Acetylcholinesterase Inhibitors from Natural Resources

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**Summary**: Acetylcholinesterase inhibitors prevent reduction of acetylcholine via inhibiting acetylcholinesterase enzyme which hydrolyzes acetylcholine in the neuronal end from which it is released. Acetylcholinesterase inhibitors play an important role in the treatment of Alzheimer’s Disease as well as Myasthenia Gravis, Glaucoma and Helminthiasis together with the mechanism of action of insecticide drugs. In this review, some compounds obtained from natural resources that have acetylcholinesterase inhibitory activity are evaluated.

**Key Words**: Acetylcholinesterase, Alzheimer’s Disease, acetylcholinesterase inhibitory activity, plant.

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INTRODUCTION

Many new natural product-originated bioactive compounds effective in treating several diseases have been isolated from different plants, fungi and microorganisms. They are unknown complex mixtures having potentially large number of secondary metabolites. Sensitive assays have been developed to screen these extracts from natural sources. The simplest assays are the ones based on the mechanism of action of a known drug. The assays have also been incorporated into efficient testing schemes that are useful for high-throughput screening (HTS). For example; one assay used for Alzheimer’s Disease (AD) is based on the inhibition of acetylcholinesterase (AChE). The development of new leads of AChE inhibitors has been realized by the Ellman method for screening biological sources. AD is one of the most common mental problems in the aged population1-3. The basal forebrain and brainstem cholinergic systems also play an important role in the regulation of cortical and thalamic electrical activity4. The findings from experimental animals, aging and AD research have provided an experimental foundation for the cholinergic hypothesis of learning and memory5-7. Based on the cholinergic hypothesis, AD results from a defect in the cholinergic system. One goal in the treatment for AD is to increase the acetylcholine level in the brain. Therefore, AChE inhibitors are being developed for the treatment of this disease.

Because of the side effects of the present drugs, recently, galanthamine isolated from Amaryllidaceae

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plants has been approved by the FDA for the treatment of AD. However, the research for new AChE inhibitors is still of interest and natural products are an important source of these compounds.

Based on the documented memory enhancing and antiaging activities in folk medicine, the following plants and isolated compounds have been chronologically described as potential leads for the development of new drugs for the treatment of AD.

### Acetylcholinesterase inhibitors from plants

**Physostigma venenosum**

Physostigmine (1), the first discovered-compound within this class of compounds, is an alkaloid isolated from *Physostigma venenosum* L. (Fabaceae). Later on, physostigmine (Synapton®,) has been a model for some drugs with acetylcholinesterase activity such as rivastigmine (Exelon®,) which was synthesized later. Although, the results of the first clinical trials with physostigmine were promising, short action duration and cholinergic side effects of physostigmine have limited its therapeutic use.

![Chemical structure of Physostigmine](image)

**Lycopodium species**

Huperzin A (2) has a special significance among the compounds with acetylcholinesterase inhibitory activity, isolated from natural resources. Huperzin A, [(5R, 9R, 11E)-5-amino-11-etilidin-5,6,9,10-tetrahidro-7-metil-5,9-metanosikloocta-[b]-piridin-2 (1H)-on], is an alkaloid isolated in 1986 by researchers of the Shanghai Institute of Materia Medica from the clubmoss *Lycopodium serratum* Thunb. (syn. *Huperzia serrata* (Thunb.) Trev) (Lycopodiaceae). This plant, called as “Qing Ceng Ta”, has been used in traditional Chinese medicine for its memory-enhancing property for centuries.

Over 100 alkaloids, a number of which are of the series of huperzin A-R, have been isolated from the genus *Lycopodium* which is very rich in alkaloid content. Of them, only huperzin A possessed remarkable acetylcholinesterase inhibitory activity.

![Chemical structure of Huperzin A](image)

The activity of Huperzin A has been found to be as high as physostigmine, galanthamine, donepezil and tacrine, the commercial drugs already used against AD, or (even greater) than. In various in vivo and ex vivo experiments, it has been shown to inhibit acetylcholinesterase reversibly and also to prevent oxidative cell damage induced by β-amiloid plaques.

In Ainge et al.’s work, Huperzin A isolated from *L. varium* showed a potent insecticidal activity against the insects *Anthrenocerus australis*, *Lucilia cuprina* and *Tineda bisselliella*. Its total synthesis was completed and now this compound is in the stage-III clinical trial in China.

Related to this subject, in our ongoing research on investigation of acetylcholinesterase inhibitory activity of some plants growing in Turkey, we screened five *Lycopodium* species (*L. annotinum*, *L. alpinum*, *L. clavatum*, *L. complanatum* subsp. *chamaecyparissus* and *L. selago*) of the Lycopodiaceae family regarding their acetylcholinesterase inhibitory activity.
using the Ellman method, which is a spectrophotometric, *in vitro* robotic screening method, and determined the responsible compound for the activity as α-onocerin (3), a triterpene-type compound, from *Lycopodium clavatum* that showed ca. 50 % activity.

![Diagram](image1)

### Corydalis ternata

In a screening study by Kim *et al.*, the methanolic extract prepared from tubers of *Corydalis ternata* (Papaveraceae) was found to have potent inhibitory activity by the Ellman method. Bioactivity-directed fractionation of this extract afforded protopine (6), an alkaloid-type compound, by the Ellman method. This result was supported by passive avoidance test, which is used to measure antiamnesic activity, in male mice.

![Diagram](image2)

### Salvia species

Perry *et al.* studied the acetylcholinesterase inhibitory activity of essential oils of *Salvia lavandulaefolia* and *S. officinalis* (Lamiaceae), the plants knowns to be used as memory-enhancing in European folk medicine, and the monoterpenes called (+) - α - PINEN, α - and β - terpineol, citronellal, δ - terpinen, R - (+) - limonen, 1,8-cineol, 1R-(+)-camphor, linalol, 1S-(-)-β-pinene and geranial, the constituents of these essential oils analyzed by GC-MS, were tested on human erythrocyte acetylcholinesterase by the Ellman method. As a result; the essential oils of *S. lavandulaefolia* and *S. officinalis* as well as camphor, 1,8-cineol, and α-pinene inhibited the enzyme in a dose-dependent manner. When compared to the standard drugs physostigmine and tacrine, the most active monoterpenes were 1,8-cineol (4) (IC₅₀= 0.67 mM) and α-pinene (5) (IC₅₀= 0.63 mM).

![Diagram](image3)

### Evodia rutaecarpa

In another screening study performed in South Korea, Park *et al.* investigated 87 extracts prepared from 29 plants in total by the Ellman method with regard to anticholinesterase activity and found that 9 of the extracts showed over 40 % inhibitory activity. These extracts and their inhibition rates are as follows: *Poncirus trifoliata* (dichloromethane extract, 91.0 %), *Evodia rutaecarpa* (dichloromethane extract, 84.3 %), *Coptis chinensis* (methanol extract, 83.3 %), *Coptis chinensis* (dichloromethane extract, 76.9 %), *Saussurea lappa* (dichloromethane extract, 70.5 %), *Angelica sinensis* (dichloromethane extract, 65.5 %), *Notopterygium incisium* (dichloromethane extract, 50.3 %), *Evodia rutaecarpa* (methanol extract, 43.8 %), *Polygala tenuifolia* (dichloromethane extract, 40.0 %).

Among them, the dichloromethane extract of *Evodia rutaecarpa* displayed inhibitory activity in the passive avoidance test in rats (Sprague-Dawley) with scopolamine-induced memory loss, and
dehydroevodiamine HCl (7) was isolated as the active component through bioactivity-directed fractionation.\(^{25,26}\)

**Buxus species**

*Buxus* species are well-known for their triterpene alkaloids having a great variety of biological activities. In a phytochemical work carried out on *Buxus hyrcana* (Buxaceae), three alkaloids, two of which were novel, were isolated and their acetylcholinesterase inhibitory activity was determined by the Ellman method. While hyrcanine, one of the novel alkaloids, was inactive against the enzyme, (+)-homo-moenjodaramine (8) and (+)-moenjodaramine (9) were found to be active. (respectively, IC\(_{50}\) = 19.2 ve 50.8 mM)\(^{27}\)

![Image](image1.png)

**Areca catechu**

In a study performed by Gilan *et al.*, a hydroalcoholic extract of *Areca catechu* (Areceaceae) inhibited acetylcholinesterase and butyrylcholinesterase in a dose-dependent manner.\(^{30}\) However, the active component has not been identified, yet.

**Amanita mappa**

In Bhattacharya *et al.*'s work, bufotenine (10), an indole alkaloid isolated previously from the skin secretion of several frog species and later from a fungus species, *Amanita mappa* (syn. *A. citrina*), displayed antiamnesic activity by passive avoidance test in rats.\(^{31,32}\)

![Image](image2.png)

**Galanthus and Narcissus species**

Galanthamine (11), an alkaloid isolated from some *Galanthus* species (Amaryllidaceae), has been recently in use in the treatment of AD. It has a reversible acetylcholinesterase inhibitory action and also modulates the nicotinic acetylcholin receptors.\(^{33-38}\) Although the most common side effect of galanthamine is nausea, it is possible to eliminate nausea by increasing the galanthamine dose gradually.\(^{39}\)

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\(^{25}\) For dehydroevodiamine HCl (7).

\(^{26}\) Isolated through bioactivity-directed fractionation.

\(^{27}\) Ellman method.

\(^{28}\) Buxaceae.

\(^{29}\) Values between 5.21-22.7 µM for acetylcholinesterase and 2.18-38.36 µM for butyrylcholinesterase.

\(^{30}\) Hydroalcoholic extract.

\(^{31,32}\) Passive avoidance test.

\(^{33-38}\) Reversibility.

\(^{39}\) Nicotinic acetylcholin receptors.
ditionally, galanthamine was shown to have no hepatotoxicity\textsuperscript{40}. Galanthamine (Nivalin\textsuperscript{®}) has been approved as HBr salt in Austria and later licensed as Reminyl\textsuperscript{®} in the USA and some European countries as well as Turkey in the treatment of AD.

In our ongoing research on acetylcholinesterase inhibitory activity of some Turkish medicinal plants, we screened some \textit{Galanthus} and \textit{Narcissus} species, namely \textit{Galanthus elwesii}, \textit{G. ikariae}, \textit{Narcissus tazetta} subsp. \textit{tazetta}, as well as two more \textit{Amaryllidaceae} plants, \textit{Leucojum aestivum} and \textit{Pancratium maritimum} in terms of their acetylcholinesterase activity by the Ellman method\textsuperscript{28,41}.

In total, six \textit{Amaryllidaceae}-type known alkaloids called lycorine, tazettine, crinine, galanthamine, 3-epi-hydroxybulbispermine and 2-demethoxymontanine from the active fractions of \textit{Galanthus ikariae} were obtained by bioactivity-directed fractionation. Lycorine, tazettine, N-nor-galanthamine, haemantamine and 3-epi-hydroxybulbispermine were also isolated from the active fractions of \textit{Narcissus tazetta} subsp. \textit{tazetta} as the common \textit{Amaryllidaceae} alkaloids. Although \textit{G. ikariae} and \textit{N. tazetta} subsp. \textit{tazetta} extracts showed 75.56 \% and 46.62 \% inhibition, respectively; it made us consider that the lower than 50 \% activity of the extracts was resulted from the synergistic interaction between the alkaloids isolated.

In a similar study by Lopez \textit{et al.}\textsuperscript{26}, extracts prepared from various \textit{Narcissus} species together with 23 pure \textit{Amaryllidaceae}-type alkaloids were screened against acetylcholinesterase and it was suggested that the alkaloids having galanthamine and lycorine skeletons possess inhibitory activity\textsuperscript{42}.

\textbf{Fumaria species}

Within our project on acetylcholinesterase inhibitors from some Turkish plants, we screened \textit{Fumaria} species from Fumarioideae subfamily (\textit{Fumaria asepala}, \textit{F. capreolata}, \textit{F. ciliica}, \textit{F. densiflora}, \textit{F. judica}, \textit{F. kralikii}, \textit{F. macrocarpa}, \textit{F. parvislora}, \textit{F. pete-ri} subsp. \textit{thuretii}, \textit{F. vaillantii}) for their acetylcholinesterase inhibitory activity by the Ellman method. The inhibitory activities of all \textit{Fumaria} species mentioned above were significantly high ranging between 84.9 \textdegree{} 96.8 \textdegree{}. Of these species, \textit{F. vaillantii} with 94.2 \% inhibition was chosen for bioactivity-directed fractionation and the common isoquinoline alkaloids named canadine, hydastine, bulbocapnine, fumarophycine, corydaline and protopine were obtained from the active fractions of \textit{Fumaria vaillantii}. Consequently, the responsible compound for inhibitory activity of \textit{F. vaillantii} extract were established as protopine. There was synergistic interaction between the alkaloids\textsuperscript{43}.

\textbf{Caragana chamlaque}

In a bioactivity-directed fractionation by Sung \textit{et al.}, the methanolic extract of the underground parts of \textit{Caragana chamlaque} (Fabaceae) with a significant acetylcholinesterase inhibitory activity resulted in the isolation of two active stilbene oligomers, (+)-\textit{α}-viniferin (12) (IC\textsubscript{50}=2.0 \textmu{}m) and kobophenol A (13) IC\textsubscript{50}=115.8 \textmu{}m), by a slightly modified Ellman method. Both compounds inhibited acetylcholinesterase in a dose-depen- dent manner while (+)-\textit{α}-viniferin showed a specific, reversible and noncompetitive inhibition\textsuperscript{44}.
Acetylcholinesterase inhibitors from the marine sponge Reniera sarai

In Sepcic et al.’s study, 3-alkylpyridinium polymer-type compounds (14,15) isolated from the water extract of the marine sponge Reniera sarai collected from the North Adriatic Sea had a potent acetylcholinesterase inhibitory activity. These compounds inhibited the acetylcholinesterase enzyme of recombinant insect, electric eel and human erythrocyte origins and butyrylcholinesterase of horse sera origin at the IC$_{50}$ values of 0.06 µM, 0.08 µM, 0.57 µM and 0.14 µM, respectively$^{45-48}$.

Acetylcholinesterase inhibitors obtained from microorganisms

Vizoltricine (16), isolated from the microorganism *Fusarium tricinctum*, is a potent acetylcholinesterase inhibitor (IC$_{50}$ = 4.0 x 10$^{-4}$ mM). In addition, its N-methyl derivative was found to have four times greater inhibition than vizoltricine itself (IC$_{50}$ = 7.0 x 10$^{-5}$ mM)$^{49,50}$.

In Chen et al.’s work, territrem B, the mycotoxin obtained from the microfungus Aspergillus terreus, was shown to have a potent and irreversible acetylcholinesterase inhibitory activity$^{51}$.

CONCLUSION

All of the known acetylcholinesterase inhibiting drugs used in the therapy of AD suffer from several side effects such as high toxicity, short duration of biological action, low bioavailability and narrow therapeutic effects. Consequently, development of new acetylcholinesterase inhibitors with less toxicity and more potent activity is compulsory. The search for new drugs, such as Huperzin A, with acetylcholinesterase inhibitory activity to be used in the treatment of AD from natural resources, also yielded some herbal-originated extracts and/or compounds such as Ginkgo biloba, Panax ginseng, Davilla rugosa, (-)-epigallocatechin, ferulic acid, etc. which act by different mechanisms$^{52-59}$. However, acetylcholinesterase inhibitors have been accepted to be the most effective for the treatment of AD, up to the present. These results show that the available biodiversity of natural sources and the isolated bioactive compounds may act as potential leads for the development of clinically useful pharmaceuticals.

REFERENCES

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