

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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Doğal Kaynaklardan Elde Edilen Asetilkolinesteraz İnhibitörleri
Özet : Asetilkolinesteraz inhibitörleri, asetilkolini salındığı sinir ucunda hidroliz eden asetilkolinesteraz enzimini inhibe etmek suretiyle, asetilkolin miktarının azalmasını önlemektedir. Asetilkolinesteraz inhibitörleri, Alzheimer hastalığının yanı sıra, myasthenia gravis, glokom ve helmintiyazis gibi hastalıkların tedavisi ile insektisit ilaçların etki mekanizmalarında da önemli rol oynayan bileşiklerdir.

Bu derlemede, doğal kaynaklardan elde edilen asetilkolinesteraz inhibitör aktiviteye sahip bazı bileşikler değerlendirilmektedir.

Anahtar kelimeler: Asetilkolinesteraz, Alzheimer hastalığı, asetilkolinesteraz inhibitör aktivite, bitki.

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

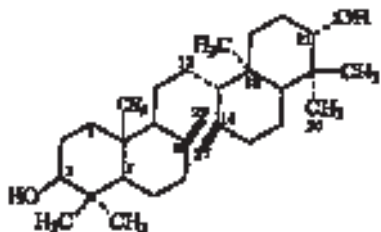
method for screening biological sources. AD is one of the most common mental problems in the aged population¹⁻³. The basal forebrain and brainstem cholinergic systems also play an important role in the regulation of cortical and thalamic electrical activity⁴. The findings from experimental animals, aging 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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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 % activity²¹.



(3)

Salvia species

Perry *et al.* studied the acetylcholinesterase inhibitory activity of essential oils of *Salvia lavandulaefolia* and *S. officinalis* (Lamiaceae), the plants known 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, 1*R*-(+)-camphor, linalol, 1*S*-(-)- β -pinen and geraniol, 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 -pinen 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) (IC_{50} = 0.67 mM) and α -pinen (5) (IC_{50} = 0.63 mM)²².



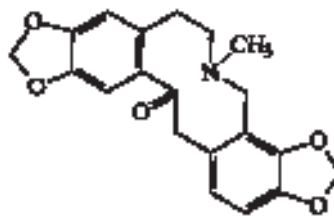
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(5)

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 anti-amnesic activity, in male mice²³.



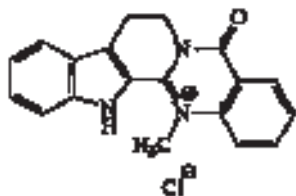
(6)

Evodia rutaecarpa

In another screening study performed in South Korea²⁴, 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 incisum* (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

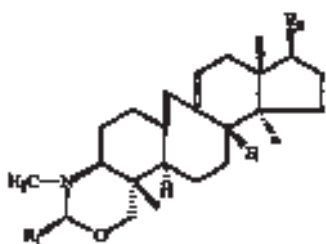
dehydroevodiamine HCl (**7**) was isolated as the active component through bioactivity-directed fractionation^{25,26}.



(7)

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.



(8) $R_1 = CH_3$, $R_2 = H_3C-CH-Nb(CH_3)_2$

(9) $R_1 = H$, $R_2 = H_3C-CH-Nb(CH_3)_2$

We investigated acetylcholinesterase inhibitory activity of *Buxus sempervirens*, a widespread plant in Turkey, by the Ellman method and its inhibition was found to be 63 % at 1 mg/ml concentration²⁸.

Sarcococca saligna

The crude alkaloidal extract of *Sarcococca saligna* (Buxaceae), which was shown to have a potent

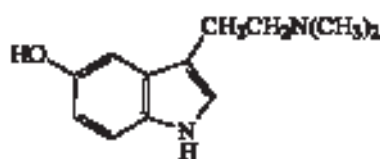
acetylcholinesterase inhibitory activity by the Ellman method against both acetylcholinesterase and butyrylcholinesterase which also plays a role possibly in the pathology of AD and 27 steroidal alkaloids of pregnane-type, 10 of which were new, were isolated by bioactivity-directed fractionation. The alkaloids inhibited both enzymes in a dose-dependent manner at IC_{50} values between 5.21-22.7 μ M for acetylcholinesterase and 2.18-38.36 μ M for butyrylcholinesterase²⁹.

Areca catechu

In a study performed by Gilan *et al.*, a hydroalcoholic extract of *Areca catechu* (Arecaceae) inhibited acetylcholinesterase and butyrylcholinesterase in a dose-dependent manner³⁰. 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 anti-amnesic activity by passive avoidance test in rats^{31,32}.

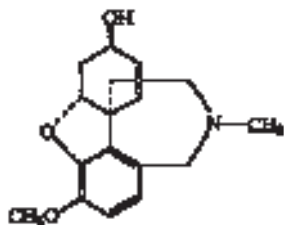


(10)

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 receptors³³⁻³⁸. Although the most common side effect of galanthamine is nausea, it is possible to eliminate nausea by increasing the galanthamine dose gradually³⁹. Ad-

ditionally, galanthamine was shown to have no hepatotoxicity⁴⁰. Galanthamine (Nivalin[®]) has been approved as HBr salt in Austria and later licensed as Reminyl[®] in the USA and some European countries as well as Turkey in the treatment of AD.



(11)

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

In total, six Amaryllidaceae-type known alkaloids called lycorine, tazettine, crinine, galanthamine, 3-epi-hydroxybulbispermine and 2-demethoxymontanine from the active fractions of *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 *Narcissus tazetta* subsp. *tazetta* as the common Amaryllidaceae alkaloids. Although *G. ikariae* and *N. tazetta* subsp. *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.

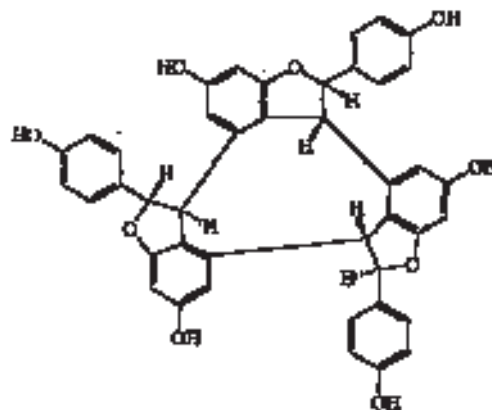
In a similar study by Lopez *et al.*,²⁶ extracts prepared from various *Narcissus* species together with 23 pure Amaryllidaceae-type alkaloids were screened against acetylcholinesterase and it was suggested that the alkaloids having galanthamine and lycorine skeletons possess inhibitory activity⁴².

Fumaria species

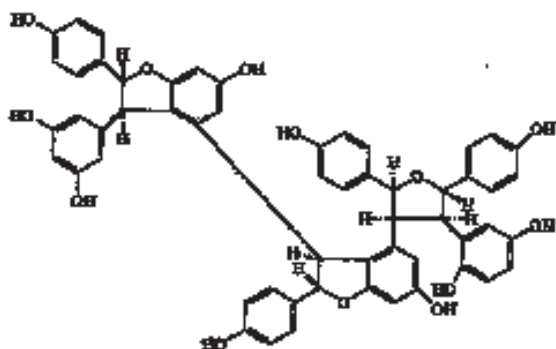
Within our project on acetylcholinesterase inhibitors from some Turkish plants, we screened *Fumaria* species from Fumarioideae subfamily (*Fumaria ase-pala*, *F. capreolata*, *F. cilicica*, *F. densiflora*, *F. juda-ica*, *F. kralikii*, *F. macrocarpa*, *F. parviflora*, *F. pette-ri* subsp. *thuretii*, *F. vaillantii*) for their acetylcholinesterase inhibitory activity by the Ellman method. The inhibitory activities of all *Fumaria* species mentioned above were significantly high ranging between 84.9 % - 96.8 %. Of these species, *F. vaillantii* with 94.2 % inhibition was chosen for bioactivity-directed fractionation and the common isoquinoline alkaloids named canadine, hydrastine, bulbocarpine, fumarophycine, corydaldine and protopine were obtained from the active fractions of *Fumaria vaillantii*. Consequently, the responsible compound for inhibitory activity of *F. vaillantii* extract were established as protopine. There was synergistic interaction between the alkaloids⁴³.

Caragana chamlague

In a bioactivity-directed fractionation by Sung *et al.*, the methanolic extract of the underground parts of *Caragana chamlague* (Fabaceae) with a significant acetylcholinesterase inhibitory activity resulted in the isolation of two active stilbene oligomers, (+)- α -viniferin (12) (IC₅₀=2.0 μ m) and kobophenol A (13) IC₅₀=115.8 μ m), by a slightly modified Ellman method. Both compounds inhibited acetylcholinesterase in a dose-dependent manner while (+)- α -viniferin showed a specific, reversible and noncompetitive inhibition⁴⁴.



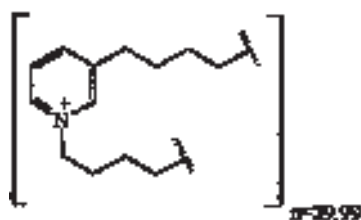
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(13)

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 IC₅₀ values of 0.06 µM, 0.08 µM, 0.57 µM and 0.14 µM, respectively⁴⁵⁻⁴⁸.



(14)

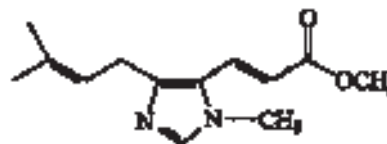


(15)

Acetylcholinesterase inhibitors obtained from microorganisms

Vizoltricine (16), isolated from the microorganism

Fusarium tricinctum, is a potent acetylcholinesterase inhibitor (IC₅₀= 4.0 x 10⁻⁴ mM). In addition, its N-methyl derivative was found to have four times greater inhibition than vizoltricine itself (IC₅₀= 7.0 x 10⁻⁵ mM)^{49,50}.



(16)

In Chen *et al.*'s work, territrein B, the mycotoxin obtained from the microfungus *Aspergillus terreus*, was shown to have a potent and irreversible acetylcholinesterase inhibitory activity⁵¹.

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 mechanisms⁵²⁻⁵⁹. 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.

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