In-vitro AND In-silico STUDIES OF AYURVEDIC MEDICINAL PLANTS PIPALI AND JYOTISHMATI FOR AChE INHIBITION: APPROACH FOR TREATMENT OF MEMORY DISORDER

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ABSTRACT
Alzheimer's is the typical type of dementia that affects Senior people and is an irrevocable, progressive neurodegenerative disorder. The characteristic symptoms are loss of memory, behavioral disturbances, mood and personality swing, followed by diminished cognitive performance. There is no permanent remedy for AD and the existing drugs in the treatment of disease have minimal effectiveness. Medicinal plants used Ayurveda have been found more productive source for lead development of drugs, and many advanced herbal products are being evaluated are in the developmental stage in a clinical trial. In fact, several studies have reported the use of various Ayurvedic medicinal plant extracts and their most active chemical constituents for the management of Alzheimer's disease. Although the accurate molecular mechanism is unknown. Pipali and Jyotishmati are extensively used in Ayurveda for various diseases and for the enhancement of memory. An attempt has been made in the current study to acknowledge the activity of extracts of Piper longum and Celastrus paniculatus, and marker compounds like Piperine on acetylcholinesterase inhibition by in-vitro and Rivastigmin as standard. In-silico AChE inhibition studies were carried out on various active constituents like Piperine, Paniculatine, Malkanguinine and Donepezil as standard. Both the enhanced extracts and the standard marker have promising AChE inhibitory action, indicating that they could be used to treat memory problems.

Keywords: Memory Disorder, Acetylcholinesterase Inhibition, Piper longum and Celastrus paniculatus.

INTRODUCTION
Memory disorder like Alzheimer's is characterized by a persistent and irreversible decline in cognitive function caused by senile plaques in the hippocampus of the brain. Alzheimers is the most frequent type of dementia among middle-aged and older adults, affecting more than 6 million Americans and by 2050 it is predicted to reach 14 million. The disease manifests itself after the age of 60, and some early-onset types are tied to a specific genetic flaw. Although the cause is uncertain, genetic factors are thought to have a role in 10% to 15% of instances. As per NIA, Alzheimer's disease (AD) is characterized by a dynamic, irrevocable decline in memory and difficulty in doing routine activities, as well as an imbalance in space orientation and time management, muddled language confused communication, narrow thinking, and failure to perform simple mathematics. Sudden swings in personality and mood is common, as well as behavioral and psychiatric disorders, are all signs. It's easy to mistake cognitive changes in Alzheimer's disease for "normal" aging, but they're actually the result of disease progression. Dementia, a common name for a group of diseases marked by a decline in cognitive function that disrupts daily routine activities and social relationships, is one of the most significant characteristics of AD. Dementia is defined by American Psychiatric Association as a progressive decline in mental skills that makes it difficult to succeed socially and professionally, whereas reversible dementia is caused by certain drugs,
lack of vitamin, or by certain bacterial or viral infections. Irreversible dementia owing to the progression of neurodegeneration.⁷

**Statistical Facts of Alzheimer's Disease**
AD was considered a rare disorder before, but now it is considered a major menace to people that strike seriously to the elderly individuals that must be considered as a challenge and need attention. AD is caused considerable damage to the neuronal system of the brain which leads to disturbances between the neurons of the brain.⁸ People with Alzheimer's disease increase approximately twofold every five years of age, resulting in about one percent of 60 years and thirty percent of 85 years old having the condition. World health organization has expected that about 44 million people are currently struggling with dementia worldwide, by 2030 will be reaching 66 million and around 116 million by the end of 2050.⁹ It has been observed that Alzheimer’s disease has around seven stages and can be identified by various symptoms. In the first stage, a person is having regular behavior and normal life. In 2nd stage appearance of memory, collapse leads to confusion and forgetfulness. In the third stage, the level of confusions increases, difficulty in recalling names of individuals increases, and is referred to as marginal disorder and is not all the time lead to Alzheimer's disease. In 4th stage, the individual is incapable to think properly which is referred to as “mild” AD. The 5th stage is referred to as "moderate" Alzheimer's, in this stage individual faces trouble recollecting the names of his own members of the family and a confused state in the mind, restlessness, anxiety is very common in this stage. In the sixth stage, "moderately severe" Alzheimers, the individual fails to do regular routine activities and need care from a family member. Speech and coordination problems are seen in the seventh stage. Death occurs in the final stages of Alzheimer's disease as a result of a variety of injuries, accidents, and hospitalisation.¹⁰,¹¹

**Acetylcholinesterase Inhibition**
AChEIs are currently used medications to manage mild to moderate Alzheimer's disease. AChEIs decrease the progress of Alzheimer's disease by enhancing acetylcholine availability in the synaptic cleft of the neuron and thus help normal neuronal function and increase brain performance. It works by inhibiting the enzyme acetylcholinesterase, which hydrolyzes acetylcholine and improves neuron transmission. Figure-1 depicts the cholinesterase inhibitor's schematic mechanism.

**Herbs having Acetylcholinesterase Inhibition Action**
*Piper longum* is also referred to as the Indian long pepper family piperaceae. In Ayurveda it is popularly called pippali. Long pepper is one of the important rejuvenator drugs (Rasayana) widely used in various ailments like respiratory disorders. Studies suggest that it enhances the bioavailability of other drugs
when administered together because of piperine. In many herbal mixtures used for memory-related disorders, long pepper is also one of the content because of its memory-enhancing properties. Studies suggest that the fruit extracts possess antioxidant and neuroprotective action by inhibiting acetylcholinesterase enzyme. 12,13

**Celastrus paniculatus**

Jyotishmati is an Ayurvedic treasured herb that is admired for its action on the memory for enhancing memory, concentration and preserving cognitive function. Aqueous extracts of seeds of *C. paniculatus* have cognitive-enhancing properties and free radical scavenging properties. The study reveals that *Celastrus paniculatus* extract protects neurons against H2O2-induced toxicity and glutamate-induced toxicity and possesses neuroprotective action. The hydroalcoholic extracts of *C. paniculatus* seed have acetylcholinesterase inhibitory activity and increase the Ach in the cortex, thereby improving memory performance in aged people. The study also reveals that *Celastrus paniculatus* seed oil is also having memory-enhancing activity and can be a potential natural medicine to overcome symptoms of Alzheimer’s disease. 14,15

**EXPERIMENTAL**

*Piper longum* and *Celastrus paniculatus* were acquired from the local market for this project. Taxonomists identified and verified the candidate plants. Herbarium specimens with voucher numbers PP575A and PP625 have been prepared and deposited in the herbarium museum of the institution.

Extraction: The authenticated plant material was properly washed and shade dried for about 14 to 15 days. Dried material of plant is then pulverized to a coarse powder and sieved with no. 22. By using a reflux condenser, the powdered drugs were extracted with hydroalcohol (80:20) followed by filtration and concentrated in a vacuum evaporator oven and then freeze-dried to powdery mass.

Marker chemical compound: Piperine was obtained from Natural Remedies Pvt. Ltd. in Bengaluru for the study.

**In-vitro AChEIs Assay**

The Amplex Red Assay Kit (A12217) was utilized for research which provides a sensitive approach for screening Acetylcholinesterase inhibition action using a fluorimetric reader. The Amplex Red reagent is a highly sensitive fluorogenic probe for H2O2 and is used to indirectly detect AChE activity in this experiment. AChE’s primary function is to convert Ach to choline. Choline oxidase then oxidizes the converted choline to produce betaine and hydrogen peroxide and will interact with 10-acetyl-3, 7-dihydroxyphenoxazine in a 1:1 stoichiometry in the presence of horseradish peroxidase to liberate the resorufin which is fluorescent in nature. The absorption & fluorescence emission maxima of Resorufin are approximately about 571 and 585 nm, respectively. 16,17

**In-silico Molecular Docking Study**

It is a useful method for filtering and screening drug molecules throughout drug discovery. It is conceivable to locate a molecule with specific properties that could be a potential lead in the creation of an effective therapeutic agent throughout the protracted quest for novel drug discovery. When a ligand or molecule interacts with a target such as enzymes or receptors, the preferred orientations of the drug molecule or ligand to create a stable complex that shows therapeutic action is predicted by molecular docking studies. The binding free energy of the ligand molecule and the protein complex of the receptor/enzyme is a critical thermodynamic parameter in the process since it verifies the predicted interactions between both the ligand and the enzyme or receptor's complex protein. The primary purpose of the study was to find the molecules that inhibit AChE using molecular modeling methods. 18,19

All of the molecular simulations were done using the Maestro interface on the Schrodinger molecular modeling software. The simulations were done on an HP computer system using a Pentium 4 processor.

The steps involved in molecular docking are as follows:

**Preparation of Protein**

Acetylcholinesterase Donepezil-bound x-ray crystal structure (protein databank ID: 4BTL) was retrieved with a 2A° resolution from PDB. Enzyme is refined by the protein preparation wizard, which has two
prime steps: first is preparation and second is refinement. The algorithm caps terminals add lacking hydrogen and add lacking residue, it’s in charge of assigning bond orders. For energy minimization, the OPLS force field was used. H2O molecules were eliminated from the area around the active site. Disulphide bridges have been built. Prime was used to fill up gaps in the chain and loops beyond the 5Å unit.

**Ligand Preparation**

The Ligprep tool, which comes with Schrodinger, was used to prepare the ligands. The force field used was OPLS 2005. The ionization states were defined using the EPIK application. Desalting was also carried out in order to identify potential tautomers. The customized chirality’s were established for ligand and processed to obtain low-energy ring conformations.

**Alignment of Ligand**

One of the criteria for better docking is the alignment of the ligand molecule. The designed ligands were properly aligned with the help of flexible ligand alignment.

**Glide Docking**

In the first step of the docking process, the receptor grid has been established. The next stage was actually docking analogs to the receptor's active site. The Schrodinger's glide TM software is used in the ligand docking procedure. Glide TM detects the most important interactions between one or more chemical substances and a receptor, which is usually a protein. Both rigid and flexible modes have been used with GlideTM. The receptor's generated grid was created using the grid generating panel in the glide utility. Using a grid-based technique, the binding pockets electrostatic and Van der Waals potentials were estimated. In the second stage, analogues were actually docked on the binding site. This was accomplished using the XP docking method option. The XP gliding approach ranks ligands' ability to bind to the bioactive conformation of protein receptors in a semi-quantitative manner. 20-22

**RESULTS AND DISCUSSION**

**In-vitro Acetylcholinesterase Inhibition Assay**

Amplex Assay Kit was used to conducting an in-vitro AChE inhibition assay on hydroalcoholic extracts of *Piper longum* and *Celastrus paniculatus*. Piperine was employed as a marker chemical compound. The Rivastigmin was used as a standard drug and results are compared with the extracts and a marker chemical compound. Table-1 summarizes the findings.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>IC50 Value</th>
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<tbody>
<tr>
<td><em>Piper longum</em> extract</td>
<td>43.06 µg</td>
</tr>
<tr>
<td><em>Piperine</em></td>
<td>46.65 µg</td>
</tr>
<tr>
<td><em>Celastrus paniculatus</em></td>
<td>111.42 µg</td>
</tr>
<tr>
<td>Rivastigmin (Standard)</td>
<td>10.32 µM</td>
</tr>
</tbody>
</table>

**In-silico Molecular Docking Studies for AChE inhibition**

**Crystal Ligand**

It binds to the AChE target protein's predicted active site. A crystal ligand that slips into AChE's active site's hydrophobic binding cavity. The interaction of the quaternary amino group has Cation-π interaction with tyrosine 337. There are two Cyclohexane rings that shows π-π interaction with tyrosine 341 and tryptophan 286. The nitrate group interacted with phenylalanine 295 as a hydrogen bond donor. The crystal ligand 2D interactions and crystal ligand 3D interactions Fig.-2 and Fig.-3.

**Piperine**

At the predicted location of acetylcholinesterase, the piperine demonstrated significant ligand binding interactions. The phenyl ring of benzofuran showed cation-π interaction with histidine 447 and carbonyl
carbon H-bond acceptor interaction with phenylalanine 295 with a docking score of -8.727. The Piperine 2D interactions and Piperine 3D interactions Fig.-4 and Fig.-5.
**Paniculatine**

At the predicted location of acetycholinesterase, paniculatine revealed strong ligand binding interactions. Quaternary ammonium group has π-cation interaction with Tyrosine 341. Hydroxyl group has H-bond interaction with Histidine 447 with a –8.998 dock score. The paniculatine 2D and 3D interaction Fig.-6 and Fig.-7.

**Fig.-5: 3D Interactions of Piperine**

**Fig.-6: 2D Interactions of Paniculatine**

**Fig.-7: 3D Interactions of Paniculatine**
Malkanguinine
At the predicted location of acetylcholinesterase, malkanguinine revealed strong ligand binding interactions. Phenyl ring cation-π interaction with tyrosine 341 and hydrogen bond donor interaction with tyrosine 124 with a -10.239 dock score. The malkanguinine 2D interactions and malkanguinine 3D interactions Fig.-8 and Fig.-9.

Donepezil
At the putative location of acetylcholinesterase, donepezil revealed strong ligand binding interactions. Tryptophan 286 shows π-cation interaction, Phenylalanine 295 interacts with carbonyl carbon and has hydrogen bond acceptor interaction, cation-π interaction of amino group with tyrosine 341 and phenyl ring π-π interaction with tyrosine 124 with a –12.947 dock score. The donepezil 2D interactions and donepezil 3D interactions Fig.-10. and Fig.-11. The results of all in-silico studies are expressed in docking scores are given in Table-2.

The pathology of AD is very complex and it is believed that the brain shrinks which affects the signal process. Cholinergic function in the cortex of the brain is disrupted and symptoms of memory loss are initiated. The IC50 value was established after extracts and markers were screened for in-vitro acetylcholinesterase inhibition. Piper longum extract shows 43.06µg, Celastrus paniculatus extract shows 111.42µg, piperine shows 46.65 µg whereas Rivastigmin shows 10.32 µM. When comparing the IC50 values of standard, Piper longum and Celastrus paniculatus extracts show significant AChE inhibition. In addition, research attempts have been made to establish molecular docking studies on markers of the
candidate plants for acetylcholinesterase inhibition using Schrodinger molecular docking software. The
dock scores were compared with Donepezil standard. Piperine, Paniculatine and Malkanguinine show -
8.727, -8.998 and -10.239 respectively whereas standard Donepezil shows -12.947.

![Fig.-10: 2D Interactions of Donepezil](image)

![Fig.-11: 3D Interactions of Donepezil](image)

<table>
<thead>
<tr>
<th>Standard Marker</th>
<th>Docking Scores</th>
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<tbody>
<tr>
<td>Piperine</td>
<td>- 8.727</td>
</tr>
<tr>
<td>Paniculatine</td>
<td>- 8.998</td>
</tr>
<tr>
<td>Malkanguinine</td>
<td>-10.239</td>
</tr>
<tr>
<td>Donepezil (Standard)</td>
<td>- 12.947</td>
</tr>
</tbody>
</table>

**CONCLUSION**

Alzheimer's is a degenerative disease that affects memory and other cognitive skills. Memory and other
critical mental functions are gradually lost as the brain cell network and the cells themselves deteriorate
and die. The primary signs of Alzheimer's disease are memory loss and confusion. Although there is no
cure for AD, drugs and management measures may help to overcome symptoms temporarily. The
currently available medications inhibit AChE, which is the most common drug since it reduces
cholinergic deficits and improves mental performance by improving neurotransmitter availability in the
brain. The primary goal of this research was to learn more about the effects of traditional herbal
medicines like *Piper longum* and *Celastrus paniculatus*. Both medications show strong inhibitory action
on acetylcholinesterase and can be considered as a drug for the management of symptoms of memory disorders like Alzheimer's and another type of dementia.

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**REFERENCES**


