

# Cholinesterase Inhibitors from Plants: Possible Treatment Strategy for Neurological Disorders – A Review

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## ABSTRACT

Dementia is a chronic progressive mental disorder, which adversely affects memory, thinking, comprehension, calculation and language. Some of the most common dementias are Alzheimer's disease, Parkinsonism, Dementia with Lewy Bodies and Myasthenia gravis. All of these disorders are related to abnormalities in the central cholinergic system, which shows a decline in acetylcholine (ACh) level due to substantial reduction in the activity of the enzyme choline acetyl transferase. A variety of strategies have been envisaged to implement the replacement of ACh, of which acetyl cholinesterase (AChE) inhibition has shown consistent positive results. Cholinesterase inhibitors act on the enzymes that hydrolyze ACh, following synaptic release. Currently several cholinesterase inhibitors such as tacrine, rivastigmine, donepezil and galanthamine have been used as first line pharmacotherapy for Alzheimer's disease. However these drugs have severe side effects like hepatotoxicity and gastrointestinal disorder, hence there is still a great interest in finding better cholinesterase inhibitors from natural sources. Natural products are significant sources of synthetic and traditional herbal medicines. A potential source of AChE inhibitors is certainly provided by the abundance of plants in nature. Huperzine, bacosides, hyperforin, desoxy-peganine and *Ginko biloba* (plant extract) are some of the natural drugs used in the treatment neurological disorders. This article aims to provide a comprehensive literature survey of plants that have been tested for AChE inhibitory activity. Numerous phytoconstituents and promising plant species as AChE inhibitors are being reported in this communication.

**Keywords:** acetylcholinesterase, butyrylcholinesterase, Alzheimer's disease, donepezil, galanthamine, phytoinhibitors

**Abbreviations:** AChE, acetylcholinesterase; AD, Alzheimer's disease; BuChE, butyrylcholinesterase; ChE, cholinesterase; ChEI, cholinesterase inhibitors; AChEI, acetylcholinesterase inhibitors; BuChEI, butyrylcholinesterase inhibitors; ACh, acetyl choline; ChAT, choline acetyl transferase; TCM, traditional Chinese medicine

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## INTRODUCTION

Neurodegenerative disease is a generic term applied to a variety of conditions arising from chronic breakdown and deterioration of the neurons, particularly those of the central nervous system (CNS). In addition, these neurons may accumulate aggregated proteins, which cause dysfunction. Most common neurodegenerative disorder in elderly people

is dementia. Dementia is a syndrome usually associated with the progressive and chronic disruption of intellectual functioning. It is characterized by the development of multiple cognitive deficits, which includes memory, orientation, comprehension, learning, thinking, language and judgement to variable extents. An estimated 24.3 million people have dementia today, with 4.6 million new cases of dementia every year (one new case every 7 seconds). The number of

people affected doubles every 20 years to 81.1 million by 2040. Most people with dementia live in developing countries (60% in 2001, rising to 71% by 2040) (Ferri *et al.* 2006). Some of the commonest dementia is Alzheimer's disease, Parkinsonism, DLB and Myasthenia gravis (Holden and Kelly 2002).

## TYPES OF DEMENTIA

### Alzheimer's disease

Alzheimer's disease (AD), a chronic, progressive, disabling organic brain disorder characterized by disturbance of multiple cortical functions, including memory, judgment, orientation, comprehension, learning capacity, and language. AD is the fourth leading disorder in the world accounting for ~60% of dementia in elderly persons. The risk of AD dramatically increases with aging, affecting 7-10% of individuals over age 65, and about 40% of persons over 80 years of age, and it is predicted that the incidence of AD will increase 3-fold within the next 50 years if no therapy intervenes (Sisodia 1999). In developed societies where life expectancy has been considerably extended, this devastating disease actually represents a major public health concern, being estimated that 22 million people worldwide will develop this progressive neurodegenerative disorder by 2025 (Sleeper and Van Duijn 2001). In USA the prevalence of AD has been estimated to be 4.5 million cases in 2000 and this number will increase by almost 3-fold i.e. 13.2 million by 2050 (Hebert 2003). Neuropathological hallmarks of AD are presence of intracellular neurofibrillary tangles and extracellular senile plaques in neurons of the hippocampal region a centre for memory and cerebral cortex, which is involved in reasoning, memory, language and other important thought processes. Cholinergic synaptic junction appears to be particularly susceptible to beta-amyloid (A $\beta$ ) peptide toxicity, and loss of synaptic vesicles on axonic terminals may precede cholinergic neuronal loss (Small *et al.* 2001). Markers for cholinergic neurons such as choline acetyltransferase (ChAT) and Acetylcholinesterase (AChE) are the enzymes responsible for synthesis and degradation of acetyl choline (ACh), respectively. These enzymes are decreased in the cortex and hippocampus areas of the AD brain. Progressive deterioration of the widespread and dense cholinergic innervations of the human cerebral cortex contributes to the salient cognitive and behavioral disturbances in AD (Wright *et al.* 1993).

### Vascular dementia and LBD

The second and third most common types of dementia are vascular dementia and dementia with Lewy bodies (LBD), respectively. Executive dysfunction is often seen in patients with vascular dementia, but memory dysfunction may be minimal or nonexistent in patients with a mild form of the disease (Roman 2003). Dementia with Lewy bodies can be characterized by symptoms of global cognitive impairment, neuropsychiatric disturbance with visual hallucinations, and Parkinsonism (Tiraboschi *et al.* 2000). The symptoms of all types of dementia are presumed to be related to impaired neurotransmission and degeneration of neuronal circuits in the brain areas affected. Cholinergic deficits occur in the brains of patients with vascular dementia and LBD. These observations suggest that impairment of cholinergic function contributes to the symptoms of all three forms of dementia and that all patients with dementia could potentially benefit from cholinergic replacement therapy (Poirier 2002).

### Parkinson's disease

Parkinson's disease is a neurodegenerative disease of the substantia nigra (an area in the basal ganglia), which involves break down of nerve cells in the motor area of the brain, also characterised by reduction in ChAT activity (White and Price 1982). More than 1.5 million people of

United States suffer from this disorder ([www.apdaparkinson.org](http://www.apdaparkinson.org)).

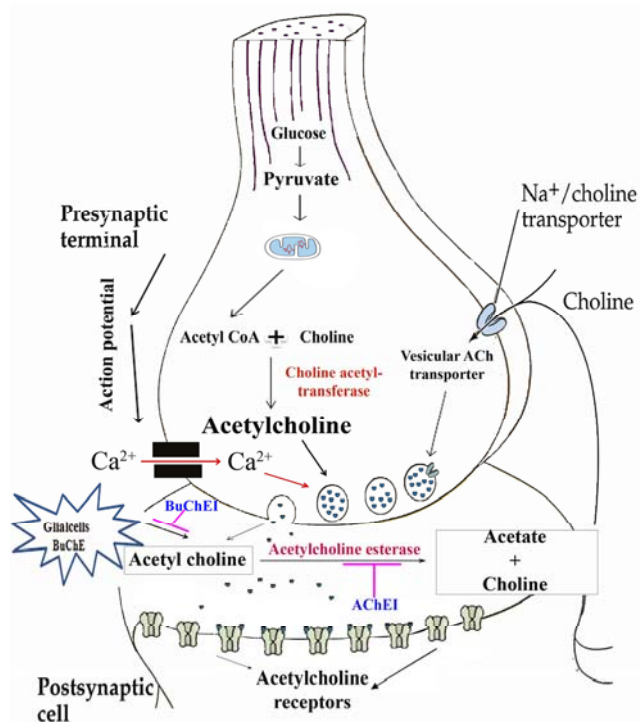
### Myasthenia gravis

Chronic autoimmune disorder characterized by production of antibodies against ACh nicotinic receptors thereby inhibiting the stimulative effect of ACh at the post synaptic neuromuscular junction (Conti-Fine *et al.* 2006). Over time, the motor end plate is destroyed leading to muscle weakness and fatigue. All these disorders are related to abnormalities in central cholinergic system, which shows a decline in ACh level. Decline in neurotransmitter ACh leads to impairment in cognitive functions. So enhancement of cholinergic neurotransmission has been put forward as the most promising strategy to improve the cognitive function.

## CHOLINESTERASE INHIBITORS – SYMPTOMATIC TREATMENT STRATEGY FOR NEUROLOGICAL DISORDERS

### Mechanism of cholinergic neurotransmission

Neurotransmitters are produced by neurons referred to as cholinergic neurons. In the peripheral nervous system ACh plays a role in skeletal muscle movement, as well as in the regulation of smooth muscle and cardiac muscle. In the central nervous system ACh is believed to be involved in learning, memory, and mood. ACh are synthesized in the presynaptic terminal from acetylcoA and choline by the action of enzyme ChAT, and stored as synaptic vesicles (50 nm diameter) in the presynaptic terminal. Synaptic transmission begins with depolarization of membrane of the presynaptic terminal. Voltage sensitive Ca<sup>+2</sup> channels, present in the presynaptic membrane, open upon depolarization and admit Ca<sup>+2</sup> into the terminals. The Ca<sup>+2</sup> facilitates the fusion of synaptic vesicles to the presynaptic membrane; the synaptic vesicle become confluent with the terminal membrane open and release their contents in to the synaptic cleft. A large number of proteins such as Vamp, Syntaxin, Synap and Synaptotagmin have been implicated in the binding of the synaptic vesicles to the presynaptic membrane. These proteins form complexes between the synaptic vesicle and presynaptic terminals, enabling the docking of the vesicles to the membrane upon the Ca<sup>+2</sup> entry and fusion of the vesicles with presynaptic membrane subsequently releasing the ACh at the neuronal junction. Once released, transmitter diffuses quickly across the synaptic cleft and binds to specific receptor proteins on the postsynaptic membrane. Cholinergic receptors are nicotinic and muscarinic. These receptors are transmembrane protein consisting of five subunits that form an aqueous channel within lipid bilayer. Nicotinic receptors are located at synapses between two neurons and at synapses between neurons and skeletal muscle cells which upon activation directly results in depolarization of the neuron, while muscarinic receptors, located at the synapses of nerves with smooth or cardiac muscle, trigger a chain of chemical events referred to as signal transduction. Once ACh binds to the receptor in the post synaptic terminal, the proteins undergo conformational change and Na<sup>+</sup>-K<sup>+</sup> channel in the receptor opens permeating the Na<sup>+</sup> ions into the post synaptic vesicles building up a positive charge inside the membrane known as excitatory postsynaptic potential (EPSP). Once the EPSP reaches a threshold, an action potential is generated in the neuron. The ACh in the post synaptic receptor is cleaved by the enzyme AChE and BuChE to acetate and choline thereby terminating the synaptic activity. Final step in neurotransmission is to restore the ACh for the subsequent chemical transmission. Choline is actively transported back into the presynaptic neurons and combines with acetylcoA to form ACh in the presence of the enzyme ChAT. Synaptic vesicles are formed by the folding of the terminal membrane away from active zones and pinching of that membrane to form vesicles (**Fig. 1**). ACh is concentrated in the synaptic vesicles and the trans-



**Fig. 1** Mechanism of transmission of acetylcholine in cholinergic neurons.

mission is complete (Dowling 2001). The first neurotransmitter defect discovered in most of the neurodegenerative disorder such as AD is ACh. As cholinergic function is required for short-term memory function, it was determined that cholinergic deficit in AD was responsible for much of the short-term memory deficit (Francis *et al.* 1999).

### Targeting acetylcholinesterase and butyrylcholinesterase in dementia

AChE (E.C. 3.1.1.7) and BuChE (acylcholine acylhydrolase; pseudocholinesterase; E.C. 3.1.1.8) are two closely related enzymes found in all vertebrate species (Lane *et al.* 2006).

#### Acetylcholinesterase

AChE a vital enzyme to mammalian life, plays a crucial role in the neuromuscular junction by terminating cholinergic neurotransmission (Soreq and Seidman 2001). It is a complex protein of  $\alpha/\beta$  hydrolase fold type having an overall ellipsoid shape containing a deep groove, usually called the gorge, which is about 20 Å deep. ACh initially binds to the “peripheral site” at the outer rim and migrates to the bottom of the gorge where hydrolysis occurs. Gorge has four main sub sites, the esteric site, the oxanion hole, anionic site and the acyl pocket. The esteric site has a catalytic triad Ser200–His440–Glu327 (Sussman *et al.* 1991) which enhances the nucleophilicity of the catalytic serine, since the strong hydrogen bond between His and Ser improves the ability of Ser to mount a nucleophilic attack on the substrate, while Glu stabilizes the histidinium cation of the transition state (Zhang *et al.* 2002). The “oxanion hole” (OH) consists of Gly118, Gly119 and Ala201 residues which contain hydrogen bond donors to stabilize the tetrahedral intermediate of ACh which is formed during the catalytic process (Ordentlich *et al.* 1998). The “anionic subsite” (choline-binding subsite or hydrophobic subsite) contains Trp84, Phe330 and Glu199, which binds to quaternary ammonium ligands of ACh by  $\pi$ -cation interactions (Kua *et al.* 2003). Trp84 is an important residue for binding ACh. The “acyl pocket” (acyl binding pocket or acyl-binding pocket) consists of Phe288 and Phe290, which are believed to play a

role in limiting the dimension of substrates that enter the active site (Zhang *et al.* 2002). The “peripheral anionic site” (PAS), which is remote from the catalytic site, has been identified by the use of inhibitor probes. AChE generally exists in three isoforms: G1 in brain; G4 in brain and the neuromuscular endplate and G2 in skeletal muscle and blood forming cells. It possesses a high specific activity and the hydrolysis rate is  $2.4 \times 10^4$  molecules per second. This hydrolytic degradation ensures that the signal does not over stimulate the post-synaptic membrane. The enzyme is located on the surface of the post-synaptic membrane and linked by a GPI anchor. Inhibition of AChE is important both medically and toxicologically. Certain substances that covalently inhibit AChE are used as insecticides and as chemical warfare agents. Nowadays some inhibitors are used to treat various types of dementia such as myasthenia gravis, AD, PD (Millard and Broomfield 1995; Taylor 1998).

#### Butyrylcholinesterase

BuChE, widely distributed in liver, intestine, heart, kidney and lung, is a tetrameric protein, which exists as isoforms like G1, G2, G3, and G4 similar to AChE, of which G4 is the predominant isoform in the mature brain. It acts on both acetyl and butyrylcholine and its active site has a catalytic triad Ser198–His438–Glu325 (Cokugras 2003). Human BuChE has an acyl pocket with Phe 288 and Phe 290 which can occupy larger substrates to fit into the active for efficient catalysis than AChE. The role of BuChE is unclear and so far no endogenous natural substrate has been identified for this enzyme. BuChE is of pharmacological and toxicological importance; because it hydrolyzes ester-containing drugs and scavenges ChEI including potent organophosphorus nerve agents before they reach their synaptic targets. BuChE acts as a detoxifying enzyme capable of metabolizing aspirin, cocaine and heroin (Raveh *et al.* 1997). Cerebrospinal fluid of AD patients has specific glycosylated form of BuChE so it is suggested that assay of this specific form of BuChE is considered as diagnostic marker for detection of AD (Sáez-Valero and Small 2001). Moreover BuChE is responsible for progression of AD as it possess peptidase activity, which cleaves amyloid protein to A $\beta$  peptide, which deposits as amyloid plaques in brain (McKenna *et al.* 1997).

#### Acetylcholinesterase and butyrylcholinesterase

At the molecular level, AChE and BuChE share 65% amino-acid sequence homology and are encoded by different genes on human chromosomes 7 (7q22) and 3 (3q26) respectively (Soreq and Zakut 1993). Structural features of the two ChE enzymes confer differences in their substrate specificity. AChE is highly selective for ACh hydrolysis, while BuChE is able to metabolize several different molecules including various neuroactive peptides (Taylor and Radic 1994). The reason for substrate diversity between these enzymes is the variation of several amino acids in the sequence determining the three-dimensional size and shape of their active site gorge. It has been proposed that the efficiency with which AChE and BuChE hydrolyse ACh is dependent on the substrate concentration. AChE has greater catalytic activity at low ACh concentrations, resulting in substrate inhibition at higher doses (Soreq and Zakut 1993; Taylor and Radic 1994). However, BuChE is more efficient at high substrate concentrations. Both AChE and BuChE appear to be simultaneously active in the synaptic hydrolysis of ACh, terminating its neurotransmitter action, and co-regulating levels of ACh (Mesulam 2003). Both the enzymes therefore represent legitimate therapeutic targets for ameliorating the cholinergic deficit considered to be responsible for the declines in cognitive, behavioral and global functioning characteristic of AD.

## Cholinesterase inhibitors in dementia

The 'cholinergic hypothesis' was the basis for the development of presynaptic, synaptic and postsynaptic treatment approaches designed to maintain and facilitate the activity of the surviving cholinergic system. Synaptic ChE inhibition has proved preferable to direct receptor agonist therapy, as ChEIs amplify the natural spatial and temporal pattern of ACh release, rather than tonically or globally stimulating either nicotinic or muscarinic ACh receptors. One of the most promising strategies for treating this disease is to enhance the ACh level in brain using ChEIs (Enz *et al.* 1993). Several AChEIs are being investigated for the treatment of AD. However, only tacrine, donepezil, rivastigmine and galanthamine have been approved by the Food and Drug Administration in the USA (Zarotsky *et al.* 2003) for the symptomatic treatment of AD. These drugs improve cognitive and neuropsychiatric symptoms, and stabilize functioning over at least 6 months during clinical trials in patients with mild to moderate AD (Farlow 2002). Although of the same drug class, ChEIs are structurally diverse (Brufani and Filocamo 2000). Donepezil and galanthamine possess relative selectivity for AChE, whereas tacrine and rivastigmine co-inhibit both AChE and BuChE. However these drugs are known to have limitations for clinical use due to their short half-lives and unfavorable side effects such as hepatotoxicity and gastrointestinal disorders. So there is still a great interest in finding better ChEIs from natural sources (Sung *et al.* 2002).

### High throughput screening methods

Several methods for screening of AChE and BuChE inhibitory activity from natural resources have been reported based on Ellman's spectrophotometric method (Ellman *et al.* 1961). Apart from spectrophotometric methods, other methods such as thin-layer chromatography (Ingkaninan *et al.* 2000; Marston *et al.* 2002) and micro-plate assay (Ingkaninan *et al.* 2000; Brühlmann *et al.* 2004) have been reported to be useful. HPLC method for detection of AChE inhibition on immobilized AChE column (Andrisano *et al.* 2001) and HPLC with on-line coupled UV-MS-biochemical detection for AChE inhibitory activity (Ingkaninan *et al.* 2000) has also been reported.

## HERBAL DRUGS AS CHOLINESTERASE INHIBITORS

Nature is a rich source of biological and chemical diversity. The unique and complex structures of natural products cannot be obtained easily by chemical synthesis. In traditional practices numerous plants have been used to treat cognitive disorders, including neurodegenerative diseases and different neuropharmacological disorders. Ethnopharmacological approach and bioassay-guided isolation have provided a lead in identifying potential AChE and BuChE inhibitors from plant sources, including those for memory disorders. This article highlights on the plants and/or their active constituents so far reported to have ChE inhibitory activity as potential leads for the development of new drugs for the treatment of ChE related neurological disorders. A variety of plants has been reported to show AChE inhibitory activity and so may be relevant to the treatment of neurodegenerative disorders such as AD. A list of plants reported to have significant AChE inhibitory activity is shown in **Table 1**.

## ALKALOIDS

Generally inhibitors can be divided into two, those that bind to the active site at the bottom of the gorge and those that bind to the peripheral anionic site. As far as the alkaloidal inhibitors are concerned, binding takes place at the active site at the bottom of the gorge and the important features of an inhibitor appear to be a positively-charged

nitrogen, which binds to the oxyanion hole area, especially the Trp84, and a region, separated by a lipophilic area from the positive charge, which can form hydrogen bonds with the serine 200 residue and others such as His 440 of the catalytic triad inhibiting AChE activity. Some inhibitors bind to both the sites (Harel *et al.* 1995).

## Indole alkaloid

### Physostigmine

Physostigmine [Fig. 2 (1)], also known as eserine, is a reversible AChEI originally isolated from the seed of calabar bean (*Physostigma venenosum* L., Fabaceae). It has been used widely for different purposes, ranging from an historical role in rituals and primitive medicine, to its present-day use for the treatment neurological disorder such as AD and Myasthenia gravis. Physostigmine has been approved by US Food and Drug Administration (FDA) as an anticholinergic drug for the treatment of mild to moderate AD. It enhances short-term memory in dementia patients (Coelho Filho and Birks 2008). Limitation factors are shorter plasma half-life (~30 min) and high incidence of adverse effects such as nausea, vomiting and diarrhea (Duvoisin 1968). Despite of limitation it has been currently used in the formulation physostigmine salicylate (Synapton<sup>®</sup>).

### Structure activity-relationship

The carbonyl group in the carbamate moiety of the drug interacts with the hydroxyl group of serine 200 present in the catalytic triad of AChE to form an ester in the urethane part of the molecule. This interferes with the AChE activity of the enzyme, and the ester is slowly hydrolyzed to regenerate the active parent form. So the carbamate group acts as key factor in the AChE inhibitory activity. Moreover, the presence of an aromatic ring and a nitrogen atom facilitates the binding of inhibitor to AChE, interfering with its activity (Houghton *et al.* 2006).

### Rivastigmine

Rivastigmine [Fig. 2 (2)], an AChEI is licensed for use in UK for the symptomatic treatment of mild-to-moderately severe AD. Chemical structure of physostigmine has provided a template for the development of Rivastigmine (Lattin and Fifer 1995). Rivastigmine is reported to inhibit AChE in the cortex and hippocampus, brain areas involved in cognition. Thus, it is apparent that plant-derived alkaloid AChEIs may be important for the development of more appropriate drug candidates for the treatment of AD (Lattin and Fifer 1995). Since the backbone of rivastigmine is provided by physostigmine the mechanism of action is the same as that of physostigmine.

### Dehydroevodiamine HCl

Twenty-nine medicinal plants from South Korea were screened for their anti-ChE activity (Kim 2002). Results showed that dichloromethane extract of *Evodia rutaecarpa* (Rutaceae) showed maximum inhibition of 84.3%. Plant extract also exhibited anti-amnesic activity in the passive avoidance test in rats (Sprague Dawley) with scopolamine-induced memory loss. Bioactive compound responsible for the activity is dehydroevodiamine-HCl [Fig. 2 (3)] (Park *et al.* 1996, 2000).

### Voacangine and voacangine hydroxyindolenine

*Tabernaemontana australis* (Apocynaceae), which flourishes in Brazil, Uruguay and Argentina, has been poorly investigated with regards to its chemical composition and pharmacological activities. TLC assay using modified Ellman method of crude chloroform extract from stalk of *T. australis* showed the presence of AChEIs. GC-MS analysis

**Table 1** Plants exhibiting acetyl cholinesterase inhibitory activity.

Family	Plants	Parts	Type of extract	Activity (% of inhibition)	Concentration	References
Acanthaceae	<i>Acanthus ebracteatus</i>	Aerial part	Methanolic	36.19 ± 8.0	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
	<i>Andrographis paniculata</i>	Aerial part	Hydroalcoholic	50	222.4 µg/ml	Mukherjee <i>et al.</i> 2007
Anacardiaceae	<i>Semecarpus anacardium</i>	Stem bark	Methanolic	56.07 ± 0.28	20 µg/ml	Vinutha <i>et al.</i> 2007
Apocynaceae	<i>Tabernaemontana divaricata</i>	Roots	Methanolic	93.50 ± 70.37	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
	<i>Geissospermum vellosii</i>	Stem bark	Ethanollic	50	2.9 µg/mL	Lima <i>et al.</i> 2009
Buxaceae	<i>Buxus sempervirens</i>	Whole	Chloroform: methanol (1: 1)	61.76 ± 0.76	1 mg/ml	Orhan <i>et al.</i> 2004
Crassulaceae	<i>Rhodiola rosea</i>	Root	Methanol	42.00 ± 3.20	10 g/l	Hillhouse <i>et al.</i> 2004
Compositae	<i>Carthamus tinctorius</i>	Flower	Methanolic	30.33 ± 9.22	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
Convolvulaceae	<i>Evalvulus alsinoides</i>	Aerial parts	Hydroalcoholic extract	50	100-150 µg/ml	Mukherjee <i>et al.</i> 2007
Cyperaceae	<i>Cyperus rotundus</i>	Whole	Methanolic	44.19 ± 2.27	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
Coniferae	<i>Ginkgo biloba</i>	Whole	Ethanollic	50%	268.33 µg/ml	Perry <i>et al.</i> 1998; Das <i>et al.</i> 2002
Combretaceae	<i>Terminalia bellirica</i>	Fruit	Methanolic	39.68 ± 8.15	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
Ericaceae	<i>Rhododendron luteum</i>	Whole	Chloroform: methanol (1: 1)	76.32 ± 0.58	1 mg/ml	Orhan <i>et al.</i> 2004
	<i>Rhododendron ponticum</i>	Whole	Chloroform: methanol (1: 1)	93.03 ± 1.12	1 mg/ml	Orhan <i>et al.</i> 2004
Ebenaceae	<i>Diospyros rhodocalyx</i>	Bark	Methanolic	15.52 ± 3.6	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
Ericaceae	<i>Rhododendron luteum</i>	Whole	Chloroform: methanol (1: 1)	76.32 ± 0.58	1 mg/ml	Orhan <i>et al.</i> 2004
	<i>Rhododendron ponticum</i>	Whole	Chloroform: methanol (1: 1)	93.03 ± 1.12	1 mg/ml	Orhan <i>et al.</i> 2004
Euphorbiaceae	<i>Euphorbia antiquorum</i>	Stem	Methanolic	42.31 ± 9.10	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
Fumariaceae	<i>Fumaria vaillantii</i>	Whole	Chloroform: methanol (1: 1)	94.23 ± 0.47	1 mg/ml	Orhan <i>et al.</i> 2004
	<i>Fumaria capreolata</i>	Whole	Chloroform: methanol (1: 1)	96.89 ± 0.17	1 mg/ml	Orhan <i>et al.</i> 2004
	<i>Fumaria kralikii</i>	Whole	Chloroform: methanol (1: 1)	84.98 ± 0.07	1 mg/ml	Orhan <i>et al.</i> 2004
	<i>Fumaria asepala</i>	Whole	Chloroform: methanol (1: 1)	91.99 ± 0.70	1 mg/ml	Orhan <i>et al.</i> 2004
	<i>Fumaria densiflora</i>	Whole	Chloroform: methanol (1: 1)	93.42 ± 0.92	1 mg/ml	Orhan <i>et al.</i> 2004
	<i>Fumaria flabellate</i>	Whole	Chloroform: methanol (1: 1)	92.14 ± 1.01	1 mg/ml	Orhan <i>et al.</i> 2004
	<i>Fumaria petterii</i>	Whole	Chloroform: methanol (1: 1)	89.45 ± 0.86	1 mg/ml	Orhan <i>et al.</i> 2004
	<i>Fumaria macrocarpa</i>	Whole	Chloroform: methanol (1: 1)	93.43 ± 0.64	1 mg/ml	Orhan <i>et al.</i> 2004
	<i>Fumaria cilicica</i>	Whole	Chloroform: methanol (1: 1)	88.03 ± 0.65	1 mg/ml	Orhan <i>et al.</i> 2004
	<i>Fumaria parviflora</i>	Whole	Chloroform: methanol (1: 1)	87.02 ± 0.31	1 mg/ml	Orhan <i>et al.</i> 2004
	<i>Fumaria judaica</i>	Whole	Chloroform: methanol (1: 1)	96.47 ± 0.63	1 mg/ml	Orhan <i>et al.</i> 2004
Fabacea	<i>Vicia faba</i> Linn.	Whole	Methanolic	45.23 ± 1.03	1 mg/ml	Orhan <i>et al.</i> 2004
Guttiferae	<i>Mammea harmandii</i>	Flower	Methanolic	33.6378.00	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
Hypericaceae	<i>Hypericum undulatum</i>	Aerial parts	Ethanollic	68.4 ± 4.7	0.5 mg/ml	Ferreira <i>et al.</i> 2006
Lamiaceae	<i>Salvia lavandulaefolia</i> Vahl.	Whole	Steam distilled oil	63.0 ± 3.7	0.1 mg/ml	Perry <i>et al.</i> 1996, 2000, 2001
	<i>Salvia officinalis</i>	Whole	Ethanol	68.2 ± 15.6	2.5 mg/ml	Perry <i>et al.</i> 1996, 2000, 2001
	<i>Salvia triloba</i>	Whole	Ethanollic	54.3 ± 3.2	2 mg/ml	Orhan <i>et al.</i> 2008
	<i>Salvia sclareoides</i>	Aerial parts	Acetone	100	1 mg/ml	Rautera <i>et al.</i> 2007
			Ethanol	77		
			Water	67		
			Butanol	100		
	<i>Teucrium polium</i>	Whole	Ethanollic	65.8 ± 3.7	2 mg/ml	Orhan <i>et al.</i> 2008
	<i>Melissa officinalis</i>	Whole	Ethanollic	39.3 ± 3.72	2 mg/ml	Orhan <i>et al.</i> 2008
	<i>Mentha suaveolens</i>	Aerial parts	Essential oil	52.4 ± 2.5	1 mg/ml	Ferreira <i>et al.</i> 2006
	<i>Isodon wightii</i>	Leaf	Acetone	50	250 µg/ml	Thirugnanasampandan <i>et al.</i> 2008
Lauraceae	<i>Laurus nobilis</i>	Leaf	Ethanollic	64 ± 0.3	1 mg/ml	Ferreira <i>et al.</i> 2006
Lycopodiaceae	<i>Lycopodium clavatum</i>	Whole	Chloroform: methanol (1: 1)	49.85 ± 1.33	1 mg/ml	Orhan <i>et al.</i> 2004
Leguminosae	<i>Albizia procera</i>	Bark	Methanolic	40.71 ± 0.46	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
	<i>Butea superba</i>	Root barks	Methanolic	55.87 ± 5.83	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
	<i>Cassia fistula</i>	Roots	Methanolic	54.13 ± 3.90	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
	<i>Derris scandens</i>	Whole	Methanolic	20.76 ± 1.17	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
	<i>Mimosa pudica</i>	Whole	Methanolic	21.40 ± 6.68	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
Malvaceae	<i>Abutilon indicum</i>	Whole	Methanolic	30.66 ± 1.06	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
Magnoliaceae	<i>Michelia champaca</i>	Leaf	Methanolic	34.88 ± 4.56	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
Mackinlayaceae	<i>Centella asiatica</i>	Aerial parts	Hydroalcoholic	50	100-150 µg/ml	Mukherjee <i>et al.</i> 2007
Musaceae	<i>Musa sapientum</i>	Fruit	Methanolic	29.14 ± 4.73	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
Menispermaceae	<i>Stephania suberosa</i>	Roots	Methanolic	91.93 ± 10.80	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
	<i>Tiliacora triandra</i>	Roots	Chloroform: methanol (1: 1)	42.29 ± 2.89	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
	<i>Tinospora cordifolia</i>	Stem	Methanolic	77.74 ± 0.38	100 µg/ml	Vinutha <i>et al.</i> 2007
Moraceae	<i>Strobilus asper</i>	Seed	Methanolic	30.51 ± 74.21	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
	<i>Ficus religiosa</i> L.	Stem bark	Methanolic	52.60 ± 2.91	80 µg/ml	Vinutha <i>et al.</i> 2007
Myrsinaceae	<i>Embelia ribes</i>	Roots	Methanolic	72.63±0.69	60 µg/ml	Vinutha <i>et al.</i> 2007
Myristicaceae	<i>Myristica fragrans</i>	Aerial parts	Hydroalcoholic	50	100-150 µg/ml	Mukherjee <i>et al.</i> 2007
Nelumbonaceae	<i>Nelumbo nucifera</i>	Stamen	Methanolic	23.77 ± 2.83	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
		Aerial parts	Hydroalcoholic extract	50	185.55 µg/ml	Mukherjee <i>et al.</i> 2007
Papaveraceae	<i>Corydalis solida</i>	Whole	Chloroform: methanol (1: 1)	87.56 ± 1.24	1 mg/ml	Orhan <i>et al.</i> 2004
	<i>Glaucium corniculatum</i>	Whole	Chloroform: methanol (1: 1)	86.55 ± 0.67	1 mg/ml	Orhan <i>et al.</i> 2004
Piperaceae	<i>Piper interruptum</i>	Stems	Methanolic	65.16 ± 8.13	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
	<i>Piper nigrum</i>	Seeds	Methanolic	58.02 ± 3.83	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003

Table 1 (Cont.)

Family	Plants	Parts	Type of extract	Activity (% of inhibition)	Concentration	References
Plumbaginaceae	<i>Plumbago indica</i>	Root	Methanolic	30.14 ± 3.28	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
Rosaceae	<i>Prunus persica</i>	Aerial parts	chloroform	50	5.6 µg/ml	Suh <i>et al.</i> 2006
	<i>Sanguisorba minor</i>	Aerial parts	Ethanollic	77.5 ± 2.2	1 mg/ml	Ferreira <i>et al.</i> 2006
Rutaceae	<i>Aegle marmelos</i>	Fruit pulp	Methanolic	44.65 ± 3.04	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
Rubiaceae	<i>Paederia linearis</i>	Whole	Methanolic	29.31 ± 6.39	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
Sapotaceae	<i>Mimusops elengi</i>	Flower	Methanolic	32.81 ± 5.36	0.1 mg/ml	Ingkaninan <i>et al.</i> 2003
Solanaceae	<i>Withania somnifera</i> Dunal	Root	Methanolic	74.37 ± 0.38	100 µg/ml	Vinutha <i>et al.</i> 2007
Scrophulariaceae	<i>Bacopa monniera</i>	Whole	Ethanollic	42.9 ± 1.2	0.1 mg/ml	Das <i>et al.</i> 2002
Tamaricaceae	<i>Myricaria elegans</i>	Aerial	Methanolic	74.8 ± 7.4	0.2 mg/ml	Ahmad <i>et al.</i> 2003
Valerianaceae	<i>Nardostachys jatamansi</i> DC	Rhizome	Methanolic	69.24 ± 0.15	100 µg/ml	Vinutha <i>et al.</i> 2007

showed that compound responsible for inhibitory activity is the presence of indole alkaloids such as coronaridine, voacangine, voacangine hydroxyindolenine and rupicoline. TLC analysis of AChE inhibitory activity showed that voacangine and voacangine hydroxyindolenine [Fig. 2 (4, 5)] exhibited detectable spot at the minimum concentration of 25 µM (Andrade *et al.* 2005).

#### Turbinatine and desoxycordifoline

Nine indole alkaloids from *Chimarrhis turbinata* (Rubiaceae) bark and leaves were screened for AChE inhibitory activity. Turbinatine (IC<sub>50</sub> 0.1 µM) and desoxycordifoline (IC<sub>50</sub> 1.0 µM), [Fig. 2 (6, 7)] showed moderate inhibitory activity (Cardoso *et al.* 2004).

#### Legucina A

Four indole alkaloids have been isolated from *Desmodium pulchellum* and *D. gangeticum* (Papilionaceae). All compounds were active against AChE with legucina A being the most active with IC<sub>50</sub> 27 µM (Ghosal *et al.* 1972).

#### Geissospermine

*Geissospermum vellosii* (Apocynaceae) is widely distributed throughout the Amazonian forest and has been frequently used by the native population for painful disorders. Screening for AChE inhibitory activity showed that alkaloid rich fraction from stem bark extract exhibited inhibitory activity to rat brain and electric eel AChE, as well as to horse serum BuChE in a concentration-dependent manner with mean IC<sub>50</sub> values of 39.3, 2.9 and 1.6 µg/ml, respectively. Bioactive guided fractionation showed that the main alkaloid with anti-ChE activity was geissospermine [Fig. 2 (8)]. On treatment with geissospermine significant reduction in scopolamine-induced amnesia in the passive avoidance and Morris water maze tests, at 30 mg/kg i.p. (given 45 min before the test sessions) was observed. These results show that compounds present in *G. vellosii* stem-bark have anti-ChE activity, and that they can revert cognitive deficits in a model of cholinergic hypo function (Lima *et al.* 2009).

#### Steriodal alkaloid

##### Galanthamine

Galanthamine [Fig. 2 (9)], an alkaloid isolated from some *Galanthus nivalis* and *Leucojum aestivum* (Amaryllidaceae), has been recently in use in the treatment of AD. It acts as reversible competitive AChEI rather than BuChE and also modulates the nicotinic ACh receptors (Thomsen and Kewitz 1990; Bores *et al.* 1996; Schrattenholz 1996). Initially derived from extracts of snowdrop and daffodil bulbs, this phenanthrene alkaloid is now synthetically produced. It provides complete oral bioavailability. Half-life period of galanthamine is 6 hr. Although the most common side effect of galanthamine is nausea, it is possible to eliminate nausea by increasing the galanthamine dose gradually (Raskind *et*

*al.* 2000). Additionally, galanthamine was shown to have no hepatotoxicity. Galanthamine (Nivalin<sup>®</sup>) has been approved as HBr salt in Austria and later licensed as Reminyl<sup>®</sup> in the USA and some European countries in the treatment of AD. Extracts from *Narcissus* and *Galanthus* spp. of Turkey were screened for AChE inhibitory activity and it was suggested that the alkaloids having galanthamine and lycorine skeletons such as assoanine, epinorgalanthamine, oxoassoanine, sanguinine, 11-hydroxygalanthamine have also been reported to possess AChE activity (Lopez *et al.* 2002). Over 2000 patients have been involved in double-blind placebo-controlled trials of galanthamine where positive effects on cognitive symptoms have been associated with significant benefits in activities of daily living (Da-Yuan *et al.* 1996).

#### Structure-activity relationship

Galanthamine binds at the base of the active site gorge of AChE, interacting with both the choline-binding site (Trp-84) and the acyl-binding pocket (Phe-288, Phe-290). The tertiary amine group of galanthamine does not interact closely with Trp-84; rather, the double bond of its cyclohexene ring stacks against the indole ring. The tertiary amine appears to make a non-conventional hydrogen bond, via its N-methyl group, to Asp-72, near the top of the gorge. The hydroxyl group of the inhibitor makes a strong hydrogen bond (2.7 Å) with Glu-199. The relatively tight binding of galanthamine to AChE appears to arise from a number of moderate to weak interactions with the protein, coupled to a low entropy cost for binding due to the rigid nature of the inhibitor (Greenblatt *et al.* 1999).

#### Homomoenjodaramine and moenjodaramine

*Buxus* spp. (Buxaceae) a widely spread plants in Turkey have long been known as rich sources of new and biologically active triterpenoidal alkaloids. In the indigenous system of medicine, the extracts of genus *Buxus* are reported to be useful in various disorders such as malaria, rheumatism and skin infections. Anti-HIV activity was also reported from the EtOH extract of *B. sempervirens*. Screening for anti-ChE activity in *B. hyrcana* showed that homomoenjodaramine and moenjodaramine [Fig. 2 (10, 11)] the steroid alkaloids are the bioactive compounds responsible for the AChE inhibitory activity with IC<sub>50</sub> value of 19.2 and 50.8 mM, respectively (Atta-ur-Rahman and Choudhary 1999). Type of inhibition is non-competitive type. *B. sempervirens* showed 50% inhibition to both AChE and BuChE at the concentration 1 mg/ml (Rahman A-ur *et al.* 1998). Triterpenoids from *B. papillosa* also exhibited inhibitory activity to both AChE and BuChE.

#### Structure-activity relationship

Structure of homomoenjodaramine and moenjodaramine differs only by the substitution at C-3 and C-20 position. Inhibitory activity of both the compounds is due to amino substituents in C-3 and C-20 position.

## Pregnane-type steroidal alkaloids

*Sarcococca hookeriana*

Bioguided phytochemical investigation of *Sarcococca hookeriana* (Buxaceae) with respect to the ChE enzyme inhibitory assay yielded two new pregnane-type steroidal alkaloids hookerianamide H and hookerianamide I [Fig. 2 (12, 13)] along with three known alkaloids *N*- $\alpha$ -methyl-

epipachysamine D, sarcovagine C and dictyophlebine [Fig. 2 (14, 15, 16)]. All compounds showed good inhibitory activities against the enzymes AChE with IC<sub>50</sub> value ranging from 2.9 to 34.1 mM and BuChE with IC<sub>50</sub> value of 0.3-3.6 mM (Khalid *et al.* 2004).

## Structure-activity relationship

Structure activity relationship shows that tigloyl amino

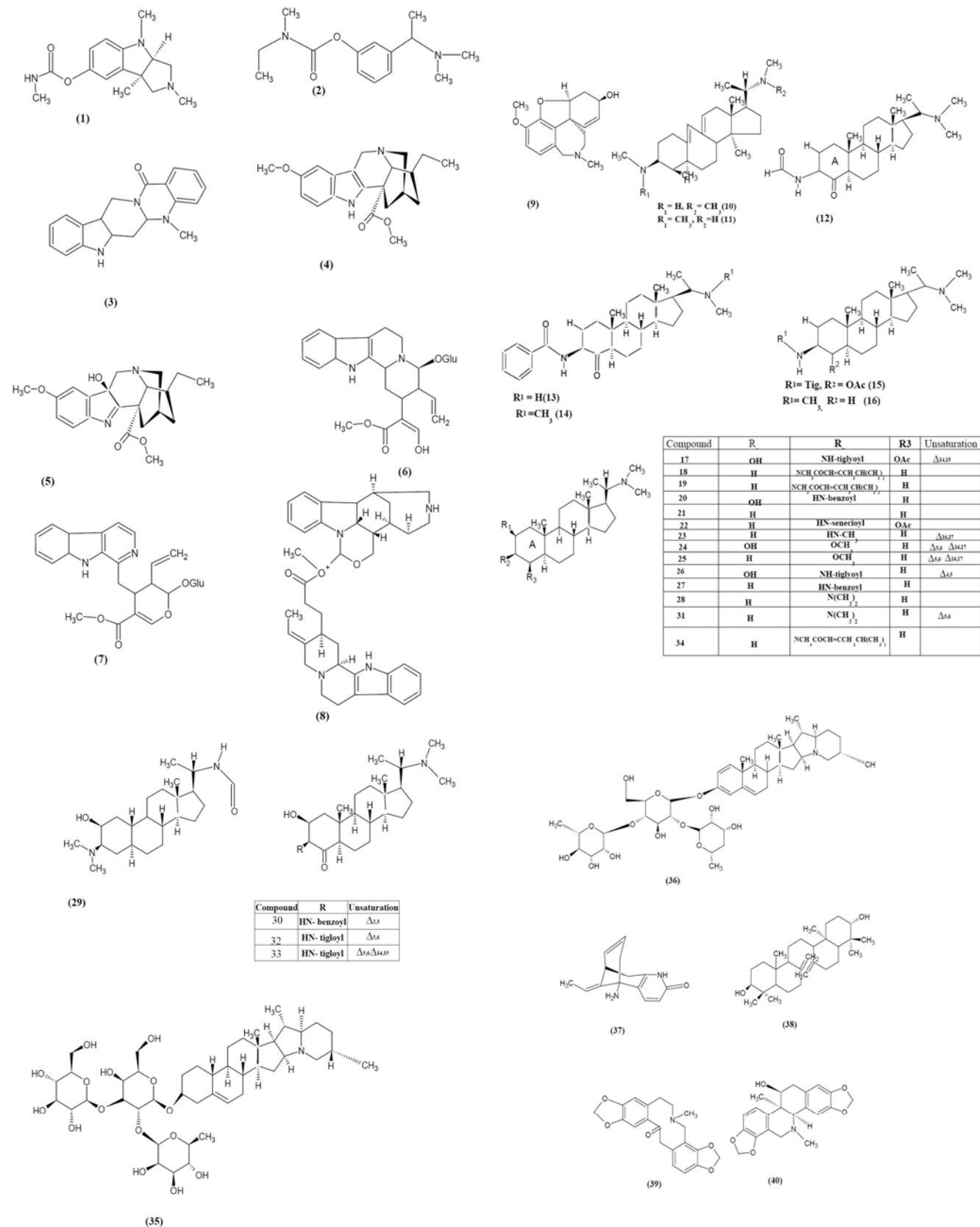


Fig. 2 (1-40) Phytoconstituents exhibiting Cholinesterase inhibitory activity.

group at position C-3 and the carbonyl moiety in ring A are responsible for inhibitory activity.

### *Sarcococca saligna*

#### **Steroidal alkaloids from *Sarcococca saligna***

The crude ethanolic extract of *Sarcococca saligna* (Buxaceae) was assessed for AChE and BuChE inhibitory activity according to the Ellman method. Bioactive guided fractionation of the extract showed the presence of several pregnane-type steroidal alkaloids which showed dual cholinergic activity. Salignenamides-C, -E and -F, Axillarine-C, Saliginnamide, Vaganine-A, 5,6-dehydrosarconidine, 2-hydroxysalignarine-E, Salignamine, 2-hydroxysalignamine-E, Epipachysamine-D, Dictyophlebine, iso-*N*-formylchone-morphine and Axillaridine-A [Fig. 2 (17-30)] inhibited both AChE and BuChE non-competitively; Sarcorine, Sarcodine [Fig. 2 (31)], Sarsalignone and Sarsalignone inhibited BuChE uncompetitively [Fig. 2 (32, 33)]; Salignenamide-A [Fig. 2 (34)] inhibited AChE uncompetitively and 2-hydroxysalignarine-E [Fig. 2 (26)] was identified as a mixed type inhibitor of AChE, producing a combination of partially competitive and non-competitive inhibition.  $K_i$  values were found to be in the range of 2.65–250.0  $\mu\text{M}$  against AChE and 1.63–30.0  $\mu\text{M}$  against BuChE.

#### **Structure-activity relationship**

The structure activity relationship studies suggested that the major interaction of the enzyme-inhibitor complexes is due to hydrophobic and cation- $\pi$  interactions inside the aromatic gorge of these ChE's. Studies shows that polycyclic compounds penetrate the aromatic gorge with ring A entering first due to its hydrophobic character, or due to increased electropositivity of the substituent in ring A. The nitrogen substituents at C-3 and C-20 which are protonated at physiological pH is an important structural feature for inhibitory potency. Moreover the stability of AChEI complex is due to formation of cation- $\pi$  interaction between amino group of the inhibitor and the Trp279, Tyr70 and Tyr121 residue at the peripheral site of AChE (Khalid *et al.* 2004, 2005).

#### **$\alpha$ -Solanine and $\alpha$ -chaconine**

The steroidal alkaloids are found in a relatively small number of plant families and they possess variety of structures. A prominent family in this respect is the Solanaceae and the toxicity of the green parts of members of *Solanum* and related species can be largely due to the presence of these  $\alpha$ -Solanine and  $\alpha$ -chaconine [Fig. 2 (35, 36)]. Both the compounds exhibited strong AChE inhibitory effect and this could well explain the gastrointestinal and CNS disturbances produced by eating plant material containing high levels of these compounds (Wierenga and Hollingworth 1992).

#### **Quinolizidine alkaloid**

##### **Huperzine A**

Huperzine A [Fig. 2 (37)], a lycopodium alkaloid isolated from the clubmoss *Lycopodium serratum* (Lycopodiaceae), has been used in traditional Chinese medicine for its memory-enhancing property for centuries (Liu *et al.* 1986). Over 100 alkaloids Huperzine A-R, have been isolated from the genus *Lycopodium* of them, only Huperzine A possessed remarkable AChE inhibitory activity. The activity of Huperzine A has been found to be as high as physostigmine, galanthamine, donepezil and tacrine, the commercial drugs already used against AD. In various *in vivo* and *ex vivo* experiments, it has been shown to inhibit AChE reversibly and also to prevent oxidative cell damage induced by  $\beta$ -amyloid plaques (Wang *et al.* 1986; Tang 1996; Jing *et al.* 1999; Tang and Han 1999; Ye *et al.* 1999).  $\alpha$ -onocerin [Fig. 2 (38)], a triterpene-type compound, from *Lycopodium cla-*

*vatum* also showed 50% activity against AChE (Orhan *et al.* 2003). Huperzine A is also a NMDA receptor antagonist which protects the brain against glutamate induced damage, and it increases nerve growth factor levels. Side effects may include breathing problems, tightness in the throat or chest, chest pain, skin hives, rash, itchy or swollen skin, upset stomach, diarrhea, vomiting, hyperactivity and insomnia.

#### **Structure-activity relationship**

In Huperzine A the  $\alpha$ -pyridine moieties interact with the "anionic" sub-site of the active site, primarily through  $\pi$ - $\pi$  stacking and through van der Waals or C-H  $\pi$ - $\pi$  interactions with Trp84 and Phe330. The carbonyl oxygen of the drug appears to repel the carbonyl oxygen of Gly117, thus causing the peptide bond between Gly117 and Gly118 to undergo a peptide flip. As a consequence, the position of the main chain nitrogen of Gly118 in the "oxyanion" hole in the native enzyme becomes occupied by the carbonyl of Gly117. Furthermore, the flipped conformation is stabilized by hydrogen bonding of oxygen molecule of Gly117 to nitrogen atom of Gly119 and Ala201, the other two functional elements of the three-pronged "oxyanion hole". Thus Huperzine A prevents the hydrolysis of ester substrates whether in charged or neutral form (Dvir *et al.* 2002).

#### **Isoquinoline alkaloid**

In alkaloids with bezylisoquinoline skeleton the AChE inhibitory activity is due to presence of a quaternary nitrogen atom at the tetrahydroisoquinoline portion of the alkaloids.

##### **Protopine**

In the course of screening Korean natural product for ChE's inhibitory activity, crude methanolic extract prepared from tubers of *Corydalis ternate* (Papaveraceae) exhibited potent AChE inhibitory activity. Bioactivity-directed fractionation afforded protopine [Fig. 2 (39)], an alkaloid-type compound which exhibited reversible competitive type inhibition with an  $\text{IC}_{50}$  value of 30.5  $\mu\text{M}$ . This result was supported by passive avoidance test, which is used to measure anti-amnesic activity, in male mice. Anti AChE activity and anti-amnesic activity of protopine increases its therapeutic value in the treatment of dementia (Davis *et al.* 1999).

##### **Corynoline**

*Corydalis incisa* (Papaveraceae), which is widely distributed in Korea, has been used as a folk medicine in China and Japan for the treatment of inflammation, skin diseases and headache. It has also been used for the treatment of stomach, liver and abdominal pains, as well as a detoxifying remedy (Kim *et al.* 1999). Methanolic extract of aerial parts of *C. incisa* exhibited AChEI activity. Corynoline [Fig. 2 (40)] an isoquinoline alkaloid exhibited reversible non-competitive inhibition with an  $\text{IC}_{50}$  value of 50  $\mu\text{M}$  which can be used for the treatment of AD (Ma *et al.* 1999).

##### **Bulbocapnine, Corydaline and Corydine**

*Corydalis cava* (Papaveraceae) has been traditionally used as memory enhancer in Danish folk medicine. Methanolic extract of tubers from *C. cava* showed significant AChE inhibitory activity in a dose-dependent manner. Activity guided fractionation of the methanolic extract resulted in the isolation of three alkaloids, bulbocapnine, corydaline and corydine [Fig. 2 (41, 42, 43)] as active constituents. Bulbocapnine inhibited AChE as well as BuChE in a dose-dependent manner with  $\text{IC}_{50}$  values of  $40 \pm 2$  and  $83 \pm 3$   $\mu\text{M}$ , respectively. Corydaline inhibited AChE in a dose-dependent manner with an  $\text{IC}_{50}$  value of  $15 \pm 3$   $\mu\text{M}$  and corydine inhibited BuChE in a dose-dependent manner with an  $\text{IC}_{50}$  value of  $52 \pm 4$   $\mu\text{M}$ . Corydaline was considered inactive against BuChE and corydine against AChE, due to



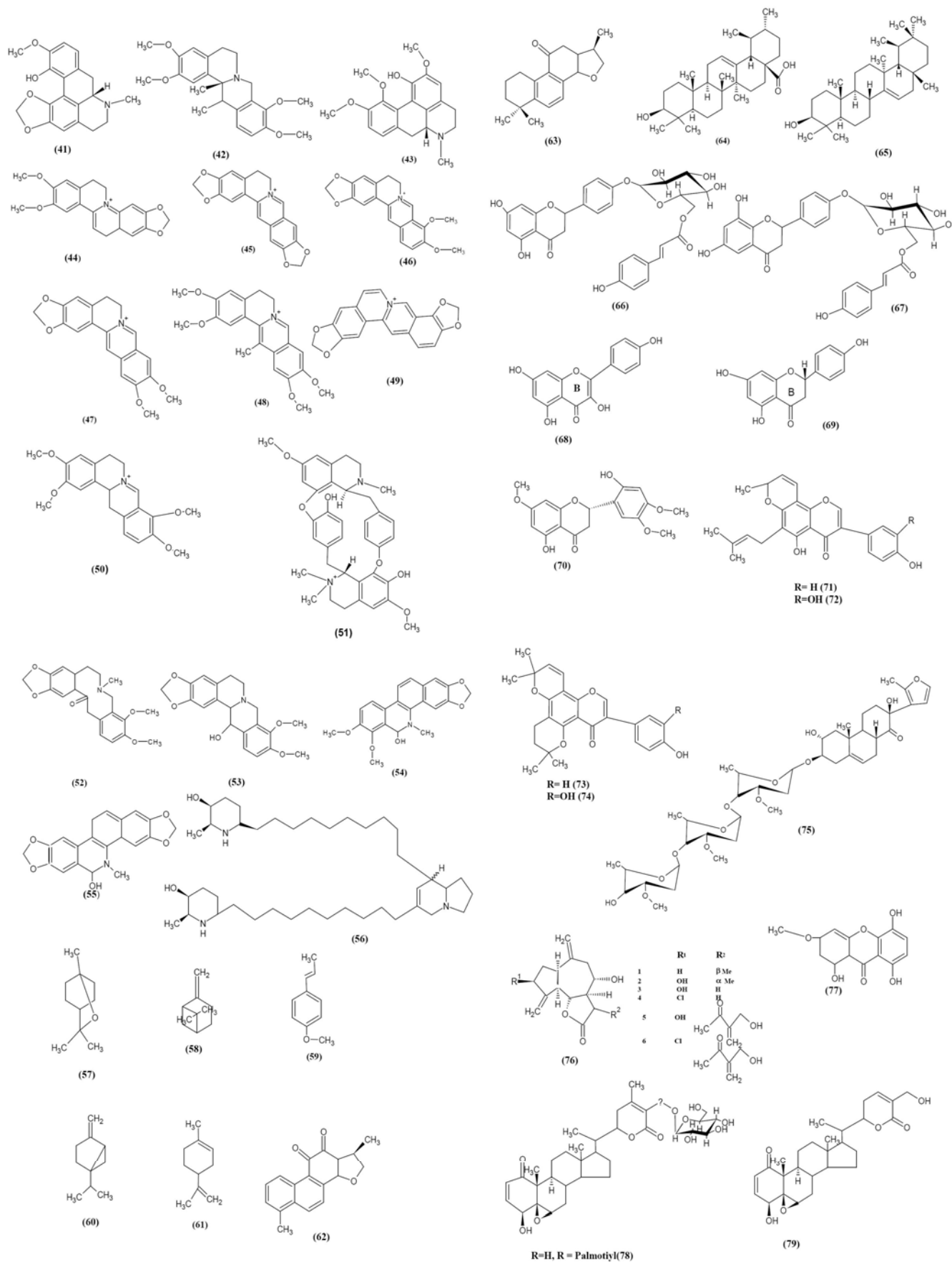


Fig. 2 (41-79) Phytoconstituents exhibiting Cholinesterase inhibitory activity.

IC<sub>50</sub> > 100 μM (Adersen *et al.* 2007).

### Berberine and its derivatives

*Corydalis turtschaninovii* (Papaveraceae) have been used in

traditional medicine for the treatment of gastric, duodenal ulcer, cardiac arrhythmia disease, rheumatism, dysmenorrheal and memory. In the course of screening plants used in Korean folk medicine as memory enhancers, a 70% ethanol extract of tuber from *C. turtschaninovii* (100 μg/ml)

showed significant AChE inhibitory activity. Bioactive guided fractionation showed that alkaloids such as 7-Epiberberine, Pseudocopsitine, Berberine, Pseudoberberine, Pseudo-dehydrocorydaline [Fig. 2, (44-48)] are responsible for inhibitory activity with IC<sub>50</sub> values of 6.5 ± 0.5, 8.4 ± 0.5, 4.7 ± 0.2, 4.3 ± 0.3, 4.5 ± 0.2 μM, respectively (Hung *et al.* 2008).

*Coptis chinensis* (Ranunculaceae) has been used in traditional Chinese medicine for several conditions including age related cognitive and memory decline. Anti-ChEI activity of methanolic extract of *C. chinensis* is due to presence of alkaloids berberine [Fig. 2 (46)], coptisine [Fig. 2 (47)] and palmatine [Fig. 2 (50)] (Shigeta *et al.* 2002). *C. chinensis* extract improved a scopolamine-induced learning and memory deficit in rats and this is likely to be due to the alkaloids present raising ACh levels. Berberine has been shown to be selectively active against AChE compared with BuChE (Kuznetsova *et al.* 2002) and it has been shown to improve scopolamine-induced amnesia in rats.

### Protoberberine alkaloids

Bioactive guided fraction of tuber extract of *Stephania venosa* (Menispermaceae) were screened for AChE inhibitory activity (Ingkaninan *et al.* 2006). Quaternary protoberberine alkaloids such as stephanine, cyclanoline and *N*-methyl stepholidine exhibited inhibitory activity on AChE with IC<sub>50</sub> values of 14.10 ± 0.81, 9.23 ± 3.47 and 31.30 ± 3.67 μM, respectively.

### Tubocurarine

*Tubocurarine* obtained from *Chondodendron tomentosum* (Menispermaceae) is a muscle relaxant used in clinical surgery. Pharmacological action of tubocurarine [Fig. 2 (51)] shows that it competes with ACh to bind with nicotinic receptors and also inhibits the AChE activity to some extent (Cousin *et al.* 1996).

### Alkaloids from *Fumaria* species

*Fumaria* species (Fumarioideae), wide-spread in Turkey and the richest source of isoquinoline alkaloids, many of which possess remarkable biological activities, were screened for ChE inhibitory activity. Results showed that all *Fumaria* spp. exhibited significantly higher activity when compared to standard galanthamine ranging from 84.9 to 96.8%. Of the 19 species *F. vaillantii* exhibited maximum inhibition of 94.2%. Bioassay guided fractionation of *F. vaillantii* revealed that many isoquinoline alkaloids are responsible for activity. Among them β-allocriptopine, ophiocarpine, berberine [Fig. 2 (52, 53, 46)], and protopine [Fig. 2 (39)], exhibited major AChEI activity and the activity of whole extract may be due to the synergistic interaction between these alkaloids, which may be of therapeutic value in the treatment of AD (Orhan *et al.* 2003).

### Alkaloids from *Chelidonium majus*

*Chelidonium majus* L. (Papaveraceae) has been traditionally used as an herbal medicine for treatment of gastric ulcer, gastric cancer, oral infection, liver disease, and general pains in Asian and European countries. Ethanolic extract of the aerial portion of *C. majus* inhibited AChE activity without a significant inhibition of BuChE. Bioactive guided fractionation showed that 8-hydroxydihydrochelerythrine, 8-hydroxydihydrosanguinarine and berberine [Fig. 2 (54, 55, 46)] exhibited potent inhibitory activity against AChE, with IC<sub>50</sub> values of 0.61-1.85 mM (Cho *et al.* 2006).

### Piperidine alkaloids

#### Juliflorine

*Juliflorine* [Fig. 2 (56)] has been isolated from the leaves of

*Prosopis juliflora* (Papilionaceae) and it noncompetitively inhibits both AChE (IC<sub>50</sub> = 0.42 μM) and BuChE (IC<sub>50</sub> = 0.12 μM). AChE-juliflorine complex is stabilized by hydrogen bonding, hydrophobic interaction and π-π interaction (Choudhary *et al.* 2005).

### TERPENOIDS

Terpenoids comprise a very large group of natural products and comprise two or more branched 5 carbon units, formed from a common precursor named mevalonic acid. Skeletons consisting of multiplets of 2, 3, 4 or 6 of these linked together in many different ways are found in a variety of mostly cyclic compounds named monoterpenes (10 carbons in the skeleton), sesquiterpenes (15 carbons), diterpenes (20 carbons) and triterpenoids (30 carbons), respectively. These compounds tend to be lipophilic, so they are able to cross the blood-brain barrier, and the monoterpenes, and some of the sesquiterpenes, are volatile, and so effects could occur through inhalation. These compounds are responsible for the strong odors and flavors of many herbs, spices and traditional medicines. Many such molecules are increasingly recognized as having a variety of roles in living organisms, including signaling between members of the same species, thus comprising part of the group of compounds known as pheromones, and protective or attractant roles in flowering plants against herbivores and pollinators respectively. An effect on CNS activity by the volatile substances in perfumes and other odoriferous materials has attracted interest in recent years and one of the first findings that monoterpenes had AChE inhibitory effects was made only in the mid 1990s in studies investigating historical records that monoterpene-containing plants were 'good for the memory'.

#### Monoterpenoids

Amongst plants that have been investigated for dementia therapy, *Salvia* is one of the most numerous genera within the family Lamiaceae and grows in many parts of the world. It has been used as memory enhancer in European folk medicine. An ethanolic extract and oil of *S. officinalis* and *S. lavandulaefolia* were investigated for anti-ChE activity and it was found that all gave inhibition of AChE at quite low concentrations (Perry *et al.* 2000). The ChE inhibition shown by the *S. lavandulaefolia* oil was shown to be partly due to the cyclic monoterpenes 1,8-cineole and α-pinene [Fig. 2 (57, 58)], which were shown to inhibit AChE *in vitro*, with some contribution from other constituents, perhaps by acting synergistically (Savelev *et al.* 2003). Anti-ChEI activity of monoterpenes is due to hydrocarbon skeleton. Since the effects of the oil were better than those of individual monoterpenes, further *in vivo* and clinical studies, described below, were carried out on the essential oils, which consist of a mixture of monoterpenes, rather than isolated compounds. Oral administration of *S. lavandulaefolia* essential oil to rat's decreased striatal AChE activity in both the striatum and the hippocampus compared to the control rats. Thus, it appeared that constituents of the *S. lavandulaefolia* oil, or their metabolites, reach the brain and inhibit AChE in select brain areas, consistent with evidence of inhibition of the brain enzyme *in vivo* (Perry *et al.* 2002). Clinical studies on human volunteers and even patients with AD have been reported in recent years. A small trial with 11 patients showing mild to moderate symptoms of AD showed that oral administration of the essential oil of *S. lavandulaefolia* significantly improved cognitive function in one of the three different methods of assessment used (Tildesley *et al.* 2003). Anti BuChE activity of *S. lavandulaefolia*, *S. fruticosa* and *S. officinalis* showed that essential oil of *S. officinalis* and *S. fruticosa* exhibited activity due to presence of β-pinene, 3-carene, sabinene and camphor in time dependent manner. Thus oils of *S. officinalis* and *S. fruticosa* which possess dual cholinergic activity can be used to treat severe AD, while *S. lavandulaefolia* can be used to treat mild AD.

*Rosmarinus officinalis* and *Melissa officinalis* (Lamiaceae) extracts was investigated for ChE inhibitory activity. Methanolic extract of *R. officinalis* showed the highest *in vitro* inhibitory activity. Bioactive-guided fractionation showed the activity is due to presence of essential oil. 1,8-Cineol (57) exhibited 44.42% and  $\alpha$ -pinene (58) showed 12.57% inhibition. Rosmarinic acid, a phenolic compound in rosemary, exhibited the highest inhibitory activity of 85.8% towards AChE (Chung *et al.* 2001).

*Pimpinella anisoides* (Apiaceae), an aromatic plant and spice widely distributed in Italy. Ethanolic extract from fruits of *P. anisoides* was assessed for ChE inhibitory activity. Results showed that the extract, exhibited activity against both AChE and BuChE, with IC<sub>50</sub> values of 227.5 and 362.1  $\mu$ g/ml, respectively. Bioactive guided fractionation showed the presence of terpenoids like *trans*-anethole, (+)-limonene and (+)-sabinene [Fig. 2 (59, 60, 61)] of which *trans*-Anethole exhibited the highest activity against AChE and BuChE with IC<sub>50</sub> values of 134.7 and 209.6  $\mu$ g/ml, respectively (Menichini *et al.* 2009). The bicyclic monoterpene (+)-sabinene exhibited a promising activity against AChE (IC<sub>50</sub> - 176.5  $\mu$ g/ml) and BuChE (IC<sub>50</sub> - 218.6  $\mu$ g/ml).

### Diterpenoids

Another sage species *S. miltiorrhiza* (Lamiaceae) which has been used in traditional Chinese medicine for heart and calm nerves has been investigated for AChE inhibitory effect. Root extract showed inhibitory effect, which is due to presence of diterpenes tanshinones. Dihydrotanshinone [Fig. 2 (62)] was shown to be the most active (IC<sub>50</sub> = 1.0  $\mu$ M) with cryptotanshinone [Fig. 2 (63)] (IC<sub>50</sub> = 7.0  $\mu$ M) also showing activity. A feature, which appears to be necessary for activity, is the saturated bond in the furan ring of the molecules (Ren *et al.* 2004).

### Triterpenoids

#### Ursolic acid

AChE inhibitory activity of *Origanum majorana* (Lamiaceae) extract is due to the presence of active component ursolic acid a triterpene, which exhibited an IC<sub>50</sub> value of 7.5 nM (Orhan *et al.* 2007). Widespread occurrence of ursolic acid [Fig. 2 (64)] accounts for the traditional use of several plant species for memory improvement and AD related conditions.

#### Taraxerol

Taraxerol [Fig. 2 (65)] is a triterpene with similar structure as ursolic acid isolated from twigs of *Vaccinium oldhami* (Ericaceae). The IC<sub>50</sub> value of taraxerol against AChE was 79  $\mu$ M (Lee *et al.* 2004).

## FLAVONOID DERIVATIVES

### Structure activity relationship

Many recent reports are available regarding the multipotent activities of flavonoid derivatives in combating Alzheimer disease, but only fewer studies are available on anti-ChEI activity. According to Ji and Zhang (2006), the AChE inhibitory activity of flavonoid derivatives is due to the presence of the catechol moiety in its structure.

#### Flavonoid glycosides

*Lecuas urticifolia* (Lamiaceae) is an annual herb which is commonly found in Karachi and other parts of Sind province of Pakistan. It is astringent, stimulant, haemostatic, anthelmintic and diuretic and is also widely used for the treatment of diarrhea, dysentery, uterine haemorrhages, dropsy, gravel, cystitis, calculus, bronchial catarrh, skin diseases, fever and various types of mental disorders. Ethano-

lic extract of whole plant exhibited potent BuChE inhibitory activity. Bioactive guided fractionation showed that Leufofolin A and Leufofolin B [Fig. 2 (66, 67)] are the compounds responsible for BuChE inhibitory activity with IC<sub>50</sub> value of  $1.6 \pm 0.98$  and  $3.6 \pm 1.7$   $\mu$ M (Atia-tun-Noor *et al.* 2007).

### Flavones

The ethyl acetate extract of whole plants of *Agrimonia pilosa* (Rosaceae) was assessed for AChE inhibitory activity. Activity is due to presence of four flavones tiliroside, 3-methoxy quercetin and quercetin. Quercetin [Fig. 2 (68)] showed twice the activity of dehydroevodiamine (DHED) (Jung and Park 2007) and its inhibitory activity is due to presence of a catechol moiety on ring B which facilitates quercetin binding to AChE.

#### Ginkgo biloba EGb 761

*Ginkgo biloba* (Ginkgoaceae) extract was assessed for ChEI's. Ginkobilbo EGb 761 fraction exhibited ChE inhibitory activity equivalent to standard drug tacrine, Donepezil (Wettstein 2000). EGb 761 is standardized extract, which contains approximately 24% flavone glycosides (primarily quercetin, kaempferol and isorhamnetin) and 6% terpene lactones (2.8-3.4% ginkgolides A, B and C, and 2.6-3.2% bilobalide). Ginkgolide B and bilobalide account for about 0.8 and 3% of the total extract, respectively (Das *et al.* 2002).

### Flavanones

Naringenin [Fig. 2 (69)] a flavanone isolated from *Citrus junos* (Rutaceae) inhibited AChE *in vitro* dose dependently (Heo *et al.* 2004). Moreover it ameliorated scopolamine-induced amnesia in mice. Hispidone [Fig. 2 (70)] isolated from *Onosma hispida* (Boraginaceae) was moderately active (IC<sub>50</sub> value = 11.6  $\mu$ M) (Ahmad *et al.* 2003).

### Isoflavonoids

*Maclura pomifera* (Moraceae) commonly referred to "Osage orange, hedge apple, bow wood, and horse apple" were used as folkloric medicine worldwide. Decoction prepared from the roots of *M. pomifera* is used for the treatment of sore eyes, while the bark of *M. tinctoria* has been reported to be used against toothache (Elvin-Lewis *et al.* 1980). Fruit of *M. pomifera* rich in isoflavonoid and xanthones (Delle Monache *et al.* 1994) possess several biological activities including antimicrobial, estrogenic, anti-inflammatory and antinociceptive activities (Mahmoud 1981; Maier *et al.* 1995). In the current study, the methanol extracts prepared from the leaf, wood, flower, twig, and stem bark of the female and male individuals as well as rhizodermis and fruit from the female tree of *M. pomifera* were examined along with its two major isoflavonoids osajin, pomiferin [Fig. 2 (71, 72)] and their semi-synthetic derivatives iso-osajin and iso-pomiferin [Fig. 2 (73, 74)] for their anti-ChE activity under *in vitro* condition. Most of the extracts did not exert remarkable anti-AChE effect at the tested concentrations when compared to galanthamine. Only, the rhizodermis and fruit extract showed inhibition over 50% at 2 mg/ml (58.02 and 52.87%, respectively). On the other hand, Osajin (IC<sub>50</sub> = 2.239 mM), Pomiferin (IC<sub>50</sub> = 0.096 mM), and its semisynthetic derivatives isoosajin (IC<sub>50</sub> = 1.35 mM), and iso-pomiferin (IC<sub>50</sub> = 2.67 mM) displayed a remarkable inhibition against AChE in a concentration-dependent manner (Orhan *et al.* 2009). Nevertheless, these compounds were completely inactive against BuChE (Ji and Zhang 2006). AChE and BuChE inhibitory activity of Osajin and isoosajin might be due to presence of catechol moiety in ring B of its structure. The structure-activity relationship of pomiferin and its semisynthetic derivative has not been elucidated.

## PREGNANE GLYCOSIDES

Roots of *Cynanchum atratum* (Asclepiadaceae) were investigated for AChE inhibitory activity and the activity is due to presence of pregnane glycoside. Of all the glycosides Cynatoside B exhibited potent inhibitory activity with IC<sub>50</sub> value of 3.6 pM. The mode of AChE inhibition by cynatoside B [Fig. 2 (75)] was reversible and non-competitive in nature. Cynatoside B (1.0 mg/kg body weight i.p.) significantly ameliorated memory impairments induced in mice by scopolamine (1.0 mg/kg body weight s. c.) as measured in the passive avoidance and the Morris water maze tests. Presence of both anti-AChE and anti-amnesic activities makes it significant in therapeutics in alleviating certain memory impairments observed in AD (Lee *et al.* 2005).

## GUAIANOLIDES

*Amberboa ramose* (Compositae) is an annual herbaceous plant grown in India and Pakistan possess cytotoxic and antibacterial activities. Chloroform soluble fraction revealed significant inhibitory activity against the BuChE and bioactive guided isolation of the extract showed the presence of six guaianolides [Fig. 2 (76)]. All of them exhibited inhibitory activity against BuChE; compounds 3-6 are more potent than 1 and 2 (Khan *et al.* 2005).

## XANTHONES

Methanol leaf extract of *Gentiana campestris* (Gentianaaceae) exhibited significant inhibition of AChE activity. Four xanthenes, bellidin, bellidifolin [Fig. 2 (77)], bellidin 8-*O*-β-glucopyranoside (norswertianolin), and bellidifolin 8-*O*-β-glucopyranoside (swertianolin), were found to be responsible for the anti-AChE activity effects (Urbain *et al.* 2004).

### Structure-activity relationship

Absence of glucopyranosyl moiety in bellidifolin increases the inhibitory activity when compared to other xanthenes, which might be due to steric factors or hydrophobicity. Presence of methoxy group at its C-3 position also enhances its inhibitory activity (Houghton *et al.* 2006).

## SITOINDOSIDES AND WITHAFERIN A

The roots of *Withania somnifera* (Solanaceae) one of the most highly regarded herbs in Ayurvedic medicine where it is known as 'ashwagandha' and has a history of use for almost 4,000 years. It is classed among the rejuvenative tonics known as 'Rasayanas'. Root extract was administered orally to mice and effect of neurotransmitter system in brain was observed. Extract showed enhanced AChE activity in the lateral septum and globus pallidus areas of the brain and also enhanced muscarinic M1 receptor binding in cortical regions. Active compound responsible for the activity is sitoindosides and withaferin A. The extract containing the active compound also reversed the reduction in cholinergic markers (e.g. ACh, ChAT) in rats (Bhattacharya *et al.* 1995). These activities could explain the reputed cognition enhancing effects of *W. somnifera* root because of preferential action on cholinergic neurotransmission in the cortical and basal forebrain, brain areas involved in cognitive function. Based on this information, it could be speculated that the sitoindosides and withaferin A [Fig. 2 (78, 79)] could have potential in AD therapy.

## LIGNANS

The hexane extract of the fruit of *Schizandra chinensis* (Schisandraceae) was found to show significant inhibition of the activity of AChE. Further studies showed that lignans were responsible for the inhibitory effect on AChE. The compounds having both aromatic methylenedioxy and hydroxyl groups on their cyclooctadiene ring, such as gomisin C,

gomisin D, gomisin G, schisandrol B and gomisin A, [Fig. 2 (80, 81, 82, 83, 84)] entirely inhibited AChE in dose-dependent manners, with IC<sub>50</sub> values of 6.71 ± 0.53, 6.55 ± 0.31, 7.84 ± 0.62, 12.57 ± 1.07 and 13.28 ± 1.68 μM, respectively. These results indicate that the lignans could potentially be a potent class of AChEI's (Ingkaninan *et al.* 2006).

### Structure-activity relationship

The presence of aromatic methylene-dioxy and hydroxyl groups attached to the cyclooctadiene ring in compounds [Fig. 2 (80, 81, 82, 83, 84)] might be responsible for AChE inhibitory activity in dose dependent manner. Gomisin D possess lesser active than gomisin G and C which is due to presence of α-hydroxyl group at C-7, side chains at C-6 and C-14 and methylene-dioxy groups at C-12 and C-13. Interestingly, schisandrol B, which has 13-hydroxyl group at C-8 and methylene-dioxy groups at C-2 and C-3 position, and gomisin A which possess 13-hydroxyl group at C-7 and methylenedioxy groups at C-12 and C-13, exhibit significantly lower inhibitory activities than compounds gomisin C, G and D. This indicates that in the presence of hydroxyl group, the β orientation might exhibit lesser inhibitory effect than the α orientation. These results indicate that aromatic methylene-dioxy and cyclooctadiene hydroxyl groups are important for the AChE inhibitory activity (Hung *et al.* 2007). Moreover the low molecular wt of the material makes it easier to cross the blood brain barrier to reach the site of action (Broadwell *et al.* 1993).

The root and stem bark of *Magnolia officinalis* (Magnoliaceae) (have been used in TCM to treat anxiety and nervous disturbances. *M. officinalis* contains the biphenolic lignans, honokiol and magnolol [Fig. 2 (85, 86)] which increased ChAT activity and inhibited AChE activity *in vitro* and increased hippocampal ACh release *in vivo* (Hou *et al.* 2000). These two compounds also appear to have antioxidant, anti-inflammatory, anxiolytic and neuroprotective properties and such polyvalency in activity is of interest in their potential use in the treatment of AD.

## POLYPHENOLS

### Vitisin A and heyneanol A

*Vitis amurensis* (Vitaceae) a wild-growing grape, in Japan, China and Korea has been widely used in traditional medicine for the treatment of cancer and various pains. Root extracts possess anti-inflammatory and anti-tumor activity. Vitisin A and heyneanol A [Fig. 2 (87, 88)], two polymers of resveratrol isolated from the butanolic root extract, inhibited both AChE and BuChE in a dose-dependent manner and exhibited higher activity against BuChE compared with that of galanthamine, a positive control (Jang *et al.* 2008).

### (+)-α-viniferin and Kobophenol A

Total methanolic extract of the underground parts of *Caragana chamlague* (Leguminosae) showed significant inhibition towards AChE. Inhibitory activity is due to bioactive compounds, polyphenolic stilbene oligomers, (+)-α-viniferin [Fig. 2 (89)] and kobophenol A [Fig. 2 (90)]. Both compounds inhibited AChE activity in a dose-dependent manner, and their IC<sub>50</sub> values are 2.0 and 115.8 mM, respectively. Type of inhibition is specific reversible non-competitive (Sung *et al.* 2002).

### Structure-activity relationship

Vitisin A has appropriately bulky structure which masks AChE and prevents ACh from binding to AChE in a non-competitive manner. In contrast in the case of heyneanol A the bulky structure completely masks AChE and lowers the accessibility of substrate to AChE.

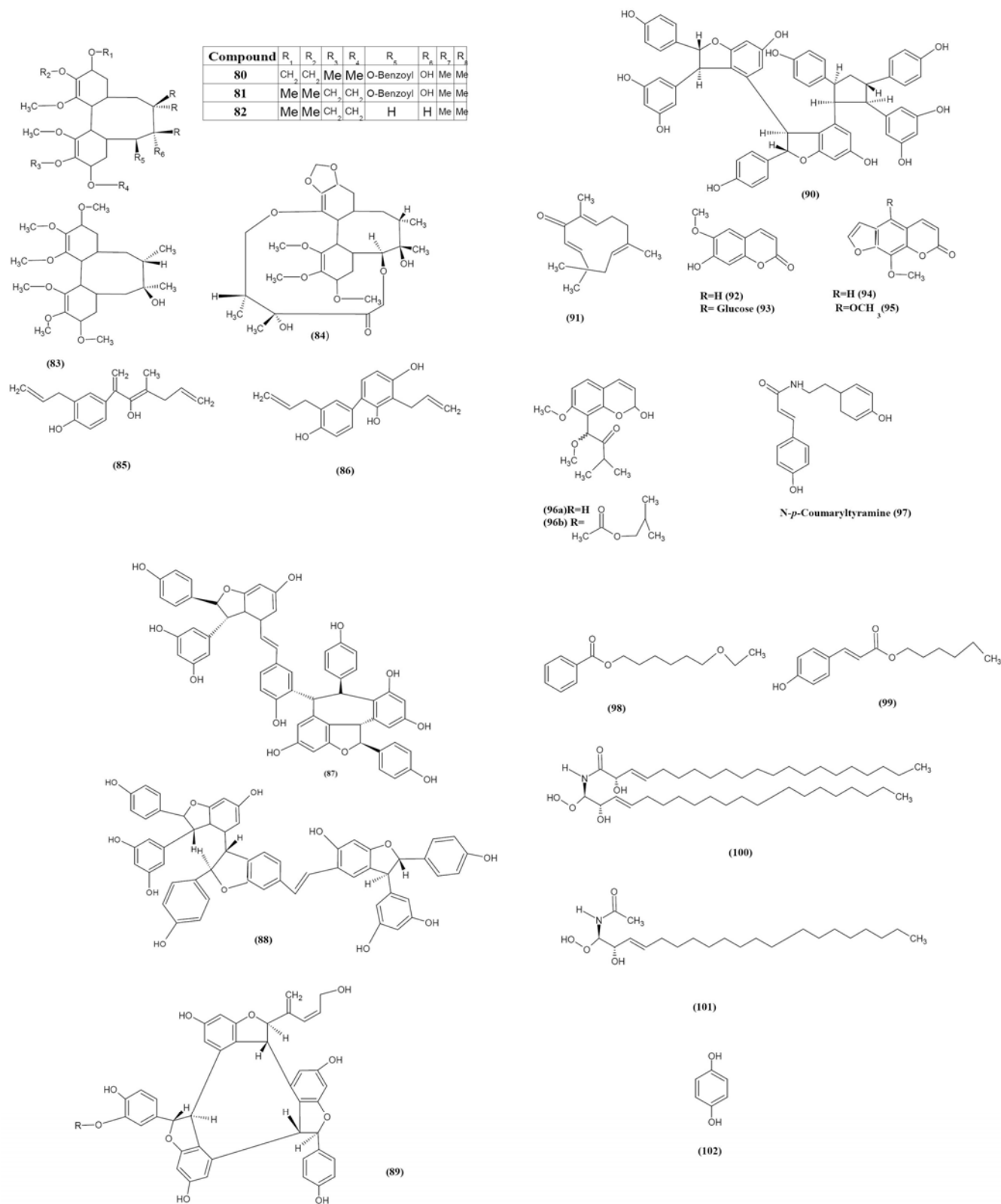


Fig. 2 (80-102) Phytoconstituents exhibiting Cholinesterase inhibitory activity.

## SESQUITERPENE

Zerumbone (ZER) [Fig. 2 (91)] is sesquiterpene from the edible plant, *Zingiber zerumbet* (Zingiberaceae) which is known to possess tremendous biological activities. In this study, the inhibitory effect of ZER towards AChE was evaluated using thin layer chromatography (TLC) bioautography and compared concurrently to tacrine, as positive control. The results obtained in this research showed that ZER has an enzymolytic effect towards AChE. It could be suggested that ZER might be a potential candidate for the

development of anti-AChE for AD treatment (Bustamam *et al.* 2008).

## LINOLEIC ACID DERIVATIVES

In a search for potential AChEI's, the ethanol extract of the bulbs of *Crinum x powellii* (Amaryllidaceae) was found to demonstrate a marked inhibition of this enzyme. Using a bio-guided isolation strategy, linoleic acid ethyl ester has been identified as the compound responsible for this inhibition (Kissling *et al.* 2005).

## SHIKIMATE-DERIVED COMPOUNDS AS AChE INHIBITORS

The shikimic acid pathway is the major pathway of producing phenolic compounds in flowering plants. Structural analysis shows the presence of one phenylpropanoid unit alone or two or more units combined most of which were found to be as AChEI.

### Coumarins

Scopoletin a coumarin isolated from methanolic extract of *Vaccinium oldhami* (Ericaceae) is the most active AChE inhibitor with IC<sub>50</sub> value of 79 μM. Scopoletin and scopolin [Fig. 2 (92, 93)] extracted from *Scopolia carniolica* (Solanaceae) was found to be active against AChE (Lee *et al.* 2004).

Furanocoumarins such as Xanthotoxin and isopimpinellin [Fig. 2 (94, 95)] from the roots of *Angelica acutiloba* (Apiaceae) exhibited significant inhibitory activity with IC<sub>50</sub> value of 0.58 and 0.32 μM respectively (Mizayawa *et al.* 2004). Most of the prenylated coumarins from *A. dahurica*, pyrano and furanocoumarins from *A. gigas* exhibited weak AChE inhibitory activity (Kim *et al.* 2002). Two pyrenlated coumarins murranganin and hainanmurpanin [Fig. 2 (96 a, b)] isolated from *Murraya paniculata* (Rutaceae) exhibited weak AChE inhibitory activity (IC<sub>50</sub> 79.1 and 31.6 μM) (Choudhary *et al.* 2002). N-*p*-Coumaryltyramine [Fig. 2 (97)], isolated from twigs of *Celtis chinensis* (Ulmaceae) which is an unusual combination of amino acid and phenylpropanoid showed weak AChE inhibitory activity with IC<sub>50</sub> value of 122 μM.

### MISCELLANEOUS

*Areca catechu* (Piperaceae) is the areca palm or areca nut palm, a species of palm which grows in much of the tropical Pacific, Asia, and parts of east Africa commonly termed as betel leaf. Hydroalcoholic extract of *A. catechu* inhibited AChE and BuChE in a dose-dependent manner (Gilani *et al.* 2004). However, the active component has not been identified, yet.

The essential oils of *Origanum ehrenbergii* and *O. syriacum* (Lamiaceae) collected in Lebanon were analysed for anti-ChEI activity. Essential oils exhibited both AChE and BuChE inhibitory activity with IC<sub>50</sub> of 0.3 μg/ml (Loizzo *et al.* 2009).

Nonyl benzoate and hexyl *p*-hydroxy cinnamate [Fig. 2 (98, 99)] isolated from *Buddleja crispa* (Buddlejaceae) exhibited weak AChE and BuChE inhibitory activity (Ahmad *et al.* 2005).

Ceramides and tanacetamides [Fig. 2 (100, 101)] isolated from whole plants of *Tanacetum artemisioides* (Asteraceae) exhibited AChE inhibitory activity *in vitro* with IC<sub>50</sub> value of 67.1 and 74.1 μM, respectively (Ahmad *et al.* 2004).

*Rodiola rosea* (Crassulaceae) commonly termed as golden roots is widely distributed in cold regions such as arctic region of the world. Pilot studies in human trails have shown that *R. rosea* improves mental performance and physical activity and reduces fatigue. Bioactive guided fractionation and purification of the ethanolic extract of rhizome of *R. rosea* showed the presence of hydroquinone [Fig. 2 (102)] which is responsible for the AChE inhibitory activity (Wang *et al.* 2007).

Curcuminoids (a mixture of curcumin, bisdemethoxycurcumin and demethoxycurcumin) share vital pharmacological properties possessed by turmeric, a well known curry spice, considered useful in AD. Curcuminoids was analysed for AChE inhibitory activity and memory enhancing activities under *in-vitro* and *ex-vivo* condition. Under *in vitro* condition curcuminoids inhibited AChE with IC<sub>50</sub> value of 19.67 μM, while bisdemethoxycurcumin, demethoxycurcumin and curcumin inhibited AChE with IC<sub>50</sub> value of 16.84, 33.14 and 67.69 μM, respectively (Ahmed and Gilani 2009).

In the *ex-vivo* AChE assay, curcuminoids and its individual components except curcumin showed dose-dependent (3–10 mg/kg) inhibition in frontal cortex and hippocampus. When studied for their effect on memory at a fixed dose (10 mg/kg), all compounds showed significant ( $p < 0.001$ ) and comparable effect in scopolamine-induced amnesia. These data indicate that curcuminoids and all individual components except curcumin possess pronounced AChE inhibitory activity. Curcumin was relatively weak in the *in-vitro* assay and without effect in the *ex-vivo* AChE model, while equally effective in memory enhancing effect, suggestive of additional mechanism(s) involved. Thus curcuminoids mixture might possess better therapeutic profile than curcumin for its medicinal use in AD.

*Cymbopogon schoenanthus* (Poaceae), commonly termed as lemon grass, was collected from the mountainous region of south Tunisia and assessed for ChE inhibitory activity. Ethyl acetate and methanol extract of shoot exhibited highest AChE inhibitory activity with IC<sub>50</sub> = 0.23 ± 0.04 mg/ml (Khadri *et al.* 2009).

### CONCLUSION

ChE inhibitors have therapeutic applications in AD, senile dementia, ataxia, myasthenia gravis and Parkinson's disease. Central cholinergic system is considered as the most important neurotransmitter system involved in the regulation of cognitive functions. Cholinergic neuronal loss in hippocampal area is the major feature of AD and enhancement of central cholinergic activity by use of anti-ChE is presently the mainstay of the pharmacotherapy of senile dementia of Alzheimer type (Enz *et al.* 1993; Siddiqui and Levey 1999). All of the known ChE 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 ChEI's inhibitors with less toxicity and more potent activity is compulsory. The search for plant derived inhibitors of ChE has accelerated in view of the benefits of these drugs not only in the treatment of AD but in other forms of dementia, such as dementia with Lewy bodies (Perry *et al.* 1994), vascular dementia (Erkinjuntti *et al.* 2002) and Down's syndrome (Kishnani *et al.* 1999). Along with the prototype inhibitor of AChE physostigmine, obtained from the plant *Physostigma venenosum*, other molecules with highly significant anti-ChE activity are Huperzine-A, galanthamine, α-viniferin and ursolic acid obtained from *Huperzia serrata*, *Galanthus nivalis* and *Narcissus* sp., *Caragana chamlague* and *Origanum majorana*, respectively. Majority of studies have focused on the anti-ChEI alkaloids, such as physostigmine and galanthamine. So far, more than 35 alkaloids have been reported to have AChE inhibitory activity. The other major classes of compound reported to have such activity are the terpenoids, glycosides and coumarins. Plants belonging to Acanthaceae, Apocynaceae, Amaryllidaceae, Angelicaceae, Araceae, Asclepiadaceae, Berberidaceae, Buxaceae, Combretaceae, Compositae, Coniferae, Cyperaceae, Ebenaceae, Ericaceae, Euphorbiaceae, Fumariaceae, Gentianaceae, Guttiferaceae, Lamiaceae, Leguminosae, Liliaceae, Lycopodiaceae, Malvaceae, Magnoliaceae, Menispermaceae, Molluginaceae, Moraceae, Musaceae, Nelumbonaceae, Papaveraceae, Piperaceae, Rubiaceae, Rutaceae, Sapotaceae, Solanaceae and Tamaricaceae families have been reported to have AChE inhibitory potential. 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. For many of the plants and compounds that have demonstrated anti-ChEI activity relevant to AD therapy, the clinical data are very limited.

Molecular docking studies of fewer compounds are done whose structure activity relationship is presented in this review. Studies on structure activity relationship of enormous compounds are lacking which are yet to be elucidated. Moreover clinical efficacy and potential toxicity of

active plants and compounds in larger trials requires further assessment, before recommendations concerning their routine use can be identified.

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## REFERENCES

- Adersen A, Kjøbye A, Dall O, Jäger AK (2007) Acetylcholinesterase and butyrylcholinesterase inhibitory compounds from *Corydalis cava*. *Journal of Ethnopharmacology* **113**, 179-182
- Ahmed T, Gilani AH (2009) Inhibitory effect of curcuminoids on acetylcholinesterase activity and attenuation of scopolamine-induced amnesia may explain medicinal use of turmeric in Alzheimer's disease. *Pharmacology Biochemistry and Behavior* **91**, 554-559
- Ahmad I, Malik A, Afza N, Anis I, Fatima I, Nawaz SA, Tareen RB, Choudhary MI (2005) Enzymes inhibitory constituents from *Buddleja crispa*. *Zeitschrift für Naturforschung B – A Journal of Chemical Sciences* **60**, 341-346
- Ahmad VU, Hussain J, Hussain H, Farooq U, Akber E, Nawaz SA, Choudhary MI (2004) Two ceramides from *Tanacetum artemesioides*. *Zeitschrift für Naturforschung B – A Journal of Chemical Sciences* **59**, 329-333
- Ahmad W, Ahmad B, Ahmad M, Iqbal Z, Nisar M, Ahmad M (2003) *In vitro* inhibition of acetylcholinesterase, butyrylcholinesterase and lipoxigenase by crude extract of *Myricaria elegans* Royle. *Journal of Biological Science* **3**, 1046-1049
- Andrade MT, Lima JA, Pinto AC, Rezende CM, Carvalho MP, Epifanio RA (2005) Indole alkaloids from *Tabernaemontana australis* (Muell. Arg) Miens that inhibit acetylcholinesterase enzyme. *Bioorganic and Medicinal Chemistry* **13**, 4092-4095
- Andrisano V, Bartolini M, Gotti R, Cavrini V, Felix G (2001) Determination of inhibitors potency (IC<sub>50</sub>) by a direct high performance liquid chromatographic method on an immobilized acetylcholinesterase column. *Journal of Chromatography B* **753**, 375-383
- Atia-tun-Noor, Fatima I, Ahmad I, Malik A, Afza N, Iqbal L, Latif M, Khan SB (2007) Leufofins A and B, potent butyrylcholinesterase-inhibiting flavonoid glucosides from *Leucas urticifolia*. *Molecules* **12**, 1447-1454
- Atta-ur-Rahman, Choudhary MI (1999) Recent studies on bioactive natural products. *Pure Applied Chemistry* **71**, 1079-1081
- Bhattacharya SK, Kumar A, Ghosal S (1995) Effects of glycowithanolides from *Withania somnifera* on an animal model of Alzheimer's disease and perturbed central cholinergic markers of cognition in rats. *Phytotherapy Research* **9**, 110-113
- Bores GM, Huger FP, Petko W (1996) Pharmacological evaluation of novel Alzheimer's disease therapeutics: Acetylcholinesterase inhibitors related to galanthamine. *Journal of Pharmacological and Experimental Therapeutics* **277**, 1728-1738
- Brühlmann C, Marston A, Hostettmann K, Carrupt PA, Testa B (2004) Screening of non-alkaloidal natural compounds as acetylcholinesterase inhibitors. *Chemistry and Biodiversity* **1**, 819-829
- Broadwell RD, Sofroniew MV (1993) Serum proteins bypass the blood-brain fluid barriers for extracellular entry to the central nervous system. *Experimental Neurology* **120**, 245-263
- Brufani M, Filocamo L (2000) Rational design of cholinesterase inhibitors. In: Giacobini E (Ed) *Cholinesterase and Cholinesterase Inhibitors*, Martin Dunitz, London, pp 27-46
- Bustaman A, Ibrahim S, al zubairi AS, Manal MET, Syam MM (2008) Zerumbone: A natural compound with anticholinesterase activity. *American Journal of Pharmacology and Toxicology* **3**, 209-211
- Cardoso CL, Castro-Gamboa I, Silva DHS, Furlan M, Epifanio RD, Pinto AD, de Rezende CM, Lima JA, Bolzani VD (2004) Indole glucoalkaloids from *Chimarrhis turbinata* and their evaluations as antioxidant agents and acetylcholinesterase inhibitors. *Journal of Natural Products* **67**, 1882-1885
- Cho KM, Yoo ID, Kim WG (2006) 8-Hydroxydihydrochelerythrine and 8-hydroxydihydroanguinarine with a potent acetylcholinesterase inhibitory activity from *Chelidonium majus*. *Biological and Pharmaceutical Bulletin* **29**, 2317-2320
- Choudhary MI, Azizuddin A, Khalid S, Ziauddin S, Atta-ur-Rahman (2002) New coumarins from *Murraya paniculata*. *Planta Medica* **68**, 81-83
- Choudhary MI, Devkota KP, Nawaz SA, Ranjit R, Atta-ur-Rahman (2005) Cholinesterase inhibitory pregnane-type steroidal alkaloids from *Sarcococca hookeriana*. *Steroids* **70**, 295-303
- Chung YK, Heo HJ, Kim EK, Kim HK, Huh TL, Lim Y, Kim SK, Shin DH (2001) Inhibitory effect of ursolic acid purified from *Origanum majorana* L. on the acetylcholinesterase. *Molecules and Cells* **11**, 137-143
- Coelho Filho JMJC, Birks J (2009) Physostigmine for dementia due to Alzheimer's disease. In: *Cochrane Database of Systematic Reviews: The Cochrane Collaboration*, John Wiley & Sons, UK, pp 1-81
- Neşe Çokuğraş A (2003) Butyrylcholinesterase: Structure and physiological importance. *Turkish Journal of Biochemistry* **28**, 54-61
- Conti-Fine BM, Milani M, Kaminski HJ (2006) Myasthenia gravis: past, present, and future. *Journal of Clinical Investigation* **116**, 2843-2854
- Cousin SB, Duval N, Bassoulie J, Bon C (1996) Cloning and expression of acetylcholinesterase from *Bungarus fasciatus* venom: a new type of COOH-terminal domain; involvement of a positively charged residue in the peripheral site. *Journal of Biological Chemistry* **271**, 15099-15108
- Das A, Shanker G, Nath C, Pal R, Singh S, Singh HK (2002) A comparative study in rodents of standardized extracts of *Bacopa monniera* and *Ginkgo biloba* anticholinesterase and cognitive enhancing activities. *Pharmacology Biochemistry and Behaviour* **73**, 893-900
- Davis KL, Mohs RC, Marin D (1999) Cholinergic markers in elderly patients with early signs of Alzheimer's disease. *The Journal of American Medical Association* **281**, 1401-1406
- Da-Yuan Z, Dong-Lu B, Xi-Can T (1996) Recent studies on traditional Chinese medicinal plants. *Drug Development Research* **39**, 147-157
- Delle MG, Scurria R, Vitali A, Botta A, Monacelli B, Pasqua G, Palocci C, Cernia E (1994) Two isoflavones and a flavone from the fruits of *Maclura pomifera*. *Phytochemistry* **37**, 893-898
- Dowling JE (Ed) (2001) The chemistry of synaptic transmission. In: *Neurons and Networks: An Introduction to Behavioral Neuroscience* (2<sup>nd</sup> Edn), Harvard University Press, Cambridge, pp 127-132
- Dvir H, Jiang HL, Wong DM, Harel M, Chetrit M, He XC, Jin GY, Yu GL, Tang XC, Silman I, Bai DL, Sussman JL (2002) X-ray structure of *Torpedo californica* acetylcholinesterase complexed with (+)-huperzine A and (-)-huperzine B: structural evidence for an active site rearrangement. *Biochemistry* **41**, 10810-10818
- Duvoisin RC (1968) Reversal of central anticholinergic syndrome in man by physostigmine. *Journal of the American Medical Association* **206**, 1963-1967
- Ellman GL, Courtney KD, Andres V, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology* **7**, 88-95
- Elvin-Lewis M, Hall JB, Adut-Tutu M, Afful Y, Asante-Appiah K, Lieberman D (1980) The dental health of chewing stick users in Southern Ghana: preliminary findings. *Journal of Preventive Dentistry* **6**, 151-159
- Enz A, Amstutz R, Boddeke H, Gmelin G, Malanowski J (1993) Brain selective inhibition of acetylcholinesterase: a novel approach to therapy for Alzheimer's disease. *Progress in Brain Research* **98**, 431-438
- Erkinjuntti T, Kurz A, Gauthier S, Bullock R, Lilienfeld S, Damaraju CV (2002) Efficacy of galantamine in probable vascular dementia and Alzheimer's disease combined with cerebrovascular disease: a randomized trial. *Lancet* **359**, 1283-1290
- Farlow M (2002) A clinical overview of cholinesterase inhibitors in Alzheimer's disease. *International Psychogeriatrics* **14**, 93-126
- Ferreira A, Proença C, Serralheiro MLM, Araújo MEM (2006) The *in vitro* screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal. *Journal of Ethnopharmacology* **108**, 31-37
- Ferri CP, Prince M, Brayne C, Brodaty H, Fratiglioni L, Ganguli M, Hall K, Hasegawa K, Hendrie H, Huang Y, Jorm A, Mathers C, Menezes PR, Rimmer E, Sczufca M (2006) Global prevalence of dementia: a Delphi consensus study. *Lancet* **366**, 2112-2117
- Francis PT, Palmer AM, Snape M, Wilcock GK (1999) The cholinergic hypothesis of Alzheimer's disease: A review of progress. *Journal of Neurology, Neurosurgery and Psychiatry* **54**, 137-147
- Ghosal S, Bhattacharya SK, Mehta R (1972) Naturally occurring and synthetic  $\beta$ -carboline as cholinesterase inhibitors. *Journal of Pharmaceutical Sciences* **61**, 808-811
- Gilani AH, Ghayur MN, Ahmed SP, Saify Z, Choudhary MI, Khalid A (2004) Presence of cholinomimetic and acetylcholinesterase inhibitory constituents in betel nut. *Life Sciences* **75**, 2377-2389
- Greenblatt HM, Kryger G, Lewis T, Silman I, Sussman JL (1999) Structure of acetylcholinesterase complexed with (-)-galanthamine at 2.3 Å resolution. *FEBS Letters* **463**, 321-326
- Harel M, Kleywegt GJ, Ravelli RB, Silman I, Sussman JL (1995) Crystal structure of an acetylcholinesterase-fasciculin complex: interaction of a three-fingered toxin from snake venom with its target. *Structure* **3**, 1355-1366
- Hebert LE, Scherr PA, Bienias JL, Bennett DA, Evans DA (2003) Alzheimer disease in the US Population prevalence estimates using the 2000 census. *Archives of Neurology* **60**, 1119-1122
- Hillhouse BJ, Ming DS, French CJ, Neil TGH (2004) Acetylcholinesterase inhibitors in *Rhodiola rosea*. *Pharmaceutical Biology* **42**, 68-72
- Heo HJ, Kim DO, Shin SC, Kim MJ, Kim BG, Shin MD (2004) Effect of antioxidant flavanone naringenin *Citrus Junos* on neuroprotection. *Journal of Agricultural and Food Chemistry* **52**, 1520-1525
- Holden M, Cornelius K (2002) Use of cholinesterase inhibitors in dementia. *Advances in Psychiatric Treatment* **8**, 89-96
- Hou YC, Chao PD, Chen SY (2000) Honokiol and magnolol increased hippocampal acetylcholine release in freely moving rats. *American Journal of Chinese Medicine* **28**, 379-384
- Houghton PJ, Ren Y, Howes MJ (2006) Acetylcholinesterase inhibitors from plants and fungi. *Natural Product Research* **23**, 181-199
- Hung TM, Na MK, Min BS, Ngoc TM, Lee IS, Zhang XF, Bae KH (2007)

- Acetylcholinesterase inhibitory effects of lignans isolated from *Schizandra chinensis*. *Archives of Pharmacol Research* **30**, 685-690
- Hung TM, Na MK, Dat NT, Ngoc TM, Youn UJ, Kim HJ, Min BS, Lee JP, Bae KH (2008) Cholinesterase inhibitory and anti-amnesic activity of alkaloids from *Corydalis turtschaninovii*. *Journal of Ethnopharmacology* **119**, 74-80
- Ingganinan K, Best D, Heijden VD, Hofte AJP, Karabatak B, Irth H, Tjaden UR, Greef VD, Verpoorte R (2000) High-performance liquid chromatography with on-line coupled UV, mass spectrometric and biochemical detection for identification of acetylcholinesterase inhibitors from natural products. *Journal of Chromatography A* **872**, 61-73
- Ingganinan K, Phengpa P, Yuenyongsawad S, Khorana N (2006) Acetylcholinesterase inhibitors from *Stephania venosa* tuber. *The Journal of Pharmacy and Pharmacology* **58**, 695-700
- Ingganinan K, Temkitthawon P, Chuenchom K, Yuyaem T, Thongnoi W (2003) Screening for acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. *Journal of Ethnopharmacology* **89**, 261-264
- Jang MH, Piao XL, Kim JM, Kwon SW, Park JH (2008) Inhibition of cholinesterase and amyloid- $\beta$  aggregation by resveratrol oligomers from *Vitis amurensis*. *Phytotherapy Research* **22**, 544-549
- Ji HF, Zhang HY (2006) Theoretical evaluation of flavonoids as multipotent agents to combat Alzheimer's disease. *Journal of Molecular Structure – THEOCHEM* **76**, 3-9
- Jing L, Zhang HY, Wang LM, Tang XC (1999) Inhibitory effects of huperzine B on cholinesterase activity in mice. *Acta Pharmacologica Sinica* **20**, 141-145
- Jung M, Park M (2007) Acetylcholinesterase inhibition by flavonoids from *Agrimonia pilosa*. *Molecules* **12**, 2130-2139
- Khadri A, Neffati M, Smiti S, Fale P, Lino ARL, Serralheiro MLM, Araújo MEM (2010) Antioxidant, antiacetylcholinesterase and antimicrobial activities of *Cymbopogon schoenanthus* L. Spreng (lemon grass) from Tunisia. *LWT - Food Science and Technology* in press
- Khalid A, Azim MK, Parveen S, Atta-ur-Rahman, Choudhary MI (2005) Structural basis of cholinesterases inhibition by triterpenoidal alkaloids. *Biochemical and Biophysical Research Communication* **331**, 1528-1532
- Khalid A, Zaheer-ul-Haq, Anjum S, Khan MR, Atta-ur-Rahman, Choudhary MI (2004) Kinetics and structure-activity relationship studies on steroidal alkaloids that inhibit cholinesterases. *Bioorganic and Medicinal Chemistry* **12**, 1995-2003
- Khan SB, Haq A, Perveen S, Afzai N, Malik A, Nawaz SA, Shah MR, Choudhary MI (2005) Butyrylcholinesterase inhibitory guaianolides from *Amberboa ramose*. *Archives of Pharmacol Research* **28**, 172-176
- Kim DK (2002) Inhibitory effect of corynoline isolated from the aerial parts of *Corydalis incise* on the acetylcholinesterase. *Archives of Pharmacol Research* **25**, 817-819
- Kim DK, Lim JP, Yang JH, Eom DO, Eun JS, Leem KH (2002) Acetylcholinesterase inhibitors from the roots of *Angelica dahurica*. *Archives of Pharmacol Research* **25**, 856-859
- Kim SR, Hwang SY, Jang YP, Park MJ, Markelonis GJ, Oh TH, Kim YC (1999) Protopine from *Corydalis ternata* has anticholinesterase and anti-amnesic activities. *Planta Medica* **65**, 218-221
- Kishnani PS, Sullivan JA, Walter BK, Spiridigliozzi GA, Doraiswamy PM, Krishnan KRR (1999) Cholinergic therapy for Down's syndrome. *Lancet* **353**, 1064-1065
- Kissling J, Loset JR, Marston A, Hostettmann K (2005) Bio-guided isolation of cholinesterase inhibitors from the bulbs of *Crinum x powellii*. *Phytotherapy research* **9**, 984-987
- Kua J, Zhang Y, Esлами AC, Butler JR, McCammon JA (2003) Studying the roles of W86, E202, Y337 in binding of acetylcholine to acetylcholinesterase using a combined molecular dynamics and multiple docking approach. *Protein Science* **12**, 2675-2684
- Kuznetsova LP, Nikolskaya EB, Sochilina EE, Faddeeva MD (2002) Inhibition of human blood acetylcholinesterase and butyrylcholinesterase by some alkaloids. *Journal of Evolutionary Biochemistry and Physiology* **38**, 35-39
- Lane RM, Potkin SG, Enz A (2006) Targeting acetylcholinesterase and butyrylcholinesterase in dementia. *International Journal of Neuropsychopharmacology* **9**, 101-124
- Latrin DL, Fifer EK (1995) Drugs affecting cholinergic neurotransmission: Pharmacodynamic agents. In: Foye WO, Lemke TL, Williams DA (Eds) *Principles of Medicinal Chemistry* (5<sup>th</sup> Edn), Williams and Wilkins, USA, pp 278-279
- Lee JH, Lee KT, Yang JH, Baek NL, Kim DK (2004) Acetylcholinesterase inhibitors from the twigs of *Vaccinium oldhami* Miquel. *Archives of Pharmacol Research* **27**, 53-56
- Lee KY, Yoon JS, Kim ES, Kang SY, Kim YC (2005) Anti-acetylcholinesterase and anti-amnesic activities of a pregnane glycoside, cynatroside B, from *Cynanchum atratum*. *Planta Medica* **71**, 7-11
- Lima J, Costa RS, Epifanio RA, Castro NG, Rocha MS, Pinto AC (2009) *Geissospermum vellosii* stem bark: Anticholinesterase activity and improvement of scopolamine-induced memory deficits. *Pharmacology Biochemistry and Behaviour* **92**, 508-513
- Liu JS, Zhu YL, Yu CM, Zhou YZ, Han YY, Wu FW, Qi BF (1986) The structures of huperzine A and B1, two new alkaloids exhibiting marked anticholinesterase activity. *Canadian Journal of Chemistry* **64**, 837-839
- Loizzo MR, Menichini F, Conforti F, Tundis R, Bonesi M, Saab AM, Statti GA, de Cindio B, Houghton PJ, Menichini F, Frega NG (2009) Chemical analysis, antioxidant, anti-inflammatory and anticholinesterase activities of *Origanum ehrenbergii* Boiss and *Origanum syriacum* L. essential oils. *Food Chemistry* **117**, 174-180
- López S, Bastida J, Viladomat F, Codina C (2002) Acetylcholinesterase inhibitory activity of some *Amaryllidaceae* alkaloids and *Narcissus* extracts. *Life Science* **71**, 2521-2529
- Ma WG, Fukushi Y, Tahara S (1999) Fungitoxic alkaloids from Hokkaido *Corydalis* species. *Fitoterapia* **70**, 258-265
- Mahmoud ZF (1981) Antimicrobial components from *Maclura pomifera* fruit. *Planta Medica* **42**, 299-301
- Maier CGA, Chapman KD, Smith DW (1995) Differential estrogenic activities of male and female plant extracts from two dioecious species. *Plant Science* **109**, 31-43
- Marston A, Kissling J, Hostettmann K (2002) A rapid TLC bioautographic method for the detection of acetylcholinesterase and butyrylcholinesterase inhibitors in plants. *Phytochemical Analysis* **13**, 51-54
- McKenna MT, Proctor GR, Young LC, Harvey AL (1997) Novel tacrine analogues for potential use against Alzheimer's disease: Potent and selective acetylcholinesterase inhibitors and 5-HT uptake inhibitors. *Journal of Medicinal Chemistry* **40**, 3516-3523
- Melzer D, Ely M, Brayne C (1997) Cognitive impairment in elderly people: Population-based estimate of the future in England, Scotland and Wales. *British Medical Journal* **315**, 462
- Menichini F, Tundis R, Loizzo MR, Bonesi M, Marrelli M, Statti GA, Menichini F, Conforti F (2009) Acetylcholinesterase and butyrylcholinesterase inhibition of ethanolic extract and monoterpenes from *Pimpinella anisoides* V. Brig. (Apiaceae). *Fitoterapia* **80**, 297-300
- Mesulam MM (2003) Butyrylcholinesterase in the normal and Alzheimer brain. In: Giacobini E (Ed) *Butyrylcholinesterase: Its Function and Inhibitors*, Martin Dunitz, London, pp 31-34
- Mizayawa M, Tsukamoto T, Anzai J, Ishikawa Y (2004) Insecticidal effect of phthalides and furanocoumarins from *Angelica acutiloba* against *Drosophila melanogaster*. *Journal of Agricultural and Food Chemistry* **52**, 4401-4405
- Mounter LA, Alexander HC, Tuck KD, Dien LTH (1957) The pH dependence and dissociation constants of esterase and proteases treated with di-isopropyl fluorophosphates. *The Journal of Biological Chemistry* **226**, 867-872
- Mukherjee PK, Kumar V, Houghton PJ (2007) Screening of Indian medicinal plants for acetylcholinesterase inhibitory activity. *Phytotherapy Research* **21**, 1142-1145
- Ordentlich A, Barak D, Kronam C, Ariel N, Segal Y, Velan B, Shafferman A (1998) Functional characteristics of the oxyanion hole in human acetylcholinesterase. *The Journal of Biological Chemistry* **273**, 19509-19517
- Orhan I, Aslan M (2009) Appraisal of scopolamine-induced anti-amnesic effect in mice and *in vitro* antiacetylcholinesterase and antioxidant activities of some traditionally used *Lamiaceae* plants. *Journal of Ethnopharmacology* **122**, 327-332
- Orhan E, Aslan S, Kartal M, Sener B, Baser KHC (2007) Inhibitory effect of Turkish *Rosmarinus officinalis* L. on acetylcholinesterase and butyrylcholinesterase enzymes. *Food Chemistry* **108**, 663-668
- Orhan I, Salih T, Bilge S (2003) An acetylcholinesterase inhibitor from *Lycopodium clavatum*. *Planta Medica* **69**, 1-3
- Orhan I, Sener B, Choudhary MI, Khalid A (2004) Acetylcholinesterase and butyrylcholinesterase inhibitory activity of some Turkish medicinal plants. *Journal of Ethnopharmacology* **91**, 57-60
- Orhan I, Şenol FS, Kartal M, Dvorska M, Žemlička M, Šmejkal K, Mokry P (2009) Cholinesterase inhibitory effects of the extracts and compounds of *Maclura pomifera* (Rafin.) Schneider. *Food and Chemical Toxicology* **47**, 1747-1751
- Orhan I, Terzioğlu S, Sener B (2003) Alpha-onocerin: an acetylcholinesterase inhibitor from *Lycopodium clavatum*. *Planta Medica* **69**, 265-267
- Park CH, Kim SH, Choi W, Lee YJ, Kim JS, Kang SS, Suh YH (1996) Novel anticholinesterase and anti-amnesic activities of dehydroevodiamine, a constituent of *Evodia rutaecarpa*. *Planta Medica* **62**, 405-409
- Park CH, Lee YJ, Lee SH, Choi SH, Kim HS, Jeong SJ, Kim SS, Suh YH (2000) Dehydroevodiamine HCl prevents impairment of learning and memory and neuronal loss in rat models of cognitive disturbance. *Journal of Neurochemistry* **74**, 244-253
- Perry EK, Haroutunian V, Davis KL (1994) Neocortical cholinergic activities differentiate Lewy body dementia from classical Alzheimer's disease. *Neuroreport* **5**, 747-749
- Perry EK, Pickering AT, Wang WW, Houghton PJ, Perry NSL (1998) Medicinal plants and Alzheimer's disease: integrating ethnobotanical and contemporary scientific evidence. *Journal of Alternative and Complementary Medicine* **4**, 419-428
- Perry N, Court G, Bidet N, Court J, Perry E (1996) European herbs with cholinergic activities: potential in dementia therapy. *International Journal of Geriatric Psychiatry* **11**, 1063-1069
- Perry NS, Houghton PJ, Jenner P, Keith A, Perry EK (2002) *Salvia lavandulaefolia* essential oil inhibits cholinesterase *in vivo*. *Phytomedicine* **9**, 48-



- Perry NSL, Houghton PJ, Theobald A, Jenner P, Perry EK (2000) *In vitro* inhibition of human erythrocyte acetylcholinesterase by *Salvia lavandulaefolia* essential oil and constituent terpenes. *Journal of Pharmacy and Pharmacology* **52**, 895-902
- Poirier J (2002) Evidence that the clinical effects of cholinesterase inhibitors are related to potency and targeting of action. *International Journal of Clinical Practice* **127**, 6-19
- Rahman A-ur, Feroz F, Naem I, Zaheer-ul-Haq, Nawaz SA, Khan N, Khan MR, Choudhary MI (2004) New pregnane-type steroidal alkaloids from *Sarcococca saligna* and their cholinesterase inhibitory activity. *Steroids* **69**, 735-741
- Rahman A-ur, Parveen S, Khalid A, Farooq A, Ayattollahi SAM, Choudhary MI (1998) Acetylcholinesterase inhibiting triterpenoidal alkaloids from *Buxus hyrcana*. *Heterocycle* **49**, 481-488
- Raskind MA, Peskind ER, Wessel T, Yuan W (2000) Galanthamine USA-1 Study Group. A randomized, placebo-controlled trial with a 6-month extension. *Neurology* **54**, 2261-2268
- Rautera AP, Branco I, Lopes RG, Justino J, Silva FVM, Noronha JP, Cabrita EJ, Brouard I, Bermejo J (2007) A new lupene triterpenetriol and anti-cholinesterase activity of *Salvia sclareoides*. *Fitoterapia* **78**, 474-481
- Raveh L, Grauver E, Grunwald J, Cohen E, Ashani Y (1997) The stoichiometry of protection against soman and VX toxicity in monkeys pretreated with human butyrylcholinesterase. *Toxicology and Applied Pharmacology* **145**, 43-53
- Ren Y, Houghton PJ, Hider RC, Howes MJ (2004) Novel diterpenoid acetylcholinesterase inhibitors from *Salvia miltiorrhiza*. *Planta Medica* **70**, 201-204
- Roman GC (2003) Vascular dementia: distinguishing characteristics, treatment and prevention. *Journal of American Geriatric Society* **51**, S296-S304
- Sáez-Valero J, Small DH (2001) Altered glycosylation of cerebrospinal fluid butyrylcholinesterase in Alzheimer's disease. *Brain Research* **889**, 247-250
- Savelev S, Okello E, Perry NSL, Wilkins RM, Perry EK (2003) Synergistic and antagonistic interactions of anticholinesterase terpenoids in *Salvia lavandulaefolia* essential oil. *Pharmacology Biochemistry and Behaviour* **75**, 661-668
- Schrattenholz A, Pereira EFR, Roth U, Weber KH, Albuquerque EX, Maelicke A (1996) A novel class of allosterically acting ligands potentiates agonist responses of neuronal nicotinic acetylcholine receptors. *Molecular Pharmacology* **49**, 1-6
- Shigeta K, Ootaki K, Tatemoto H, Tsutomu N, Akria I, Norio M (2002) Potentiation of nerve growth factor-induced neurite outgrowth in PC12 cells by a *Coptidis rhizome* extract and protoberberine alkaloids. *Bioscience, Biotechnology and Biochemistry* **66**, 249-254
- Siddiqui MF, Levey AI (1999) Cholinergic therapies in Alzheimer's disease. *Drugs of Future* **24**, 417-444
- Sisodia SS (1999) Series Introduction: Alzheimer's disease: perspectives for the new millennium. *The Journal of Clinical Investigation* **104**, 1169-1170
- Soreq H, Zakut H (1993) *Human Cholinesterases and Anticholinesterases*, Academic Press, San Diego, pp 1-300
- Slegers K, van Duijn CM (2001) Alzheimer disease: Genes, pathogenesis and risk prediction. *Community Genetics* **4**, 197-203
- Small DH, Mok SS, Bornstein JC (2001) Alzheimer's disease and A $\beta$  toxicity: from top to bottom. *Nature Reviews Neuroscience* **2**, 595-598
- Soreq H, Seidman S (2001) Acetylcholinesterase – new roles for an old actor. *Neuroscience* **2**, 294-302
- Suh SJ, Koo BS, Jin UH, Hwang MJ, Lee IS, Kim CH (2006) Pharmacological characterization of orally active cholinesterase inhibitory activity of *Prunus persica* L. Batsch in rats. *Journal of Molecular Neuroscience* **29**, 101-107
- Sung SH, Kang SY, Lee KY, Park MJ, Kim JH, Park JH, Kim YC, Kim J, Kim YC (2002) (+)- $\alpha$ -Vinifrin, a stilbene trimer from *Caragana chamlague*, inhibits acetylcholinesterase. *Biological and Pharmaceutical Bulletin* **25**, 125-127
- Sussman JL, Harel M, Frolow F, Oefner C, Goldman A, Toker L, Silman I (1991) Atomic structure of acetylcholinesterase from *Torpedo californica*: a prototypic acetylcholine-binding protein. *Science* **253**, 872-879
- Tang XC (1996) Huperzine A (Shuangyiping): A promising drug for Alzheimer's disease. *Acta Pharmacologica Sinica* **17**, 481-484
- Tang XC, Han YF (1999) Pharmacological profile of huperzine A, a novel acetylcholinesterase inhibitor from Chinese herb. *CNS Drug Reviews* **5**, 281-300
- Taylor P, Radic Z (1994) The cholinesterase: from genes to proteins. *Annual Review of Pharmacological Toxicology* **34**, 281-320
- Taylor P (1998) Development of acetylcholinesterase inhibitors in the therapy of AD. *Neurology* **51**, 30-35
- Thomsen T, Kewitz H (1990) Selective inhibition of human acetylcholinesterase by galanthamine *in vitro* and *in vivo*. *Life Science* **46**, 1553-1558
- Thirugnanasampandan R, Jayakumar R, Narmatha Bai V, Martin E, Rajendra Prasad KJ (2008) Antiacetylcholinesterase and antioxidant Kaurene diterpenoid melissoides from *Isodon wightii*. *Natural Product Research* **22**, 681-688
- Tildesley NT, Kennedy DO, Perry EK, Ballard CG, Savelev S, Wesnes KA, Scholey AB (2003) *Salvia lavandulaefolia* (Spanish sage) enhances memory in healthy young volunteers. *Pharmacology Biochemistry and Behaviour* **75**, 669-674
- Tiraboschi P, Hansen LA, Alford M, Sabbagh MN, Schoos DO, Masliah E (2000) Cholinergic dysfunction in diseases with Lewy bodies. *Neurology* **54**, 407-411
- Urbain A, Marston A, Queiroz EF, Ndjoko K, Hostettmann K (2004) Xanthenes from *Gentiana campestris* as new acetylcholinesterase inhibitors. *Planta Medica* **70**, 1011-1014
- Vinutha B, Prashanth D, Salma K, Sreeja SL, Pratiti D, Padmaja R, Radhika S, Amita A, Venkateshwarlu K, Deepak M (2007) Screening of selected Indian medicinal plants for acetylcholinesterase inhibitory activity. *Journal of Ethnopharmacology* **109**, 359-363
- Wang HH, Chou CJ, Liao JF, Chen CF (2001) Dehydroevodiamine attenuates beta-amyloid peptide-induced amnesia in mice. *European Journal of Pharmacology* **413**, 221-225
- Wang YE, Yue DX, Tang XC (1986) Anticholinesterase activity of huperzine A. *Acta Pharmacologica Sinica* **7**, 110-113
- Wang H, Zhou G, Gao X, Wang Y, Yao W (2007) Acetylcholinesterase inhibitory-active components of *Rhodiola rosea* L. *Food Chemistry* **105**, 24-27
- Wettstein A (2000) Cholinesterase inhibitors and *Ginkgo* extracts are they comparable in the treatment of dementia? Comparison of published placebo-controlled efficacy studies of at least six months' duration. *Phytomedicine* **6**, 393-401
- Whitehouse PJ, Price DL, Struble RG, Clark AW, Coyle JT, Delon MR (1982) Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science* **215**, 1237-1239
- Wierenga JM, Hollingworth RM (1992) Inhibition of insect acetylcholinesterase by the potato glycoalkaloid  $\alpha$ -chaconine. *Natural Toxins* **1**, 96-99
- Wright CI, Geula C, Mesulam MM (1993) Neurological cholinesterases in the normal brain and in Alzheimer's disease: relationship to plaques, tangles and patterns on selective vulnerability. *Annals of Neurology* **34**, 373-384
- Ye JW, Cai JX, Wang LM, Tang XC (1999) Improving effects of huperzine A on spatial working memory in aged monkeys and young adult monkeys with experimental cognitive impairment. *Journal of Pharmacological and Experimental Therapeutics* **288**, 814-819
- Yong LK, Seon YJ, Sil KM, Young KS, Choong KY (2005) Anti-acetylcholinesterase and anti-amnesic activities of a pregnane glycoside, cynatroside B, from *Cynanchum atratum*. *Planta Medica* **71**, 7-11
- Zarotsky V, Sramek JJ, Cutler NR (2003) Galanthamine hydro-bromide: an agent for Alzheimer's disease. *American Journal of Health-System Pharmacy* **60**, 446-452
- Zhang Y, Kua J, McCammon JA (2002) Role of the catalytic triad and oxyanion hole in acetylcholinesterase catalysis: An *ab initio* QM/MM Study. *Journal of the American Chemical Society* **124**, 10572-10577