

## DRUG DISCOVERY FOR ACETYLCHOLINESTERASE

Ms. NIKHITHA SELVARAJ

Department of  
Artificial Intelligence and  
Machine Learning  
Sri Shakthi Institute of  
Engineering and Technology

Ms. NANDHANA S

Department of  
Artificial Intelligence and  
Machine Learning  
Sri Shakthi Institute of  
Engineering and Technology,

Mrs. S. Hemalatha

Head of the Department  
Artificial Intelligence and  
Machine Learning  
Sri Shakthi Institute of  
Engineering and Technology

**Abstract:** Acetylcholinesterase (AChE), the predominant cholinesterase in the brain, hydrolyzes ACh to choline and acetate, thereby terminating the effect of this neurotransmitter at cholinergic synapses. Therefore, AChE is the target of cholinesterase inhibitors used for addressing the cholinergic deficit in Alzheimer's disease (AD) patients. Despite decades of research and advances in our understanding of its aetiology and pathogenesis, current pharmacotherapeutic options for AD are still very limited and represent an area of need that is currently unmet. The leading AD therapeutics involves AChE inhibitors, resulting in increased acetylcholine concentrations in the synaptic cleft and enhanced cholinergic transmission. Compounds showing an AChE inhibitory effect are also used for the treatment of senile dementia, myasthenia gravis, Parkinson's disease and ataxia. Taking into account that the inhibition of AChE has been one of the most used strategies for treating AD and that existing drugs are effective only against mild to moderate type of disease while presenting considerable side effects, the search for new sources of effective and selective anti acetylcholinesterase agents with fewer side effects is imperative. Various plants and phytochemical substances have demonstrated AChE inhibitory activity and thus could be beneficial in the treatment of neurodegenerative disorders such as AD.

**Keywords:** Alzheimer's disease (AD); Acetylcholine (ACh); Acetylcholinesterase (AChE); Medicinal Plants.

### Introduction

Inhibition of Cholinesterases, mainly Acetylcholinesterase (AChE) and therefore prevention of acetylcholine degradation in synapses of cholinergic system is one of the most accepted palliative therapy opportunities for Alzheimer's disease (AD) today [1]. Since the introduction of the first cholinesterase inhibitor in 1997, most clinicians would consider the cholinergic drugs, donepezil, rivastigmine [2], and galantamine [3], to be the first line pharmacotherapy for mild and moderate AD. The most that these drugs could achieve is to modify the manifestations of AD. Due to a lack of selectivity of cholinesterase inhibitor drugs on the market, AD-patients suffer from side effects like nausea or vomiting.

The enzyme acetylcholinesterase (AChE) catalyses the hydrolysis of the ester bond of acetylcholine (ACh) to terminate the impulse transmitted action of ACh through cholinergic synapses [4]. Although the basic reason of Alzheimer's disease (AD) is not clear so far, AD is firmly associated with impairment in cholinergic transmission. A number of AChE inhibitors have been considered as candidates for the symptomatic treatment of AD as the most useful relieving strategy [5].

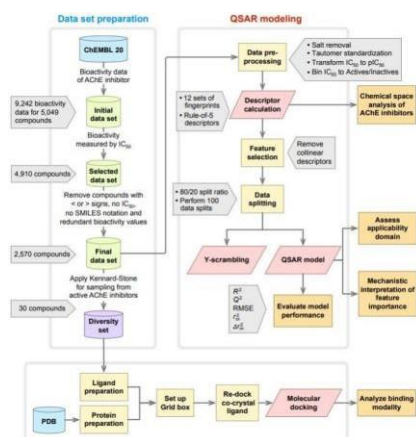
Principal role of acetylcholinesterase (AChE) is the termination of nerve impulse transmission at the cholinergic synapses by rapid hydrolysis of acetylcholine (ACh). Inhibition of AChE serves as a strategy for the treatment of Alzheimer's disease

(AD), senile dementia, ataxia, myasthenia gravis and Parkinson's disease [6,7,8]. There are a few synthetic medicines, e.g. tacrine, donepezil, and the natural product based rivastigmine for treatment of cognitive dysfunction and memory loss associated with AD [9]. These compounds have been reported to have their adverse effects including gastrointestinal disturbances and problems associated with bioavailability [10,11], which necessitates the interest in finding better AChE inhibitors from natural resources.

**Acetylcholinesterase inhibitors from Plants** Several reviews on the newly discovered AChE inhibitors obtained from plants, fungus and marine organisms have also been published over the last years [12-15]. The majority of these AChE inhibitors belong to the alkaloid group, including indole, isoquinoline, quinolizidine, piperidine and steroidal alkaloids. On the other hand, several non-alkaloidal and potent AChE inhibitors have been obtained from natural sources, including terpenoids, flavonoids and other phenolic compounds. Interestingly, although literature demonstrates to be rich in the study on AChE inhibitors obtained from plants, this issue keeps on being the centre of attention for research as confirmed by the increasing number of studies published every year.

Methanolic extracts of seven herbs *Acorus calamus*, *Acorus gramineus*, *Bupleurum falcatum*, *Dioscorea batatas*, *Epimedium koreanum*, *Poria cocos* and *Zizyphi jujuba*,

used in traditional Korean medicine for improvement of memory and cognition in old age have been tested for cholinesterase inhibitory properties and significant inhibition of the enzyme was shown by extracts from *Acorus calamus* and *E. koreanum* [9,16,17] screened the methanolic extracts of 32 plants used in Thai traditional rejuvenating and neurotonic remedies, for inhibitory activity on AChE and found that the extracts from roots of *Stephania suberosa* and *Tabernaemontana divaricata* showed significant inhibitory activity.



Aqueous and methanol extracts of 11 plants, used in Danish folk medicine for improvement of memory and cognition, and three *Corydalis* species have been tested for their AChE inhibitory activity [18]. The authors reported significant dose-dependent inhibitory activity for extracts of the *Corydalis* species whereas only moderate inhibition of the enzyme was observed for extracts of *Ruta graveolens* L., *Lavandula angustifolia* Miller, *Rosmarinus officinalis* L., *Petroselinum crispum* (Mil.) Nym. ex A. W. Hill., and *Mentha spicata* L. The latter five species contain essential oils with terpenes, a group of compounds reported to have AChE inhibitory activity [19,18].

The history of drug discovery has shown that plants contain active compounds that have become new sources to investigate for the pharmaceutical industry. Plant constituents may not only act synergistically with other constituents from the same plant but may also enhance the activity of compounds or counteract toxic effects of compounds from other plant species [20]. In traditional practices, numerous plants have been used to treat cognitive disorders, including neurodegenerative diseases and different neuropharmacological disorders [21]. Yokukansan, a Chinese herbal remedy which is used to treat various neurological states has been reported as being effective with no adverse effects [22]. Also, galanthamine, an alkaloid from snowdrop, has been approved by the Food and Drug Administration in the United States for use in the treatment of Alzheimer's disease [17,23]. Since AD has become a public health burden, and the commonly available

synthetic drugs have undesirable side-effects, new treatment strategies based on medicinal plants have been the subject of current focus.

Black pepper (*Piper nigrum*), is one of the oldest and most popular spices in the world. It belongs to the family Piperaceae and is used in many Asian countries. It is used in folk medicine for stomach disorders, digestive problems, and neuralgia and as CNS depressant [24]. Its active constituent, piperine, has been reported to significantly improve memory impairment and neurodegeneration in hippocampus. Moreover, piperine also demonstrated the neurotrophic effect in hippocampus [25]. Cumin (*Cuminum cyminum*) is an

aromatic plant, belongs to Apiaceae family. It is used to flavour foods and used in many medicinal preparations. It has been used as an astringent, as carminative and eupeptic as well as an analgesic agent [26]. Cumin extract has also been known to possess antistress, antioxidant and memory-enhancing activity [27].

Sedge or *Cyperus rotundus* belongs to the family Cyperaceae. The rhizome of *Cyperus* is rich in essential oils that contain pinene, a little cineole, terpenes, and a new alcohol called isociprol. Several chemical components have been isolated from ethanol extract of *C. rotundus* rhizome and some of these chemicals possess anti-AChE activity [28]. *Ginkgo biloba* extract significantly inhibit the AChE activity in the brain. The inhibition of AChE activity can be correlated with improvement observed in scopolamine-induced deficits in passive avoidance by *G. biloba* extract. The decrease in AChE activity indicates an increase in the basal level of acetylcholine [29].

*Morinda citrifolia* (*M. citrifolia*) fruit and extract have analgesic, anti-inflammatory and antioxidant properties [30]. Ethyl acetate extract of *M. citrifolia* prevented memory deficit and oxidative stress induced by amyloid beta peptide in mice [31]. Treatment with chloroform, ethyl acetate and butanol extracts of *M. citrifolia* in a dose-dependent manner reduced AChE enzyme activity in the brain of scopolamine-treated mice. In addition, scopolamine-induced amnesia is associated with decreased cerebral blood flow and increased oxidative stress and AChE activity in rat brain, and treatment with the *M. citrifolia* extract improved scopolamine induced memory impairment in the shuttle box test [30].

*Desmodium gangeticum* DC belongs to family Leguminosae. Extract of the root and leaves of *D. gangeticum* inhibited acetyl-cholinesterase enzyme activity in mid brain, cerebellum, medulla and cortex of mice brain [32]. *Desmodium gangeticum*, commonly known as "Salpan", "Salpani" in Hindi and "Shalparni" in Sanskrit, is used in Ayurveda, Siddha and Unani systems of medicine. It is a bitter tonic, febrifuge, digestive, anticatarrhal, antiemetic

also used in inflammatory conditions of chest and in various other inflammatory conditions which are due to “vata” disorder. Its roots have been used as expectorant and in snake bite and scorpion sting. It is an ingredient of Ayurvedic preparations like “Dashmoolarishta” and “Dashmoolakwaath” recommended for post-natal care to avoid secondary complications [33]. *Embelia ribes* Burm belongs to family Myrsinaceae also inhibit acetylcholinesterase. *Azadirachta indica* belongs to family Meliaceae, aqueous extract from bark of *A. indica* inhibit the AChE activity by *in vitro* methods [34]. *Ficus racemosa* belongs to family Moraceae; cold

used for constructing the models (Wright & Ziegler,

#### VALIDATION OF QSAR MODELS Model

validation is an important process, which should be performed to ensure that a fitted model can accurately predict responses for future or unknown subjects. Two statistical parameters were used to evaluate the performance of the QSAR models consisting of Pearson’s correlation coefficient (r) and root mean squared error (RMSE). The r value is a commonly used metric to represent the degree of relationship between two variables of interest. It can range from -1 to +1 in which negative values are indicative of negative correlation between two variables and vice versa. RMSE is a commonly used parameter to assess the relative error of the predictive model. The predictive performance of the QSAR models was verified by 10-fold cross validation, external validation and Y-scrambling test. The 10-fold cross-validation technique does not use the entire data set to build predictive model. Instead, it splits the data into training and testing data set by allowing model that is built with training data set us allow to assess the performance of the model on the testing data set. By performing repeats of the 10-fold validation, the average accuracies can be used to truly assess the performance of the predictive model. Y-scrambling test was used to ensure the robustness of the predictive model

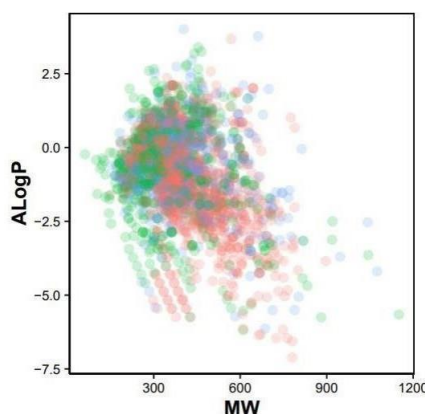
#### PROCESSING

**Feature selection:** Collinearity is a condition where descriptor pairs are known to have intercorrelation, which not only add complexity to the model but could potentially give rise to bias. To remedy this, the cor function from the R package stats was used to find the pairwise correlation among descriptors, and descriptors in a pair with a Pearson’s correlation coefficient greater than the threshold of 0.7 was filtered out using the find Correlation function from the R package caret to obtain a smaller subset of descriptors (Kuhn, 2008).

**Data splitting:** To avoid the possibility of bias that may arise from a single data split when building predictive models (Puzyn et al., 2011), predictive models were constructed from 100 independent data splits and the mean and standard deviation values of statistical parameters were reported. The data set was split into internal and external sets in which the former comprises

#### MULTIVARIATE ANALYSIS

Supervised learning is to learn a model from labeled training data which can be used to make prediction about unseen or future data (James et al., 2013). This study constructs regression models, which affords the prediction of the continuous response variable (i.e., pIC50) as a function of predictors (i.e., fingerprint descriptors). Random forest (RF) is an ensemble classifier that is composed of several decision trees (Breiman, 2001). Briefly, the main idea behind RF is that instead of building a deep decision tree with an ever-growing number of nodes, which may be at risk for overfitting and overtraining of the data, rather multiple trees are generated as to minimize the variance instead of maximizing the accuracy. As such, the results will be more noisier when compared to a well- trained decision tree, yet these results are usually reliable and robust. The ranger function from the R package ranger, which is a fast implementation of the RF algorithm that was



and hot aqueous extracts of *Ficus racemosa* stem bark showed AChE inhibitory activity against rat brain acetylcholinesterase *in vitro* [35].

*Cinnamomum zeylanicum* has been used as a spice as well as traditional medicine for many centuries. It possesses many unique medicinal properties such as sugar control, anti-oxidant, anti-inflammatory and antimicrobial activity [36]. Cinnamon extract has been found to have an inhibitory effect on tau aggregation related to Alzheimer's disease (AD). The extract can also promote complete disassembly of recombinant tau filaments and cause substantial alteration of the morphology of paired-helical filaments isolated from AD brain [37]. In a recent study, orally administered cinnamon extract has been found to reduce  $\beta$ -amyloid oligomerization and correct cognitive impairment in AD animal models [38]. *Eugenia caryophyllus* (clove), belonging to the family Myrtaceae, has a number of medicinal properties. Clove has been reported to possess a potent anti-oxidant activity *in vitro*, which reduces the oxidative stress in the body [39]. Clove oil has been reported to reverse learning and memory deficits in scopolamine treated mice [40]. The aqueous extract of *Eugenia caryophyllus* has also been found to possess AChE inhibitory activity in rats. It reduces the hydrolysis of ACh by AChE [41].

The chief chemical constituent of the *Galanthus nivalis* L. (common snowdrop) is Galanthamine, and this is an isoquinoline alkaloid. Galanthamine has been found to be the long-acting and specific inhibitor of the AChE enzyme and to potentiate cholinergic nicotinic neurotransmission by allosterically modulating the nicotinic acetylcholine receptors, which may be of additional value in the treatment of AD [42,43]. *Huperzia serrata* (Thunb. ex Murray) is one of the genera in the Huperziaceae family (syn. Lycopodiaceae family). This genus, has been used for its memory-enhancing effect since ages in the Traditional Chinese Medicinal system (TCM), and is known to contain a large group of alkaloids called 'Lycopodium alkaloids'. Huperzine A, a novel Lycopodium alkaloid extracted from *Huperzia serrata*, is well known as a reversible, potent, and selective AChE inhibitor. It is also known as 'Qian Ceng Ta' in China, and Huperzine A has been used as a therapeutic agent for AD from centuries [44]. *Tinospora Cordifolia* (Guduchi) possesses a memory enhancing property for learning and memory in normal and memory-deficient animals. *Tinospora Cordifolia*'s mechanism for cognitive enhancement is by immunostimulation and synthesis of acetylcholine, this supplementation of choline enhances the cognitive function [45].

### Conclusion

Acetylcholine is a neurotransmitter that plays a key role in cognitive function and reasoning. The brains of those with mild-to-moderate Alzheimer's disease,

a progressive type of dementia, have abnormally low acetylcholine concentrations. This means that any compound that enhances the cholinergic system in the brain may be useful in treating Alzheimer's disease and similar brain malfunctions.

Herbs may play a promising role in the early treatment of Alzheimer's and other conditions involving poor memory and dementia. One of the chief benefits is that they have a low toxicity compared to pharmaceutical agents. A review of the literature indicates that the sooner the treatment is started; the better will be the outcome. Therefore, if clients have family members with a history of Alzheimer's disease, or other states involving poor memory, they may start taking these remedies prior to the onset of symptoms, to delay or possibly prevent the advent of the symptoms. The use of herbal medicines in the treatment of AD should be compared with the pharmacological treatment currently in use. Such studies should include identification of the active principle in order to improve the validation of the clinical trial. Further large-scale, multicenter studies are necessary to determine the effectiveness of these substances in the cognitive deterioration of AD. Until then, this review provides some evidence of the benefit of a wide range of herbs (included in the Indian Medicine System, Chinese Medicine System, European Medicine System, etc.) in the treatment of AD.

### Acknowledgments

The Authors are thankful to University Grants Commission, New Delhi for grant as Major Research Project. File No-43-114/2014(SR).

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