenoids, steroids, amino acids, proteins and carbs. Thus, an assessment of the anti-Alzheimer properties of the ethanolic extract from *Clerodendreme inerme* and C-Phycocyanin from *Spirulina platensis* was conducted. **Materials and Methods:** The study was designed to evaluate prophylactic effect of *Spirulina platensis* and *Clerodendreme inerme* against Scopolamine induced Alzheimer's in mice. Piracetam (200 mg/kg) was used as a standard. The extracts from the plant and leaves of *Spirulina platensis* and *Clerodendreme inerme* were screened for anti-alzheimer potential in scopolamine induced Alzheimer's in mice by administering 0.4 mg/kg b.w/day i.p. as test dose.Behavioral Assessments as well as serum levels of AchE, *in vitro* and Bio-chemical parameters were assessed. **Results:** Treated with ethanolic extracts of *Clerodendreme inerme*, C-PC of *Spirulina platensis*, it demonstrated a dose-dependent reduction in escape latency time and an increase in time spent in the target quadrant. *Clerodendreme inerme* and C-PC of *Spirulina platensis* both reduce the escape latency time and transfer latency time in EPM in a dose-dependent manner. AD rodents treated with ethanolic extracts of *Clerodendreme inerme*, C-PC of *Spirulina platensis* demonstrated a dose-dependent rise in percentage change in the Y maze test. Apart from behavioral assessments, extracts from ethanolic extract of *Clerodendreme inerme*, SP demonstrated a dose-dependent decrease in AchE level, since the loss of ACh resulting from AChE's hydrolytic action causes cognitive impairment. AD rodents that were pretreated with ethanolic extract of *Clerodendreme inerme*, SP there is an increase in catalase and lipid peroxidation**. Conclusion:** The study concludes that *Spirulina platensis* and *Clerodendreme inerme* extracts have a significant anti-Alzheimer activity. This is likely because the extracts contain a variety of nutrients, including saponins, oil, fat, phenolic compounds, tannins, alkaloids, flavonoids, glycosides, terpenoids, amino acids, steroids, proteins and carbs.

Keywords: Anti-Alzheimer activity, *Clerodendreme inerme*, C-PC, Anti-oxidants, Dementia, Alzheimer's disease, Scopolamine.

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INTRODUCTION

One of the common diseases of modern civilization is AD. This progressive neurodegenerative disease, which affects 10% of the world's population, causes unimaginable suffering for people and costs US$100 billion in medical expenses each year. Even though amyloid plaque is recognized to be the primary cause of AD, it is unclear how these plaques form in the brain.[1-3] Therapy is, therefore, restricted to increasing the cerebral concentration of acetylcholine through the use of medications such as tacrine, donepezil, rivastigmine, or galantamine to alleviate the symptoms of memory loss. Even after much investigation, the pathophysiology of AD is still unclear.[4] Gene polymorphisms, inflammation, cerebral lesions and hypoperfusion of brain cells comprise the pathology. Our findings suggest that ECs

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(Endothelial Cells) could affect the cortical cells' continuous degeneration in AD. Alzheimer's disease is caused by a neurotoxin that specifically damages cortical cells and precursors of β-amyloid plaque released by the brain's endothelium. AG activates a large number of endothelial cells in response to inflammation, ischemia, and cerebral hypoxia.[5] The development of antiAG drugs that target aberrant ECs in the brain may enhance AD prevention and treatment if AD is, as suggested, an AG-dependent illness. In 1906, Dr. Alois Alzheimer noted pathological neurological findings that were classified and further identified as indicators of AD; these included neural plaques, which are masses primarily, composed of beta-Amyloid (Aβ) peptides and neurofibrilar tangles, which are primarily calm as intraneuronal hyperphosphorylated tau-masses. The kDa peptide Aβ is produced when the α and γ secretase enzymes break down the Amyloid Precursor Protein (APP).[6,7] Abnormal Aβ mass (Aβ1-42) is caused by β secretase breakdown resulting from mutations in the APP gene or the secretase enzyme. These Aβ masses generate oligomers, which then multimerize to form solid core amyloid plaques through multimerization.[8-10]

The BBB (Blood Brain Barrier) is a barrier that exists between the blood vessels in the cerebral area and other regions of the central nervous system, preventing fluid and particles from entering the brain through blood circulation.[11-13] The first evidence of this BBB's dysfunction came from AD animal trials[14] and was then recognized as the prime yet unclear path of AD.[15-20] Therefore, a promising target for treating AD is the pathway that disrupts the BBB. These two hallmark pathologies are the classical characteristics of this neurodegenerative disease process: Deposition of β-amyloid plaque and hyperphosphorylated tau neurofibrillary tangles. Neutrophil formation results from the degradation of amyloid precursor protein derivative β-amyloid protein, which is also referred to as dendritic, amyloid plaques, or senile. Nerve cells include twisted protein fibers in addition to several other components. These fibers include the protein tau, which is normally present in neurons. Tau molecules cluster together to create neurofibrillary tangles when they are processed improperly. This research addresses the experimental paradigm of AD type dementia induced by scopolanine. Scopolamine is an antimuscarinic drug that blocks acetylcholine's effect on muscarinic receptors by highly affinity-binding to postsynaptic receptor sites. This raises hippocampus and cortex AChE activity. Because of cholinergic hypofunction brought on by scopolamine, cerebral blood flow is reduced. Additionally, scopolamine releases ROS (Reactive Oxygen Species), which worsens the antioxidant status in the group treated with scopolamine and causes free radical damage and a raise in brain levels of MDA. Through a rise in pro-inflammatory cytokines and oxidative stress, scopolamine causes neuroinflammation in the hippocampal region. It is established that scopolamine raises APP and Tau levels. In an animal model of dementia, scopolamine administration has been used to assess the efficacy of possible novel Alzheimer's disease therapeutic agents.

MATERIALS AND METHODS

Collection and authentication of plant

Purchased from Perrys Neutraceuticals in Chennai, *Spirulina platensis* and *Clerodendreme inerme* were verified by Dr. V. Sampath Kumar, Scientist D in-charge at the Botanical Survey of India, "Southern Regional Center, Coimbatore, Tamil Nadu", India.

Experimental animals and maintenance

Animals were sustained on a 12:12-hr dark-light cycle at 45% humidity and controlled temperature of 22-25°C. Every procedure was performed in compliance with the CPCSEA, following a protocol that was authorized by the IAEC ("Institutional Animal Ethics Committee").

Preparation of Plant Extracts

C. inerme leaves were cleaned with tap water, dried at 500°C and then blended. Ground leaves (1.2 kg) were macerated in 12 L of ethanol for five days at room temperature, stirring them occasionally, before being filtered via Whatman filter paper number 3.

The plant residue had been then twice removed using ethanol, as previously mentioned. The filtrates were combined, evaporating *in vacuo*.

The residues of the "water (WF, 98.5 g, 58.3% w/w), ethyl acetate (EF, 26.7 g, 15.8% w/w) as well as hexane (HF, 36.7 g, 21.7% w/w) were obtained by partitional separation from the ethanol extract (EE, 195 g, 16.2% w/w).

Fractions were stored at 200°C and protected from light till the anti-inflammatory properties were studied. It was selected for more research. Because ethyl acetate fraction had a strong inhibitory impact on NO generation in LPS-induced macrophages. Column chromatography was used to isolate the ethyl acetate fraction (EF, 17 g)" with silica gel as the stationary phase. Subfractions 1-8 (F1-F8) have been separated from EF with a gradient of a solvent solution ("dichloromethane: methanol") from 100:0-50:50 v/v.

Eight subfractions (SF5.1-5.8) were obtained by subjecting the subfraction F5 (1.3549 g, 0.11% w/w) to a silica gel column along with a step gradient of hexane and ethyl acetate (30:70, 50:50 and 70:30 v/v) based on anti-alzheimer's activity. Subfractions SF5.1 and 5.2 were combined (0.810 g, 0.07% w/w) based on their anti-angiogenic efficacy. Compounds 1 (119 mg), 2 (129.7 mg), as well as 3 (6 mg) were obtained by eluting these on silica gel column chromatography.

Four subfractions (SF8.1-8.4) were obtained by eluting "subfraction F8 (2.930 g, 0.24% w/w) to a silica gel column using

a dichloromethane gradient: methanol (99:1-80:20, v/v). SF8.1 (0.1662 g, 0.01% w/w) was further sent to a silica gel column with compound 1 (4.1 mg) obtained from a dichloromethane gradient: methanol (97:3-92:8, v/v) as the mobile phase based on its anti-inflammatory properties. SF 8.2 (0.2648 g, 0.02% w/w) was separated using hexane-ethyl acetate (70:30-95:5, v/v)" gradient in a silica gel column to get Compound 2 (12.6 mg). Compounds 1, 2 and 3 have respective yields of 0.0103%, 0.0118% and 0.0005%.

Extraction Procedures of C-PC from *Spirulina* platensis

- Distilled water extraction.
- Homogenization method.
- Freezing and Thawing method.
- Acid extraction process.
- Heating.
- Every One Hour Freezing and Thawing.

The supernatant that was left behind after centrifugation was utilized in each of the processes as mentioned above to estimate C-PC.

Experimental Design

Group	Control	Treatment
Group I	Normal control	Distilled water or saline (2 mL/kg).
Group II	Diseased	Scopolamine Hydrobromide IP (0.4 mg/kg).
Group III	Standard	Piracetam (200 mg/kg) by oral route.
Group IV	Test-I (*Spirulina platensis*)	Low-dose extract (100 mg/kg).
Group V	Test-II (*Spirulina platensis*)	High-dose extract (200 mg/kg).
Group VI	Test-I (*Clerodendrum inerme*)	Low dose extract (200 mg/kg).
Group VII	Test-II (*Clerodendrum inerme*)	High dose extract (400 mg/kg).

Anti-Alzheimer's Study

Elevated plus maze method

This method entails testing lab animals for anxiety, typically rodents, with the primary goal of screening for anxiogenic or potentially anxieogenic compounds. The animal's anxiety is expressed as a function of how much time it spends in the arms using this approach.

It is made up of a raised, plus-shaped device with 2 closed and 2 open arms. It involves rodents' adaptation to open spaces, which results in a behaviour known as thigmotaxis, a guide for staying inside enclosed spaces or close to an edge of bounded space. The amount of anxiety reduced in this maze is shown by the longer time spent in their arms and by their suggestion of entry. Time in open arms /Total time in closed or open arms

Entries in open arms/Total entries into closed or open arms.[21-23]

T Maze

The T-maze test was applied to determine working memory capacity. It involved recording spontaneous change activity in a single session in a painted black wood T-maze. Each arm was 12 cm in height and 40 cm in length, with 10 cm at the top and 3 cm at the bottom, converging in an equilateral triangular middle section. For eight minutes, every mouse was positioned at the end of an arm and given unrestricted access to the maze. The mice must be aware of which arm they have already visited to be able to switch. Visual documentation of the sequence of arm entries-comprising any potential returns into the same arm-will be made. Recording spontaneous alternation behaviour allowed researchers to evaluate the performance of immediate working memory. When the mouse's hind paws have fully entered the arm, entry is deemed to have been completed. The term "alternation" was defined as consecutive entries on overlapping triplet sets into the 3 distinct arms (A, B and C). To assess short-term memory, the proportion of trials that included all 3 arms-that is, ABC, BCA, or CAB but not BAB-was noted as an alternation. After the last dose arm was treated on the ninth day, entries were made and the percentage change was computed.

Morris water maze

It is the approach that is most frequently used to assess physiological and neurological mechanisms. The technique entails submerging animals in a sizable circular pool of water that allows them to escape into a concealed platform whose location can only be determined by unique memory. It does not offer far-reaching local cues.

All of the animals' brains were gathered after they were sacrificed. Brains are triturated in the prepared solution according to brain weight and then centrifuged. The collected supernatant was used in the following investigations.[24]

Estimation of the brain's acetylcholinesterase enzyme levels

A cuvette comprising 2.6 mL of "phosphate buffer (0.1 M, pH 8) was 100 µL of DTNB filled with 0.4 mL of the homogenate aliquot. The absorbance was determined in a spectrophotometer

at 412 nm" after completely mixing the contents of the cuvette with bubbling air. The absorbance was measured as the basal measurement once it stabilized at that value. The absorbance altered and was recorded with the addition of 20 µL of the substrate, acetylthiocholine. Therefore, the absorbance change/min was calculated.[25]

Catalase (CAT) Principal Determination

The Aebi H technique was used to test the catalase activity. 0.1 mL of supernatant was added to a cuvette comprising 1.9 mL of 50mM phosphate buffer (pH 7.0). A newly made 30 mM H_2O_2 solution containing 1.0 mL was added to initiate the reaction. The rate of H_2O_2 decomposition was examined by monitoring variations in absorbance at 240 nm using spectrophotometry. Units/mg of protein were used to represent the catalase activity. The addition of H_2O_2 causes the reaction to occur right away. The solutions were well combined and after 15 sec (t1), initial absorbance (A1) and 30 sec (t2) were measured.[26]

The Reduced Glutathione (GSH) Principal's Estimation

GSH is an important endogenous antioxidant and non-protein thiol that counters free radical-induced damage. It contributes to the detoxification response, redox equilibrium maintenance and quenching of free radicals to protect normal cell structure and function.[27]

Lipid Peroxidation (LPO) Principle's Estimation

The peroxidation of cellular lipids is linked to oxidative stress and may be measured using TBARS ("Thiobarbituric Acid Reacting Substance"). The content of LPO products may show the oxidative stress level. OFRs ("Oxygen Free Radicals"), which damage polyunsaturated unsaturated fatty acids in cell membranes and induce LPO, are produced in response to elevated TBARS levels. The MDA (Malondialdehyde) content-a gauge of lipid peroxidation-was determined using TBARS.[28]

Determination of antioxidant activity

DPPH radical scavenging activities

Extracts as well as isolated compounds were studied for their ability to scavenge DPPH radicals by Ohkawa *et al.* In a total amount of 1 mL, all samples were combined with 0.2 mL of the newly prepared 0.1 mM "DPPH (2, 2-diphenyl-1-picrylhydrazyl)" solution. After 20 mins at room temperature and vigorously agitating the reaction liquid, the absorbance at 210 nm was determined.[24] For the creation of the control sample, neither the test compounds nor the reference antioxidants were employed; instead, DMSO (Dimethyl Sulfoxide) was utilized. In all studies, BHT (Butylated Hydroxytoluene) was employed as a reference antioxidant and acted as a positive control. A decrease in DPPH absorbance was utilized to evaluate radical scavenging activity.

$$scavenging\ effect\ (\%) = [\frac{A\ control - A\ sample}{} \times 100]$$

Where *A* control indicates the control absorbance and *A* sample represents the absorbance of the standard or extract or fractions.

Antioxidants function as radical scavengers because of their capacity to donate hydrogen, transforming the stable radical DPPH (purple) to non-radical DPPH-H (yellow). The table displays the findings of the scavenging activity of DPPH for every sample tested. The activity of the SP and CI extract and ASC ("Ascorbic Acid") both increased with the rise in sample concentration (100-500 µg mL^{-1}). SP and CI came up with IC_{50} values of 181.77 µg/mL^{-1} (Y=0.2332 x+7.61) and 195.34 µg/mL^{-1} (Y=0.217x+1.028) respectively. These results establish the SP as a major antioxidant with the capacity to block free-radical damage in the body and as an efficient free-radical inhibitor.

Statistical analysis

Every value was shown as mean±SEM. Dunnett's *t*-test was applied to perform a statistical analysis of the data after a one-way ANOVA. ANOVA was used to assess the behavioural and biochemical parameter data and Dunnett's *t*-test was then conducted. *p*-values <0.05 were considered to be significant.

RESULTS

Experimental Results

Elevated plus maze

These findings demonstrate the raised plus maze test's sensitivity to a range of experimental and procedural factors and suggest that behaviour in the improved plus maze most likely reflects state responses instead of trait responses (Andreatini and Bacellar, 2000). An animal's behaviour at the start of a test may change significantly from its behaviour at the conclusion (Carobrez and Bertoglio, 2005).

T-maze alternation

It is usually conducted in a modified cross-maze, with one arm removed to create a T-shaped maze (Figure 2). Animals may complete repeated trials over several days by being moderately deprived of food and given sweet rewards.

Time spent in the target quadrant

Morris water maze test

It assesses deficits in small rats' visual-spatial abilities and visual short-term memory. The mice in the water maze test are submerged in a large, circular pool of opaque water and given the task to swim to a platform that could be concealed or clearly visible. In both the control group and the knockout mice, there was no discernible reduction in their ability to swim. The training allowed both normal littermates and knockout mice to find

the concealed platform. Figure illustrates a typical littermate's training experiment.

DPPH radical scavenging activities

The outcomes of this activity for every tested sample are revealed in the table. The "scavenging activity" of the SP and CI extract and ASC ("Ascorbic Acid") both increased with the rise in sample concentration (100-500 µg mL^{-1}). SP and CI came up with IC_{50} values of 195.34 µg/mL^{-1} (Y=0.217x+1.028) and 181.77 µg/mL^{-1} (Y=0.2332x+7.61), respectively. These results establish the SP as a major antioxidant with the capacity to block free-radical damage in the body and as an efficient free-radical inhibitor

The *in vitro* lipid peroxidation inhibition activity

Animals that have been drinking heavily have much higher levels of MDA substances and lower GSH activity. Pretreatment with Piracetam (200 mg/kg b.wt) and SP and CI (1.75% to 3.5% 200 mg/kg, 400 mg/kg b.wt) successfully maintained the growth at levels of MDA and brought them towards the normal range, whereas GSH levels were enhanced generally (p<0.01), offering assurance against Scopalamine toxicity.

Animals have much higher levels of MDA substance and lower GSH activity. GSH or glut levels were elevated globally (p<0.01), giving assurance against diclofenac toxicity and growth of Malondialdehyde (MDA) levels was successfully maintained and transferred within the normal range after pre-treatment with Piracetam (100 mg/kg b.wt).:

In vitro inhibition of Catalase (CAT) levels

Biochemical parameter: brain sample collection

The animals in the same group that were employed on the final day were chosen to measure the activity of acetylcholinesterase. The animals were sacrificed on day nine via cervical dislocation while receiving extremely small dosages of anesthesia. The entire brain is created apart from the skeletal skull. A homogenizer was used to create brain homogenates.

Table 1: 8th Day Results.

Group	Treatment	Dose	Time spent (sec)	
			Open arm	Closed Arm
Group I	Normal control	Distilled water or saline (2 mL/kg).	236	64
Group II	Diseased	Scopolamine Hydrobromide IP (0.4 mg/kg).	92	208
Group III	Standard	Piracetam (200 mg/kg) by oral route.	192	108
Group IV	Test-I (*Spirulina platensis*).	Low dose extract (100 mg/kg).	110	190
Group V	Test-II (*Spirulina platensis*).	High dose extract (200 mg/kg).	144	156
Group VI	Test-I (*Clerodendrum inerme*).	Low dose extract (200 mg/kg).	123	177
Group VII	Test-II (*Clerodendrum inerme*).	High-dose extract (400 mg/kg).	164	136

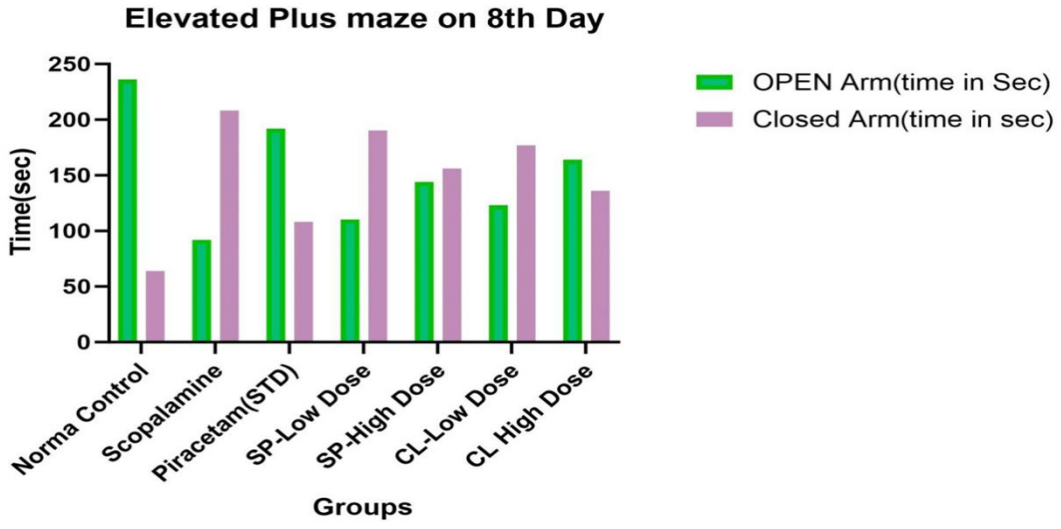

Figure 1: Results of EPM on 8th day.

Table 2: 9th Day Results.

Group	Treatment	Open arm	Closed Arm
Group I	Normal control	42	58
Group II	Diseased	9	91
Group III	Standard	36	74
Group IV	Test-I (*Spirulina platensis*)	19	81
Group V	Test-II (*Spirulina platensis*)	27	73
Group VI	Test-I (*Clerodendrum inerme*)	22	88
Group VII	Test-II (*Clerodendrum inerme*)	29	71

Table 3: 8th Day and 9th Day Results.

Group	Control	Treatment	Time spent in target zone (sec)	
			8th day	9th day
Group I	Normal control	Distilled water or saline (2 mL/kg).	48	52
Group II	Diseased	Scopolamine Hydrobromide IP (0.4 mg/kg).	14	19
Group III	Standard	Piracetam (200 mg/kg) by oral route.	42.67	37
Group IV	Test-I (*Spirulina platensis*).	Low-dose extract (100 mg/kg).	11.3	12
Group V	Test-II (*Spirulina platensis*).	High-dose extract (200 mg/kg).	23.7	32
Group VI	Test-I (*Clerodendrum inerme*).	Low dose extract (200 mg/kg).	16.3	21
Group VII	Test-II (*Clerodendrum inerme*).	High dose extract (400 mg/kg).	34	25

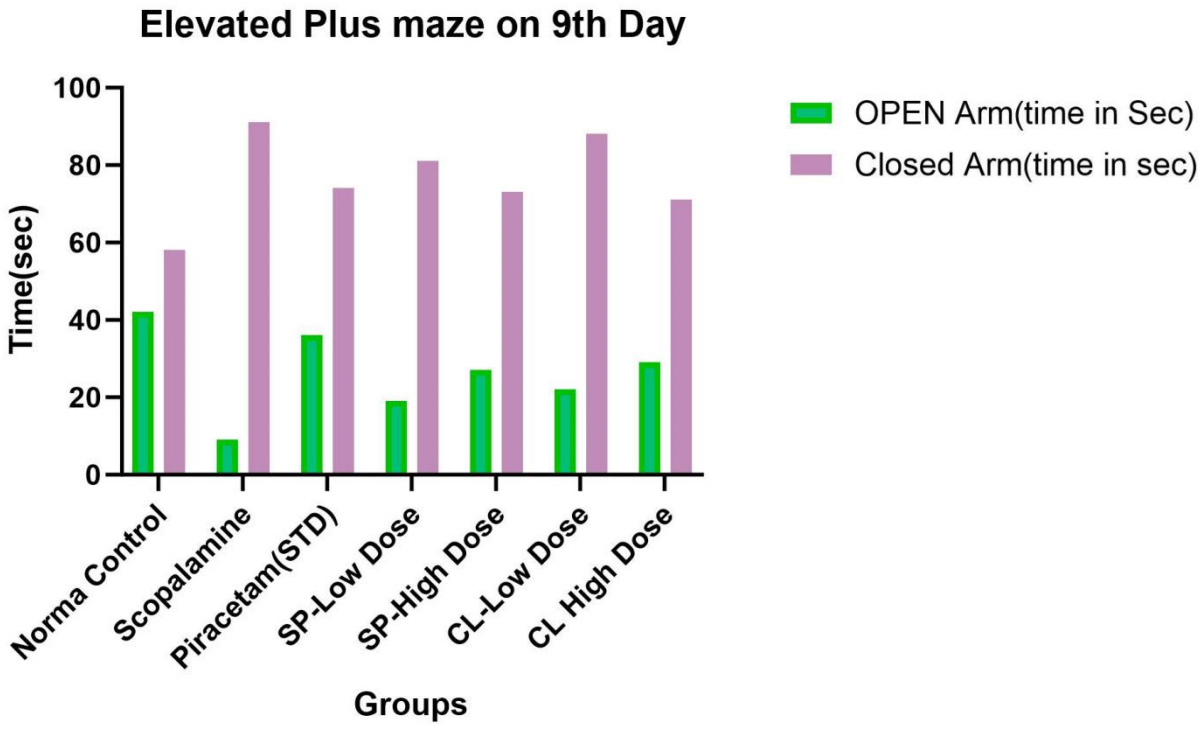

Figure 2: Results of EPM on 9th day.

Table 4: Results of the "Morris water maze test".

Group	Control	Treatment	Escaping latency in (sec)	
			8th day	9th day
Group I	Normal control	Distilled water or saline (2 mL/kg).	13	9
Group II	Diseased	Scopolamine Hydrobromide IP (0.4 mg/kg).	53	44
Group III	Standard	Piracetam (200 mg/kg) by oral route.	18	14
Group IV	Test-I (*Spirulina platensis*).	Low-dose extract (100 mg/kg).	37	36
Group V	Test-II (*Spirulina platensis*).	High-dose extract (200 mg/kg).	28	25
Group VI	Test-I (*Clerodendrum inerme*).	Low-dose extract (200 mg/kg).	32	33
Group VII	Test-II (*Clerodendrum inerme*).	High-dose extract (400 mg/kg).	22	21

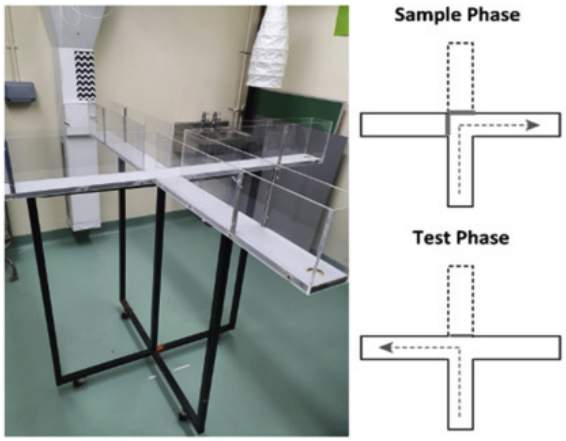

Figure 3: T-shaped maze.

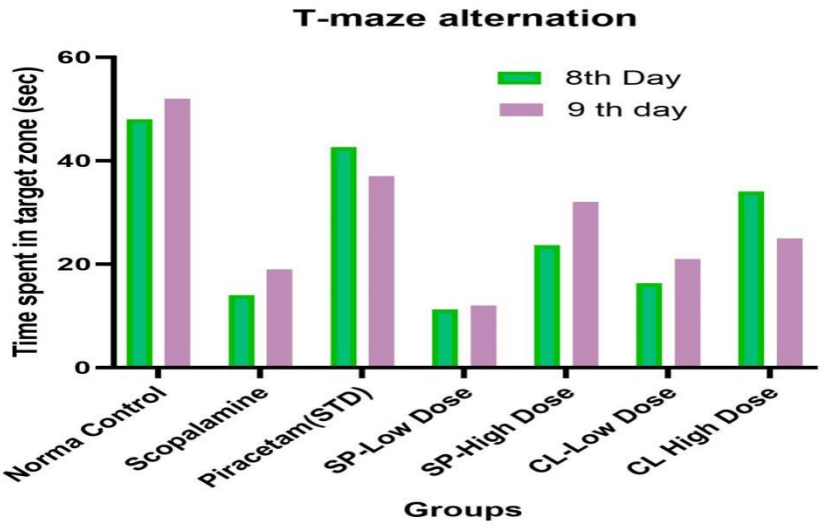

Figure 4: T -Maze alternation.

DISCUSSION

A neurodegenerative condition linked to a reduction in cognitive function is AD. AD can strike at any age, even as young as 40. Patients also commonly experience non-cognitive symptoms that interfere with day-to-day functioning, such as depression, apathy and psychosis. The dense deposits of cellular debris and protein that build up outside and around nerve cells are recognized as beta-amyloid plaques and they are the primary cause of it.

Table 5: Percentage of DPPH radical scavenging activity inhibition for CI and SP extracts.

Name of Sample	Concentration	% Inhibition	Regression equation	IC_{50} (µg/mL)
Spirulina platensis (SP)	100	14.87±1.12	Y= 0.217x+1.028	195.34
	200	35.54±1.36		
	300	48.43±2.21		
	400	65.23±0.98		
	500	74.21±2.28		
Clerodendrum inerme (CI)	100	22.20±1.10	Y= 0.2332x+7.61	181.77
	200	48.82±1.29		
	300	59.76±5.38		
	400	74.21±0.62		
	500	88.28±2.23		

Table 6: Effect of GSH and LPO, Scopalamine induced damage in rodents.

Groups	LPO (nM MDA/mg protein)		GSH (µg/mg protein)	
Group-I	0.83	0.12	5.21	0.89
Group-II	1.93	0.99	6.71	1.22
Group-III	5.18	0.98	3.16	1.32
Group-IV	3.89	2.27	6.98	2.12
Group-V	3.11	1.07	4.98	1.10
Group-VI	3.21	1.11	5.40	0.98
Group-VII	3.55	1.03	5.84	0.89

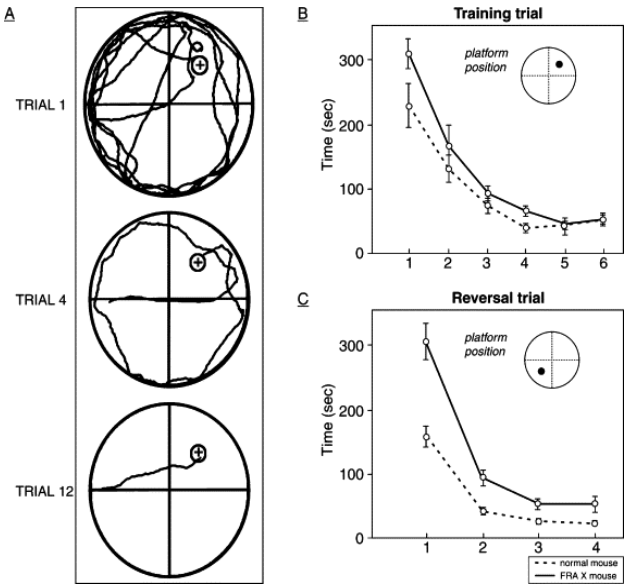

Figure 5: Morris water maze training experiment.

Twisted fibres called neurofibrillary tangles to accumulate inside nerve cells. Previous illness has been linked to a decrease in short-term memory, an inability to pick up new information, mood swings and trouble recognizing terms, identity loss and item loss. Medications known as "disease-modifying" drugs, which can prevent AD or at least efficiently modify its course, are still the subject of extensive research. The Morris water maze task is a commonly used assessment tool for memory and spatial learning in mice. *Clerodendreme inerme* and C-PC of *Spirulina platensis* both reduce the escape latency time and transfer latency time in EPM in a dose-dependent manner (Figures 1 and 2, Tables 1 and 2). In preclinical research, the T maze is frequently

used to evaluate behavioural tasks related to spatial learning and memory (Figure 3). AD rodents treated with ethanolic extracts of *Clerodendreme inerme*, C-PC of *Spirulina platensis* demonstrated a dose-dependent rise in percentage change in the T maze test (Figures 4, 5, Table 3).When treated with ethanolic extracts of *Clerodendreme inerme*, C-PC of *Spirulina platensis*, it demonstrated a dose-dependent reduction in escape latency time and an increase in time spent in the target quadrant (Figure 6, Table 4).The results establish the SP as a major antioxidant with the capacity to block free-radical damage in the body and as an efficient free-radical inhibitor (Figure 7, Table 5).Pretreatment with Piracetam (200 mg/kg b.wt) and SP and CI (1.75% to 3.5% 200 mg/kg, 400 mg/kg b.wt) successfully maintained the growth at levels of MDA and brought them towards the normal range, whereas GSH levels were enhanced generally (p<0.01), offering assurance against Scopalamine toxicity. (Figure 8, Table 6). AD rodents that were pretreated with ethanolic extract of

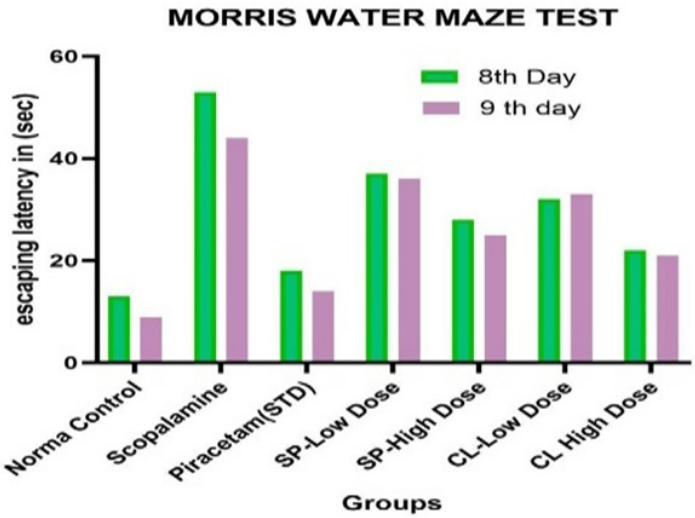

Figure 6: Results of the Morris water maze test.

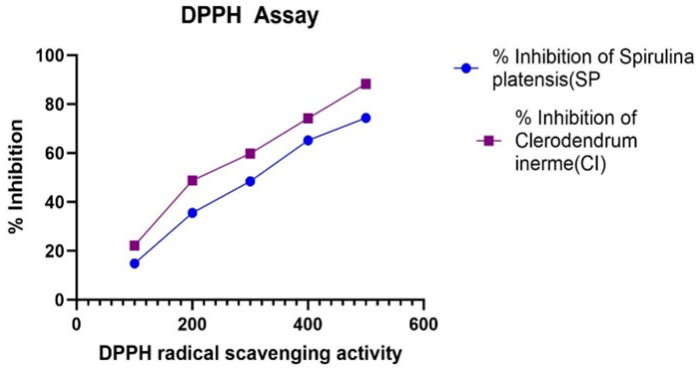

Figure 7: DPPH assay Free radical scavenging activity.

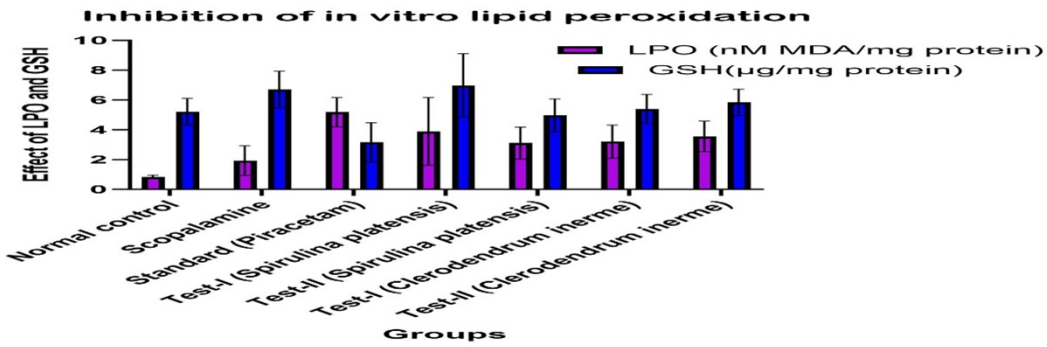

Figure 8: Inhibition of *in vitro* lipid peroxidation.

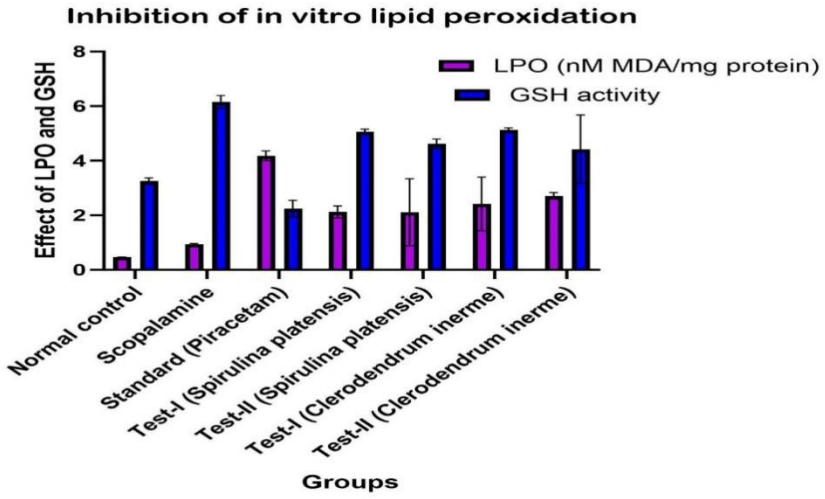

Figure 9: *In vitro* inhibition of LPO, GSH Levels.

Table 7: ***In vitro*** **inhibition of lipid peroxidation and GSH levels.**

Groups	LPO (nM MDA/mg protein)	GSH (µg/mg protein)
Group-I	0.46±0.01	3.25±0.12
Group-II	0.93±0.03	6.15±0.24
Group-III	4.18±0.18[a]	2.24±0.30[a]
Group-IV	2.15±0.22***	5.06±0.10***
Group-V	2.11±1.23***	4.62±0.19***
Group-VI	2.42±0.98***	5.12±0.08***
Group-VII	2.71±0.12***	4.42±1.26***

Every value presents "the mean±SEM. n=6 indicates the number of animals in every group. [a]p<0.001 versus vehicle control, *p<0.05, **p<0.01, ***p<0.001 comparison with respective DC treated control" group.

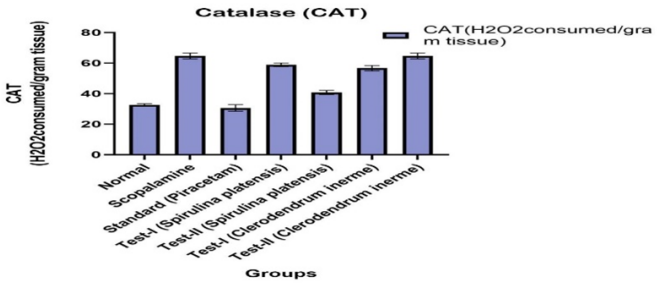

Figure 10: *In vitro* inhibition of catalase levels.

Table 8: **Catalase (CAT).**

Groups	CAT (H_2O_2 consumed/gram tissue)
Group-I	32.54±0.8
Group-II	64.68±1.9
Group-III	30.63±2.12###
Group-IV	58.85±1.22***
Group-V	40.75±1.37**
Group-VI	56.67±1.76***
Group-VII	64.68±1.9

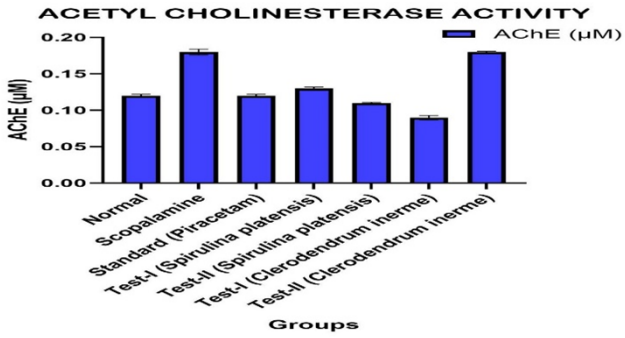

Figure 11: Acetyl cholinesterase activity using EPM.

Clerodendreme inerme and C-PC, there is an increase in lipid peroxidation and GSH levels (Figure 9, Table 7).

Due to the fact that catalase is an antioxidant enzyme that is constantly created by a variety of metabolic processes, AD rodents that were pretreated with ethanolic extract of *Clerodendreme inerme*, there is an increase in catalase (Figure 10, Table 8). Apart from behavioral assessments, extracts from ethanolic extract of *Clerodendreme inerme*,and C-PC of Spirulina demonstrated a dose-dependent decrease in AchE level (Figure 11, Table 9), since the loss of ACh resulting from AChE's hydrolytic action causes cognitive impairment. The "scavenging activity" of the SP and CI

Table 9: AchE activity using EPM.

"Group	Treatment	Dose	AChE (µM)
I	Normal control	10 mL/kg	0.12±0.0020
II	Scopolamine	0.4 mg/kg (i.p)	0.18±0.0046***
III	Piracetam	200 (mg/kg)	0.12±0.0025###
IV	Test-1(SP)	100 mg/kg	0.13±0.0023###
V	Test-2(SP)	200 mg/kg	0.11±0.0013aaa
VI	Test-1(CI)	200 mg/kg (p.o.)	0.09±0.0036***
VII	Test-2(CI)	400 mg/kg (p.o.)	0.18±0.0014***

extract and ASC ("Ascorbic Acid") both increased with the rise in sample concentration (100-500 µg mL^{-1}). A drop in the lipid peroxidation product levels indicates elevated oxidative stress; GSH is an important endogenous antioxidant.

CONCLUSION

The study concludes that *Spirulina platensis* and *Clerodendreme inerme* extracts have a significant anti-Alzheimer activity. This is likely because the extracts contain a variety of nutrients, including saponins, oil, fat, phenolic compounds, tannins, alkaloids, flavonoids, glycosides, terpenoids, amino acids, steroids, proteins and carbs. However, the ethanolic extract of *Clerodendreme inerme*, C-PC(C-Phycocyanin) of *Spirulina platensis* contains antioxidants and anti-angiogenic properties may be the cause of their anti-amnesic properties. The use of plant extracts from *Spirulina platensis* and *Clerodendreme inerme* is suggested by this study as a potential therapy for AD treatment.

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ETHICAL APPROVAL

All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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