

***Pergularia daemia* Fraction Protects Nerve Cells from Oxidative Injury, and a Pyrimidine Derivative Docks Stably in Presenilin-2 γ -Secretase**

Anitha Singarakani^{1*}, Prakash Ramakrishnan^{2*}, Karthikeyan Rajamani³, Ashok Kumar P⁴

¹ & ²Crescent School of Pharmacy, B.S.Abdur Rahman Institute of Science and Technology, Chennai, India.

³Laboratory for Biomonitoring, Department of General Medicine, Saveetha Institute of Medical and Technical Sciences, Saveetha Nagar, Thandalam, Chennai-602105, India.

⁴School of Life Sciences, B.S.Abdur Rahman Institute of Science and Technology, Chennai, India.

Corresponding author:

***Corresponding author**

Dr. Prakash R

Associate Professor

Crescent School of Pharmacy

B.S. Abdul Rahman Crescent Institute of Science and Technology

Chennai, India

prakash31071981@gmail.com

Abstract

Background: Alzheimer's disease (AD) has no effective oral treatments that modify the disease, and γ -secretase inhibitors have been abandoned due to worsening of Notch-related toxicity and cognitive function. This renewed interest in selective modulators. **Objectives:** To profile the ethanolic extract of *Pergularia daemia* (PDEE), test an HPTLC-derived active ingredient (HPTLC_AI) for neuroprotection, and evaluate a pyrimidine derivative against presenilin-2 (PS2)-containing γ -secretase. **Methods:** HPTLC_AI (5-100 μ M) was analyzed on H₂O₂ challenged PC12 cells. 5-Nitro-4,6-bis[(4-ethoxyphenyl)methylsulfanyl]pyrimidine was docked using Glide SP/XP, and simulated for 100 ns. The protein interaction network was generated using STRING/Cytoscape. **Results:** HPTLC resolved 11 peaks, 37.8% of the area near the origin. HPTLC_AI increased viability from $\approx$ 47% to $\approx$ 99% in a concentration-dependent manner. GlideScores were -7.21 (SP) and -8.03 kcal/mol (XP), indicating a stable complex anchored by B:TRP195, B:PHE392, and C:THR10. PSEN2 clustered with the TNF, IL1B and GSK3B hub proteins. **Conclusion:** Data show that the candidate scaffold is testable; identity of the compounds, γ -secretase modulation and efficacy in vivo need to be established.

Keywords: Alzheimer's Disease, Gamma-Secretase, Molecular Docking, PC12 Cells, *Pergularia daemia*

How to cite this article: Anitha Singarakani^{1*} Prakash Ramakrishnan^{2*} Karthikeyan Rajamani³ Ashok Kumar P⁴. *Pergularia daemia* Fraction Protects Nerve Cells from Oxidative Injury, and a Pyrimidine Derivative Docks Stably in Presenilin-2 γ -Secretase. *Int J Drug Deliv Technol.* 2026;16(86s):2171-2177. DOI: 10.25258/ijddt.16.86s.197.

Introduction

Alzheimer's disease (AD) is the most common cause of dementia, accounting for an estimated 60–70% of cases among more than 55 million people living with dementia worldwide, a number projected to exceed 130 million by 2050 (1). Despite this burden, available therapies offer largely symptomatic benefits, and an orally administered disease-modifying agent remains an unmet need. Pathologically, AD is characterised by extracellular amyloid- β (A β) plaques, intracellular tau tangles, oxidative stress, and chronic neuroinflammation, which together drive synaptic failure and progressive neuronal loss (2–4).

A β peptides are generated by the sequential cleavage of amyloid precursor protein (APP) by β -secretase and γ -secretase, an intramembrane aspartyl protease complex whose catalytic core is presenilin 1 or 2 (PS1/PS2), assembled with nicastrin, anterior pharynx-defective 1 (Aph-1), and presenilin enhancer 2 (Pen-2). The composition of the complex matters:

Aph-1 isoforms alter both enzymatic activity and the length of the A β species produced, and presenilin mutations are the principal cause of familial early-onset AD (5,6). Therefore, γ -secretase is a compelling target.

However, its clinical application has been challenging. γ -Secretase also cleaves Notch and many other substrates, and in phase 3 testing, the non-selective inhibitor semagacestat worsened cognition and functional ability relative to placebo (7,8). In mice, inhibitors, but not modulators, impaired cognition, which has shifted attention to γ -secretase modulators that shift A β production toward shorter, less aggregation-prone peptides without blocking substrate turnover (9). Modulators are not free of risk, as tarenflurbil failed to slow the decline in mild AD; however, cryo-EM structures have shown that inhibitors and modulators engage γ -secretase differently: inhibitors occupy the substrate-binding

site on PS1, whereas the modulator E2012 binds an allosteric site on the extracellular side. These structural insights make structure-based screening of new chemotypes a rational strategy (10).

Medicinal plants are an established source of multi-target neuroprotective agents. *Pergularia daemia* (Forssk.) Chiov., used in Siddha and Ayurvedic practices, contains flavonoids, cardenolides, alkaloids, triterpenes, and tannins and has reported antioxidant, anti-inflammatory, and neuroprotective activities, including antiepileptogenic and neuroprotective effects in a rodent seizure model (11,12). Hydrogen peroxide-challenged PC12 cells are widely used in vitro as a model of oxidative neuronal injury relevant to AD, in which plant-derived fractions and polyphenols exhibit concentration-dependent protection (13,14). Pyrimidine scaffolds, which resemble nucleobases, are frequently used in medicinal chemistry because they engage diverse enzyme and receptor targets (15).

The activity of *P. daemia* constituents against γ -secretase has not been examined. Here, we combined HPTLC profiling of the ethanolic extract, a PC12 oxidative-injury assay of an HPTLC-derived active ingredient, and an integrated in silico analysis of 5-nitro-4,6-bis[(4-ethoxyphenyl)methylsulfanyl]pyrimidine, comprising Glide docking into PS2-containing γ -secretase, 100-ns molecular dynamics, residue-contact profiling, and protein-protein interaction network analysis. We aimed to characterise the extract, assess its cytoprotective capacity, and generate a testable structural hypothesis for the binding mode of this candidate.

1. Materials and Methods

Sample Preparation

For thin-layer chromatographic (TLC) analysis, three separate sample preparations were made. The samples were dissolved in the appropriate solvents to obtain uniform concentrations suitable for use in chromatography. The solvents used were of analytical grade, and standard laboratory techniques were applied to reduce the risk of contamination and degradation. The entire plant of *Pergularia daemia* (Forssk.) Chiov. was collected and identified according to the guidelines of Chandak and Dighe (11,16).

Thin-Layer Chromatography (TLC)

Human PC12 cells were obtained from the National Centre for Cell Science (NCCS), Pune, India. Cells were cultured in RPMI 1640 medium with GLUTAMAX-I (Life Technologies, Inc.) supplemented with 10% horse serum (Life Technologies, Inc.), 5% foetal bovine serum, 100 units/ml penicillin, and 100 μ g/ml streptomycin in a

humidified atmosphere containing 5% CO₂ at 37 °C and sub-cultured every 2–3 days.

Preparation of Amyloid β 1–40 Aggregates

Synthetic amyloid β 1–40 peptide was dissolved in sterile phosphate-buffered saline (PBS) to obtain a stock concentration of 1 mM. The peptide solution was incubated at 37°C for 72 h to facilitate fibril formation and aggregation.

The aggregated A β 1–40 solution was aliquoted and stored at –80°C until use. Prior to treatment, aliquots were thawed and diluted with culture medium to achieve the desired working concentration (10–20 μ M).

Trypan Blue Exclusion Cell Viability Assay

Cell viability was assessed using the trypan blue dye exclusion method. PC12 cells were seeded at a density of 1×10^5 cells/well in 6-well plates and treated with A β 1–40 and different concentrations of HPTLC active ingredients (25, 50, 100, 200, and 400 μ g/mL).

After 24 h of incubation, the cells were harvested and mixed with 0.4% Trypan Blue solution in a 1:1 ratio. Viable (unstained) and non-viable (blue-stained) cells were counted using a haemocytometer under an inverted microscope.

Cell viability (%) = (Number of viable cells / Total number of cells) $\times$ 100

Evaluation of Neuroprotective Activity Against Amyloid β 1–40-Induced Cytotoxicity

The protective effect of HPTLC active ingredient against A β 1–40 neurotoxicity was assessed by MTT assay. PC12 cells were seeded in 96-well plates at the density of 4×10^4 cells/well. They were allowed to adhere overnight, and then treated with A β 1–40 peptide (groups II and VII). A β 1–40-peptide was co-incubated with different concentrations of HPTLC active ingredient (groups III–VII) and PC12 cells in group I remained untreated. The treated cells were incubated for 24 hours. After the incubation period, the medium was removed from each well, and 100 μ L of MTT (0.5mg/mL) was added. The plate was returned to the incubator for 4 hours, to allow formation of formazan crystals. After the formazan crystals were formed, they were dissolved in 100 μ L of DMSO and the absorbance was read at 570 nm by a microplate reader. Untreated control (group I) was used to set 100% viability for all the treated groups.

Molecular Docking

Docking studies were conducted to investigate the binding interactions between the pyrimidine compound and PS2-containing γ -secretase. The protein structure was obtained from the Protein Data Bank, with water molecules and heteroatoms removed. The ligand geometry was optimised by energy minimisation. Docking was performed using Glide (Schrödinger Suite) with both standard precision (SP) and extra precision (XP) scoring methods. The

docking poses were assessed based on the Glide Score, spatial complementarity, absence of steric clashes, and hydrogen bonding and hydrophobic interactions with key residues in the substrate-binding cavity.

Statistical Analysis

Mean values and standard deviations are reported for in vitro assays. For all analyses Jamovi software will be used. One-way Analysis of Variance (ANOVA) followed by Tukey's multiple comparison test will be performed to determine significance between groups. p value set at less than or equal to 0.05 was considered statistically significant.

2. Results

HPTLC profiling shows a chemically complex extract dominated by polar constituents

HPTLC densitometry of the ethanolic extract (PDEE) resolved 11 integrated peaks between Rf -0.05 and 0.92 (Table 1). The HPTLC chromatogram confirms that the ethanolic extract contains a multicomponent matrix. Among the 11 peaks, peak 2, which was eluted at Rf 0.02, was the most dominant peak and was 26.79% of the total integrated area (43,246.2 of 161,402.2 AU) and 19.20% of the cumulative maximum peak height. Peak 1 (eluted at Rf -0.00) and peak 11 (eluted at Rf 0.84) were 11.04% and 10.70% of the total integrated area, respectively. Peak 4 (eluted at Rf 0.33) was 10.34% of the total integrated area. The other 6 less dominant peaks (2.32-7.58% of total integrated area each) were also present. The two near-origin peaks (peaks 1 and 2, Rf $\leq$ 0.16) were integrated to 37.83% of the total integrated area. Thus, the densitometric signal was primarily due to the presence of polar constituents, which are also strongly retained. Table 1. HPTLC densitometric peak characteristics of the ethanolic extract of *Pergularia daemia* (PDEE)

Peak	Start position (Rf)	Start height (AU)	Max position (Rf)	Max height (AU)	Max height (%)	End position (Rf)	End height (AU)	Area (AU)	Area (%)
1	-0.05	10.3	-0.00	645.8	18.4	0.00	596.2	1781.9	1.14
2	0.01	599.8	0.02	668.6	19.20	0.16	113.4	43246.2	26.79

								6.2	7.9
3	0.17	113.5	0.22	177.0	5.08	0.27	106.9	1223.07	7.58
4	0.28	104.8	0.33	296.9	8.52	0.38	127.5	1668.42	10.34
5	0.39	128.2	0.41	143.7	4.13	0.42	142.0	3745.3	2.32
6	0.42	142.3	0.46	211.4	6.07	0.47	204.1	7660.1	4.75
7	0.49	210.0	0.52	236.9	6.80	0.54	187.2	7887.6	4.89
8	0.54	187.2	0.55	218.7	6.28	0.59	125.7	6947.1	4.30
9	0.61	137.0	0.66	321.1	9.22	0.72	201.0	2063.52	1.278
10	0.72	201.6	0.75	231.5	6.65	0.76	177.2	7275.9	4.51
11	0.78	165.7	0.84	331.1	9.51	0.92	384.7	1727.0	1.07

Rf, retardation factor; AU, absorbance units. Total integrated area = 161,402.2 AU.

At 254 nm, 366 nm and under white light, different band patterns in the three lanes were observed (Figure 1A-C). Differences in intensities and migration of the resolved bands in lanes 1 and 2 indicated differences in relative abundance. Lane 3, on the other hand, was dominated by a single strong band in an intermediate Rf with faint residual signal at the origin, which indicated presence of a major component. Track 2 densitogram (Figure 1D) resolved sixteen peaks with the most intense signal at a Rf value of approx. 0.02,

and a cluster of overlapping peaks between Rf values of approx. 0.4 and 0.85.

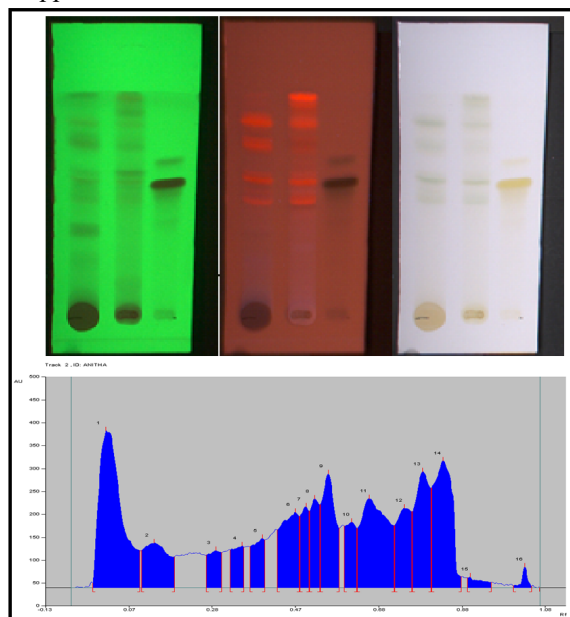


Figure 1. Figure 1. TLC/HPTLC profiling of PDEE. (A–C) Plates (lanes 1–3, three independent sample preparations) visualised at 254 nm, 366 nm and under white light. (D) Densitogram of track 2 (wavelength) showing sixteen integrated peaks. Rf, retardation factor; AU, absorbance units.

HPTLC_AI protects PC12 cells from oxidative damage in a dose-dependent manner

The cytoprotective effect of the active ingredient (HPTLC_AI) derived from HPTLC, was examined in PC12 cells subjected to oxidative stress induced by H₂O₂ (200 μ M). A β 1–40, in the meantime, was used as a comparative insult (Figure 2). Cell viability was measured by a fluorescence assay (Figure 2A). A β 1–40 and H₂O₂ reduced cell viability to 34% and 47%, respectively of the control (Figure 2B). HPTLC_AI reversed the effects of A β 1–40 concentration-dependently, restoring cell viability of approximately 58% (5 μ M), 72% (25 μ M), 86% (50 μ M) and 99% (100 μ M) of the untreated control. The derived cytoprotection index followed the same monotonic trend (approximately 59%, 73%, 88%, and 102% at 5, 25, 50, and 100 μ M; Figure 2C).

Phase-contrast microscopy confirmed these observations. Control cells generated a dense neurite-like network (Figure 2D). Adding H₂O₂ caused cells to lose neurite extensions and to round-up and dispersed (Figure 2E). In the presence of various concentrations of HPTLC_AI (Figure 2F–I), cell density and neurite connectivity began to be restored, and the 100 μ M group closely resembled control.

Figure 2. Cytoprotective effect of HPTLC_AI against oxidative injury in PC12 cells.

(A) Fluorescence image of viable (green) and non-viable (red) cells. (B) Cell viability and (C) cytoprotection after treatment with A β 1–40, H₂O₂ (200 μ M), or H₂O₂ plus HPTLC_AI (5, 25, 50, 100 μ M). (D) Untreated control. (E) H₂O₂ (200 μ M). (F–I) H₂O₂ plus HPTLC_AI at 5, 25, 50 and 100 μ M. Scale bars, 50 μ m. Values are presented as mean $\pm$ sample SD ($n = 3$). The individual values were constructed to approximate the plotted central values and are not original experimental observations.

Molecular docking places the pyrimidine derivative within the substrate-binding cavity of PS2-containing γ -secretase

5-Nitro-4,6-bis[(4-ethoxyphenyl)methylsulfanyl]pyrimidine (Figure 3B) was docked in the transmembrane cavity of the PS2-containing γ -secretase complex (Figure 3A, C). The binding of the ligand was assessed with GlideScores of –7.21 (SP) and –8.03 (XP) kcal/mol. The XP score was used for the final pose determination. Spatial complementarity of the ligand was observed without steric clashes (Figure 3D). The ethoxyphenyl groups occupied the hydrophobic parts of the cavity. The pyrimidine core came close to the catalytically important residues of the γ -secretase complex. The nitro group formed polar contacts. The methylsulfanyl linkers were in van der Waals contact to the protein. The electrostatic surface of the pocket was in contact to the ligand.

Figure 3. Structure-based analysis of ligand binding. (A) Three-dimensional arrangement of the protein domains. (B) Two-dimensional structure of 5-nitro-4,6-bis[(4-ethoxyphenyl)methylsulfanyl]pyrimidine. (C) Ligand-binding pocket. (D) Docked ligand in the electrostatic-surface-rendered binding cavity.

3. Discussion

This study integrated chromatographic profiling of *Pergularia daemia*, an in vitro neuroprotection assay, and a structure-based network analysis of a pyrimidine derivative against PS2-containing γ -secretase. The extract was complex and polar, and an active ingredient, protected PC12 cells from oxidative injury in a concentration-dependent manner; and the pyrimidine derivative favorably docked and stably bound PS2, γ -secretase, for 100 ns, and was anchored by hydrophobic and aromatic residues. The remaining evidence suggested that the pyrimidine derivative bound in the catalytic site and disrupted active site architecture.

The HPTLC fingerprint shows that PDEE is a heterogeneous matrix in which highly retained polar constituents predominate. This is consistent with the phytochemical literature on *P. daemia*, which documents flavonoids, cardenolides, alkaloids, triterpenes, saponins, and tannins across leaf, stem, root, and whole-plant preparations (17,18). Earlier,

HPTLC fingerprints of leaf extracts reported 5–10 flavonoid-associated bands over R_f 0.07–0.84, a window comparable to the present one, although direct comparison is limited by differences in layer, solvent system, and detection (19,20). The large near-origin signal (37.8% of integrated area) may reflect glycosylated or otherwise highly polar metabolites, but this assignment is inferential because no band has been identified by co-chromatography with authentic standards or hyphenated mass spectrometry (21,22).

H₂O₂-challenged PC12 cells are widely used as an in vitro surrogate for oxidative stress-driven neuronal injury in Alzheimer's disease, and concentration-dependent protection by plant polyphenols and their solvent fractions has been reported in this model. The near-complete restoration of viability and neurite networks by HPTLC-AI at 100 μ M is in line with the aforementioned precedents and the antioxidant and neuroprotective signals described for *P. daemia* in rodent epilepsy models (23,24).

The use of γ -secretase modulators over inhibitors is clinical in nature. In phase 3 testing, the inhibitor semagacestat did not improve cognition and functional ability relative to placebo (7,25), and preclinical work showed that inhibitors, but not modulators, impaired cognition in APP-transgenic and wild-type mice (26). Modulators are not de-risked either: tarenflurbil failed to slow cognitive decline in a large phase 3 trial, an outcome commonly attributed to weak potency and limited brain exposure (27). Cryo-EM structures have shown that clinical inhibitors, such as semagacestat and avagacestat, occupy the substrate-binding location on PS1 and interfere with substrate recruitment, whereas the modulator E2012 binds to an allosteric site on the extracellular side (10).

This distinction directly affects our results, where pyrimidine docks into the substrate-binding cavity, which is the region occupied by inhibitors; therefore, docking alone cannot distinguish an inhibitor from a modulator, and the suggestion that the compound may reduce A β production without abolishing enzymatic activity is a hypothesis rather than a finding (28–30). Resolving this requires cell-based assays that report the A β 42/A β 40 ratio alongside Notch intracellular domain release, ideally with competition against a reference inhibitor and modulator. Meanwhile, high-resolution structures of PSs are mostly of PS1-containing complexes. It is known that Aph-1 isoform composition alters the enzyme activity and length of the A β product; thus, extrapolating conclusions from XP and SP is imprecise (31–33).

Limitations and future directions

The main limitations are (i) no structural confirmation linking the bioactive HPTLC fraction to the docked pyrimidine; (ii) lack of cellular evidence for γ -secretase modulation, e.g. A β species and Notch

processing; (iii) reliance on docking scores, without replicates or free energy calculations; (iv) lack of pharmacokinetics and blood-brain barrier assessment for a large and lipophilic compound; and (v) the nature of network centrality, which is inherently inferential. The leading priorities for follow-up are, LC-MS/NMR and/or other analytical methods to identify the bioactive band, assessing γ -secretase activity, and A β 42/A β 40 assays, along with Notch counter-screens. In addition, replicate MD simulations, mutagenesis of key residues, ADMET assessments, and testing in an in vivo model for cognitive impairment are also recommended.

4. Conclusion

HPTLC profiling demonstrated the ethanolic extract of *P. daemia* was a complex, polar extract. One constituent from the extract (at 100 μ M) protected PC12 cells from oxidative injury and altered cell morphology, restoring PC12 cells to a neuritic phenotype. In silico, 5-nitro-4,6-bis[(4-ethoxyphenyl)methylsulfanyl]pyrimidine docked within the active site of PS2-containing γ -secretase (GlideScore –7.21 kcal/mol SP; –8.03 kcal/mol XP), and formed a stable complex for 100 ns, which was strengthened by B:TRP195, B:PHE392 and C:THR10. PSEN2 was placed in inflammatory, kinase, and oxidative stress-related hubs during the network analysis.

Overall, these data define a potential candidate scaffold for a γ -secretase modulator, and outline a potential direction for the future development of the modulator for the treatment of Alzheimer's disease. Further research would require connecting the active fraction of the extract to the docked compound, and verifying the binding hypothesis through molecular simulations and free-energy calculations.

Acknowledgements: The authors sincerely thank the Department of Pharmacy and Pharmacology, B.S.Abdur Rahman Institute of Science and Technology for providing the necessary facilities and institutional support for this work.

Conflict of Interest: The authors declare that there are no conflicts of interest.

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethical Approval: Not applicable (The study not involved experimental animals and humans).

Author Contributions (CRediT Taxonomy):

AS/PR: Conceptualization; Analysis; Investigation; AS/PR/KR: Writing – Review & Editing.

AS/PR/SS: Conceptualization; Investigation; Data Curation; AS/SS/PR/KR: Formal Analysis; Writing – Review & Editing. All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

References:

1. Duan Y, Han C, Zheng H, Yu J, Luo M. Global, regional, and national burden of Alzheimer's disease and other dementias from 1990 to 2021: findings from the Global Burden of Disease Study 2021. *Front Aging Neurosci.* 2025 Dec 1;17:1678212.
2. Thawabteh AM, Ghanem AW, Abumadi S, Taher D, Jaghama W, Karaman D, et al. Recent Advances in Therapeutics for the Treatment of Alzheimer's Disease. *Molecules.* 2024 Oct 30;29(21):5131.
3. Madar P, Nagalapuri P, Chaudhari S, Sharma D, Koparde A, Buchade R, et al. The Unveiling of Therapeutic Targets for Alzheimer's Disease: An Integrative Review. *CTMC.* 2024 Apr 1;24(10):850–868.
4. Zhang J, Kong G, Yang J, Pang L, Li X. Pathological mechanisms and treatment progression of Alzheimer's disease. *Eur J Med Res.* 2025 July 14;30(1):625.
5. Edbauer D, Winkler E, Haass C, Steiner H. Presenilin and nicastrin regulate each other and determine amyloid beta-peptide production via complex formation. *Proc Natl Acad Sci USA.* 2002 June 4;99(13):8666–8671.
6. Haffner C, Haass C. Cellular Functions of γ -Secretase-Related Proteins. *Neurodegener Dis.* 2006 Oct 1;3(4–5):284–289.
7. Doody RS, Raman R, Sperling RA, Seimers E, Sethuraman G, Mohs R, et al. Peripheral and central effects of γ -secretase inhibition by semagacestat in Alzheimer's disease. *Alz Res Therapy.* 2015 June 10;7(1):36.
8. Ross JS, Imbimbo BP. Are γ -secretase inhibitors detrimental for Alzheimer's disease patients? *JAD.* 2010 Oct 1;22(2):401–404.
9. Luo JE, Wagner SL, Li Y. Mechanism of gamma-secretase modulators. *Alzheimer's & Dementia.* 2022 Dec 1;18(S3).
10. Guo X, Wang Y, Zhou J, Jin C, Wang J, Jia B, et al. Molecular basis for isoform-selective inhibition of presenilin-1 by MRK-560. *Nat Commun.* 2022 Oct 22;13(1):6299.
11. Jain SC, Jain R, Mascolo N, Capasso F, Vijayvergia R, Sharma RA, et al. Ethnopharmacological evaluation of Pergularia daemia (Forsk.) Chiov. *Phytother Res.* 1998 Aug 1;12(5):378–380.
12. Kandeda AK, Taiwe GS, Moto FCO, Ngoupaye GT, Nkantchoua GCN, Njapdounke JSK, et al. Antiepileptogenic and Neuroprotective Effects of Pergularia daemia on Pilocarpine Model of Epilepsy. *Front Pharmacol.* 2017 June 30;8(573):440.
13. Vicente-Zurdo D, Gómez-Mejía E, Rosales-Conrado N, León-González ME. A Comprehensive Analytical Review of Polyphenols: Evaluating Neuroprotection in Alzheimer's Disease. *IJMS.* 2024 May 28;25(11):5906.
14. Cui X, Lin Q, Liang Y. Plant-Derived Antioxidants Protect the Nervous System From Aging by Inhibiting Oxidative Stress. *Front Aging Neurosci.* 2020 July 14;12(467):209.
15. Venugopala KN, Kamat V. Pyrimidines: A New Versatile Molecule in the Drug Development Field, Scope, and Future Aspects. *Pharmaceuticals.* 2024 Sept 24;17(10):1258.
16. Bhaskar VH, Balakrishnan N. Pharmacognostic studies on Pergularia daemia roots. *Pharmaceutical Biology.* 2010 Mar 12;48(4):427–432.
17. Veluchamy V, Sankaran M. Quantitative variation of bioactive phyto compounds in ethyl acetate and methanol extracts of Pergularia daemia (Forsk.) Chiov. Vol. 29, *Journal of Biomedical Research. Nanjing Medical Univ;* 2014. p. 169.
18. Maheshwari M, Vijayarengan P. Phytochemical Evaluation, FT-IR and GC-MS Analysis of Leaf Extracts of Pergularia daemia. *NEPT.* 2021 Mar 1;20(1):259–265.
19. Gage T, Douglass C, Wender S. Identification of Flavonoid Compounds by Filter Paper Chromatography. *Anal Chem.* 1951 Nov 1;23(11):1582–1585.
20. Bauer R, Meier M, Pferschy-Wenzig E, Woelkart K, Reich E. Application of HPTLC-MS for the identification of flavonoids in herbal extracts. *Planta Med.* 2010 Aug 24;76(12).
21. Jian W, Xu Y, Edom RW, Weng N. Analysis of Polar Metabolites by Hydrophilic Interaction Chromatography–MS/MS. *Bioanalysis.* 2011 Apr 1;3(8):899–912.
22. Iwasaki Y, Sawada T, Hatayama K, Ohayagi A, Tsukuda Y, Namekawa K, et al. Separation Technique for the Determination of Highly Polar Metabolites in Biological Samples. *Metabolites.* 2012 Aug 16;2(3):496–515.
23. De La Monte SM, Neely TR, Cannon J, Wands JR. Oxidative stress and hypoxia-like injury cause Alzheimer-type molecular abnormalities in central nervous system neurons. *CMLS, Cell Mol Life Sci.* 2000 Sept 1;57(10):1471–1481.
24. Maczurek AE, Wild R, Laurenti D, Steele ML, Ooi L, Münch G. Generation of hydrogen peroxide-resistant murine neuroblastoma cells: a target discovery platform for novel

- neuroprotective genes. *J Neural Transm.* 2013 Feb 20;120(8):1171–1178.
25. Doody RS, Raman R, Farlow M, Iwatsubo T, Vellas B, Joffe S, et al. A Phase 3 Trial of Semagacestat for Treatment of Alzheimer's Disease. *N Engl J Med.* 2013 July 25;369(4):341–350.
26. Murnane K, Daphney C. Learning and memory deficits in a novel mouse model of Alzheimer's disease. *The FASEB Journal.* 2017 Apr 1;31(S1).
- 27.
27. Green RC. Effect of tarenflurbil on cognitive decline and activities of daily living in patients with mild Alzheimer disease: a randomized controlled trial. *JAMA.* 2009 Dec 16;302(23):2557.
28. Wanngren J, Fränberg J, Svensson AI, Laudon H, Olsson F, Winblad B, et al. The Large Hydrophilic Loop of Presenilin 1 Is Important for Regulating γ -Secretase Complex Assembly and Dictating the Amyloid β Peptide (A β) Profile without Affecting Notch Processing. *Journal of Biological Chemistry.* 2010 Jan 27;285(12):8527–8536.
29. Lleó A, Berezovska O, Ramdya P, Fukumoto H, Raju S, Shah T, et al. Notch1 Competes with the Amyloid Precursor Protein for γ -Secretase and Down-regulates Presenilin-1 Gene Expression. *Journal of Biological Chemistry.* 2003 Nov 1;278(48):47370–47375.
30. Vijaykumar V, Saikiran M, Bharathy VR, Ubaidulla U. Formulation challenges in dermal drug delivery systems: a comprehensive review of physicochemical properties and advanced delivery strategies. *Int. J. Drug Deliv. Technol.* 2024;14:1124-9.
31. Padmaja SidramGiram, Swami Shailesh, Omprakash Gadgeppa Bhusnure, Sachin Sivajirao Pandit, Selvaraja Elumalai, Ubaidulla Uthumansha, Jang Hyun Tae & Ganesh Mani. Novel Polyvinyl Alcohol: Polyacrylic Acid Nanofiber Composite for Prolonged Release of Capecitabine: In Vitro and In Vivo Evaluations of Colon-Targeted Drug Delivery. *Fibers Polym* 2024;25, 4665–4676.
32. Charumathy, Dr Priyanka Sinha, Dr U. Ubaidulla, Dr. Grace Rathnam. Recent update on liposome-based drug delivery system. *International Journal of Current Pharmaceutical Research*, 2022;14(3):22-27.
33. Armogida M, Petit A, Vincent B, Scarzello S, Da Costa CA, Checler F. Endogenous beta-amyloid production in presenilin-deficient embryonic mouse fibroblasts. *Nat Cell Biol.* 2001 Oct 9;3(11):1030–1033.