Plants as Potential Sources for Drug Development against Alzheimer’s Disease

Keyvan Dastmalchi • H. J. Damien Dorman* • Heikki Vuorela • Raimo Hiltunen

ABSTRACT

Alzheimer’s disease (AD) is a progressive, neurodegenerative disorder that primarily affects the elderly population and is considered to be responsible for ca. 60% of all dementia in people aged 65 or older. Due to its debilitating nature, an enormous social and economic burden is placed on society. The significance of AD is further compounded as the number of identified cases is estimated to double or triple by 2050. Currently there is no cure for the disorder and much of the treatments available have been able to only delay the progression of the disease or provide symptomatic relief for a short period of time. Therefore there is a need for a different approach to the treatment of these diseases. Plants have been used since antiquity in the treatment of various diseases including cognitive disorders, such as AD. Therefore ethnopharmacological screening of plants may provide useful leads in the discovery of new drugs for AD therapy. This article reviews screening of the plants, belonging to 21 families, used in traditional systems of medicine (e.g. Chinese, Indian and European) for treatment of cognitive dysfunction. Electronic data bases were used for searching information related to the studies done on the plants in the last 20 years. Phytochemical substances such as alkaloids, biphenolic lignans, curcuminoids, caffeic acid derivatives, diterpenes, triterpenoid saponins, triterpene lactones, stilbenes and withanolides with pharmacological activities relevant to AD treatment are discussed in this review. Compounds of potential interest for further drug development studies have been highlighted.

Keywords: antioxidant, anti-inflammatory activity, Amyloid β peptide, cholinesterase inhibition, ethnopharmacology, iron chelation, lipid peroxidation, traditional medicine

Abbreviations: AD, Alzheimer’s disease; AChE, acetylcholinesterase; Aβ peptide, Amyloid β peptide; AM, Ayurvedic medicine; BuChE, butyrylcholinesterase; ChAT, choline acetyltransferase; COX, cyclooxygenase; 5-HT, 5-hydroxytryptamine; KUT, Kami-utan-to; NGF, nerve growth factor; NMBA, N-methyl-D-aspartate; ROS, reactive oxygen species; RNS, reactive nitrogen species; TNF-α, Tumour Necrosis Factor-α; TCM, traditional Chinese medicine

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Invited Review
INTRODUCTION

A reduction in birth rates and increased life expectancy has resulted in a quantitative increase in the mean population age. There has also been an increase in the incidence of age-associated diseases, e.g. arthritis, cardiovascular disease, diabetes, neurodegeneration, etc. Perhaps the most debilitating of these conditions are those that affect the nervous system. While the aetiology and pathogenesis of many age-related conditions may be affected by lifestyle changes or can be managed through pharmacological intervention, neurodegenerative disorders are either poorly responsive to such approaches or their progression appears unabatable. Of the neurodegenerative disorders, Alzheimer’s disease (AD) is considered to be responsible for ca. 60% of all dementia in people aged 65 or older (Francis et al. 1999). Due to its debilitating nature, an enormous social and economic burden is placed on society. The significance of AD is further compounded as the number of identified cases is estimated to double or triple by 2050 (Fillit 2000).

None of the pharmacological lines of intervention have so far been able to stop the progression of AD (Small and Mayeux 2005); thus, a need for an alternative approach was believed necessary to make progress with particular emphasis on plants. Plants have been used since antiquity in traditional medicinal systems for the treatment of memory dysfunction. Studies carried out on some species have resulted in the identification of compounds which are currently used in clinical practice or templates for further drug discovery, e.g. galantamine, an alkaloid isolated from Galanthus nivalis L. (Amaryllidaceae). Galantamine was approved by the FDA in 2004 for use as an acetylcholinesterase inhibitor in the treatment of AD (Jones et al. 2006). It was the traditional use of G. nivalis L. in Bulgaria and Turkey for neurological conditions that led to the development of this drug (Shu 1998).

The importance of plant-derived compounds in drug discovery is evident from a glance at the Prescription Drug Audit (Jones et al. 2006): 35 natural product related drugs originally discovered from vascular plants were among the 150 top selling drugs in 1993 (Jones et al. 2006). The majority of plant-related drugs were discovered from ten plant species, nine of which had been used traditionally for medicinal properties that were related to the current therapeutic indication (Jones et al. 2006). This shows a clear correlation between the ethnomedical uses of the plants and the current use of their derived drugs. The strategy used for discovery of new drugs based on the screening of plants having medicinal uses relevant to the treatment of a particular disease, is referred to as ethnopharmacological screening (Samuelsson 2004a). This appears to be a particularly rewarding approach because it has been reported that, of 122 drugs derived from medicinal plant which are in use world wide, 80% can be traced back to their ethnomedical uses (Jones et al. 2006).

There have been previous reviews on the plants demonstrating pharmacological and clinical effects of potential interest in AD therapy, including Clement et al. (2004), Howes and Houghton (2003), Howes et al. (2003), Kumar (2006) and Zhang (2004). By studying the reviews it becomes clear that ethnopharmacological screening is one of the main approaches used in drug discovery. Some of the preclinical and clinical studies related to AD, carried out with medicinal plants have been mentioned in Table 1. The following is a review of the plants and the phytochemical substances which have shown to be of therapeutic potential in AD therapy.

ALZHEIMER’S DISEASE AND MAJOR PHARMACOLOGICAL INTERVENTIONS

Alzheimer’s disease is a progressive neurodegenerative disorder characterised by impairment in learning and memory followed by more global cognitive deficits and behavioural disturbances (i.e. depression, agitation and psychosis), which become progressively more severe. The pathology of AD is
<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Extract</th>
<th>Extraction</th>
<th>Material</th>
<th>Dosage</th>
<th>RA</th>
<th>Model</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acorus calamus</td>
<td>50% EtOH&lt;sup&gt;b&lt;/sup&gt;</td>
<td>N.S.&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Rhizome</td>
<td>25 mg/kg/day</td>
<td>p.o&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Animal</td>
<td>Shukla et al. 2002</td>
</tr>
<tr>
<td>Bacopa monniera</td>
<td>EtOH</td>
<td>N.S.</td>
<td>N.S.</td>
<td>5/10 mg/kg/day</td>
<td>p.o</td>
<td>Animal</td>
<td>Vohora et al. 2000</td>
</tr>
<tr>
<td>Curcuma longa</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;O Distillation</td>
<td>Plant</td>
<td>30 mg/kg/day</td>
<td>Post. op&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Animal</td>
<td>Russo and Borelli et al. 2005</td>
<td></td>
</tr>
<tr>
<td>Crocus sativus</td>
<td>50% EtOH</td>
<td>N.S.</td>
<td>Stem/leaf</td>
<td>5/10 mg/kg/day</td>
<td>p.o</td>
<td>Animal</td>
<td>Bhattacharya et al. 2000</td>
</tr>
<tr>
<td>Salvia lavandulaefolia</td>
<td>EtOH</td>
<td>N.S.</td>
<td>Rhizome</td>
<td>300 mg/kg/day</td>
<td>p.o</td>
<td>Animal</td>
<td>Stough et al. 2001</td>
</tr>
<tr>
<td>Salvia miltiorrhiza</td>
<td>EtOH</td>
<td>N.S.</td>
<td>Rhizome</td>
<td>5/10 mg/kg/day</td>
<td>i.c.v.&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Animal</td>
<td>Bhattacharya et al. 1999</td>
</tr>
<tr>
<td>Polygala tenuifolia</td>
<td>N.S.</td>
<td>Rhizome</td>
<td>300/400 mg/day</td>
<td>p.o</td>
<td>Animal</td>
<td>Kumar 2006</td>
<td></td>
</tr>
<tr>
<td>Piper methysticum</td>
<td>EtOH</td>
<td>N.S.</td>
<td>Seed</td>
<td>250/500 mg/kg/day</td>
<td>p.o</td>
<td>Animal</td>
<td>Nishiyama et al. 1995b</td>
</tr>
<tr>
<td>Melissa officinalis</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;O Infusion</td>
<td>Plant</td>
<td>100/200/300 mg/kg/day</td>
<td>p.o</td>
<td>Animal</td>
<td>Kumar and Gupta 2002a</td>
<td></td>
</tr>
<tr>
<td>Ginkgo biloba</td>
<td>50% EtOH</td>
<td>N.S.</td>
<td>Stem/leaf</td>
<td>60 drops/day</td>
<td>50% EtOH</td>
<td>Animal</td>
<td>Nishiyama 1997</td>
</tr>
<tr>
<td>Ginkgo biloba</td>
<td>80% MeOH</td>
<td>Reflux/LLE&lt;sup&gt;i&lt;/sup&gt;</td>
<td>N.S.</td>
<td>100 mg/kg</td>
<td>p.o&lt;sup&gt;j&lt;/sup&gt;</td>
<td>Animal</td>
<td>Park et al. 1996</td>
</tr>
<tr>
<td>Hypericum perforatum</td>
<td>50% EtOH</td>
<td>N.S.</td>
<td>Stem/leaf</td>
<td>60 drops/day</td>
<td>p.o</td>
<td>Human</td>
<td>Maurer et al. 1997</td>
</tr>
<tr>
<td>Hypericum perforatum</td>
<td>80% EtOH</td>
<td>N.S.</td>
<td>Rhizome</td>
<td>100/200 mg/kg/day</td>
<td>p.o</td>
<td>Animal</td>
<td>Kumar et al. 2000, 2002c</td>
</tr>
<tr>
<td>Hypericum perforatum</td>
<td>Crude powder</td>
<td>Eq.Hy&lt;sup&gt;k&lt;/sup&gt;, 4.3/13μg/kg/day</td>
<td>p.o</td>
<td>Animal</td>
<td>Tyszkielewicz et al. 2002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypericum perforatum</td>
<td>45% EtOH</td>
<td>N.S.</td>
<td>Leaf</td>
<td>100/200 mg/kg/day</td>
<td>p.o</td>
<td>Animal</td>
<td>Schindowski et al. 2000</td>
</tr>
<tr>
<td>Hypericum perforatum</td>
<td>30% MeOH</td>
<td>N.S.</td>
<td>Leaf</td>
<td>300/600/900 mg/kg/day</td>
<td>p.o</td>
<td>Human</td>
<td>Kennedy et al. 2002</td>
</tr>
<tr>
<td>Piper methysticum</td>
<td>N.S.</td>
<td>Rhizome</td>
<td>300mg</td>
<td>p.o</td>
<td>Human</td>
<td>Thompson et al. 2004</td>
<td></td>
</tr>
<tr>
<td>Polygala tenuifolia</td>
<td>80% EtOH</td>
<td>N.S.</td>
<td>Rhizome</td>
<td>10 mg/kg</td>
<td>i.p</td>
<td>Animal</td>
<td>Park et al. 2002</td>
</tr>
<tr>
<td>Salvia lavandulaefolia</td>
<td>Essential oil</td>
<td>N.S.</td>
<td>N.S.</td>
<td>25/50 μl/visit</td>
<td>p.o</td>
<td>Human</td>
<td>Tildesley et al. 2005</td>
</tr>
<tr>
<td>Salvia miltiorrhiza</td>
<td>MeOH</td>
<td>N.S.</td>
<td>Rhizome</td>
<td>0.5/1 g/kg</td>
<td>p.o</td>
<td>Animal</td>
<td>Hsieh et al. 2000</td>
</tr>
<tr>
<td>Salvia officinalis</td>
<td>CHCl&lt;sub&gt;3&lt;/sub&gt;, EtOAc&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Soxhlet</td>
<td>Leaf</td>
<td>50-1000 μg/cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Topical</td>
<td>Animal</td>
<td>Baricic et al. 2001</td>
</tr>
<tr>
<td>Withania somnifera</td>
<td>45% EtOH</td>
<td>N.S.</td>
<td>Leaf</td>
<td>60 drops/day</td>
<td>p.o</td>
<td>Human</td>
<td>Akhondzadeh et al. 2003a</td>
</tr>
<tr>
<td>Withania somnifera</td>
<td>Rhizome powder</td>
<td>N.S.</td>
<td></td>
<td>1000 mg/kg/day</td>
<td>p.o</td>
<td>Animal</td>
<td>Rasool and Varalakshmi 2007</td>
</tr>
</tbody>
</table>

Abbreviations: RA, route of administration; EtOH, ethanol; N.S., non specified; p.o, per os; Post.op, post operatively; i.c.v., intracerebroventricular; %i.d., per in die; *Aer., aerial parts; MeOH, methanol; LLE, liquid-liquid extraction; i.p, intraperitoneal; *Aq. ROH, aqueous alcohol; *Ac. ACN: aqueous acetone; *An. MeOH, anhydrous methanol; Eq. Hy, equivalent hypericin; CHCl<sub>3</sub>, chloroform; *EtOAc, ethylacetate
a complex and a multifaceted one with several pathogenic pathways are believed to contribute to the progression of the disease, viz., senile plaque deposition, neurofibrillary tangle formation, inflammatory cascade, oxidative stress and cholinergic deficit (Small and Mayeux 2005). Based on these pathological hallmarks, several lines of pharmacological treatments have been investigated which are discussed.

**Senile plaque deposition and anti-amyloid agents**

A major component of senile plaques is the Amyloid β (Aβ) peptide which is formed as a result of proteolytic cleavage of the amyloid precursor protein (APP) by the secretases. According to the ‘β-amyloid theory’, it has been proposed that Aβ peptide deposits or even the partially aggregated soluble form are responsible for triggering a neurotoxic cascade of events which ultimately results in neurodegeneration (Castro et al. 2002). Therefore, modulating the chain of events starting from the production of Aβ peptide fragments from APP to its deposition in the form of extracellular plaques or even clearance of the already formed plaques are believed to be possible approaches toward the treatment of AD.

**α-Secretase activity enhancers**

α-Secretase is a membrane bound enzyme that hydrolyses APP within the Aβ domain thereby there is no Aβ peptide formed as a result of proteolysis. This is the major pathway of APP processing and is referred to as the non-amyloidogenic pathway (Blennow 2006). Promoting this pathway by enhancing the activity of α-secretase can be considered as a line of therapy; however, the problem is information about α-secretase enzyme is limited.

**β- and γ-Secratease inhibitors**

Another proteolytic pathway of APP processing is the amyloidogenic route. This involves cleavage of the APP at the extracellular and transmembrane domains by β- and γ-secretases, respectively. The result of the two proteolytic steps is the formation of Aβ(1-40) and Aβ(1-42) fragments with the former being the most abundant of the two species (Scorer 2001). The Aβ(1-42) fragment is the pathogenic species which aggregates more readily and forms amyloid fibrils (Scorer 2001). Attempts at inhibition of these enzymes have been made, but the compound under investigation are in the early stages of testing and it will take several years before they reach clinical trials.

**Aβ immunisation**

One strategy used for targeting the Aβ peptide is the immunotherapy. The approach is based on immunisation of the subject against the peptide with the result that plaque deposition and the subsequent related hallmarks related to it are prevented. The problem with this approach, however, is that despite no adverse effects were reported in initial patient safety tests, during phase II trials signs of inflammation development in the CNS were observed.

**Neurofibrillary tangle formation and tau inhibitors**

One of the pathological hallmarks of AD is the formation of intracellular neurofibrillary tangles which consists of hyperphosphorylated tau protein. Tau is an axonal protein which binds to microtubules and by doing so promotes their assembly and stability. Phosphorylation of tau protein is regulated by the balance between multiple kinases (e.g. GSK-3β and CDK5) and phosphatases (e.g. PP-1 and PP-2A) (Blennow 2006). Hyperphosphorylation of tau protein starts in AD intracellularly and leads to sequestration of the protein and other microtubule associated proteins, thus preventing microtubule assembly and impairing axonal transport. This results in neuronal function being compromised which ultimately precipitates neuronal death (Blennow 2006). Based on this pathogenic cascade, two principal lines of investigation have been proposed: (i) prevention of tau hyperphosphorylation and (ii) prevention of tau aggregation.

**Preventing tau hyperphosphorylation**

This approach address the imbalance in the kinase and phosphatase activities by inhibiting the action of protein kinases involved in phosphorylation of tau protein. There are several kinases implicated in tau phosphorylation such as glycogen synthetase kinase-3β (GSK-3β) and cyclin-dependent kinase 5 (CDK5) (Blennow 2006) which can be potential drug targets. The search for protein kinase inhibitors is an active one, however, to date, no kinase inhibitors have been launched as drug products (Castro et al. 2002).

Among the compounds which have demonstrated GSK-3β inhibitory activity, lithium, bisindolylmaleimides I (1) and IX (2) and thiadiazolidinones derivatives can be mentioned. Hymenialdisine, indirubin and paullones and even bisindolylmaleimides I and IV have shown CDK5 inhibitory activity (Castro et al. 2002).

One interesting development is that M1 muscarinic agonists AF102B (3), AF150(S) (4) and AF267B (5) are reported to have GSK-3β inhibitory activity (Fisher 2000). This unique feature of having the ability to alter different aspects of AD pathophysiology is of considerable importance and further research on the nature of activity of these compounds is recommended.

**Preventing tau aggregation**

It is important to develop compounds that could be used to facilitate the proteolytic degradation of tau aggregates and prevent propagation of neurofibrillary tangles. Research has shown that selective inhibitors of cathepsin D are capable of preventing the formation for hyperphosphorylated tau fragments in a dose dependent fashion. Cathepsin D is a pro tease which is capable of cleaving tau protein at neutral pH and could be useful in regulating the formation of the precursors to neurofibrillary tangles (Bi et al. 2000). There is also selective inhibition of tau aggregation by dianmophenotiazines reported (Wischik et al. 1996). However there is more need for pharmacokinetic and toxicological studies.

**Oxidative stress and antioxidant activity**

The vulnerability of CNS to oxidative damage is due to a number of factors such as excessive oxygen uptake and high unsaturated lipid content. Under normal physiological conditions, damage by reactive oxygen species (ROS) is kept in check by antioxidant defence cascade consisting of enzymatic and non-enzymatic components (Valko et al. 2007). However, during degenerative processes there is an imbalance between ROS and cellular antioxidant defences which leads to critical failure of biological functions. One of the sources of oxidative stress in AD is the disturbance in metal homeostasis such as iron, copper, zinc and aluminium, metals capable of catalyzing reactions that produce free radicals (Sayre et al. 2001). Mitochondrial dysfunction, as source of ROS generation, has been proposed to be associated with variety of degenerative pathways leading to AD progression (Law et al. 2001). Aβ peptides are another important source of oxidative damage, producing neurotoxic effects directly by inducing more ROS and indirectly by activating microglia (Varadarajan et al. 2000). It has been proposed that Aβ peptides in the presence of transition metal ions produces ROS such as superoxide anion radical and hydrogen peroxide which are known to be responsible for oxidative damage in vivo (Varadarajan et al. 2000). Microglial activation leads to a massive production of inflammatory cytokines, ROS and reactive nitrogen species (RNS), thereby contributing to oxidative damage (Scorer 2001). Therefore, oxidative stress and the inflammatory cascade working in
concert with each other have been proposed to play a significant role in the pathogenesis of AD.

Taking into account the various sources of oxidative stress, previously discussed, several pharmacological opportunities for influencing the disease can be suggested. One class of chemicals are those scavenging free radicals before they can bring about their deleterious effects. These include chemicals such as vitamins E (6) and C (7), selegiline (5-methoxytryptamines) (8), dopamine (amine neurohormone) (9) and idebenone (a coenzyme Q analogue) (10), which have been used for different therapeutic purposes and have produced clinically significant results in AD studies (Castro et al. 2002). Neuroprotective effects of non-estriadiol anti-inflammatory drugs by direct scavenging of nitric oxide radicals has opened up another avenue for treatment of the disease. Another group of antioxidants being investigated for the therapeutic potential are metal chelators (Castro et al. 2002). There are two aspects of their activity which are important: (i) by chelating the transition metal ions present in brain tissues, they inhibit their catalytic activity thereby preventing associated free radical generation which is termed as secondary antioxidant effect (Gordon et al. 1990) and (ii) by chelating the metals they prevent their subsequent binding to Aβ which prevents senile plaque formation (Doraiswamy et al. 2002). In a study carried out by Cherry et al. (1999), a copper/zinc chelator was capable of dissolving Aβ plaques. It has been shown that a mild to moderate chelating activity, which prevents metal ion binding to Aβ peptide, is preferred to strong chelating activity (Bush 2003; Ji and Zhang 2005). The former is referred to in the literature as the metal attenuation. A very good example is clioquinol (11) which showed desirable therapeutic efficacy in the initial stages of clinical trials (Doraiswamy 2002; Rosenberg 2003). AGE-inhibitors such as Tenilsetam (12) (are capable of inhibiting protein binding with sugars and the resultant sugar-derived oxidation (Dury et al. 1999). Another line of investigation is the inhibition of membrane lipid peroxidation, Lazabemide (13) being an example of such an inhibitor. Thus compounds can inhibit propagation of free radicals by partitioning into the hydrophobic membrane domain (Mason et al. 2000).

So far no antioxidant compound has been approved for clinical use, but clinical studies of these compounds continue in the hope of finding a suitable treatment in the near future. Some plants and their constituents which possess potent antioxidant activity have shown effects upon the CNS that are of relevance in the treatment of AD.

**Inflammatory cascade and anti-inflammatory activity.**

Hallmarks of inflammation such as activated microglial cells and pro-inflammatory cytokines have been found in the post mortem brains of the AD patients. Clinical studies have also pointed out an increase in the level of inflammatory markers (Doraiswamy et al. 1997). Treatment of culture systems with fibrillier Aβ has lead to microglial activation (Doraiswamy et al. 2002). Several epidemiological studies pointed to an association between the use of NSAIDs and reduced risk of developing AD (Rich et al. 1995; Doraiswamy et al. 1997; Veld et al. 2001). Some of these studies have suggested that they may affect the age and onset of the disease. Many mechanisms have been proposed for their activity ranging from COX inhibition (Patricó and Trojanowski 2000) to lowering of amyloidogenic Aβ–42 peptide (Scorer 2001). Unfortunately, recent studies with NSAIDs such as celecoxib (14) and rofecoxib (15) were not beneficial in the treatment (Scorer 2001; Doraivaswamy 2002). This shows that, it is unclear which anti-inflammatory targets are more relevant in the treatment of AD. Attention should be focused on clarification. Once resolved, there is a need for identification of novel molecular moieties with fewer adverse effects than the currently available drugs which are more effective in stopping the progression of the disease. Natural products can be a potential source for such novel molecules. For example, numerous plant constituents have demonstrated anti-inflammatory properties (Handa et al. 1992; Bingöl and Şener 1995).

**Cholinergic deficit and neurotransmitter replacement therapy**

The selective degeneration of cholinergic neurons that originate in the basal forebrain and projects to the cortex and hippocampus results in the loss of all known cholinergic markers, such as choline acetyltransferase (ChAT), acetylcholine (ACh) levels and acetylcholinesterase (AChE). ACh is associated with cognition and it is the deficit of this neurotransmitter which contributes to cognitive dysfunction. The degeneration of these cholinergic neurons has been proposed to be a result of amyloid fibril-induced neuronal injury, ROS/RNS or astrocyte phagocytic activity (Small and Mayeux 2005).

On the other hand, ACh is known to promote non-amyloidogenic processing and reduce tau phosphorylation by reducing the activity of protein kinase which phosphorylates tau. Therefore, disruption of cholinergic signalling may lead to a feedback loop that increases production Aβ through altered APP processing, increasing phosphorylation of tau protein, thereby contributing to the progression of AD pathology (Lahiri et al. 2003).

Based on what has been mentioned above, restoration of the central cholinergic function may significantly improve cognitive impairment and may inhibit AD progression in patients. There are 3 principal approaches by which the cholinergic deficit can be addressed: (i) nicotinic receptor stimulation, (ii) muscarinic receptor stimulation and (iii) cholinesterase inhibition.

**Nicotinic receptor stimulation**

It has been reported that smoking may have protective effect against AD and nicotine (16) administration improved cognitive functions in AD patients as well as healthy elderly people (Newhouse and Kelton 2000; Min et al. 2001). It is also reported that nicotine increased the ACh level in vivo (Whitehouse and Kalaria 1995; Balfour and Fagerström 1996), thereby enhancing cholinergic neurotransmission in AD patients. However, nicotine is not the only compound and therefore nicotinic receptor agonists have been used for the restoration of ACh levels (Houghton and Howes 2005). In a study carried out by Potter et al. (1999) ABT-418 (17), a novel nicotinic agonist, significantly improved declining cognitive function. Several studies indicating that stimulation of central nicotinic receptors has an acute cognitive benefit (Kihara and Shimohama 2004). Currently, there is no nicotinic receptor agonist available for the treatment of AD patients, however there is research going in this field.

**Muscarinic receptor stimulation**

The rationale behind using compounds for their muscarinic agonistic action is to compensate for the low levels of ACh associated with AD. In addition to addressing the cholinergic deficits these agents also inhibit the fibrillary tangle formation and Aβ production (Houghton and Howes 2005). Currently there is no chlorogenic substance with muscarinic stimulation which has been marketed. However, research is going on in this field and some of the muscarinic compounds have shown promising results in animal experiments. Arecoline (18) and pilocarpine (19) are examples of
muscarnic agonist which have been tested for their cogni
tive enhancing function. Both the chemical are plant-de
rived alkaloids. They have provided template for further
drug development research (Houghton and Howes 2005).

Cholinesterase inhibitors

Two types of cholinesterases, AChE and BuChE, are pre
sent in a wide variety of tissues. AChE, which is the predo
minant cholinesterase in the brain, hydrolyzes ACh to cho
line and acetate, thereby terminating the effect of this neu
rotransmitter at cholinergic synapses (Small and Mayeux
2005). AChE is, therefore, the target of cholinesterase in
hibitors which are used for addressing the cholinergic defi
cit in AD patients.

Over the last two decades, cholinesterase inhibition has be
come the most widely studied and effective clinical ap
proach to treat the symptoms of AD. Four cholinesterase in
hibitors, tacrine (20), donepezil (21), rivastigmine (22) and
galantamine (23) and have been approved by the United
States Food and Drug Administration (FDA) for treating
symptoms of AD. All these drugs are centrally active and
were shown to improve memory and cognition in some
patients with mild to moderate AD. However, this approach
is limited, in principle, to patients who have intact func
tionally active presynaptic neurons that are capable of syn
thesizing and releasing ACh. Therefore, AChEIs so far are
only useful in the early stages of AD and lose effectiveness
over time.

Increasingly, research has indicated the possibility that
cholinesterase inhibitors in addition to providing symptom
atic relief are having modulatory effects upon plaque depo
sition. Several recent studies using cell culture and animal
models have shed light upon the effects of cholinesterase in
hibitors at the level of Aβ peptide. Specific cholinesterase in
hibitors exert amyloid lowering effect as a consequence of
both their cholinergic and non-cholinergic activities: (i)
cholinesterase inhibition results in an increase in ACh
which as mentioned before will promote non-amyloidoge
nic processing (Lahiri et al. 2003) (ii) APP expression is sup
pressed with the result that the quantity of its proteolytic
product, Aβ peptide, will also decrease (Shaw et al. 2001).

Butryrl cholinesterase inhibitory agents may be especi
ally critical in light of co-localization of BuChE and amy
loid plaques, Aβ peptide, NFTs and dystrophic neurons, all
pathological hallmarks associated with AD pathology (Cas
tro et al. 2002; Lahiri et al. 2003).

PLANTS AND PHYTOCHEMICALS OF POTENTIAL
INTEREST IN ALZHEIMER’S DISEASE THERAPY

Medicinal plants

Acorus calamus L. (Araceae)

The plant A. calamus commonly know as sweet flag, is a
perennial herb which grows mainly in swamps, marshes and
river banks. In Ayurvedic medicine (AM), the rhizome has
been used for the treatment of memory loss (Manyam
1999). Two rhizome extracts, ethanolic and hydroethanolic, exer
sedative and neuroprotective effects in vivo respectively
(Vohora et al. 1990; Shukla et al. 2002) (Table 1).

Angelica archangelica L. (Umbelliferae)

A. archangelica is a perennial herbaceous plant used in tra
ditional Chinese medicine (TCM) for treatment of cerebral
diseases (Yang et al. 2005). An ethanol extract of the dried
plant roots was capable of displacing nicotine from nicotine
binding receptors in a concentration-dependent manner
(Perry et al. 1996). Park et al. (1996) showed that a di
chloromethane subfraction of a methanol extract inhibited
AChE activity in vitro.

Bacopa monniera Wettst. (Scrophulariaceae)

B. monniera (Plate 1A), commonly known as water hyssop,
is an annual plant found throughout the Indian subcontinent
in wet, damp and marshy areas. In AM, the plant is used to
improve memory and intellect. In India, this plant is locally
known referred to as Brahmi or Jalaninab (Chopra et al.
1956). Ethanol extracts of aerial parts and rhizome from the
plant possessed nootropitic activity (Stough et al. 2001; Rus
so and Borrelli 2005; Kumar 2006) (Table 1). It has been
suggested that this may be due to the bacosides being able
to induce membrane dephosphorylation with a concomitant
increase in protein and RNA turnover in specific brain areas
(Singh et al. 1988). Alternative propositions include: (i) en
hancement of protein kinase activity in the hippocampus
(Singh and Dhawan 1997) and (ii) cognitive enhancement
via its modulatory effect on the cholinergic system (Stough
et al. 2001) (Table 1).

Bhattacharya et al. (2001a) (Table 1) showed that a
standardised bacside-rich extract from the leaf and stem of
B. monniera reversed cognitive deficits induced by colchi
cine and ibotenic acid. In the same study the extract re
versed the depletion of ACh, the reduction in ChAT activity
and decreased muscarinic receptor binding in the frontal
cortex and hippocampus. A similar extract of the plant de
monstrated antioxidant activity in the rat frontal cortex, stri
atum and hippocampus (Bhattacharya et al. 2000) (Table 1).

A methanol extract of the plant inhibited NO-induced
toxicity and prevented hydrogen peroxide-induced DNA

Biota orientalis L. (Coniferae) Cupressaceae

The plant B. orientalis (Plate 1B) is an evergreen tree that
grows mainly in South East Asia. The seeds of the plant have
been used in TCM to relieve mental strain and to treat
insomnia and amnesia (Nishiyama et al. 1995a; Lin et
al. 2003). In a study carried out by Nishiyama et al. (1995b)
(Table 1), S-113m (a herbal preparation composed of B.
orientalis, Panax ginseng and Schizendra chinesis) prefer
entially improved memory registration and consolidation in
mice. An ethanol extract of B. orientalis seeds improved
memory dysfunction induced by amygdala and basal fore
brain lesions in mice (Nishiyama et al. 1992, 1995a) (Table
1).

Celastrus paniculatus Wild. (Celastraceae)

The plant C. paniculatus, commonly know as black-oil tree
is a large woody climbing shrub. In India it is known as
Malkangni and has been mentioned in ancient Indian lite
rature as an intelligence promoter (Nalini et al. 1995; Gattu
et al. 1997) (Table 1). The seeds and seed oil have been
used in AM as a memory enhancer (Nadkarni 1976). Nalini
et al. (1995) reported that the seed oil reduced the levels of
noradrenaline, dopamine and 5-hydroxytryptamine (5-HT)
in vivo (Table 1). In another study, the seed oil reversed
scopolamine-induced task deficit (Gattu et al. 1997) (Table
1). Nalini et al. (1986) reported that treatment of mentally
retarded children with the oil produced an improvement in
their IQ scores.

An aqueous seed extract showed antioxidant effect in
rat brain, which may be contribute to cognitive enhancing
activity observed in vivo (Kumar and Gupta 2002a) (Table
1).

Ahmad et al. (1994) reported that a methanol extract of
the inflorescences showed anti-inflammatory effect which
may be relevant to AD therapy. A methanol extract was as
sessed for N-methyl-D-aspartate (NMDA) and γ-aminobu
tyric acid (GABA) binding activities and nerve growth fac
tor (NGF) effects but did not show any response (Dev 1997).
A possible explanation may be that the extraction solvent
was polar and the seed oil and hydrophobic constituent may
be responsible for the cognitive enhancing effects of C.
paniculatus.
Centella asiatica L. (Umbelliferae)

*C. asiatica* (Plate 1C) is a slender perennial creeper which grows throughout the tropical regions in the world. The leaf, known locally as Gotu Kola, has been used in AM for revitalising and strengthening nervous function and memory. For example, an Ayurvedic formulation composed of 4 herbs, including *C. asiatica* is used as a restorative and for the prevention of dementia (Manyam 1999). In TCM, it is also used to combat physical and mental exhaustion (Duke and Ayensu 1985; Brinkhaus et al. 2000).

An alcoholic extract of the plant possessed tranquilising and potentially cholinomimetic activities in vivo, which may be due to the presence of the triterpenoid brahminsode (Sakina and Dandiya 1990) (Table 1). An aqueous leaf extract modulated dopaminergic, serotonergic and adrenergic systems in vivo and improved learning and memory (Nalini et al. 1992).

The essential oil from the plant is reported to contain monoterpenes e.g. β-pinene and γ-terpinene (Brinkhaus et al. 2000), which have demonstrated AchE inhibitory activity, though not as potent as the standard reference substance (Perry et al. 2000).

Clitoria ternatea L. (Leguminosae)

*C. ternatea* (Plate 1D), commonly known as butterfly-pea, is a persistence herbaceous perennial legume. The rhizome has been used in AM as a brain tonic and is reputed to pro-
mote memory and intellect (Misra 1998). In a study carried out by Taranalli and Cheeramkuzhy (2000) (Table 1), ethanol extracts of the rhizome and aerial parts exerted memory enhancing effects in vivo. These effects were associated with increased levels of ChAT and ACh in vivo. However, there was no associated increase in AChE inhibitory activity. In another study, an aqueous rhizome extract increased the level of ACh in rat hippocampus, which has been proposed to be due to an increase in ChAT (Rai et al. 2002) (Table 1).

An ethanol extract obtained from the stem, flowers, leaves and fruits of the plant was reported to be sedative in mice (Kulkarni et al. 1988).

**Codonopsis pilosula Franch. (Campanulaceae)**

*C. pilosula* (Plate 1E), know locally by the name Dang Shen, is a perennial climber which is commonly found in North East Asia. In TCM, the root is used as remedy for amnesia and is believed to improve circulation and increase vitality (Kulkarni et al. 1988). An n-butanol extract reduced impairment of memory acquisition in mice, induced by scopolamine, cycloheximide and ethanol, respectively. This showed that the extract had nootropic effect (Zhang and Liu 2001).

**Convulvulus pluricaulis Chois. (Convulvulaceae)**

*C. pluriculis*, commonly known as Shahkpushpi, is a fulvous hairy herb that has been prescribed by Ayurvedic practitioners for the treatment of nervous disorders and as anti-aging remedy (Kulkarni et al. 1988). An n-butanol extract reduced impairment of memory acquisition in mice, induced by scopolamine, cycloheximide and ethanol, respectively. This showed that the extract had nootropic effect (Zhang and Liu 1996).

**Coptis chinensis Franch. (Ranunculaceae)**

*C. chinensis*, known commonly as Huang Lian, is an evergreen perennial plant that has been used in TCM for several conditions. In a study carried out by Park et al. (1996), dichloromethane and methanol extracts demonstrated AChE inhibitory activity. Shigeta et al. (2002) reported that a methanol extract of the rhizome possessed NGF-enhancing activity. Methanol extracts of the plant are reported to have MAO inhibitory activity and nootropic activities in vivo and in vitro antioxidant activity (Hsiieh et al. 2000; Kong et al. 2001; Schinella et al. 2002) (Table 1). Liu and Ng (2000) reported that an aqueous extract showed in vitro antioxidant activity. An ethanol extract of the whole plant demonstrated anti-inflammatory effect in vivo (Cuellar et al. 2001) (Table 1).

**Crocus sativus L. (Iridaceae)**

*C. sativus*, commonly known as saffron, is small bulbous perennial that has been cultivated throughout the world for its culinary properties. The plant is used in TCM for treating disorders of the nervous system. An alcohol extract of pistils of *C. sativus* and the component crocin improved ethanol-induced impaired learning and behaviour in mice (Sugiuara et al. 1995a; Abe and Saito 2000) (Table 1). This may have been achieved by the inhibiting the impairment of hippocampal synaptic plasticity (Sugiuara et al. 1995a, 1995b). A hydroalcoholic extract of dried stigmas inhibited AP fibrillogenesis and exerted antioxidant effect in vitro (Papandreou et al. 2006).

**Curcuma longa L. (Zingiberaceae)**

Rhizomes of *C. longa*, commonly known as turmeric, have been used extensively for their culinary properties in Indian cooking and are used in AM as a remedy against aging. An aqueous extract of the rhizome demonstrated antidepressant activity in mice following oral administration, which was associated with inhibition of brain MAO type A (Yu et al. 2002) (Table 1). Antidepressant activity is of significant importance in the management of AD.

**Eudra rutaecarpa (Juss.) Benth. (Rutaceae)**

*E. rutaecarpa* (Plate 1F) is a deciduous small tree that is used in TCM for cardiotonic, restorative and anti-asthmatic effects (Howes and Hougham 2003; Howes et al. 2003). There are also TCM prescriptions which have been used in CNS disorders. A TCM preparation, Oren-gedoku-to, demonstrated antioxidant (Fushitani et al. 1995; Ohta et al. 1997; Hayashi et al. 2001), anti-inflammatory (Wang and Mineshita 1996; Dai et al. 1999; Fukutake et al. 2000) and neuroprotective (Kabuto et al. 1997; Kondo et al. 2000) activities. However, there are TCM preparations of the plant which, despite their claim failed to improve declining memory. An example is Nao Li Su which, failed to improve cognitive dysfunction in a double blind placebo controlled crossover trial (Iversen et al. 1997).

A dichloromethane extract of *E. rutaecarpa* strongly inhibited AChE in vitro and reversed scopolamine-induced memory impairment in rats (Park et al. 1996) (Table 1).

**Ginkgo biloba L. (Ginkgoaceae)**

*G. biloba* (Plate 2A) is dioecious perennial tree that is indigenous to East Asia, that has been used in TCM for the improvement of memory loss associated with abnormalities in the blood circulation (Samuelsson 2004b). Administration of plant extracts to both AD and non-AD patients in various randomised, double-blind, placebo controlled, multicentre trials resulted in improvement of cognitive functions (Hoffberth 1994; Kanowski et al. 1997; le Bars et al. 1997; Rigney et al. 1999) (Table 1).

Since early pharmacological studies revealed that the flavonoids from *G. biloba* modulated contractile movement of vascular smooth muscles, attempts were made to prepare a standardised extract rich in flavonoids, the outcome of which is EGb 761 (Kumar 2006). EGb 761 showed cognitive enhancing activity in number of clinical studies (Hoffberth 1994; le Bars et al. 1997; Maurer et al. 1997; Kanowski et al. 1997) (Table 1). The extract showed neuroprotective effect against Aβ and nitric oxide (NO) induced toxicity in the neuronal cell culture (Bastianetto et al. 2000a, 2000b) and could reduce apoptosis both in vitro and in vivo (Schindowski et al. 2001; Yao et al. 2001) (Table 1). EGb 761 showed protective effect against ischaemia-induced neurotoxicity (Chandrasekaran et al. 2001). The extract also demonstrated in vitro and in vivo antioxidant activities (Barth et al. 1991; Marcocci et al. 1994; Topol 2002). The extract improved blood supply to the brain, thereby ensuring its efficient functioning and enhanced cognitive performance (Heiss and Zeiler 1978; Loffler 1994; le Bars et al. 1997) (Table 1). Modulation of muscarinic cholinergic system enhanced performance of spatial task (Kristofikova et al. 1992).

**Hypericum perforatum L. (Clusiaceae) (Hypericaceae)**

*H. perforatum* (Plate 2B) commonly known as St. John’s Wort is a herbaceous perennial plant that has been used in Portuguese and Turkish folklore medicine for the treatment of neurological disorders (Ross 2001). The dried crude herb standardised to hypericins improved memory and learning dysfunction (Widy-Tyskievicz et al. 2002; Trofimuk et al. 2005) (Table 1). Lu et al. (2001) reported that a standard extract of *H. perforatum* (hypericin) possessed neuroprotective activity. It is reported that extracts of *H. perforatum*, which have been standardised to hypericin and hyperforin respectively, showed in vitro antioxidant activity (Hunt et al. 2001; Zheng and Wang 2001), in vivo anti-inflammatory effects (Kumar et al. 2001).

Hydroalcoholic extracts of aerial parts of *H. perforatum*,
demonstrated nootropic activity in vivo, which may due to adrenergic (α and β receptor) and serotonergic (5HT1A) antagonistic activity (Khalifa 2001; Kumar et al. 2002c, 2000) (Table 1). Re et al. (2003) suggested that a hydroalcoholic extract of the plant could reduce the rate of degradation of ACh.

**Magnolia officinalis Rehd. & Wils. (Magnoliaceae)**

* M. officinalis (Plate 2C) is a deciduous tree originally from East Asia that has been used in TCM for treating nervous disorders. Ethanolic extract of *M. officinalis*, magnolol and honokiol are reported to have antioxidant activity in vitro and in vivo (Lo et al. 1994; Chiu et al. 1997; Jie et al. 2000; Kong et al. 2000; Chen et al. 2001). Li and Weng (2005) demonstrated the in vitro antioxidant activity of various Soxhlet and supercritical fluid extracts, with the ethyl acetate-soluble Soxhlet extract being the most active.

**Melissa officinalis L. (Lamiaceae)**

* M. officinalis (Plate 2D), commonly known as lemon balm, is a perennial herb native of West Asia and eastern Mediterranean region that has been used in the European traditional system of medicine as a remedy for improving memory (Bisset 1994; Perry et al. 1996; Howes et al. 2003). The volatile oil has been reported to possess in vitro AChE inhibitory (Perry et al. 1996; Ferreira et al. 2006) and antioxidant activities (Mimica-Dukic et al. 2004; de Sousa et al. 2004; Ferreira et al. 2006). Its constituent monoterpenes were reported to possess weak AChE inhibitory activity (Ryan and Byrne 1988), while it has been suggested its anti-
oxidant activity is due to presence of oxygenated monoterpenes and sesquiterpene hydrocarbons (Mimica-Dukic et al. 2004). A wide range non-polar and polar extracts have displayed antioxidant activity (Triantaphyllou et al. 2001; Mordoniu et al. 2004; Venskutonis et al. 2005; Ivanova et al. 2005; Ferreira et al. 2006; Dastmalchi et al. 2008). In case of polar extracts, it has been proposed that the active constituents contributing to the activity of the extracts are the polyphenolic substances (Ivanova et al. 2005; Dastmalchi et al. 2008).

Ethanol and decoction extracts of aerial parts of the plant also showed in vitro AChE inhibitory activity (Ferreira et al. 2006). Ethanol extracts obtained from the leaf material were reported possessed nicotine and muscarinic receptor binding properties (Perry et al. 1996; Wake et al. 2000). A methanolic extract of the plant leaves was clinically capable of improving the mood and accuracy of attention Kennedy et al. (2002) (Table 1). However, there was a decline in memory function. Furthermore, in vitro nicotinic and muscarinic binding were low in comparison to that found by Wake et al. (2000). This difference may be due to loss of volatile component during the manufacturing process (Wake et al. 2000). Based on the reports of Kennedy et al. (2002a) and Wake et al. (2000), a clinical study was conducted by Akhondzadeh et al. (2003a) in which, a hydro-alcoholic leaf extract was effective in improving cognitive functions in mild to moderate AD patients (Table 1).

Piper methysticum Frost. (Piperaceae)

P. methysticum (Plate 2E), commonly known as Kava, is a perennial shrub that has been used in Polynesia, Melanisia and Micronesia occupies in preparation of a drink to be consumed for ritual and social purposes (Samuelsson 2004c; Shinomiya 2005). In a clinical trial carried out by Thompson et al. (2004) (Table 1), a standardised rhizome extract (kavalactones) elevated the mood and enhanced cognition performance.

Polygala tenuifolia Wild. (Polygalaceae)

P. tenuifolia, commonly known as Senega, is a perennial herb, which according to the Chinese Materia Medica its rhizome has been used as a sedative, tranquilliser and for the treatment of amnesia, forgetfulness, neuritis, nightmares and insomnia (Duke and Ayensu 1985). There have been many studies carried out on the preparation used in TCM containing P. tenuifolia one of which is DX-9386. This formulation demonstrated in vitro antioxidant activity, and improved memory dysfunction in mice (Nishiyama et al. 1994a, 1994b, 1994c; Zhang et al. 1994).

P. tenuifolia is also a component of Kami-utan-to (KUT), a traditional Japanese preparation used in the treatment of psychoneurological diseases. KUT up-regulated ChAT activity and increased NGF secretion in vitro, it also induced ChAT activity in the cerebral cortex of aged rats and in the scopolamine induced memory impaired rats (Yabe et al. 1997; Yamada and Yabe 1997). The effect of the preparation in up regulation of ChAT activity and increased NGF secretion was not as significant when P. tenuifolia was absent, however, the rhizome extract did not contribute to the effects (Yabe et al. 1997; Yamada and Yabe 1997). It was demonstrated in a clinical study that KUT treatment in AD patients improved memory-related behavior (Nakamura and Yabe 1997). It is suggested that, cinnamic acid derivatives may be contributing to the beneficial effects of KUT (Yabe et al. 1997).

A dichloromethane subfraction of a methanol rhizome extract demonstrated in vitro AChE inhibitory activity (Park et al. 1996). In another study, an ethanol rhizome extract improved cognitive dysfunction, and exerted protective effect against glutamate and APP toxic metabolites induced neurotoxicity in vitro (Park et al. 2002) (Table 1).

Aqueous extract of the rhizome showed in vitro anti-inflammatory properties (Kim et al. 1998; Koo et al. 2000). The aqueous extract also demonstrated tranquillizing activity (Tang and Eisenbrand 1992; Chang and But 2001).

Rheum spp. L. (Polygonaceae)

It is usually common to refer to Rheum palmatum L. and other species and hybrids of the genus Rheum, except Rheum rhaponticum, as rhubarb (Samuelsson 2004d). The dried rhizome of rhubarb has been used in TCM for the treatment of blood stagnation syndrome (Matsuda et al. 2001).

In a study carried out by Kageura et al. (2001), a methanol extract obtained from the rhizome of Korean rhubarb, Rheum undulatum, demonstrated in vitro antioxidant activity. In another study, methanol extracts of rhizomes from five Rheum species (R. palmatum, R. tangerinum, R. officinale, R. coreanum and R. undulatum) exhibited in vitro antioxidant properties (Matsuda et al. 2001).

Salvia lavandulaeefolia Vahl. (Lamiaceae)

S. lavandulaeefolia, known by the common name Spanish Sage, is a perennial shrub which along with Salvia officinalis has been used in European traditional medicine for enhancement of memory (Perry et al. 1998). Volatile oil obtained from S. lavandulaeefolia showed strong AChE inhibitory activity (Perry et al. 1996). The activity is believed to be due to the presence of the cyclic monoterpenes 1,8-cineole and α-pinene, with some contribution from other constituents perhaps by acting synergistically (Perry et al. 2001). Administration of S. lavandulaeefolia volatile oil decreased AChE activity in vivo (Perry et al. 2001). Components of the oil were also screened for antioxidant activity. 1,8-Cineole, α-pinene and β-pinene exerted antioxidant effect, however, camphor showed pro-oxidant activity (Perry et al. 2001).

The ethanol S. lavandulaeefolia extract showed weak activity when compared against antioxidant propyl gallate. Water and chloroform subfractions of this extract demonstrated similar activity (Perry et al. 2001). An ethanol extract of the plant demonstrated in vitro anti-inflammatory properties (Perry et al. 2001). In a clinical study carried out by Tildesley et al. (2005) (Table 1), administration of a standardised essential oil extract resulted in mood elevation and improvements of memory.

Salvia miltiorrhiza Bung. (Lamiaceae)

S. miltiorrhiza (Plate 2F), commonly known as Dan-Shen, is a perennial herb which its rhizomes have been used for the treatment of diseases and pathological conditions such as cardiovascular disorders, insomnia, neurasthenia, inflammation (Tang and Eisenbrand 1992; Huang 1993). Moon et al. (1998) reported that a methanol extract of the plant demonstrated in vitro anti-inflammatory activity. The extract was fractionated further and among all the fractions ethyl acetate fraction displayed the strongest anti-inflammatory activity. In a study carried out by Hsieh et al. (2000) (Table 1), the methanol extract improved cognitive dysfunction in rats.

Aqueous leaf and rhizome extracts of the plant, demonstrated in vitro antioxidant properties (Koo et al. 2004; Zhao et al. 2006).

Salvia officinalis L. (Lamiaceae)

S. officinalis is a perennial shrub native of Mediterranean region, and is believed by many to be the plant sage which has a reputation in the European and other traditional and folklore medicine for promoting intellect (Perry et al. 1998). Essential oil obtained from the plant exhibited in vitro AChE and BuChE inhibitory activities (Perry et al. 1996; Savelev et al. 2004). Eun-A et al. (2004) reported that hexane and ethyl ace-
tate extracts of the plant showed in vitro anti-inflammatory properties. In another study hexane and chloroform extracts of the leaves were reported to possess in vivo anti-inflammatory activity (Barievic et al. 2001) (Table 1). Miliauskas et al. (2004) demonstrated that ethyl acetate, acetone extracts obtained from the aerial parts of the plant possessed in vitro antioxidant properties.

Methanol extracts of the leaf material and the aerial parts also showed in vitro antioxidant activity (Hohmann et al. 1999; Pizzarelli et al. 2000). Ethanolic leaf extract of S. officinalis demonstrated in vitro AChE and BuChE inhibitory activities (Perry et al. 1996; Kennedy et al. 2006). A hydroalcoholic extract form the leaves demonstrated in vitro protective effect against Aβ induced neurotoxicity (Iuvone et al. 2006). In a clinical study carried out by Akhondzadeh et al. (2003b) (Table 1), a hydroalcoholic leaf extract was effective in the management of mild to moderate AD. Aqueous extracts of the leaves, obtained by hydrodistillation and hot water extraction displayed in vitro antioxidant activity (Ol-lanketo et al. 2002; Dorman et al. 2003).

**Terminalia chebula L. (Combretaceae)**

The ripe fruit of T. chebula is reputed to enhance memory and to promote longevity (Misra 1998; Manyam 1999). However, there is no hard data substantiating the reputed effects of this plant in the AM. A methanol extract is reported to bind NMDA and GABA receptors, but did not show any cholinesterase inhibitory activity (Dev 1997). In a study carried out by Naik et al. (2004), the aqueous extract of dried fruits T. chebula demonstrated in vitro antioxidant activity.

**Withania somnifera L. (Solonaceae)**

The root of the plant W. somnifera known by the name Ashwagandha is one of the most valuable herbs used in AM. It is used rejuvenative tonics (‘Rasyanas’), and enhancement of memory and intellect in AM (Upton 2000).

Administration of the standardised root extracts improved cognitive dysfunction in vivo (Dhuley 2001; Naidu et al. 2006) (Table 1). A hydroalcoholic extract of the roots standardised for withanolides and withanoleic acid showed neuroprotective effect in vivo (Jain et al. 2001) (Table 1). Hydroalcoholic and ethanolic root extracts demonstrated in vitro and in vivo antioxidant and anti-inflammatory properties (Dhuley 1997; Chaurasia et al. 2000; Gacche and Dhole (2006) (Table 1).

A methanol root extract promoted the formation of dendrites in a culture of human neuroblastoma cells (Tohda et al. 2000). Bhatnagar et al. (2005) reported that the methanolic extract possessed in vivo antioxidant properties. W. somnifera root powder demonstrated in vivo antioxidant and anti-inflammatory effects (Rasool and Varalakshmi 2007) (Table 1).

**PHYTOCHEMICALS**

**Arecoline**

The alkaloid aracoline is isolated from the betel nut of Areca catechu L. (Areaceae), which is used as a maastica
tory throughout the Indian subcontinent and other parts of southeast Asia. Administration of aracoline resulted in improvement of memory in rats (Bratt et al. 1996). Arecoline has exhibited muscarinic (M4) binding activity (Yang et al. 2000). In a clinical study aracoline demonstrated memory enhancing effect in AD patients (Soncrant et al. 1993). Despite initial success with in vitro studies the compounds failed to improve the cognitive functions in mild to moderate AD patients (Houghton and Howes 2005). However, research on synthetic analogue of aracoline such as Lu 25-109 (24) and talsacline (25) appears to be promising (Houghton and Howes 2005).

**Asiaticosides**

Lee et al. (2000) reported the triterpene Asiatic acid (26) and its derivatives protected cortical neuronal cell against glutamate induced toxicity in vitro. Asiaticoside derivatives were assessed in vitro for their neuroprotective activity against β-amyloid toxicity death (Mook-Jung et al. 1999). Of 28 asiaticoside derivatives, three components including Asiatic acid and its derivatives, showed strong inhibition of β-amyloid and free radical-induced cell death (Mook-Jung et al. 1999). These derivatives may potentially be candidates in AD treatment.

**Bacosides**

Bacosides, which are dammarane triterpenoid saponins isolated from Bacopa monniera, showed nootropic activity (Russo and Borrelli 2005; Kumar 2006). These compounds such as bacoside A (3) (27), demonstrated in vitro antioxidant activity (Pawar et al. 2001).

**Biphenolic lignans**

Biphenolic lignans isolated from Magnolia officinalis, honokiol (28) and magnolol (29), have demonstrated the ability to increase ChAT activity and inhibit AChE activity in vitro and have also shown to release hippocampal ACh in vivo (Hou et al. 2000). Both the compounds showed in vivo antioxidant activities (Lo et al. 1994). Magnolol demonstrated in vitro neuroprotective effect (Lee et al. 1998). The compound also showed anti-inflammatory activity in vitro and in vivo (Wang et al. 1992, 1995). Liou et al. (2003) demonstrated that honokiol exerted in vivo anti-inflammatory effect by inhibiting ROS formation.

**Caffeic acid derivatives**

Salvianolic acids A (30) and B (31) isolated from Salvia miltiorrhiza offered protection against cerebral ischemia induced memory impairment in mice (Du and Zhang; 1997 Du et al. 2000). Lin et al. (2006) reported that salvianolic acid B prevented Aβ (25-35) induced neurotoxicity in vitro. This effect was accompanied by decreased formation ROS, suggesting the antioxidant activity being behind the neuroprotective effect. Rosmarinic (32) a well known antioxidant isolated from Salvia and other Lamiaceae species (Jiang et al. 2005; Imanshahidi et al. 2006; Dastmalchi et al. 2008) demonstrated in vitro protective effect against Aβ induced neurotoxicity (Iuvone et al. 2006).

Sinapic acid (33) isolated from Polygala tenuifolia increased the activity of ChAT in the frontal cortex of brain lesioned rats (Yabe et al. 1997).

**Crocin**

Crocin (34) isolated from Crocus sativus demonstrated cognition enhancing activity in mice (Sugiyama et al. 1995a; Abe and Saiio 2000). The compound possessed in vitro antiinflammatory and antiamyloidogenic properties (Papandreou et al. 2006), furthermore it suppressed TNF-α induced apoptosis in vitro (Soeda et al. 2001).

**Curcuminoinds**

Curcuminoinds from Curcuma longa; curcumin (35), demethoxycurcumin (36), bisdemethoxycurcumin (37) and calebin-A (38) (and some of its synthetic analogues), showed neuroprotective activity against Aβ-induced toxicity (Kim and Kim 2001; Park and Kim 2002). It was suggested that this activity may be due to an antioxidant effect (Kim et al. 2001). Among the curcuminoinds present in C. longa, curcu-

min has been the subject of most research (Xu et al. 2006). The antioxidant activity of curcumin has been reported in various studies (Priyadarshini 1997; Scartezzini and Spe- roni 2000; Das and Das 2002; Miquel et al. 2002). It de-
monstrated neuroprotective activity against ethanol-induced brain injury in vivo. It was reported that this effect was related to its in vivo antioxidant activity (Rajakrishnan et al. 1999). A number of studies have demonstrated that curcumin possesses anti-inflammatory activity (Srivastava et al. 1995; Ramsewak et al. 2000; Skrzypczak-Jankun et al. 2000; Miquel et al. 2002). Using computational software Balasubramanian (2006) demonstrated that curcumin as a result of containing an enolic centre and two phenolic polar groups separated by a conjugated hydrocarbon chain, exhibits unique hydrophobic and hydrophilic features. The former property facilitates its partition into the blood brain barrier and the later enables its binding to the Aβ peptide. Further studies also show that the enol isomer has all the properties for an ideal antioxidant.

**Galantamine**

The alkaloid galantamine is found in members of Amaryllidaceae family including the Chinese medicinal plant *Lycoris radiata* Herb. and the European *Gallantus nivalis* Herb. and Narcissus spp. Galantamine is licensed in Europe for AD treatment and has been reported to significantly improve the cognitive functions when administered to the patients in multicentre randomised clinical trials (Wilcock 2000; Wilkinson and Murray 2001). This alkaloid, which is also isolated from is shown to be more selective inhibitor for AChE than BuChE and provide complete oral bioavailability (Bickel et al. 1991; Harvey 1995; Fulton and Benfield 1996). The alkaloid is also capable of stimulating nicotinic receptors which is believed to further enhance cognition and memory (Pearson 2001; Woodruff-Pak et al. 2001). This is a therapeutic advantage over that of other AChE inhibitors.

Clinical studies have also shown that the alkaloid improves the symptoms of cerebral haemorrhage induced hemiplagia (Chang and But 2001). This may be of value in vascular dements.

**Huperzine A**

Huperzine A (39), a quinolizidine alkaloid isolated from *Huperzia serrata* Brenh. (Lycopodiaceae), has demonstrated in a number of in vitro and in vivo studies its ability to reversibly inhibit AChE (Wang et al. 1986; Laganière et al. 1991; McKinney et al. 1991; Ashani et al. 1992).

In a number of animal studies, administration of the alkaloid showed it has improved working and spatial memory (Lu et al. 1988; Xiong and Tang 1995; Wang and Tang 1998; Ye et al. 1999; Wang et al. 2000; Lian et al. 2001). Huperzine A improved cognitive functions in chronically hypoperfused rats (Wang et al. 2000) and in gerbils following ischaemia (Zhou et al. 2001a, 2001b). It is suggested that the cerebrovascular effects of the extract may be contributing to cognitive enhancing action.

In a double blind clinical trial Huperzine A improved behaviour and memory in AD patients, and it was more selective for acetylcholinesterase (AChE) than butyrylcholinesterase (BuChE) (Small et al. 1997; Shu 1998). The alkaloid was less toxic than the synthetic cholinesterase inhibitors such as tacrine and donepezil.

Huperzine A extracts have been shown to have neuroprotective activity against Aβ2335-induced neurotoxicity (Xiao et al. 2002), scavenging free radicals (Xiao et al. 1999) and possess antagonistic NMDA receptor activity in the cell culture model (Wang et al. 1999). Zhou and Tang (2002) reported that huperzine A also inhibited apoptosis by modulating the mitochondrial caspase pathway. This compound has currently been introduced in China for the management of AD patients, while phase II clinical studies are being conducted in US.

**Hyperforine**

The vast majority of the reports on the pharmacological uses of the plant extracts and their therapeutic potential revolves around the phytochemical constituents hyperforine (40) (Kumar et al. 2000; Lu et al. 2001; Widy-Tyszkiezwicz et al. 2002; Kumar et al. 2002c; Trofimiuk et al. 2005; Kumar 2006). The phytochemical substance is also reported to possess NMDA receptor antagonistic activity, thereby inhibiting glutamate induced neurotoxicity (Kumar 2006). In a study carried out by Klusa et al. (2001) hyperforin completely reversed scopolamine-induced amnesia in mice, thereby showing its cognitive enhancing action.

**Pilocarpine**

The alkaloid pilocarpine is isolated from the species belonging to the plant genus *Pilocarpus* found mainly in South America. The molecular structure of the alkaloid bears similarities with ACh since the positively charged nitrogen atom and the lactone binding to the serine are the same distance apart and this is proposed to be the reason behind its muscarinic binding activity (Houghton and Howes 2005). The alkaloid has demonstrated nootropic activity in the rat (Levin and Torry 1996); however, no studies have been done in humans due to its poor pharmacokinetic profile (Houghton and Howes 2005).

**Protoberberine alkaloids**

Shigeta et al. (2002) reported that alkaloids berberine (41), coptisine (42) and palmatine (43) isolated from *Coptis chinensis* possessed AChE inhibitory and NGF-enhancing activities in vitro.

**Stilbenes**

Resveratrol (44), rhaponticin (45) and rhapontigenin (46) isolated from rhubarb demonstrated in vitro neuroprotective action against Aβ induced toxicity (Misiti et al. 2006), furthermore these compound possessed in vitro antioxidant properties (Kageura et al. 2001; Matsuda et al. 2001). Resveratrol inhibited Aβ fibril formation (Rivière et al. 2007) and promoted Aβ clearance in vitro (Marambaud et al. 2005). The compound improved cognitive dysfunction, which is proposed to be related to its in vivo antioxidant and AChE inhibitory activities. (Sharma and Gupta 2002; Luo and Huang 2006).

**Tanshinones**

Tanshinones isolated from *S. miltiorrhiza*, viz., tanshinone I (47), dihydrotanshinone (48), methylenetanshiniquinone (49) and cryptotanshinone (50), demonstrated significant antioxidant effect in lard (Zhang et al. 1990; Weng and Gordon 1992). Tanshinon I, dihydrotanshinone, and cryptotanshinone showed anti-inflammatory activity in vitro and in vivo (Kang et al. 2000; Kim et al. 2002).

Ren et al. (2004) demonstrated that tanshinone I and tanshinone IIa (51), dihydrotanshinone, cryptotanshinone, exerted AChE inhibitory activity in vitro. Tanshinone improved changes induced by Aβ (1-42) in rats, including a decrease in AChE positive fibres (Li et al. 2004).

A screening method based on Aβ induced neurotoxicity, have been used to identify Aβ-peptide inhibitor, tanshinone IIa (Hu et al. 2007). Both the screening method and the inhibitor have been patented in China (Hu et al. 2007).

Tanshinones followed demonstrated a wide range of pharmacological and therefore of relevance to AD therapy, therefore they are potential targets for further drug discovery studies. The fact that tanshinone IIA has already been patented shows research on *S. miltiorrhiza* has proved promising.

**Terpenoid indole alkaloid**

Dehydroevodiamine (52) strongly inhibited AChE in vitro and reversed scopolamine-induced memory impairment in the...
Terpenoid trilactones

In addition to flavonoids, there are terpene lactones, i.e. bilobalide (54) and ginkgolides present in Ginkgo biloba, that have been classified as nootropic agents (Kumar 2006). Some of the research showed that bilobalide, was successful in inhibiting phospholipids breakdown and cholinesterase release under hypoxic conditions (Klein et al. 1997). This group has also established that bilobalide inhibited glutamatergic excitotoxid membrane breakdown both in vivo and in vitro, an effect of great relevance to neuronal hyperactivity and neurodegeneration (Weichsel et al. 1999). Recently another group has reported that bilobalide inhibited an NMDA-induced chloride flux through glycine/GABA-operated channels, thereby preventing NMDA induced breakdown of membrane phospholipids (Klein et al. 2003). Bilobalide showed protective effect against ischemia-induced neurotoxicity (Chandrasekaran et al. 2001).

Wu et al. (2006) reported that ginkgolides alleviates Aβ induced pathological behaviour. Ginkgolide B (55) demonstrated neuroprotective activity against Aβ induced toxicity (Bate et al. 2004). Ginkgolides also reversed Aβ suppression of ACh release in vivo (Lee et al. 2004).

It should be mentioned that despite the structural similarities between ginkgolides and bilobalide, few analogues between their CNS activities profiles can be detected (Kumar 2006). In a structure activity study, Chatterjee et al. (2003) have indicated that the difference in the existing molecular space around the (tart-butylated substituted cyclopentane ring) dictate their activity profile (Chatterjee et al. 2003).

Withanolides

There have been numerous studies on W. somnifera and its constituents. The sitoindosides IX (56) and X (57) isolated from the plant, augmented learning acquisition and memory in both young and old rats (Ghosal et al. 1989). It has been suggested that the mechanism for this effect may involve modulation of cholinergic neurotransmission. Administration of a mixture containing sitoindosides VIII-X and withaferin A (58) to mice resulted in enhanced AChE activity in the lateral septum and globus pallidus and decreased AChE activity in the vertical diagonal band, enhanced muscarinic M1 receptor binding in the lateral and medial septum and in frontal cortices, and increased muscarinic M2 receptor binding sites in the cortical regions (Schliebs et al. 1997). The mixture improved ibotenic acid-induced cognitive dysfunction and reduction in the cholinergic markers in rats (Bhattacharya and Kumar 1995). The compounds glycywithanolides and sitoindosides are believed to be responsible for antioxidant activity of W. somnifera because they demonstrated their effect both in vitro and in vivo (Bhattacharya et al. 1997, Chaurasia et al. 2000; Bhattacharya et al. 2001b).

Zeatin

Zeatin (59), isolated from F. villosa, exerted AChE inhibitory effect in vitro (Letham et al. 1967; Hoe et al. 2002).

CONCLUSION

By looking at the pharmacological activities of the plant extracts investigated it can be concluded that essential oils and non-polar extracts of a wide range of plant species such as Angelica archangelica, Centella asiatica, Celastrus paniculatus, Coptis chinensis, Evodia rutacarpa, Melissa officinalis, Polygala tenufolia, Salvia officinalis, Salvia lavandulaefolia and Salvia miltiorrhiza at differing dosages demonstrated AChE inhibitory activity. The extracts were prepared from the rhizome, seeds and aerial parts of the plants, however in some cases the plant parts used in the extraction were not specified. In few cases the phytochemical constituents contributing to the activity have been isolated. These include alkaloids, monoterpenes, diterpenes and triterpenoids. The non polar extracts and essential oils from C. asiatica, Melissa officinalis and Salvia species possessed antioxidant and anti-inflammatory properties. However, some of the phytochemicals responsible for the activities of the extracts have not been identified, therefore, it is suggested that the extracts be subjected to activity guided fractionation. It is also proposed that the compounds, which have already been isolated to be investigated further in models of AD.

Another interesting trend is that the polar extracts of the plant species mentioned above and other medicinal plants showed antioxidant and anti-inflammatory activities. It has been proposed that the activities are due to the presence of flavonoids, cinnamic acid derivatives, triterpenoid saponins, bacosides, curcuminoids, zeatin, crocin, anraaquinoine glycosides, dimeric anraaquinoine derivatives, phloroglucinol derivatives, naphthaquinone glycosides and stilbenes.

There are some standardised extracts which have proven to be effective in the clinical studies and currently they are being investigated for their pharmacodynamic and pharmacokinetic properties. The Ginkgo biloba extract EGB 761, and hypericum extract are such examples.

Based on phytochemical and pharmacological studies carried out there are several phytoconstituents which can be potential drug targets for AD treatment. These include asiatic acid, berberine, coptisine, palmistine, crocin, rutacarpine, dehydroevodiamine, curcumin, hyperforin, hypericin, honokiol, magnolol, sinapic acid, rhamnoticin, rhapontigenin, resveratrol, tanshinones, salviannolic acids, arecoline and pilocarpine. However there are some phytochemical substance which have already been launched or in the clinical trial phase. It should be also mentioned that these substances, examples of which galantamine and huperzine A are only being used in the management of AD patients.

Therefore, one can conclude that extracts of medicinal plants having a wide range of polarity and different classes of phytochemical substances have demonstrated pharmacological activities relevant to the treatment of AD.

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Plants in the treatment of Alzheimer’s Disease. Dastmalchi et al.
Huperzine A (39)  
Berberine (41)  
Hyperforin (40)  
Resveratrol (44)  
Coptisine (42)  
Palmatine (43)  
Rasvontigenin (46)  
Tanshinone I (47)  
Raponticin (45)  
Methylene tanshiquinone (49)  
Dihydrotanshinone (48)  
Dehydroevodiamine (52)  
Rutaecarpine (53)  
Tanshinone IIA (51)  
Bilobalide (54)  
Ginkgolide B (55)
Plants in the treatment of Alzheimer’s Disease. Dastmalchi et al.

Sitoindoside IX (56)

Sitoindoside X (57)

Withaferin A (58)

Zeatin (59)