RESEARCH PAPER

"Neuroprotective Potential of Hydroalcoholic Leaf Extract of Ixora finlaysoniana: In Vitro Acetylcholinesterase Inhibition and Antioxidant Activity"

Suvarna Yadav[1], Saranya U[2], Saranya Balan[3], Jisha James[4], Sunith DK[5], Jimmy Alexander[6], Shikha suresh[7], Ayshath Mubashira .k.M[8]

[1,2,4,5,6,7]Faculty,MGM college of harmacy,Kannur,Kerala
[3]Faculty,Rajiv Gandhi institute of pharmaceutical sciences and research, Kasaragod,Kerala
[8]Student, Department of pharmacology,MGM College of Pharmacy, Kannur,Kerala.

Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by memory impairment, cognitive decline, and cholinergic dysfunction. Acetylcholinesterase (AChE) inhibition is a well-established therapeutic strategy for enhancing cholinergic neurotransmission and alleviating the symptoms associated with AD. The present study aimed to investigate the in vitro acetylcholinesterase inhibitory and antioxidant activities of the hydroalcoholic leaf extract of Ixora finlaysoniana to explore its potential as a natural neuroprotective agent.

Fresh leaves of Ixora finlaysoniana were collected, authenticated, and extracted using a hydroalcoholic solvent system. Preliminary phytochemical screening demonstrated the presence of several bioactive constituents, including flavonoids, phenolic compounds, alkaloids, saponins, triterpenoids, steroids, and glycosides. Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, whereas acetylcholinesterase inhibitory activity was determined using a standard in vitro enzyme inhibition assay.

The hydroalcoholic extract exhibited concentration-dependent antioxidant activity, achieving a maximum DPPH radical scavenging effect of 76.5% at 200 µg/mL, with an IC_{50} value of 53.3 µg/mL, compared with the reference standard ascorbic acid (IC_{50} = 25.87 µg/mL). The extract also demonstrated significant acetylcholinesterase inhibitory activity, producing 62.5% inhibition at 100 µg/mL with an IC_{50} value of 55.6 µg/mL. The observed biological activities are likely attributable to the synergistic effects of the phytoconstituents present in the extract, particularly phenolic compounds and flavonoids, which are known to possess antioxidant and neuroprotective properties.

Overall, the findings suggest that the hydroalcoholic leaf extract of Ixora finlaysoniana possesses promising antioxidant and acetylcholinesterase inhibitory activities, supporting its potential as a natural source of neuroprotective phytochemicals for the management of Alzheimer's disease. However, further phytochemical characterization, mechanistic investigations, and in vivo pharmacological studies are necessary to identify the active constituents and establish their therapeutic efficacy and safety.

Keywords: Alzheimer's disease; Ixora finlaysoniana; Acetylcholinesterase inhibition; Antioxidant activity; DPPH assay; Phytochemicals; Hydroalcoholic extract.

How to cite this article: Yadav S, Saranya U, Balan S, James J, Sunith DK, Alexander J, Suresh S, Mubashira KM A. Neuroprotective Potential of Hydroalcoholic Leaf Extract of Ixora finlaysoniana: In Vitro Acetylcholinesterase Inhibition and Antioxidant Activity. Int J Drug Deliv Technol. 2026;16(72s): 836-845. DOI: 10.25258/ijddt.16.72s.93

1. Introduction

Alzheimer's disease (AD) is the leading cause of dementia and one of the fastest-growing neurodegenerative disorders worldwide. It is characterised by the progressive deterioration of memory, cognition, and behaviour, imposing profound clinical, social, and economic burdens on patients, caregivers, and healthcare systems [3]. The pathological hallmarks of AD include extracellular amyloid-β (Aβ) plaque deposition, intracellular neurofibrillary tangles formed by hyperphosphorylated tau protein, synaptic dysfunction, oxidative stress, and progressive neuronal loss [11]. Despite significant advances in understanding the pathogenesis of AD, effective therapeutic strategies to prevent or reverse disease progression remain limited [20].

Among the various hypotheses proposed to explain AD pathogenesis, the cholinergic hypothesis remains one of the most extensively validated therapeutic concepts. Degeneration of cholinergic neurons in the basal forebrain leads to depletion of acetylcholine, resulting in impaired learning, memory, and cognitive function. Consequently, acetylcholinesterase (AChE), the enzyme responsible for hydrolysing acetylcholine within cholinergic synapses, has become an established pharmacological target for the symptomatic treatment of AD [5,10].

*Author for Correspondence: Suvarna Yadav

Currently approved AChE inhibitors, including donepezil, rivastigmine, and galantamine, improve cognitive symptoms by enhancing central cholinergic neurotransmission [2]. However, these drugs neither arrest disease progression nor adequately address the multifactorial pathology of AD. Moreover, prolonged therapy is frequently associated with adverse effects such as gastrointestinal disturbances, dizziness, bradycardia, and hepatotoxicity [16], highlighting the need for safer and more effective therapeutic alternatives. Recent clinical evidence and post-marketing studies further emphasise that although AChE inhibitors provide symptomatic benefits, their long-term efficacy remains modest [23].

Natural products have emerged as promising sources of multi-target therapeutic agents for Alzheimer's disease because of their ability to simultaneously modulate oxidative stress, cholinergic dysfunction, and other pathological processes. Numerous plant-derived phytochemicals, particularly flavonoids and phenolic compounds, exhibit significant antioxidant and acetylcholinesterase inhibitory activities, thereby protecting neurons from oxidative damage and improving cholinergic neurotransmission. Consequently, medicinal plants continue to represent an important resource for the discovery of novel anti-Alzheimer's agents [7,27].

Ixora finlaysoniana Hook. f., a member of the Rubiaceae family, is known to contain diverse phytoconstituents including flavonoids, phenolic compounds, alkaloids, saponins, triterpenoids, steroids, and glycosides, many of which have been associated with antioxidant and neuroprotective properties[14]. Nevertheless, the acetylcholinesterase inhibitory potential of I. finlaysoniana has not been scientifically investigated.

Therefore, the present study aimed to evaluate the in vitro acetylcholinesterase inhibitory activity of the hydroalcoholic leaf extract of Ixora finlaysoniana. The antioxidant activity of the extract was also assessed to investigate its potential as a natural therapeutic candidate for the management of Alzheimer's disease.

2. Materials and methods

2.1 Plant Profile

Fig 1. Ixora Finlaysoniana Wall. ex G. Don. (leaves and flower) [12]

Botanical Name: Ixora Finlaysoniana Wall. ex G. Don

Family: Rubiaceae

Synonyms: Ixora denticulate Pierre ex Pit, Ixora findlayna B.S Williams, Ixora merguensis var. parvifolia F.N Williams. [25]

Taxonomical Classification

Kingdom: Plantae
Phylum: Streptophyta.
Class: Equisetopsida.
Order: Gentianales.
Family: Rubiaceae
Genus: Ixora
Species: Ixora Finlaysoniana Wall. ex G. Don [25]

Vernacular names

Hindi: Sada chaphra, sita-Rangani.
Marathi: Pandhara Rukhmini.
Tamil: Pasumaiyana Pudar
Malayalam: Vella thechi.
Telugu: Tella thechi.
Kannada: Bili thechi
Sanskrit: Kundapushpa.
Tulu: Boli Kepula. [17]

Morphology

Growth Form are evergreen shrub or small tree, typically 1–3 meters tall. Leaves are Opposite, elliptic to oblong, 6–12 cm long, with leathery texture and entire margins. Flowers are Small, white, fragrant, tubular; arranged in dense terminal corymbs or clusters. Fruits are Small, globose drupes that turn red to black upon ripening. Stem is Woody and smooth; young stems are greenish and flexible.[12]

Phytochemical Profile[14]

Apigenin-4'-o-D-glucopyranoside,11-hydroxy-dodec-5-en-2-one, palmitic acid, oleic acid, stearic acid, linoleic acid, protocatechuic acid, ferulic acid, gallic acid, beta-sitosterol, sitosterol-3-o- beta-D-glycoside, nonacosanol, α-amyrin, β-sitosterol 3-hydroxyhexan-5olide, protocatechuic acid, gallic acid, β- sitostreol glucoside, parasorboside, D-1-Omethyl-myo-inostiol and galactitol, dammarine triterpenoids.

Medicinal uses [17]

White Ixora (Ixora finlaysoniana), known as Boli Thechi or Vella Chethi, is valued in folk medicine for its healing and cooling effects. Its leaves and flowers are used to treat wounds, skin rashes, and fever, while decoctions help with indigestion and body heat.
The plant is also used to ease menstrual pain, reduce joint swelling, and heal mouth ulcers, making it a gentle multipurpose remedy in traditional healing practices.

2.2 Collection and Authentication of Leaves
Fresh leaves of Ixora finlaysoniana were collected from their natural habitat at Vilayancode, Kannur, Kerala, India, during October. The plant material was authenticated by Dr K. P. Prasanth, Associate Professor, Department of Botany, Sree Narayana College, Kannur. A voucher specimen was prepared and preserved for future reference.
Following authentication, the collected leaves were thoroughly cleaned to remove any adhering impurities and shade-dried at room temperature. The dried leaves were then pulverised using a mechanical grinder to obtain a coarse powder, which was stored appropriately and used for subsequent analyses.

2.3Preparation of Extract (Hydroalcoholic Extraction) [4,13,14,28]
The collected leaves of Ixora finlaysoniana were shade-dried at room temperature and coarsely powdered using a mechanical grinder. The powdered plant material was subjected to hydroalcoholic extraction by the maceration method using 70% ethanol in water (1:5 w/v) for 48–72 hours. The mixture was filtered, and the filtrate was concentrated by partial evaporation of the solvent under reduced pressure (600 mmHg) at 40 ± 1°C using a rotary evaporator. The concentrated extract was further dried in a hot air oven to obtain the hydroalcoholic extract. The dried extract was protected from light and stored at 2–8°C until further use.

2.4. Phytochemical Screening [1,14,15,18,26]
2.4.1. Test for Alkaloids
a) Hager's Test:
The extract was treated with Hager's reagent (saturated picric acid solution). The appearance of a yellow precipitate indicated the presence of alkaloids.
b) Mayer's Test:
The extract was mixed with Mayer's reagent (potassium mercuric iodide solution). Formation of a cream-coloured precipitate confirmed the presence of alkaloids.
c) Dragendorff's Test:
The extract was treated with Dragendorff's reagent (potassium bismuth iodide solution). An orange-brown precipitate indicated the presence of alkaloids.
d) Wagner's Test:
The extract was treated with Wagner's reagent (iodine-potassium iodide solution). Formation of a reddish-brown precipitate confirmed the presence of alkaloids.

2.4.2. Test for Carbohydrates
a) Molisch's Test:
The extract was treated with Molisch's reagent (α-naphthol in ethanol), followed by the careful addition of concentrated sulfuric acid along the side of the test tube. The formation of a violet ring at the junction of the two layers indicated the presence of carbohydrates.
b) Fehling's Test:
The extract was mixed with equal volumes of Fehling's solutions A and B and heated in a water bath. The formation of a brick-red precipitate indicated the presence of reducing sugars.
c) Barfoed's Test:
The extract was treated with Barfoed's reagent and heated in a water bath. The appearance of a red precipitate confirmed the presence of monosaccharides.
d) Benedict's Test:
The extract was mixed with Benedict's reagent and heated for 10 minutes. The development of a brick-red precipitate indicated the presence of reducing sugars.

2.4.3. Test for Proteins and Amino Acids
a) Biuret Test:
The extract was treated with 10% sodium hydroxide solution, followed by a few drops of 1% copper sulfate solution. The appearance of a violet colour indicated the presence of proteins.
b) Millon's Test:
The extract was treated with Millon's reagent and gently heated. The development of a brick-red colour or precipitate confirmed the presence of proteins.

c) Xanthoproteic Test:
The extract was treated with concentrated nitric acid. The formation of a yellow colour, which intensified to orange upon the addition of alkali, indicated the presence of aromatic amino acids in proteins.
d) Ninhydrin Test:
The extract was treated with 0.1% ninhydrin solution and heated in a boiling water bath for 10 minutes. The development of a purple or blue-violet colour confirmed the presence of free amino acids or proteins.

2.4.4. Test for Flavonoids
a) Ferric Chloride Test:
The extract was treated with a few drops of ferric chloride solution. The appearance of a blackish-blue or green colour indicated the presence of flavonoids.
b) Lead Acetate Test:
The extract was treated with lead acetate solution. Formation of a yellow precipitate confirmed the presence of flavonoids.
c) Alkaline Reagent Test:
The extract was treated with sodium hydroxide solution. An intense yellow colour, which disappeared upon the addition of dilute acid, indicated the presence of flavonoids.
d) Ammonia Test:
A filter paper soaked in the alcoholic extract was exposed to ammonia vapours. The development of a yellow colour indicated the presence of flavonoids.

2.4.5. Test for Saponins
a) Froth Test:

One millilitre of the extract was diluted with distilled water to a final volume of 20 mL and shaken vigorously for 15 minutes. The formation of a stable froth approximately 1 cm in height indicated the presence of saponins.

b) Haemolytic Test:

An equal volume of red blood cell suspension in normal saline was mixed with the extract prepared in normal saline. The occurrence of haemolysis, producing a clear red solution, confirmed the presence of saponins.

2.4.6. Test for Triterpenoids

Hirschhorn Test:

The extract was warmed with trichloroacetic acid. A yellow colour changing to red indicated the presence of triterpenoids.

2.4.7. Test for Steroids

Salkowski Test:

The extract was mixed with chloroform, followed by concentrated sulfuric acid. The appearance of a deep red or purple colour in the chloroform layer and green fluorescence in the acid layer confirmed the presence of steroids.

2.4.8. Test for Tannins and Phenolic Compounds

The extract was treated separately with various reagents, and the following observations indicated the presence of tannins and phenolic compounds:

- Ferric chloride solution: Deep blue-black or green colour.
- Lead acetate solution: White precipitate.
- Gelatin solution: White precipitate.
- Bromine water: Decolourisation of bromine water.
- Acetic acid solution: Red colour.
- Potassium dichromate solution: Red precipitate.
- Dilute iodine solution: Transient red colour.
- Dilute nitric acid: Reddish-yellow colour.

2.4.9. Test for Glycosides

a) General Test for Glycosides:

The extract was hydrolysed using dilute sulfuric acid, neutralised with sodium hydroxide, and treated with Fehling's solutions A and B. Formation of a brick-red precipitate after hydrolysis indicated the presence of glycosides. A parallel test without hydrolysis was performed for comparison.

b) Legal's Test (Cardiac Glycosides):

The extract was treated with pyridine and sodium nitroprusside solution. Development of a pink to red colour indicated the presence of cardenolide glycosides.

c) Keller–Killiani Test (Deoxysugars):

The extract was mixed with glacial acetic acid containing ferric chloride, followed by concentrated sulfuric acid. Formation of a reddish-brown ring at the interface and a bluish-green upper layer confirmed the presence of deoxysugars associated with cardiac glycosides.

d) Borntrager's Test (Anthraquinone Glycosides):

The extract was heated with an organic solvent, filtered, and the filtrate was treated with sodium hydroxide or ammonia solution. Development of a pink, red, or violet colour in the alkaline layer indicated the presence of anthraquinone glycosides.

2.5. In Vitro Antioxidant Study

2.5.1. DPPH Radical Scavenging Assay [6,8,9,19,22]

The antioxidant activity of the extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Antioxidants inhibit or retard the oxidation of biomolecules such as lipids and proteins by interrupting free radical chain reactions. The DPPH assay is a simple, rapid, and widely employed method for assessing the free radical scavenging potential of plant extracts and bioactive compounds.

DPPH is a stable free radical that exhibits a deep violet colour in solution with a characteristic absorption maximum at 517 nm. Upon reaction with an antioxidant capable of donating a hydrogen atom or an electron, DPPH is reduced to its non-radical form (DPPH-H), resulting in a colour change from violet to pale yellow. The decrease in absorbance at 517 nm is directly proportional to the radical scavenging activity of the test sample.

The reaction can be represented as follows:

$DPPH\bullet + AH \rightarrow DPPH\text{-}H + A\bullet$

Where AH represents the antioxidant molecule.

Reagent Preparation

A 0.1 mM DPPH solution was prepared by dissolving 4 mg of DPPH in 100 mL of ethanol. The solution was prepared freshly and protected from light until use.

Procedure

Different concentrations of the test extract (12.5, 25, 50, 100, and 200 µg/mL) were prepared from a stock solution of 10 mg/mL using dimethyl sulfoxide (DMSO) as the solvent. An aliquot (20 µL) of each concentration was transferred into separate test tubes. Subsequently, 1.48 mL of freshly prepared 0.1 mM DPPH solution was added to each tube to obtain the reaction mixture.

A control was prepared by replacing the sample solution with an equal volume of DMSO while maintaining all other experimental conditions. The reaction mixtures were incubated in the dark at room temperature for 20 minutes to prevent photo-degradation of DPPH. After incubation, the absorbance was measured at 517 nm using a UV–Visible spectrophotometer (Shimadzu UV-1900i).

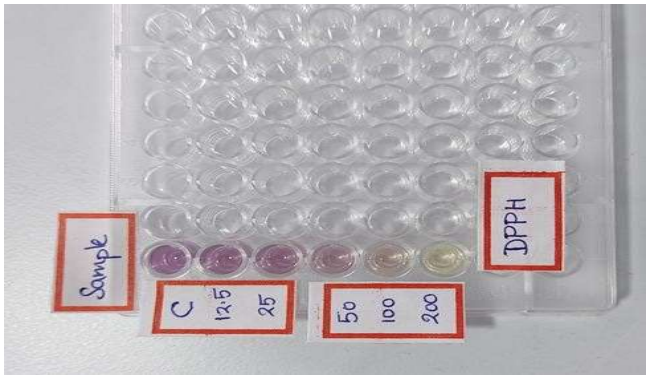

Fig 2. Antioxidant study of Ixora finlaysoniana by DPPH assay

The free radical scavenging activity of the samples was expressed as percentage inhibition and calculated using the following equation:

% Inhibition = [(A Control - A Sample) / A Control)] × 100

where:

- Acontrol = Absorbance of the control
- Asample = Absorbance of the test sample

A higher percentage inhibition indicates greater free radical scavenging activity and stronger antioxidant potential of the test sample.

2.6. In Vitro Acetylcholinesterase (AChE) Inhibitory Activity Using the Microplate Assay[10,21,24]

Principle

The acetylcholinesterase (AChE) inhibitory activity of the test extract was determined using a modified 96-well microplate method based on Ellman's colourimetric assay. Acetylcholinesterase catalyses the hydrolysis of acetylthiocholine iodide (ATCI) to produce thiocholine and acetate. The liberated thiocholine reacts with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, Ellman's reagent), resulting in the formation of the yellow-coloured 5-thio-2-nitrobenzoate (TNB) anion. The intensity of the yellow colour is directly proportional to the enzyme activity and is measured spectrophotometrically at 412 nm. In the presence of an AChE inhibitor, the formation of TNB decreases, resulting in reduced absorbance.

Reagent Preparation

A 50 mM Tris-HCl buffer (pH 8.0) was used throughout the experiment unless otherwise specified. Acetylcholinesterase obtained from Electrophorus electricus (electric eel) was used as the enzyme source. The enzyme stock solution (222 U/mL) was stored at −80°C, and working enzyme solutions were prepared by diluting the stock in 0.1% bovine serum albumin (BSA) prepared in Tris-HCl buffer.

Ellman's reagent (DTNB) was prepared in Tris-HCl buffer containing 0.1 M sodium chloride (NaCl) and 0.02 M magnesium chloride ($MgCl_2$). Acetylthiocholine iodide (ATCI) was dissolved in deionised water immediately before use.

Procedure

The assay was carried out in a 96-well microplate. Each well received 100 µL of 3 mM DTNB solution, 20 µL of acetylcholinesterase solution (0.26 U/mL), 40 µL of 50 mM Tris-HCl buffer (pH 8.0), and 20 µL of the test sample at different concentrations (25, 50, and 100 µg/mL) prepared in buffer.

The reaction mixture was gently mixed and incubated at 25°C for 15 minutes. The initial absorbance was recorded at 412 nm using a microplate reader. The enzymatic reaction was initiated by adding 20 µL of acetylthiocholine iodide (ATCI) solution to each well. After thorough mixing, the absorbance was measured again at 5 and 20 minutes.

A blank containing all reagents except the test sample was prepared under identical experimental conditions. The inhibitory activity of the samples against acetylcholinesterase was expressed as percentage inhibition and calculated using the following equation:

% inhibition = {(E-ΔOD) / E} *100

where:

- E = Absorbance of the blank (enzyme control)
- ΔOD = Absorbance of the test sample

A higher percentage inhibition indicates stronger acetylcholinesterase inhibitory activity of the test extract.

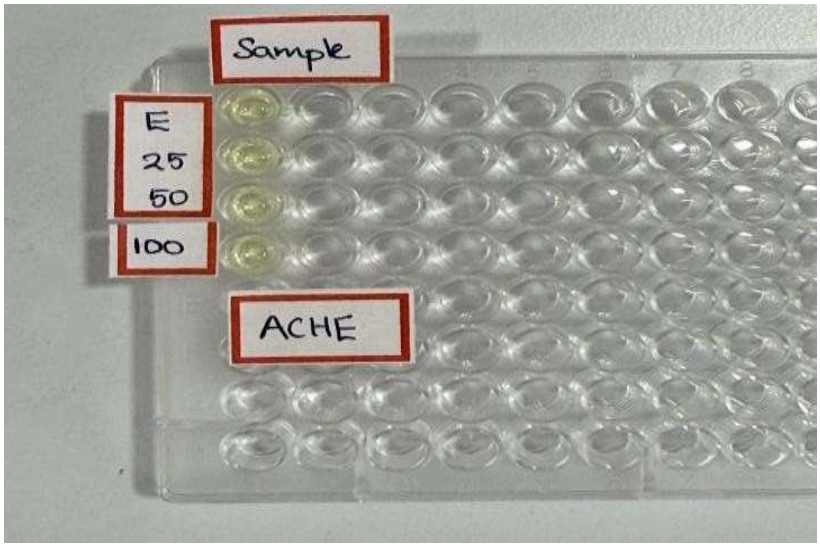

Fig 3. AchE inhibitory assay of Ixora finlaysoniana

3. Results And Discussion

3.1 Pharmacognostic Evaluation

3.1.1 Preliminary phytochemical analysis

In the phytochemical tests, hydro alcoholic extract of Ixora finlaysoniana. Wall. ex G. Don. Revealed the presence of alkaloids, flavonoids, phenols, carbohydrate, tannins, saponins, glycosides as well as terpenes.

Table 1. Phytochemical constituents of Hydroalcoholic leaf extract of Ixora finlaysoniana

CONSTITUENTS	HYDRO - ALCOHLIC EXTRACT OBSERVATION
CARBOHYDRATE	+
ALKALOIDS	+
PROTEINS AND AMINO ACIDS	-
FLAVANOIDS	+
SAPONINS	+
TERPENOIDS	+
TANNINS AND PHENOLIC COMPOUNDS	+
GLYCOSIDES	+

Present (+), Absent (-)

3.2. Pharmacological Evaluation

3.2.1 In Vitro Antioxidant Study by DPPH Radical Scavenging Assay

Table 2. DPPH radical scavenging activity of standard and sample

SI. No	Groups	Concentration (µg/ml)	Absorbance	Percentage Inhibition (%)	IC50
1.	Control		0.715		
2.	Ascorbic acid	12.5	0.44	38.4 ± 0.1000	

		25	0.36	49.6 ± 0.0500	
		50	0.18	60.8± 0.0500	
		100	0.08	88.8 ± 0.1000	25.87µg/ml
		200	0.04	94.4± 0.1500	
3.	Hydro alcoholic leaf extract of Ixora finlaysoniana	12.5	0.489	31.6 ± 0.1000	
		25	0.412	42.3 ± 0.0200	
		50	0.363	49.2± 0.0400	
		100	0.276	61.3 ± 0.1500	53.3µg/ml
		200	0.168	76.5± 0.1000	

Antioxidant activity of the Hydroalcoholic leaf extract of Ixora finlaysoniana was evaluated using DPPH radical scavenging assay. The extract exhibited antioxidant activity in dose dependent manner. The Ixora finlaysoniana and standard, ascorbic acid exhibited the maximum inhibition at a concentration of 200 µg/ml. The IC50 value of ascorbic acid was found to be 25.87 µg/ml and Ixora finlaysoniana was calculated as 53.3 µg/ml. The results were compared to standard ascorbic acid and the values are shown Table No:2 and graphically represented in Figure No:4.

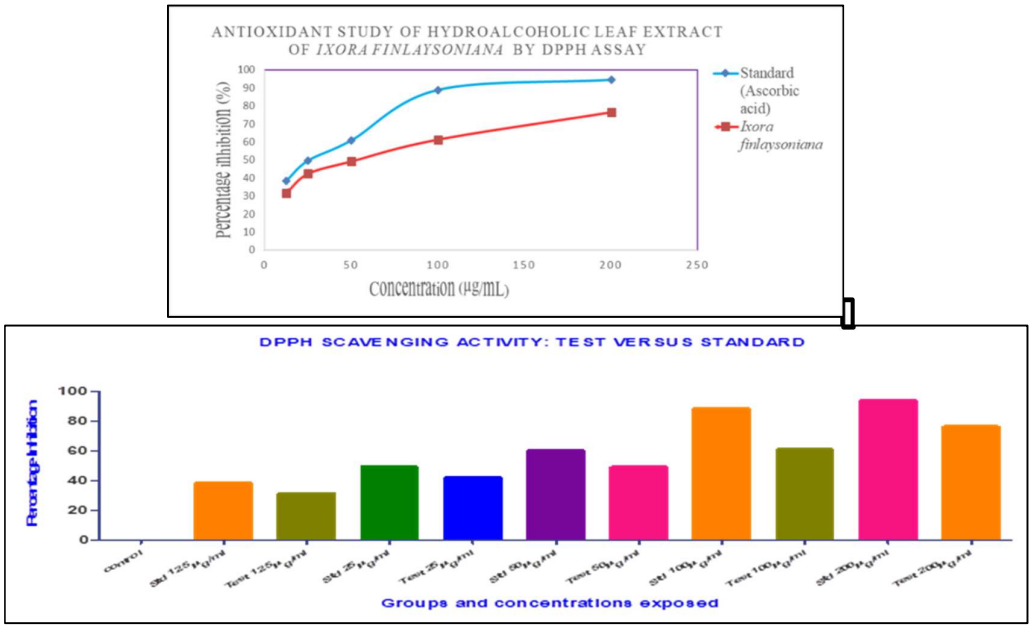

Fig 4. Graphical representation depicting DPPH Radical scavenging assay of sample and standard.

Statistical Analysis

All experimental data were expressed as mean ± SEM (n = 3). Statistical significance was analysed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. One-way ANOVA showed a statistically significant difference among treatment groups ($F(10,22)=260000$, $p<0.0001$), with all Ixora finlaysoniana extract and standard concentrations exhibiting significantly increased DPPH radical scavenging activity compared to control.

3.2.2. In Vitro Acetylcholinesterase Inhibitory Assay

Table 3. Acetylcholinesterase inhibitory activity of Hydro alcoholic leaf extract of Ixora finlaysoniana

SI. No	Groups	Concentration (µg/ml)	Absorbance	Percentage Inhibition (%)	IC50
1.	Blank		0.163±0.0005		
2.	Physostigmine	25	0.092±0.0015	39.2	35.7µg/ml
		50	0.058±0.0001	64.4	

		100	0.025±0.00001	84.6	
3.	Hydro alcoholic leaf extract of Ixora finlaysoniana	25	0.099±0.00058	42.9	55.6µg/ml
		50	0.084±0.0002	48.4	
		100	0.061±0.00005	62.5	

In acetylcholinesterase inhibitory activity it is observed that the hydro alcoholic leaves extract of Ixora finlaysoniana. Wall. ex G. Don have dose dependent increase in the AchE inhibitory activity. At 100 µg, the standard drug and Ixora finlaysoniana showed maximum activity. The IC50 value of standard drug Physostigmine was found to be 35.7 µg/ml and IC50 value of hydroalcoholic extract of Ixora finlaysoniana was 55.6 µg/ml. The results were compared to standard Physostigmine and the values are shown Table No:3 and graphically represented in Figure No: 5.

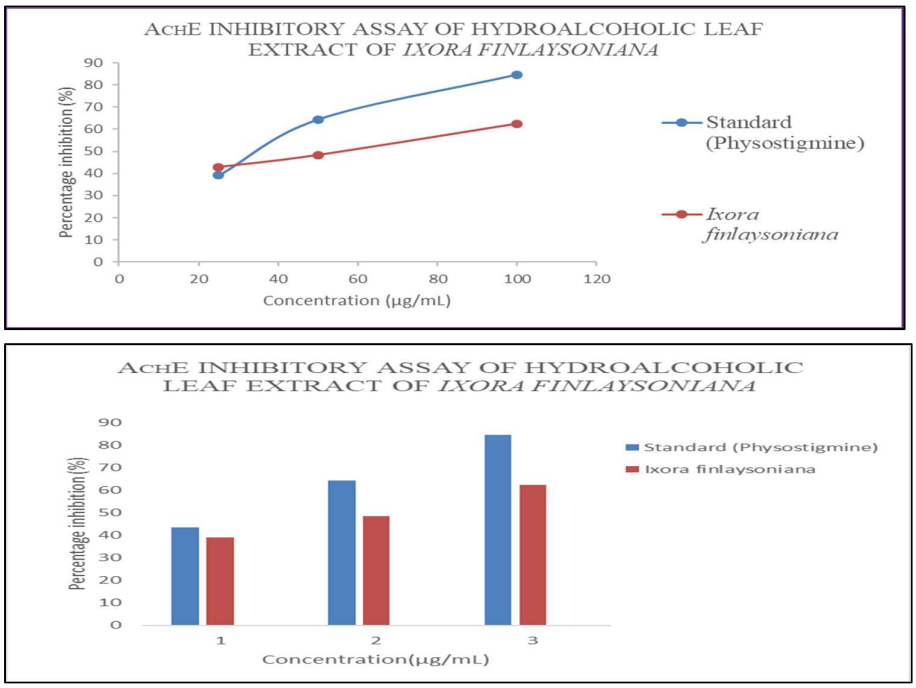

Fig 5. Graphical representation depicting AChE Inhibitory activity of Sample and Standard.

Statistical Analysis

All experimental data were expressed as mean ± SEM (n = 3). Statistical significance was analysed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Statistical analysis using one-way ANOVA demonstrated significant differences among groups ($F(6,14)=13000$, $p<0.05$), indicating concentration-dependent and significant acetylcholinesterase inhibitory activity of both test and standard samples.

4. Conclusion

The present study demonstrates that the hydroalcoholic leaf extract of Ixora finlaysoniana possesses promising in vitro neuroprotective properties, as evidenced by its significant antioxidant and acetylcholinesterase (AChE) inhibitory activities. The extract exhibited concentration-dependent free radical scavenging activity and effective inhibition of AChE, indicating its potential to reduce oxidative stress and improve cholinergic neurotransmission, two major pathological mechanisms implicated in the progression of Alzheimer's disease.

The phytochemical screening revealed the presence of flavonoids, phenolic compounds, alkaloids, saponins, triterpenoids, steroids, and glycosides, which may collectively contribute to the observed biological activities through synergistic interactions. Among these, phenolic compounds and flavonoids are well recognized for their antioxidant, anti-inflammatory, and neuroprotective effects, suggesting that they may play a key role in the extract's pharmacological activity. The dual antioxidant and anti-acetylcholinesterase effects observed in this study highlight the potential of Ixora finlaysoniana as a multifunctional natural therapeutic candidate for addressing the multifactorial pathology of Alzheimer's disease.

Although the extract demonstrated encouraging in vitro activity, these findings should be interpreted with caution, as in vitro assays alone cannot predict clinical efficacy. Further studies are required to isolate and

identify the bioactive constituents responsible for these effects using advanced phytochemical techniques such as HPLC, LC–MS/MS, GC–MS, and NMR spectroscopy. In addition, mechanistic studies should be conducted to elucidate the molecular pathways underlying the neuroprotective effects, including investigations into oxidative stress modulation, cholinergic signaling, neuroinflammation, mitochondrial function, and apoptosis.

Moreover, comprehensive in vivo studies using appropriate animal models of Alzheimer's disease are necessary to evaluate the extract's pharmacological efficacy, pharmacokinetic profile, blood–brain barrier permeability, toxicity, and long-term safety. These investigations will be essential for establishing the therapeutic relevance of Ixora finlaysoniana and assessing its suitability for further preclinical and clinical development.

In conclusion, the findings of the present study provide scientific evidence supporting the traditional medicinal value of Ixora finlaysoniana and demonstrate its potential as a valuable source of neuroprotective phytochemicals. The combined antioxidant and acetylcholinesterase inhibitory activities observed suggest that this plant may serve as a promising lead for the development of novel plant-derived therapeutic agents or phytopharmaceutical formulations aimed at the prevention and management of Alzheimer's disease. Future multidisciplinary studies integrating phytochemistry, pharmacology, toxicology, and molecular biology will be crucial for translating these preliminary findings into effective therapeutic applications.

References

1. Aiyegoro OA, Okoh AI. Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of Helichrysum longifolium DC. BMC Complement Altern Med. 2010 May 10;10(21). doi:10.1186/1472-6882-10-21
2. ALNasser MN, Alboraiy GM, Alsowig EM, Alqattan FM. Cholinesterase inhibitors from plants and their potential in Alzheimer's treatment: systematic review. Brain Sci. 2025;15(2):215. doi:10.3390/brainsci15020215
3. Alzheimer's Association. 2024 Alzheimer's disease facts and figures. Alzheimers Dement. 2024;20(5):3708-3821.
4. Azwanida NN. A review on the extraction methods used in medicinal plants, principles, strengths, and limitations. Med Aromat Plants. 2015;4(3):196.
5. Bartus RT, Dean RL 3rd, Beer B, Lippa AS. The cholinergic hypothesis of geriatric memory dysfunction. Science. 1982;217(4558):408-414.
6. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181:1199–1200.
7. Bordoloi S, et al. Some promising medicinal plants used in Alzheimer's disease: an ethnopharmacological perspective. Discover Applied Sciences. 2024;6:215.
8. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol. 1995;28(1):25–30.
9. Chang ST, Wu JH, Wang SY, Kang PL, Yang NS, Shyur LF. Antioxidant activity of extracts from Acacia confusa bark and heartwood. J Agric Food Chem. 2001;49(7):3420–3424.
10. Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM. A new and rapid colourimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7(2):88-95.
11. Engelhardt E, Resende EPF, Gomes KB. Physiopathological mechanisms underlying Alzheimer's disease: a narrative review. Dement Neuropsychol. 2024;18:e2024VR01. doi:10.1590/1980-5764-DN-2024-VR01
12. Gamble JS, Fischer CEC. Flora of the Presidency of Madras. Vol 2. London: Adlard and Son; 1935. p. 783–785.
13. Handa SS, Khanuja SPS, Longo G, Rakesh DD. Extraction Technologies for Medicinal and Aromatic Plants. Trieste: United Nations Industrial Development Organization; 2008.
14. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. London: Chapman and Hall; 1998. p. 6–12, 120–145.
15. Iqbal E, Salim KA, Lim LBL. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of Goniothalamus velutinus (Airy Shaw) from Brunei Darussalam. J King Saud Univ Sci. 2015 Jul;27(3):224-232. doi:10.1016/j.jksus.2015.02.003
16. Khoury R, Rajamanickam J, Grossberg GT. An update on the safety of current therapies for Alzheimer's disease: focus on rivastigmine. Ther Adv Drug Saf. 2018;9(3):171-178.
17. Kirtikar KR, Basu BD. Indian Medicinal Plants. Vol 2. Dehradun: International Book Distributors; 2005. p. 1247–1249.
18. Kokate CK. Practical Pharmacognosy. 4th ed. New Delhi: Vallabh Prakashan; 2005.
19. Kpemissi M, Kantati YT, Veerapur VP, Eklu-Gadegbeku K, Hassan Z. Anticholinesterase, anti-inflammatory and antioxidant properties of Combretum micranthum G. Don: potential implications in neurodegenerative disease. IBRO Neurosci Rep. 2023;14:21–27.
20. Liu E, Zhang Y, Wang JZ. Updates in Alzheimer's disease: from basic research to diagnosis and therapies. Transl Neurodegener. 2024;13:45.
21. Mathew M, Subramanian S. In vitro screening for anti-cholinesterase and antioxidant activity of methanolic extracts of ayurvedic medicinal plants used for cognitive disorders. PLOS ONE. 2014;9(1):e86804. doi:10.1371/journal.pone.0086804
22. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol. 2004;26(2):211–219.
23. Rea F, et al. Acetyl-cholinesterase-inhibitors reconsidered. A narrative review of post-marketing

studies on Alzheimer's disease. Ageing Clin Exp Res. 2024;36:23.
24. Rhee IK, van Rijn RM, Verpoorte R. Qualitative determination of false-positive effects in the acetylcholinesterase assay using thin-layer chromatography. Phytochem Anal. 2003;14(3):127–131.
25. Royal Botanic Gardens, Kew. Ixora finlaysoniana Wall. ex G.Don. Plants of the World Online [Internet]. Kew: Royal Botanic Gardens; 2024 [cited 2026 Jul 15]. Available from: https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:753956-1
26. Senguttuvan J, Paulsamy S, Karthika K. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, Hypochaeris radicata L., for in vitro antioxidant activities. Asian Pac J Trop Biomed. 2014 May;4(Suppl 1):S359–S367. doi:10.12980/APJTB.4.2014C1030
27. Sharma S, et al. Natural acetylcholinesterase inhibitors: A multi-targeted therapeutic potential in Alzheimer's disease. Eur J Med Chem Rep. 2024;11:100154.
28. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. Int Pharm Sci. 2011;1(1):98–106.
29. Alexander J, Rashid M, Jayaraman S, Venkatachalam T, Chitra A. Analysing The Effect Of Metal Complexes With Cefuroxime On Some Selected Bacteria. Journal of Advanced Zoology. 2024 Jan 1;45(1):476.
30. Alexander J, Parthiban S, Boopathi T, Jayaraman S, Rathinavel G. Isolation of Quercetin from Physalis Lagascae and Its In-Vitro Anticancer Activity. World Journal of Pharmaceutical Research. 2021 Sep 8;10(13):1196-209.
31. Alex J, Thomas J, Lalu E, Sana FK, Suhana KP, Avandhika U, et al. Design, Synthesis, Characterization And Biological Evaluation Of Novel Pyrazole-Isonazid Hybrids As Dual-Activity Anti-Inflammatory And Anti-Microbial Agent. Int J Drug Deliv Technol. 2026;16(66s):1524-1536. DOI: 10.25258/ijddt.16.66s.162
32. Nath G, Ghosh M, Shergojry SA, DK S, Alexander J. TUMOR MICROENVIRONMENT DYNAMICS: MOLECULAR AND GENETIC DETERMINANTS OF CANCER PROGRESSION AND THERAPY RESPONSE. Genetics and Molecular Research. 2026 Jul 15.
33. Arul Kumar R, Indrapriyadharshini C, Parthiban S, Boopathi T, Alexander J. Review on properties of boswellia serrata in inflammatory and rheumatoid arthritis (RA) management. World J Pharm Res. 2022 May 5;11(9):118-28.
34. Jimmy A. Characterization of Physalis Lagascae for its In Vitro Anti-Cancer Activity (Doctoral dissertation, Annai JKK Sampoorani Ammal College of Pharmacy, Komarapalayam).