

MEDICINAL PLANTS HAVING ANTI-CHOLINESTERASE ACTIVITY FROM DIFFERENT REGIONS OF THE WORLD

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ABSTRACT

Cholinesterase inhibition has been one the main strategy to provide symptomatic relief to Alzheimer's patients based on cholinergic hypothesis. The main problems associated with currently used cholinesterase inhibitors are their associated side effects such as hepatotoxicity nausea, vomiting and many more. In view of this, many researchers across the world are looking for safer, cheaper and readily bioavailable cholinesterase inhibitors. Plants are considered as one of the major source of cholinesterase inhibitors. Many plants have given new leads in deriving novel molecules that can be used as cholinesterase inhibitors in last few years. However, more such studies are required to explore the cholinesterase inhibitors from unexplored plant sources. This paper deals with recently explored plants along with their bioactive constituents that might be used for the

symptomatic relief and treatment of Alzheimer's disease from different regions of the world. Plants are selected mainly on the basis of traditional knowledge. Plants from various folk medicinal systems such as from Chinese, Egyptian, Unani, and Ayurvedas are used to explore cholinesterase inhibitors. The papers have been reviewed from different databases such as Pubmed and Google scholar.

KEYWORDS: Alzheimer's disease, Anti-cholinesterase, folk medicines.

INTRODUCTION

Alzheimer's disease (AD) is a cognitive neurodegenerative disorder and one of the major forms of dementia representing 60-80% of the patients.^[1] By the end of 2015, it is estimated 53 million people would be affected and this would almost double by 2025 and triple by

2050.^[2] During AD the hippocampus and the neocortex region of the brain are affected due to the deposition of amyloid β in senile plaques and neurofibrillary tangles along with the decline of neuronal synapses and pyramidal neurons. These changes result in symptoms such as memory loss resulting in disrupted daily life, confusion with place and time, problem with speaking and writing leading to AD. Cholinergic hypothesis is the most conventional theory proposed for AD. According to this hypothesis the depletion of acetylcholine (ACh) by the action of enzyme acetylcholinesterase (AChE) results in termination of nerve transmission leading to increased cognition and memory related effects. The binding of AChE causes decrease in the binding of ACh to muscarinic receptor and nicotinic receptors.^[3] Inhibition of cholinesterase is one of the main therapeutic strategies to deal with AD. Two groups of cholinesterase inhibitors are used acetylcholinesterase inhibitors (AChI) and butyrylcholinesterase inhibitors (BuChI) to prevent the breakage of acetylcholine and butyrylcholine (BuCh), which results in an increased concentration of the former two and hence leading to an increased communication between nerve cells and may temporarily improve or stabilize the symptoms of AD.^[4] Most of these cholinesterase inhibitor (ChI) inhibitor drugs are synthetically derived, which includes tacrine, donepezine, galantamine and rivastigmine.^[5,6,7,8] Synthetic drugs have many side effects such as nausea, vomiting, diarrhea, weight loss, decreased appetite, muscle weakness, muscle cramps, fatigue, dizziness and headache. To overcome these limitations scientists all over the world are looking for ChI derived from natural products specifically plants. Plant based medicines are used by 70-80% of the world population for primary health care because of better compatibility with the human body, better cultural acceptability, lesser side effects, effectiveness with chronic conditions, widespread availability and lower cost. In recent years plant parts and their phytoconstituents for AChE inhibition have been discovered in different regions of the world. This paper would discuss different plants recently evaluated from these different geographical locations.

PLANTS AS AChE INHIBITORS

A large number of plants have been screened for their anticholinesterase activity based on their use in different traditional medicines like Chinese, Unani, Ayurvedic and Egyptian. Plants of different regions like in Asia, Africa, America and Europe have been screened on the basis of phylogenetic analysis, family similarity or the constituents present in the plants. Below are discussed few of the most recently studied plants and their constitutive compounds based on their regional distribution.

ASIA

The flora rich regions of Asia are mostly the South East Asian region comprising of India, China, Thailand, Vietnam, Bangladesh, Pakistan and the Middle East Asia comprising of Libya Syria and other countries.

Alpinia officinarum

This plant belongs to the Zingiberaceae family. It is mostly cultivated in India, Vietnam, Thailand and China.^[9] The rhizomes of the plant contain flavonoids, and glycosides.^[10] Different antioxidants possess AChE inhibitory activity.^[11]

Mentha longifolia

This plant belongs to Lamiaceae family. Species of this family show AChE inhibition. The leaf extracts of *M. longifolia* showed a moderate AChE inhibition of $62.82 \pm 0.005\%$ at $25 \mu\text{g/ml}$ and the mechanism of action reported was mixed non-competitive inhibition.^[12]

Cinnamomum zeylanicum

It belongs to Lauraceae family. GS-MS was done to study the different components of cinnamon oil. Methanolic extract of the dried leaves and oil, eugenol quantified from cinnamon oil showed AChE and BuChE inhibition.^[13]

Kopsia arborea

A plant belonging to Apocynaceae family is native to India, China, Thailand and other southern Asian countries. The twigs of this plant contain monoterpenes and indole alkaloids. Alkaloids isolated from this plant namely kopsamine and decarbomethoxy-kopsiline showed mild AChE inhibition.^[14]

Camellia japonica

This plant belongs to Theaceae family. The seed cakes showed mild AChE inhibition.^[15]

Tripleurospermum disciforme

belongs to Asteraceae family. The flowering tops of the plants showed potent AChE inhibition of $71.18 \pm 4.9\%$ at $5 \mu\text{g/ml}$ final concentration of the extract. The extract of the plant was also reported for its neuroprotective activity in PC12 cell lines against A β toxicity.^[16]

Dracocephalum multicaule

belongs to Lamiaceae family. The flowering part of this plant showed potent AChE inhibition $79.06 \pm 3.1\%$ at $5 \mu\text{g/ml}$ final concentration of the extract.^[16] Chinese Agarwood has also shown weak AChE activity.^[17]

Cynometra cauliflora

It belongs to Fabaceae family. Methanolic, n-butanolic and an aqueous extract of the leaf extracts showed AChE inhibition with IC_{50} of $0.065 \pm 0.0008 \text{ mg/ml}$; $0.031 \pm 0.021 \text{ mg/ml}$; $0.011 \pm 0.005 \text{ mg/ml}$ and $0.052 \pm 0.0152 \text{ mg/ml}$ respectively.^[8]

Piper bavinum

This plant belongs to Piperaceae family. Six fractions were reported from silica gel chromatography isolation which includes bavinol, ampelosin and violanthin showed $29.80 \pm 0.26 \mu\text{M}$; $59.47 \pm 0.078 \mu\text{M}$ and $79.86 \pm 3.01 \mu\text{M}$ AChE inhibition respectively and BuChE inhibition as $19.25 \pm 0.43 \mu\text{M}$; $99.15 \pm 1.03 \mu\text{M}$ for bavinol and ampelosin respectively.^[19]

Canarium patentinervium

This plant belongs to Burseraceae family and is indigenous to Malaysia. The leaves and the bark were used for preparing hexane, chloroform and ethanolic extracts. The chloroform extracts of the leaves were purified using silica gel chromatography, thin layer chromatography and HPLC. Scoparone, Scopoletin, (+)-catechin, vomifoliol, lioxin, syringic acid were isolated, vomifoliol, Scopoletin, syringic acid and scoparone showed AChE inhibition with IC_{50} less than $100 \mu\text{g/ml}$.^[20]

Allium stipitatum

This plant belongs to Amaryllidaceae family, is an indigenous traditional medicine of Turkey, Iran and central Asia. The constituents of *Allium stipitatum* were purified and showed IC_{50} lesser than $100 \mu\text{M}$ for AChE inhibition.^[21]

Daphne holosericea

This plant belongs to Thymelaeaceae family and used in traditional Chinese medicine, two new sesquiterpenoids were isolated from the bark of the plant- holosericin A and holosericin B, which showed a mild AChE inhibition of 31% .^[22]

Excoecaria acertiflia

This plant belongs to Euphorbaceae family. Seventeen constituents the butanol extracts of the stem, four showed AChE inhibitory activity major ones are exocarinal F, (7S,8S)-nitidanin, (7S,8S)-5-hydroxynitidanin and 1,2- bis (4-hydroxy-3- methoxyphenyl) propane-1,3-diol of 9.4, 12.2, 27.4 and 59.0% respectively at 50 μ M.^[23]

Garcinia atroviridis

This plant belongs to Clusiaceae family. A methanolic, chloroform and ethyl acetate stem extract were used to isolate two compounds garcineflavanone A and garcineflavanol A. Both showed more than 50% inhibition for AChE enzyme but only garcineflavanol A had more than 50% BuChE enzyme inhibition activity. Garcineflavanone A and garcineflavanol A showed AChE inhibition with IC₅₀ of 28.52 \pm 5.23 and 14.04 \pm 0.77 μ g/ml respectively.^[24]

Lycopodiastrum casuarinoides

The plant belongs to Licopodiaceae family, used as a folklore medicine. Five active compounds *N*-demethyl huperzine, huperzine C, huperzine B and lycoparin C showed significant AChE inhibition.^[25]

Lycopodiella cernua

This plant also belongs to Licopodiaceae family, and is widely used in Vietnamese folk medicine. The methanolic extract reported to contain alkaloid VLC showed AChE inhibition with IC₅₀ 26.7 \pm 5.5 μ g/ml.^[26]

Garcinia mangostana

This plant belongs to Clusiaceae family. The chloroform fraction reported to have highest AChE inhibition activity (92.50 \pm 3.5% at 5 μ g/ml) and 57.33 \pm 3.94% inhibition was reported for BuChE enzyme. The original extract of methanol showed an inhibitory activity of 82.19 \pm 2.89% for AChE and 44.96 \pm 6.3% for BuChE. The plant bioactives purified were α Mangostin, γ Magostin and Garcinone C showed highest cholinesterase inhibition. Garcinone C and α mangostin showed mixed mode of inhibition whereas γ mangostin showed a non-competitive inhibition.^[27]

Marsupella alpine

The member of the family Gymnomitriaceae, was isolated for six different sesquiterpenoids showed moderate to weak AChE inhibitory activity.^[28]

Polygonum hydropiper

This plant belongs to Polygonaceae family. The crude plant along with its fractions of chloroform, ethyl acetate, n butanol, n hexane, aqueous and saponins were evaluated, out of these the highest AChE inhibitory activity was observed by saponins 87.58%, followed by n-hexane 87.49% and crude extract 86.87%. For BuChE, n hexane showed the highest inhibition of 90.30%, then the aqueous extract with 87.62% followed by chloroform extract with 85.94%.^[29]

Momordica charantia

The plant belongs to Cucurbitaceae family. The triterpenoids from this plant showed AChE inhibition with IC₅₀ 32.20 µM in a non competitive mode of inhibition.^[30]

Piper betle

This plant belongs to Piperaceae family. An aqueous and an ethanolic extract of the leaves were prepared for AChE and BuChE assay. The ethanolic extract showed more BuChE inhibition than aqueous.^[31]

Essential oils

Essential oils have been evaluated and screened for cholinesterase inhibition. Significant inhibitors are *Citrus aurantium* (Rutaceae), *Foeniculum vulgare* (Apiaceae), *Cupressus sempervirens* (Cupressaceae), *Eucalyptus globules* (Myrtaceae), *Thymus vulgaris* (Lamiaceae) and *Pinus nigra* ssp. *Dalmatica* (Pinaceae).^[32, 33] *Eugenia brasiliensis* (Myrtaceae) showed weak inhibitory action against AChE.^[34] The essential oil from *Tragopogon latifolius* var. *angustifolius* (Asteraceae) has also shown AChE inhibition of 55.40% and BuChE at 46.50%.^[35] *Lycopsis orientalis* (Boraginaceae) oil also has mild AChE inhibition of 48.49% and 43.65% for AChE and BuChE respectively.^[35]

Table1: List of plants showing AChE and BuChE enzyme inhibitory activity.

S.No	Plant	Part used	Family	AChE Inhibition	IC ₅₀ (µg/ml)	Reference
1.	<i>Withania somnifera</i>	Roots	Solanaceae	86%	150	[36]
2.	<i>Acorus calamus</i>	Rhizomes	Araceae	43.796±7.2%	176±9.6	[37]
3.	<i>Bacopa monniera</i>	Whole plant	Scrophulariaceae	15.156±0.97	523±39.7	[37]
4.	<i>Cedrus deodera</i>	Stem bark	Pinaceae	7.136±0.89%	>1000	[37]
5.	<i>Celastrus paniculatus</i>	Seeds	Celastraceae	23.136±2.3%	>1000	[37]
6.	<i>Centella asiatica</i>	Whole plant	Apiaceae	30.76±2.9%	890±67.4	[37]
7.	<i>Convolvulus pluricaulis</i>	Whole plant	Convolvulaceae	40.6±5.4%	234±38	[37]
8.	<i>Coriandrum sativum</i>	Leaves	Umbelliferae	36.256±5.3%	>1000	[37]
9.	<i>Cuminum cyminum</i>	Seeds	Umbelliferae	76.90±0.003%	539.69	[38]
10.	<i>Emblica officinalis</i>	Friut	Euphorbiaceae	71.96±0.005%; 61.22±0.001% ^a	53.5±8.9	[39]
11.	<i>Evolvulus alsinoides</i>	Whole plant	Convolvulaceae	38.036±3.5%	245±32.4	[37]
12.	<i>Glycyrrhiza glabra</i>	Roots	Leguminosae	35.016±4.6%	418±30.7	[37]
13.	<i>Nardostachys jatamansi</i>	Rhizomes	Valerianaceae	83.836±9.2%	40.5±7.1	[37]
14.	<i>Nelumbo nucifera</i>	Flower	Nelumbonaceae	61.736±7.6%	76±9.2	[37]
15.	<i>Polygonum minus</i>	Leaves	Polygonaceae	68%	40.0	[40]
16.	<i>Punica granatum</i>	Whole fruit	Punicaceae	62.46±5.3%	77±6.2	[37]
17.	<i>Rauvolfia serpentina</i>	Roots	Apocynaceae	84.96±9.5%	22±4.9	[37]
18.	<i>Saussurea lappa</i>	Roots	Asteraceae	12.156±9.7%	>1000	[37]
19.	<i>Shorea robusta</i>	leaves	Dipterocarpacea	63.11±0.09% ^a	70	[41]
20.	<i>Terminalia chebula</i>	Whole fruit	Combretaceae	41.066±5.6%	180±14.6	[37]
21.	<i>Tinospora cordifolia</i>	Stem	Menispermaceae	27.756±3.5%	230±17.95	[37]
22.	<i>Trigonella foenum graceum</i>	Seeds	Fabaceae	6±0.9%	>1000	[37]
23.	<i>Valeriana wallichii</i>	Roots	Valerianaceae	8.82±0.7%	124±9.8	[37]

a. BuChE Inhibition.

AFRICA

The Mediterranean basin's rich flora has given many AChE inhibitory plants. Plants used in Egyptian, South African folk medicines have also been reported for AChI activity.

Physostigma venenosum

This plant belongs to Fabaceae family. Plant seeds were used to prepare chloroform and water extracts. Using LC-MS, different constituent compound of the plant were purified. Chloroform and methanolic extracts showed moderate AChE inhibition.^[42]

Croton species

The genus of Euphorbiaceae family, Croton, is widely used as a folk medicine. The leaves of two croton species *C. gratissimum* and *C. zambesicus* were taken and crude methanol, hexane, ethyl alcohol and butanol extract were made. The extracts of ethyl acetate and

butanol of *C. gratissimus* showed lowest AChE inhibition with IC₅₀ value of 66.9±0.24 and 64.5±5.2 µg/ml respectively.^[43] Another species *C. sparsiflorus* also showed potent AChE inhibition.^[44]

Pistacia terebinthus* and *Pistacia khinjuk

The members of Anacardiaceae family *P. khinjuk* seeds and the fruit of *P. terebinthus* were used. An extract of n hexane, dicholoromethane, ethanol and water-ethanol was prepared. Both the plants showed a higher AChE activity for ethanol and water-ethanol extracts. These were higher than galanthamine, which was used as a positive control in these studies. Significant inhibition was also observed for n hexane and dicholomethane.^[45]

Essential oils

Essential oils have been found to be effective as AChI. *Afromomum melegueta* (Zingiberaceae) had an IC₅₀ of 28.79mg/L, 15.27mg/L and 16mg/L for its stem, leaf and rhizome respectively. *Crassocephalum crepidioides* (Asteraceae) was also studied for leaf (IC₅₀ 10.96mg/l) & stem extract (12.15mg/L), *Monodora myristica* (Annonaceae) for seed (IC₅₀ 14.9 mg/L), stem (6.54mg/L) and *Ocimum gratissimum* (Lamiaceae) for leaf (6.54 mg/L) and seed (6.71mg/L).^[46]

AUSTRALIA

Nauclea officinalis

This plant belongs to Rubiaceae family. Monoterpenoids from *Nauclea officinalis* were reported for AChE and BuChE inhibition. Different monoterpenoids namely, angustine, nauclefine, angustidine, harmane and angustoline showed greater BuChE inhibition than galanthamine. Of these angustidine showed significant AChE and BuChE enzyme inhibitory activity.^[47]

EUOROPE

Europe has also a wide variety of plants that have shown AChE inhibitory activity.

Cirsium leucopsis*, *C. sipyleum* and *C. erioplurum

These plants belong to Asteraceae family, native to turkey have been found to have moderate AChE inhibition potential, the chemical constituents of these plants have also been successfully identified and the activity of these were studied. Taraxa-terol was found to have a significant AChE and BuChE inhibitory activity.^[48]

Biscutella raphanifolia

A member of Brassicaceae family was tested for AChE and BuChE inhibition. Petroleum ether and dichloromethane extracts of aerial parts of the plant showed mild AChE and BuChE inhibition.^[49]

Dittrichia viscosa

It belongs to Asteraceae family. Extracts of flower (hexane, diethyl ether), leaves (hexane), roots (ethyl acetate and methanol) and stem (diethyl ether) showed spots on silica gel having confirmed inhibitory activity against AChE.^[50]

Vitis vinifera

A common plant of Vitaceae family, grape skin is rich in Anthocyanin, these are also rich in antioxidants. The AChE inhibition was reported at 500µg/ml concentration with 55.58% inhibition.^[51] The leaves of grapes and pomegranate show potent AChE inhibition.^[52, 53] Anthocyanins of blueberry and grapes have also reported neuroprotective activity.^[54, 55, 56]

Salvia lavandulaefolia

A common European plant, belonging to Lamiaceae family. The essential oil has shown AChE inhibitory activity.^[57]

SOUTH AMERICA

The Amazon basin is another rich source of flora. South America has been one of the most diverse landmass on earth with a variety of flora and fauna.

Annonaceae family

The Annonaceae family is native to Brazil and its members have been used extensively in traditional folk medication. Four species of this family *A. crassiflora*; *A. coriacea*; *A. sylvatica*; *A. cacaous* and *D. furfuracea* were studied for AChE inhibition. The methanolic extracts of the leaves, stem and the capitulum were prepared. Stem showed the highest AChE inhibition.^[58]

Jacaranda acutifoliasis

It belongs to Bignoniaceae family. The methanolic extract of the leaves were studied for AChE inhibition both *in vivo* and *in vitro* conditions. The *in-vitro* assay gave an enzyme inhibitory activity of 3.5955 ± 0.6067 µmol/min in 0.2ml of extract as compared to the control

of 4.3582 ± 0.0929 $\mu\text{mol}/\text{min}$. 0.6ml of extract showed less inhibition of only 15.07%. The *in-vivo* analysis for AChE inhibition showed 38.93% inhibitory activity.^[59]

Amaryllidaceae family

Five species from this family were taken, *Crinum bulbispermum*, *C. jagus*, *Hippeastrum barbatum*, *Hymenocallis caribaea* and *Zephyranthes carinata* and were purified using GS-MS. The alkaloids were detected, identified and quantified. 46 different types of lycorine type alkaloids and galanthamines were identified. The alkaloids from *C. jagus* and *Z. carinata* showed AChE inhibition with IC_{50} 18.28 ± 0.29 $\mu\text{g}/\text{ml}$ and 17.96 ± 1.22 $\mu\text{g}/\text{ml}$ respectively.^[60] An endemic species of this family *Sternbergia candida* was also studied for its AChE inhibition.^[61] The bulb of *Habranthus tubispathus* and the aerial parts along with the bulb of *H. jamesonii* were also studied. The alkaloids were also extracted from these plants, maximum AChE inhibition and BuChE inhibition was observed for the aerial parts of *H. jamesonii* with IC_{50} value of $0.7 \mu\text{g}/\text{ml}$ and $6.7 \mu\text{g}/\text{ml}$ respectively.^[62]

FOOD SUPPLEMENTS AS A CURE FOR ALZHEIMER'S DISEASE

Many spices and fruits which have been mentioned are taken on daily basis, but other plants which are not a part of daily routine are sometimes difficult to get, since these plants are those which are only used as folk medicines. Hence it is more important to find plants used regularly having AChI. Few food recipes, diets and other ready to uptake items have also been evaluated for AChE inhibition. Lemon juices in blend with some berries have been found to have good anticholinesterase activity.^[63] Blends of lemon juice and papaya and lemon juice along with mori have AChE inhibitory activity and it is possible for more than one plant to be blended together resulting in greater inhibition with lower IC_{50} .^[64]

CONCLUSION

In conclusion, large number of plants has been studied for cholinesterase inhibition during last few years. These plants belong to varying families and region having different climatic requirements and used in different traditional medicines. Different folklore of the world has their own kind of plant for neurological disorders, memory enhancement and cognitive related problems. The work on cholinesterase has gathered lot of pace across the globe in recent times. This will be beneficial for whole mankind specifically those who are suffering from disease like Alzheimer's which don't have any cure till date. This research on exploration of various plant species will not only help in deriving novel molecules but also give new leads for the novel drugs for treatment of AD in near future.

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