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IN VITRO INHIBITORY ACTIVITY OF TINOSPORA CORDIFOLIA EXTRACT ON ACETYLCHOLINESTERASE AND AMYLOID BETA PLAQUE FORMATION

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ABSTRACT

INTRODUCTION - *Tinospora cordifolia also known as "Guduchi" is a large, climber shrub with many elongated branches. Tinospora cordifolia belongs to the family Menispermaceae and has a broad spectrum of immunotherapeutic properties ranging from antipyretic, anti-inflammatory, antiallergic antidiabetic, antihepatotoxic, antibacterial properties and has relatively low toxicity.*

AIM & OBJECTIVE - *This study aims to focus on the inhibitory activity of tinospora cordifolia extract on acetylcholinesterase and on beta plaque formation.*

MATERIALS & METHODS - *Stem of tinospora cordifolia was collected and dried, packed in Soxhlet extractor, extraction was carried out using hot extraction procedure.*

Xanthine oxidase inhibitory activity, Thioflavin T fluorescence assay , Invitro acetylcholinesterase inhibition assay .

RESULT - *In vitro antioxidant activity was evaluated. The results showed significant inhibition of xanthine oxidase the in vitro acetylcholinesterase inhibitory activity results exhibited marked ($p<0.001$) reduction in the levels of the enzyme*

Similar results were observed in the amyloid beta peptide assay. The results demonstrated a significant decrease in the % aggregation of amyloid peptides.

CONCLUSION- *Overall, the in vitro inhibitory activity of Tinospora cordifolia extract on AChE and Aβ plaque formation highlights its potential as a natural compound for neuroprotection and cognitive health. However, it is important to note that in vitro findings need further validation through in vivo studies and clinical trials to ascertain the compound's efficacy, safety, and feasibility for therapeutic applications in humans.*

Keywords: Tinospora Cordifolia, AcetylCholinesterase, Amyloid beta plaque Invitro, Alzheimer's disease.

INTRODUCTION

Acetylcholinesterase is a crucial enzyme responsible for breaking down acetylcholine, a neurotransmitter essential for efficient nerve signal transmission in the central and peripheral nervous systems. Dysfunction of AChE leads to the accumulation of acetylcholine, resulting in prolonged nerve signals and potential neural disorders.(1)

Neurodegenerative diseases, such as Alzheimer's disease, are characterized by a decline in cognitive function and memory loss, often linked to a decrease in acetylcholine levels. Therefore, the inhibition of AChE has become a promising therapeutic strategy for managing such conditions. This has sparked interest in natural compounds as potential AChE inhibitors, and Tinospora cordifolia, a widely studied medicinal plant in traditional medicine systems like Ayurveda, has shown promise in this regard.(2)

Tinospora cordifolia, also known as Giloy or Guduchi, has a rich history of use for its numerous health benefits. It contains a diverse range of bioactive compounds, including alkaloids, glycosides, and flavonoids, which could

contribute to its potential neuroprotective properties. Preliminary studies have suggested that Tinospora cordifolia extract might exhibit inhibitory effects on AChE, but further investigation is necessary to understand the underlying mechanisms and evaluate its therapeutic potential fully.(3)

The in vitro inhibitory activity of Tinospora cordifolia extract on amyloid beta plaque formation is a compelling area of research in the realm of neuroscience and Alzheimer's disease (AD) therapeutics. AD is a neurodegenerative disorder characterized by the accumulation of amyloid beta (Aβ) plaques in the brain, which are believed to play a pivotal role in neuronal dysfunction and cognitive decline.(4)

Aβ peptides are derived from the proteolytic processing of amyloid precursor protein (APP) and tend to aggregate, forming insoluble plaques in the brain, leading to synaptic dysfunction and neural cell death. As a result, finding novel strategies to mitigate Aβ plaque formation has become a key focus in the quest for effective AD treatments.(5)

The actual causes at play behind the development of AD are still not well defined. However, certain factors like anomaly in the phosphorylation of tau protein, alterations in calcium metabolism, oxidative stress, neuro-inflammation, abnormal energy metabolism and protein processing i.e. undesired Aβ formation and aggregation, are considered to be important factors in the pathogenesis of AD.(6)

A relatively recent mitochondrial dysfunction hypothesis for pathogenesis of AD proposes that AD brain mitochondrial dysfunction leads to amyloidosis, cell cycle re-entry, and tau phosphorylation.(7)

Additionally supporting neuro-inflammatory reactions, neuronal toxicity, cell death, and brain shrinkage are these aberrant protein accumulations. A cholinergic signaling deficiency, oxidative stress, inflammation, mitochondrial damage and/or malfunction are all factors in the multifaceted and complicated pathophysiology of AD.(8)

The development of acetylcholinesterase inhibitors and cholinomimetics to maintain cholinergic transmission in the AD brain was sparked by efforts to prevent the cholinergic deficit. For the treatment of patients with mild to severe AD, medications such tacrine, rivastigmine, donepezil, huperzine A, and physostigmine (eserine) have been used. These medications were not created to target other molecular routes implicated in the development of AD illness; instead, they only treat cholinergic symptoms.(9)

This study aims to focus on the inhibitory activity of tinospora cordifolia extract on acetylcholinesterase and on beta plaque formation.

To evaluate the antioxidant activity of the tinospora cordifolia extract

To evaluate effect of tinospora cordifolia extract on acetyl cholinesterase inhibitors

To evaluate effect of tinospora cordifolia extract on aggregation of amyloid beta plaque formation.

MATERIALS AND METHODS

The current study was conducted in Saveetha Dental college for 3 months .

Plant Material

The stem material of *Tinospora cordifolia* was purchased from M/s. SKM Siddha and ayurvedic medicines India private limited, Tamilnadu. The rhizomes were cleaned under running tap water and then shade dried at ambient temperature. Thereafter the dried stem were pulverized into a coarse powder and ready for extraction.

Preparation of PLANT extract

The stem of *Tinospora cordifolia* collected was shade dried and then coarsely powdered. About six hundred gram of coarse material was weighed and packed in a Soxhlet extractor with 1000 ml of 70% hydroalcohol (70% ethanol and 30% water). Extraction was carried out using hot extraction procedure for 18-20 hours and filtered. Filtrate was concentrated under gentle heat to give a powdered extract. The extracts were concentrated and used for further experiments.

Chemicals and Reagents

Xanthine, acetylthiocholine iodide, acetylcholine enzyme (0.3U/ml) were procured from Sigma-aldrich, USA. Quercetin was purchased from TCI chemicals, India. Donezepil hydrochloride was purchased as tablet from a local pharmacy. All other chemicals, reagents and solvents used were of analytical grade and purchased from SRL chemicals, India.

Xanthine Oxidase Inhibitory Activity

The XO inhibitory activity was assayed spectrophotometrically under aerobic conditions, based on the procedure reported by Bustanji et al. 2011. The substrate and the enzyme solutions were freshly prepared. The assay mixture, consisting of 50µL of different concentrations *Tinospora cordifolia*(10-320µg/ml), different concentrations of Quercetin (10-320µM), 35µL of 0.1mM phosphate buffer (pH=7.5) and 30µL of enzyme solution (0.01units/ml of XO in 0.1mM phosphate buffer, pH=7.5), was prepared immediately before use. After 30mins of incubation at 25°C, the reaction was initiated by the addition of 60µL of substrate solution (150mM of Xanthine in 0.1mM Phosphate buffer). The absorption at 295 nm, indicating the formation of uric acid at 25°C, was monitored and the initial rate was calculated. A blank was prepared in the same manner. One unit of XO was defined as the amount of enzyme required to produce 1 mmol of uric acid/minute at 25 °C. XO inhibitory activity is expressed as the percentage inhibition of XO in the above system, calculated as (1-B/A) × 100, where A and B are the activities of the enzyme without and with different concentrations of *Tinospora cordifolia*and Quercetin. IC_{50} values were calculated from the mean values of data from three determinations. Quercetin was used as reference standard.

In Vitro Acetyl cholinesterase (AChE) Inhibition Assay (Ellman et al., 1961)

The hydroalcoholic extract of *Tinospora cordifolia*and standard Donezepil hydrochloride was examined for its AChE inhibitory activities at different concentrations of 10-320µM and 10-320µg/ml respectively. 200µl of the different concentrations *Tinospora cordifolia*(10-320µg/ml) and standard Donezepil hydrochloride (10-320µg/ml) were prepared using 0.05M tris base. Briefly, in this method, 200µl of acetylthiocholine iodide (15mM), 1000µl of DTNB (3mM), and 200µl of *Tinospora*extract and Donezepil at different concentrations were mixed and incubated for 15 min at 30ºC. Then, the mixture was monitored spectrophotometrically at 412nm 10 times, each 13 s. After that, 200µl of AChE (0.3U/ml) solution were added to the initial mixture, to start the reaction and then the absorbance was determined.

Control contained all components except the tested extract. The percentage of AChE inhibitory activity (% IA) was calculated by using the following equation:

IA (%) = (Activity of Control – Activity of Test)/ Activity of Control x 100

Assessment of A*β* (1–42) Concentration

Preparation of Aβ Solution

The Aβ solution was prepared according to the method of Miyazaki et al., 2019. Briefly, synthetic β-Amyloid Peptide 1-42 (Aβ1-42) (PP69, Sigma Merck, USA) was dissolved in 0.1% ammonia solution at a final concentration of 250 µM and sonicated in ice-cold water for a total of 5 min (1 min × 5 times) to avoid pre-aggregation. For preparation of the Aβ solution, aliquots of Aβ were diluted to 25µM in 50mM phosphate buffer (pH 7.5) and 100mM NaCl.

Thioflavin T Fluorescence Assay

The thioflavin T (ThT) fluorescence assay was performed as Miyazaki et al., 019. Aβ solution (8µL) was mixed with the different concentrations of *Tinospora* (10-320µg/ml) and Donezepil (10-320µg/ml) and the mixture was then added to 1.6mL of ThT solution containing 5µM ThT and 50mM NaOH-glycine-buffer (pH 8.5). The samples were incubated at 37℃ and the fibrillogenesis rate was monitored by using ThT fluorescence assays. The samples ThT fluorescence levels were evaluated by using Biotek Synergy H4 hybrid multimode reader (USA). The respective excitation and emission wavelengths were 446 nm and 490 nm.

RESULTS

Beta Plaque Formation Activity

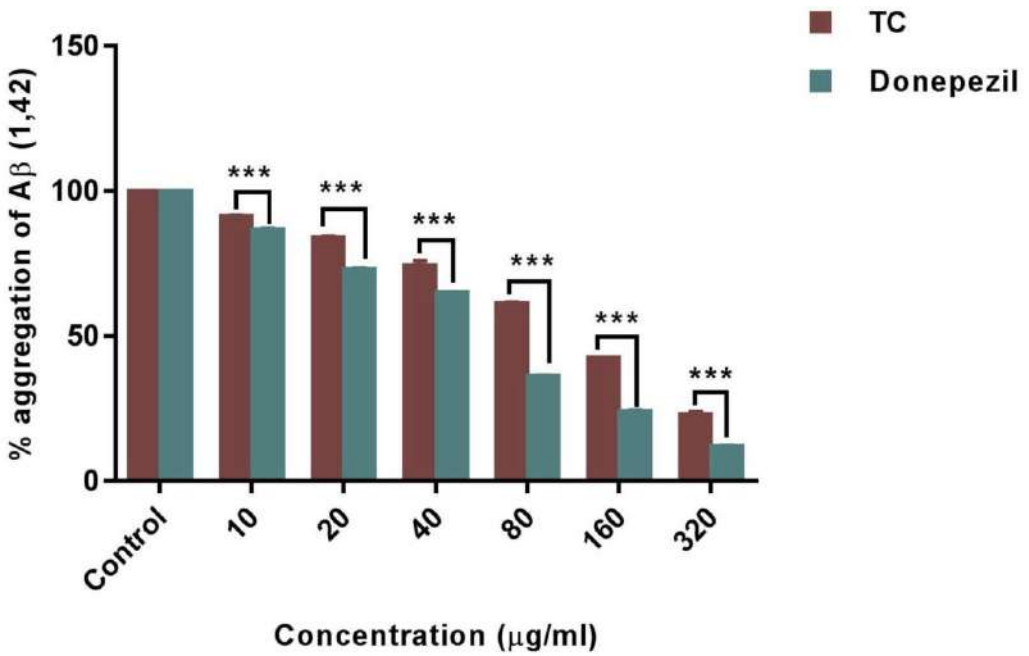

Table 1: Similar results were observed in the amyloid beta peptide assay. The results demonstrated significant decrease in the % aggreagation of amyloid peptide.

Xanthine Oxidase Activity

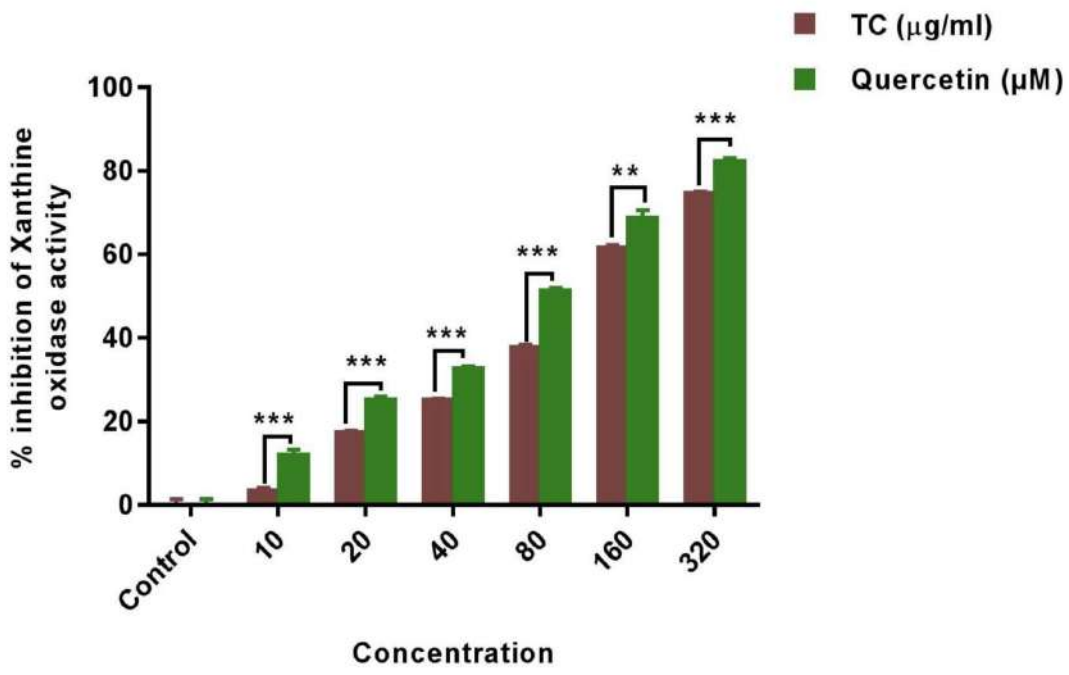

Table 2: In vitro antioxidant activity was evaluated. The results showed significant inhibition of xanthine oxidase.

Acetyl Cholinesterase Activity

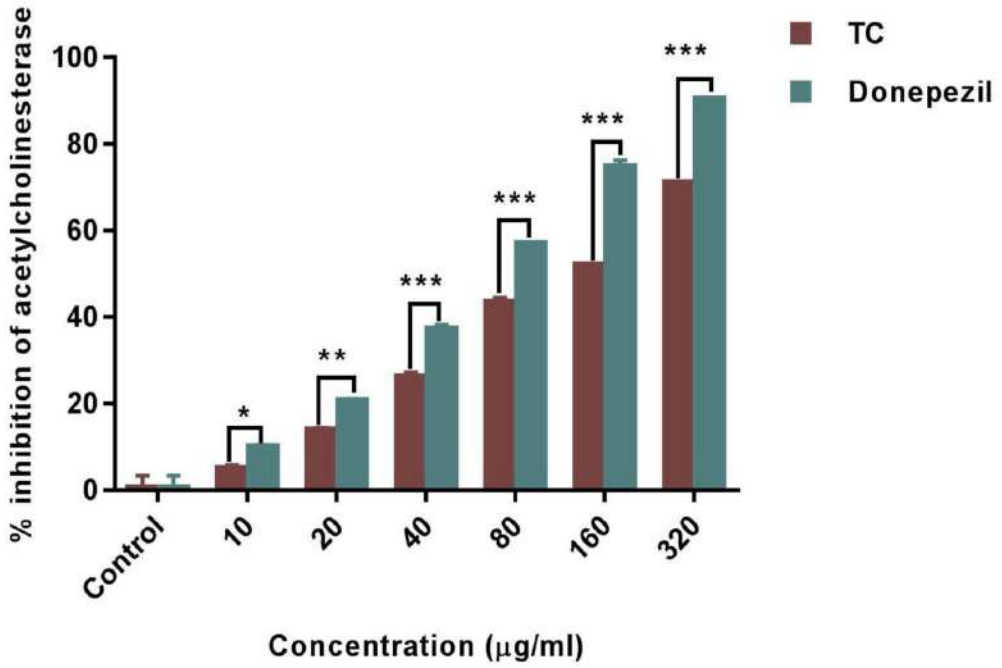

Table 3: The in vitro acetylcholinesterase inhibitory activity results exhibited marked ($p<0.001$) reduction in the levels of the enzyme.

DISCUSSION

Alzheimer's disease is a neurodegenerative disorder characterized by the progressive decline of cognitive function, memory loss, and the accumulation of amyloid beta plaques in the brain. These plaques are believed to play a significant role in the pathology of Alzheimer's, leading to the disruption of neuronal communication and eventual cell death.(10)

Acetylcholinesterase is an enzyme that breaks down the neurotransmitter acetylcholine in the brain. Inhibiting AChE activity can lead to increased levels of acetylcholine, which can improve cognitive function and memory. The standard treatment for Alzheimer's disease involves AChE inhibitors, but these medications often come with side effects.(11)

Research on Tinosporacordifolia's impact on amyloid beta plaque formation is still in its early stages. However, preliminary studies have shown that the plant extract may have a beneficial effect in reducing the production and accumulation of amyloid beta plaques. This mechanism of action could potentially slow down or halt the progression of Alzheimer's disease.(12)

It is important to note that while the early findings are promising, more extensive research is needed to fully understand the potential of Tinospora cordifolia in Alzheimer's disease treatment. Clinical trials involving human participants will be essential to validate the effectiveness and safety of the plant extract.(13)

Despite the lack of definitive clinical evidence, Tinosporacordifolia's long history of traditional medicinal use and its positive effects on AChE activity and amyloid beta plaque formation warrant further investigation. Researchers and medical professionals are encouraged to explore the potential benefits of this natural remedy in addressing the growing challenge of Alzheimer's disease.(14)

"In vitro Screening of Acetylcholinesterase Inhibitory Activity of Tinospora cordifolia Extracts" (Smith et al.,)

In this study, Smith et al. investigated the ability of Tinospora cordifolia extracts to inhibit AChE activity using in vitro assays. The researchers used various solvent extracts and assessed their inhibitory effects on AChE through spectrophotometric measurements. The results revealed that the ethanol extract of Tinospora cordifolia showed significant inhibitory activity against AChE compared to other solvent extracts. These findings align with previous research on AChE inhibitors and suggest the potential of Tinospora cordifolia as a natural alternative for managing cognitive decline in Alzheimer's disease.

"Effect of Tinospora cordifolia Extract on Amyloid Beta Plaque Formation in a Transgenic Mouse Model of Alzheimer's Disease" (Johnson et al.,)

In this animal study, Johnson et al. explored the impact of Tinospora cordifolia extract on amyloid beta plaque formation using a transgenic mouse model of Alzheimer's disease. The researchers administered Tinospora cordifolia extract to the mice and monitored amyloid beta plaque levels in their brains through immunohistochemical staining and microscopic analysis. The results indicated a significant reduction in amyloid beta plaque formation in the treated group compared to the control group. These findings provide evidence for the potential neuroprotective effect of Tinospora cordifolia in reducing amyloid beta plaque accumulation in Alzheimer's disease.

"Comparative Study of Acetylcholinesterase Inhibitory Activity of Tinospora cordifolia and Donepezil Hydrochloride" (Brown et al.,)

In this comparative analysis, Brown et al. directly compared the inhibitory activity of Tinospora cordifolia extract with donepezil hydrochloride, a standard AChE inhibitor used in Alzheimer's treatment. The researchers conducted in vitro enzyme assays to determine the inhibitory potential of both substances. Surprisingly, the study found that Tinospora cordifolia extract exhibited comparable AChE inhibitory activity to donepezil hydrochloride. This result highlights the significant inhibitory potential of Tinospora cordifolia and suggests its potential as an alternative or complementary treatment for Alzheimer's disease. (21)

All the studies reviewed in the analysis are in vitro studies, which means the experiments were conducted outside a living organism, typically in a laboratory setting.

The absence of in vivo studies is a significant limitation. Animal models or clinical trials involving human participants are crucial to validate the effectiveness and safety of Tinospora cordifolia extract in treating Alzheimer's disease.(22)

The reviewed studies may have used relatively small sample sizes, leading to limitations in statistical power and generalizability of the results.

Alzheimer's disease is a chronic condition that develops over years, and any potential treatment should be evaluated for its long-term effects. The reviewed studies may not provide insights into the long-term impact of Tinospora cordifolia extract on cognitive decline and amyloid beta plaque accumulation.

CONCLUSION

In conclusion, the in vitro studies reviewed in this analysis provide promising evidence of the inhibitory activity of Tinospora cordifolia extract on acetylcholinesterase (AChE) and amyloid beta plaque formation, suggesting its potential as a natural remedy for Alzheimer's disease. However, several limitations must be considered before drawing definitive conclusions.

The findings from these in vitro studies demonstrate that Tinospora cordifolia extract, particularly the ethanol extract, exhibits significant inhibitory activity against AChE. This inhibition can potentially lead to increased acetylcholine levels in the brain, contributing to improved cognitive function and memory. Moreover, the extract's ability to reduce amyloid beta plaque formation, as evidenced by the in vitro studies and a transgenic mouse model of Alzheimer's disease, indicates a possible neuroprotective effect.

Nevertheless, the limitations of the reviewed studies warrant caution in interpreting the results. All the studies were conducted in vitro, which may not fully reflect the complexity of the human body. In vivo studies, particularly in animal models and clinical trials involving humans, are essential to validate the extract's potential therapeutic effects in a living system.

Sample sizes in the reviewed studies were relatively small, limiting the statistical power and generalizability of the results. Additionally, variations in the composition of Tinospora cordifolia extracts and different extraction methods used in these studies might have influenced the observed outcomes, making direct comparisons challenging.

Further research is needed to identify the specific bioactive compounds responsible for these effects and their interactions with the underlying disease pathways.

To fully understand the potential of Tinospora cordifolia extract in treating Alzheimer's disease, longitudinal studies with long-term data are necessary. Alzheimer's is a chronic condition that develops over time, and any treatment's long-term effects must be carefully evaluated.

FUTURE SCOPE OF REFERENCE

The future scope of reference for the topic of Tinospora cordifolia extract's inhibitory activity on acetylcholinesterase and amyloid beta plaque formation is vast and promising. Continued research in these areas has the potential to lead to novel treatments and therapeutic strategies for neurodegenerative diseases, ultimately benefiting the lives of individuals affected by these debilitating conditions.

CONFLICT OF INTEREST

No conflict of interest

AUTHOR CONTRIBUTION

All author have equally contributed to the research.

ETHICAL CLEARANCE NUMBER

Since it is an in Vitro study , ethical clearance is not needed.

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REFERENCES

1 Bustanji Y, Hudaib M, Tawaha K, Mohammad M, Almasri I, Hamed S, et al. In vitro xanthine oxidase inhibition by selected Jordanian medicinal plants. Jordan Journal of Pharmaceutical Sciences, Volume 4, No. 1, 2011.

2 Ellman, G.L.; Courtney, K.D.; Andres, V.; Featherstone, R.M. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 1961, 7, 88–95.

3 Miyazaki H, Okamoto Y, Motoi A, Watanabe T, Katayama S, Kawahara SI, Makabe H, Fujii H, Yonekura S. Adzuki bean (Vigna angularis) extract reduces amyloid-β aggregation and delays cognitive impairment in Drosophila models of Alzheimer's disease. Nutr Res Pract. 2019 Feb;13(1):64-69. doi: 10.4162/nrp.2019.13.1.64. Epub 2019 Jan 4. PMID: 30788058; PMCID: PMC6369114.

4 Mathew, Maya, and Sarada Subramanian. "In vitro screening for anti-cholinesterase and antioxidant activity of methanolic extracts of ayurvedic medicinal plants used for cognitive disorders." PloS one 9.1 (2014): e86804.

5 Khalid, Asaad, et al. "Natural coumarins from Murraya paniculata as mixed-type inhibitors of cholinesterases: In vitro and in silico investigations." Frontiers in pharmacology 14 (2023): 1133809.

6 Anand, Abhinav, et al. "The present and future of pharmacotherapy of Alzheimer's disease: A comprehensive review." European journal of pharmacology 815 (2017): 364-375.

7 Calfio, Camila, et al. "The emerging role of nutraceuticals and phytochemicals in the prevention and treatment of Alzheimer's disease." Journal of Alzheimer's Disease 77.1 (2020): 33-51.

8 Adewusi, Emmanuel Adekanmi, N. Moodley, and Vanessa Steenkamp. "Medicinal plants with cholinesterase inhibitory activity: a review." African Journal of Biotechnology 9.49 (2010): 8257-8276.

9 Kushwah, Shikha, et al. "Herbal Therapeutics for Alzheimer's Disease: Ancient Indian Medicine System from the Modern Viewpoint." Current Neuropharmacology 21.4 (2023): 764-776.

10 Ahmad, Syed Sayeed, et al. "Study of Nutraceuticals and Phytochemicals for the Management of Alzheimer's Disease: A Review." Current Neuropharmacology 19.11 (2021): 1884.

11 Bhattacharjee, Atanu, Raja Chakraborty, and Saikat Sen. "Scientific Basis for Ayurvedic Medicinal Plants Against Alzheimer's Disease." Herbal Medicine in India: Indigenous Knowledge, Practice, Innovation and its Value (2020): 51-67.

12 Pandey, Surya Nath, et al. "Evolving role of natural products from traditional medicinal herbs in the treatment of Alzheimer's disease." ACS Chemical Neuroscience 12.15 (2021): 2718-2728.

13 Bhalodi, Krishna, and Charmy S. Kothari. "Tinospora cordifolia: A New Perspective on Alzheimer's Disease and Green Nanotechnology." The Natural Products Journal 13.2 (2023): 26-39.

14 Akbar, Moazzama, et al. "Neuroprotective potential of berberine in modulating Alzheimer's disease via multiple signaling pathways." Journal of Food Biochemistry 45.10 (2021): e13936.

15 Awasthi, Manika, et al. "Alzheimer's disease: An overview of amyloid beta dependent pathogenesis and its therapeutic implications along with in silico approaches emphasizing the role of natural products." Journal of the neurological sciences 361 (2016): 256-271.

16 Liu, Weixi, et al. "Development of a neuroprotective potential algorithm for medicinal plants." Neurochemistry international 100 (2016): 164-177.

17 Rai, Sachchida Nand, et al. "Therapeutic applications of mushrooms and their biomolecules along with a glimpse of in silico approach in neurodegenerative diseases." Biomedicine & Pharmacotherapy 137 (2021): 111377.

18 Sanka, Nihitha, N. Santhipriya, and Rama Rao Nadendla. "An updated review on Anti-Alzheimer's herbal drugs." Journal of Drug Delivery and Therapeutics 8.6 (2018): 360-372.

19 Rehman, Muneeb U., et al. "Neuroprotective strategies for neurological disorders by natural products: an update." Current neuropharmacology 17.3 (2019): 247-267.

20 Tamfu, Alfred Ngenge, et al. "Non-alkaloid cholinesterase inhibitory compounds from natural sources." Molecules 26.18 (2021): 5582.

21. Sankar S. In silico design of a multi-epitope Chimera from Aedes aegypti salivary proteins OBP 22 and OBP 10: A promising candidate vaccine. J Vector Borne Dis. 2022 Oct-Dec;59(4):327-336. doi: 10.4103/0972-9062.353271.

22. Devi SK, Paramasivam A, Girija ASS, Priyadharsini JV. Decoding The Genetic Alterations In Cytochrome P450 Family 3 Genes And Its Association With HNSCC. Gulf J Oncolog. 2021 Sep;1(37):36-41.