Phytomedicines as potential inhibitors of β amyloid aggregation: significance to Alzheimer's disease

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[ABSTRACT]

Throughout the history of drug development, plants have been an important source for the discovery of novel therapeutically active compounds for many diseases. The ethnopharmacological approach has provided several leads to identify potential new drugs from plant sources, including those for memory disorders. For the treatment of Alzheimer's disease the drug discovery focus shifted from cholinesterase inhibitors, to other targets primarily based on two key neuropathological hallmarks, namely the hyperphosphorylation of the tau protein resulting in the formation of neurofibrillary tangles (NFTs), and the increased formation and aggregation of amyloid-beta peptide (Aβ) derived from amyloid precursor protein (APP). The present article aims to provide a comprehensive literature survey of plants and their constituents that have been tested for Aβ aggregation, thus possibly relieving several features of Alzheimer’s disease (AD).

[KEY WORDS] Amyloid-beta peptide; Medicinal plants; Thioflavin T; β amyloid aggregation; Plant-derived pharmaceuticals

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Introduction

Alzheimer’s disease (AD) is a neurodegenerative disease that mostly afflicts the elderly. It has been estimated that 115.4 million people will suffer from AD by 2050 [1]. AD is pathologically characterized by extracellular senile plaques, intracellular neurofibrillary tangles and extensive neuronal loss [2]. The amyloid precursor protein (APP) and the microtubule-associated protein, tau, are two major proteins involved in AD pathology. Extensive evidence indicates that the brains of individuals with AD are characterized by exaggerated oxidative stress [3-4], and the overproduction of Aβ leads to Aβ-associated free-radical production and cell death [5]. The principal component of amyloid plaque extracted from AD patients revealed the presence of characteristic 40 and 42 amino acid sequences, termed the amyloid β (Aβ) peptide [5-7]. The imbalance between the production and clearance of Aβ peptides released proteolytically from the membrane-bound amyloid precursor protein (APP) in the brain appears to be directly related to the development of AD [8]. It was historically unclear whether the accumulating Aβ plaques in the brains of patients with dementia caused the dementia, or simply indicated the presence of dying neurons. Indeed, studies of head trauma and brain ischemia in humans and animals demonstrate transient increases in brain Aβ deposition [9-10]. The precise physiological function of APP is not known, and remains one of the vexing issues in the field. In most studies, APP overexpression shows a positive effect on cell health and growth. Currently there is no approved therapeutic agent directed towards the inhibition of the formation of these Aβ fibrillar assemblies.

One possible approach is the use of small molecules that specifically and efficiently inhibit the fibrillogenesis process. Several plant-based compounds such as polyphenols, curcumin, rosmarinic acid, tannic acid, catechin, and quercetin have demonstrated the ability to inhibit the formation of fibrillar assembly in vitro [11]. Towards the development of new Aβ aggregation block-
ers, a number of methods for screening putative small-molecule inhibitors of Aβ aggregation have been developed [12-15]. The majority of these assays have been performed in vitro using either turbidity or binding of thioflavin T (ThT). Since AD has become a public health burden, and the commonly available synthetic drugs have undesirable side-effects, new treatment strategies based on medicinal plants have been the subject of current focus. Sourcing the ethnomedical information may also be useful as a starting point for the discovery of new drugs for the treatment of AD and cognitive disorders.

**Plant Based Pharmaceuticals**

Natural products are an important source of new structures leading to drugs in all major disease areas. Testing and identifying biologically active natural products from microbial or plant extracts in today’s high-throughput screening (HTS) environment poses several challenges, such as sample preparation and single component identification. The various indigenous systems, such as Siddha, Ayurveda, Unani and Allopathy, use several plant species to treat different ailments [16]. The use of plant-based medicine has become more popular due to the toxicity and side effects of allopathic medicines. This has led to a sudden increase in the number of plant drug manufacturers [17]. Plant-derived pharmaceuticals hold great promise for helping to develop new ways to treat a wide range of diseases and conditions, including cancer, arthritis, and cystic fibrosis. These metabolites are in various stages of development for use in the production of existing, as well as new, treatments for a range of therapeutic needs. Plants and plant-based medicaments are the foundation of many of the modern pharmaceuticals used today for various ailments [18-19]. At one time, nearly all medicines were derived from biological resources. Even today they remain vital and as much as 67%–70% of modern medicines are derived from natural products [20-21]. Nearly 80% of the developing world population relies on traditional medicines for primary health care, most of which involve plant extracts [22]. In India, almost 95% of the prescriptions are plant-based in the traditional systems of Unani, Ayurveda, Homoeopathy, and Siddha [23].

Today there are at least 120 distinct chemical substances derived from plants that are considered as important drugs currently in use in one or more countries in the world. Several of the drugs sold today are simple synthetic modifications or copies of the naturally obtained substances [24]. For example, taxol (paclitaxel), an important anticancer drug, is isolated from the Pacific Yew tree. It is used against various types of tumors today in the U.S. and in many other countries. Therefore plants are important source of medicines and presently about 25% of pharmaceutical prescriptions in the United States contain at least one plant-derived ingredient. Despite a period in which pharmaceutical companies cut back on their use of natural products in drug discovery, there are many promising drug candidates in the current development pipeline that are of natural origin [25].

**Drug discovery & challenges in the research on natural medicines for AD**

Recently, there have been an increasing number of drug discovery efforts on medicinal plants to find potential drug candidates to treat neurologic dysfunctions such as AD [26]. The World Health Organization has estimated that 3.4 billion people in the developing world use plants as a source of medicine [27]. Use of plants in treating diseases is as old as civilization [28] and traditional medicines are still a major part of habitual treatments of different maladies [29]. Plants are considered as one of the main sources of biologically active materials. Recent records reported that medicinal herbs are used by 80% of the people living in rural areas as their primary healthcare system [30]. In spite of the recent domination of synthetic chemistry as a method to discover and produce drugs, the potential of bioactive plants or their extracts to provide new and novel products for disease treatment and prevention is still enormous [31]. Compared with chemical synthesis, plant-derived natural products represent an attractive source of biologically active agents since they are natural and available at affordable prices [32]. Approximately 75% of these substances were discovered as a result of chemical studies focused on the isolation of active substances from plants used in traditional medicine [33,35]. Despite the recent interest in molecular modeling, combinatorial chemistry, and other synthetic chemistry techniques by pharmaceutical companies and funding organizations, natural products, and particularly medicinal plants, remain an important source of new drugs, new drug leads, and new chemical entities (NCEs) [36-38]. In traditional practices, numerous plants have been used to treat cognitive disorders, including neurodegenerative diseases and different neuropharmacological disorders [39]. Galantamine (1) is a natural product discovered through an ethnobotanical lead and first isolated from snowdrop, has been approved by the Food and Drug Administration in the United States for use in the treatment of Alzheimer’s disease, slowing the process of neurological degeneration by inhibiting acetylcholinesterase (AChE) [40-41].

The rapid increase in the number of dementia patients urgently demands effective prevention and treatment. Current approaches to dementia-related neurodegenerative diseases still rely highly on relieving symptoms. As some Chinese medicinal plants have been used in treating dementia, many studies are now turning to Chinese medicine for identifying potential neuroprotective agents or disease modifying agents. Natural products provide a starting point for new synthetic compounds, with diverse structures and often possess multiple stereocenters that can be challenging synthetically [42]. With the increasing acceptance that the chemical diversity of natural products is well suited to provide the core scaffolds for future drugs, there will be further developments in the use of novel natural products and chemical libraries based on natural products in drug discovery campaigns.

4’-(3’-Methoxy-4’-hydroxyphenyl)-2’-oxo-3’-enebutanoyl-3-(3’-methoxy-4’hydroxyphenyl)propenoate (calebin-A) (24)

1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,4,6-heptatrien-3-one (25)

1,7-Bis(4-hydroxyphenyl)-1-heptene-3,5-dione (30)

1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one (31)

1-Hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-6-heptene-3,5-dione (29)

1,5-Bis(4-hydroxy-3-methoxyphenyl)-1,4-pentadien-3-one (32)

Luteolin (36)

Ginsenoside Rg1 (33)

Ginsenoside Rg3 (34)

Ginsenoside RE (35)

Vitisin A (37)
Nowadays, the major challenge of any pharmaceutical scientist is to concern the overall quality, safety, and efficacy of phytomedicines. Usually, a plant contains hundreds of chemical constituents, but only a few components are bioactive, and it is essential to determine all of the bioactive constituents of medicinal plant to ensure the reliability and repeatability of clinical research and enhance quality control from the pharmacologically beneficial and/or hazardous actives. However, the inherent complexity and variability of botanical extracts has presented significant challenges for separation and detection methods enabling rapid analysis of the chemical composition of medicinal plants. Furthermore, in pharmacokinetic and pharmacodynamic studies, determination and quantification of trace metabolites of natural products in complex biological matrices requires sophisticated analytical methods with high sensitivity and selectivity [43].

Despite their many challenges, plant-based medicines afford clinical and research opportunities that should not be neglected when greater regulation of these products is considered. Without doubt, the therapeutic potential of many plants is yet to be fully discovered. The discovery of artemisins (new class of antimalarials drugs) in Chinese herbs supports this assertion [44]. Nevertheless, if plant-based medicines are to assume a respected place in contemporary health care, the quality of the data and the quality of the products themselves, as well as their regulatory control must improve greatly. To demonstrate that plants produce as much beneficial effect as modern medicine by scientific means remains a challenge, particularly when using criteria applied for assessing chemical drugs which are consumed in a purified and concentrated form. Biotechnology companies working in the fields of combinatorial biosynthesis, genetic engineering, and metagenomic approaches to identify novel natural product lead molecules have had limited success [45]. The rigorous implementation of Good Agricultural and Collection Practices (GACP) and Good Manufacturing Practices (GMP) would undoubtedly reduce the risk of these issues. Through the support of modern analytical methods and pharmaceutical techniques, previously unsolved internal issues have become solvable. Standardized plant-based products can be manufactured from standard plant extracts. Undeclared chemical or synthetic substances or other active ingredients are the adulterants which are common in raw material trade of medicinal plants. Adverse event reports are often due to the presence of unintended plants and this has affected the promotion of plant products. Adultera-
tion of plant drugs with one or more synthetic drugs is reported from different parts of the world. There is widespread concern on the unintended/harmful effects of plant drugs, with a greater number of studies carried out on plant drug reactions in the developed countries. This is challenging the notion that long traditional use is an indication of safety of plant medicines. Manufacturers, particularly in South East Asia, face the challenge of proving the authenticity and purity of plant drug preparations during commercialization. Marker based standards are becoming popular for the identification/authentication of plant drug components [46].

**β** Amyloid aggregation inhibitors from medicinal plants

The traditional medicinal plants with dependable ethno-pharmacological properties have recently been demonstrated to possess neurotrophic and neuroprotective abilities, which can be useful in preventing various forms of neuronal cell loss in neurodegenerative and neuroinflammatory diseases. Aggregation of Aβ peptides in the brain tissue is believed to be an exclusively pathological process. Therefore, blocking the initial stages of Aβ peptide aggregation with small molecules could hold considerable promise as the starting point for the development of new therapies for AD [37]. Based on this, several medicinal plants have been tested for their inhibitory actions on neuroinflammation to improve memory and treat neurodegenerative disorders. A brief description of plants, extracts, and phytoconstituents is given in Table 1.

Table 1  Plants and phytoconstituents with **β** Amyloid Aggregation inhibitory activity

<table>
<thead>
<tr>
<th>Plants</th>
<th>Phytoconstituents</th>
<th>Cell lines</th>
<th>Type of extract</th>
<th>Family</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salvia officinalis</td>
<td>Rosmarinic acid (2)</td>
<td>PC12</td>
<td>Hydroalcoholic extract</td>
<td>Lamiaceae [40]</td>
<td></td>
</tr>
<tr>
<td>Paeonia suffruticosa</td>
<td>1,2,3,4,6-Penta-O-galloyl-β-D-glucopyranose (PGG) (3)</td>
<td>SK-N-SH</td>
<td>Water, methanol and ethanol extracts</td>
<td>Paeoniaceae [49]</td>
<td></td>
</tr>
<tr>
<td>Dipsacus asper</td>
<td>Akebia saponin D (4), Loganic acid ethyl ester (5), Chlorogenic acid (6), Caffeic acid (7), Logann (8), Canteleoside (9) and Syringaresinol-4', 4'-O-bis-β-D-glucopyranosyl (10)</td>
<td>PC 12</td>
<td>Water extract, methanol extract</td>
<td>Dipsacaceae [59, 62]</td>
<td></td>
</tr>
<tr>
<td>Withania somnifera</td>
<td>Withaferin A (11), Withanamides A (WA) (12) and C (WC) (13)</td>
<td>PC12</td>
<td>Aqueous extract, methanol extract</td>
<td>Solanaceae [5, 53-54]</td>
<td></td>
</tr>
<tr>
<td>Angelica sinensis</td>
<td>Z-ligustilide (17), 11-Angeloylsenkyunolide F (18), Coniferyl ferulate (19) and Ferulic acid (20)</td>
<td>dPC12; Neuro 2A</td>
<td>Methanol; ethanol extract</td>
<td>Apiaceae [57]</td>
<td></td>
</tr>
<tr>
<td>Salvia miltiorrhiza</td>
<td>Salvianolic acid B (21) and Danshensu (22)</td>
<td>PC-12</td>
<td>Aqueous/ethanol extracts</td>
<td>Lamiaceae [59-60]</td>
<td></td>
</tr>
<tr>
<td>Curcuma longa</td>
<td>4''-(3''-Methoxy-4''-hydroxyphenyl)-2''-oxo-3''-methanal 1(3''-methoxy-4''-hydroxyphenyl)propenoate (calebin-A) (24) and 1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,4,6-heptatrien-3-one (25), Curcumin (26), Demethoxycurcumin (27), Bisdemethoxycurcumin (28), 1-Hydroxy-1, 7-bis(4-hydroxy-3-methoxyphenyl)-6-heptene-3, 5-dione (29), 1,7-Bis(4-hydroxyphenyl)-1-heptene-3,5-dione (30), 1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one (31), and 1,5-Bis(4-hydroxy-3-methoxyphenyl)-1, 4-pentadien-3-one (32)</td>
<td>PC12</td>
<td>Methanol</td>
<td>Zingiberaceae [62]</td>
<td></td>
</tr>
<tr>
<td><em>Elsholtzia rugulosa</em></td>
<td>Luteolin (36)</td>
<td>Human neuroblastoma SH-SYSY</td>
<td>Ethanol extract</td>
<td>Lamiaceae [71]</td>
<td></td>
</tr>
<tr>
<td><em>Magnolia officinalis</em></td>
<td>Magnolol (46), Honokiol (49), and 4-O-Methylhonokiol (89)</td>
<td>PC12</td>
<td>Ethanol extract</td>
<td>Magnoliaceae [73, 111]</td>
<td></td>
</tr>
<tr>
<td><em>Polygala tenuifolia</em></td>
<td>Tenuigenin (52), Tenuifolin (53)</td>
<td>SH-SYSY APP695; COS-7</td>
<td>Water</td>
<td>Polygalaceae [82-83]</td>
<td></td>
</tr>
<tr>
<td><em>Punica granatum</em></td>
<td></td>
<td>PC12</td>
<td>Ethanol extract</td>
<td>Lythraceae [86]</td>
<td></td>
</tr>
<tr>
<td><em>Scutellaria baicalensis</em></td>
<td>Oroxylin A (59), Baicalin (60)</td>
<td>SH-SYSY</td>
<td>Ethanol extract</td>
<td>Lamiaceae [94-96]</td>
<td></td>
</tr>
<tr>
<td><em>Pueraria lobata</em></td>
<td>Genistein (66), Biochanin A (67), Sissostrin (68), and Puerol B (69), 2-O-α-D-Methylallyl)coumestrol (70)</td>
<td>PC12</td>
<td>Ethyl acetate-soluble extract</td>
<td>Fabaceae [107]</td>
<td></td>
</tr>
<tr>
<td><em>Eragrostis furginea</em></td>
<td>7-Demethylageconyflavone A (71), Tricin (72), Ageconyflavone A (73), Corylin (74), Nectandrin B (75), and 4-Ketopinoresinol (76)</td>
<td>PC12</td>
<td>Methanol extract</td>
<td>Poaceae [108]</td>
<td></td>
</tr>
<tr>
<td><em>Schisandra chinensis</em></td>
<td>Schisandrin (77), Schisantherin A (78), Schisandrin B (79) and Schisandrin C (80)</td>
<td>PC12</td>
<td>Hexane extract</td>
<td>Schisandraceae [109]</td>
<td></td>
</tr>
<tr>
<td><em>Callistemon lanceolatus</em></td>
<td>4''-5-Dihydroxy-6,8-dimethyl-7-methoxyflavanone (81), Eucalyptin (82), 8-Demethyleucalyptin (83), Sideroxylin (84), Syzaflerin (85), and Quecetin (86)</td>
<td>PC12</td>
<td>Methanol extract</td>
<td>Myrtaceae [110]</td>
<td></td>
</tr>
</tbody>
</table>
Muthaiyah et al. (2011) reported on the effects of walnut extract against Aβ peptide-induced cell death and oxidative stress in PC12 Cells. The results suggest that walnut extract offers protection against Aβ-mediated cell death by reducing the generation of free radicals, inhibiting membrane damage and attenuating DNA damage. A. officinalis fibril formation was inhibited by 10 μmol·L⁻¹ of the water (48.5% ± 0.3%), methanol (40.1% ± 2.8%), and ethanol (34.6% ± 0.2%) extracts of Paeonia suffruticosa. Aβ₁₋₄₂ fibril formation was also inhibited by each of the three different extracts (10 μmol·L⁻¹), although the inhibitory concentration was lower than for Aβ₁₋₄₀. 1, 2, 3, 4, 6-penta-O-galloyl-β-D-glucopyranose (PGG) (3 μmol·L⁻¹) inhibited Aβ₁₋₄₂ fibril formation by more than 50%. Fluorescence derived from thioflavin T was decreased in a dose-dependent manner after the addition of PGG to pre-formed Aβ fibrils to an extent similar to that seen for the inhibition of Aβ aggregation. The protective effects of Dipsacus asper extract were evaluated against the cognitive impairment and overexpression of hippocampal β-amyloid protein (Aβ) induced by chronic aluminium (Al) exposure in rats. The extract reduces the cognitive impairment and overexpression of hippocampal Aβ immunoreactivity induced by Al exposure.

Chloroform extracts of Curcuma aromatica and Zingiber officinale effectively protected cells from Aβ₁₋₄₂ insult at EC₅₀ of 18–24 μg·mL⁻¹ under MTT reduction assay condition, followed by Gingko biloba extract (EC₅₀ = 69–78 μg·mL⁻¹). Methanol extracts of C. aromatica and Z. officinale required higher concentration to achieve EC₅₀ = 38–54 μg·mL⁻¹; and G. biloba, EC₅₀ = 98–109 μg·mL⁻¹. Several extracts showed cytotoxicity at high concentration (150 μg·mL⁻¹), whereas other extracts did not at all protect cells from Aβ₁₋₄₂ insult. Kumar et al. (2012) showed that an aqueous extract of W. somnifera (Table 1) strongly inhibited Aβ fibril (50% at 50 μmol·L⁻¹) formation in a concentration-dependent manner, when compared with control. Jayaprakasam et al. (2010) tested two major withanamides A (WA) and C (WC) for their ability to protect PC-12 cells. Molecular modeling studies showed that withanamides A and C uniquely bind to the active motif of β-amyloid and suggest that withanamides have the ability to prevent the fibril formation. Patil et al. (2010) studied withanolide A (14) and asiatic acid (15) for their potential activities against multiple targets associated with Aβ pathways. Berberine, an isoquinoline alkaloid isolated from Coptidis rhizoma, effectively reduced the levels of Aβ₁₋₄₀ (47.1% ± 11.5% at 5 μmol·L⁻¹; 35.3% ± 8.1% at 50 μmol·L⁻¹) and Aβ₁₋₄₂ (49.1 ± 13.6% at 5 μmol·L⁻¹; 35.3% ± 10.2% at 50 μmol·L⁻¹); its 50% inhibition concentration (IC₅₀) for extracellular Aβ production was around 5 μmol·L⁻¹. Four compounds isolated from Angelica sinensis (AS) (Table 1) significantly inhibit Aβ₁₋₄₀ toxicity on dPC-12 cells at lower concentrations (1–10 μg·mL⁻¹), but at high concentrations (> 50 μg·mL⁻¹) they were toxic to the dPC-12 cells, except 11-angeloylsenkyunolide f. Huang et al. (2009) reported the effect of the alcohol extract from the root of Angelica sinensis (Table 1) on beta-amyloid peptide induced toxicity. It decreased viability of Neuro 2A cells in a concentration-dependent manner with an IC₅₀ of 14.9 μmol·L⁻¹.

Salvianolic acid B (SalB) (21) isolated from Salvia miltiorrhiza (Table 1) was administered to animals with drug-induced amnesia by administering scopolamine, diazepam, muscimol, or amyloid-β(25-35) peptide. SalB (10 mg·kg⁻¹, p.o.) was found to significantly reverse the cognitive impairments induced by scopolamine (1 mg·kg⁻¹, i.p.) or Aβ(25-35) (10 nmol/5 mL, i.c.v.) injection. The effects of Salvia miltiorrhiza root (RSM) (Table 1) aqueous/ethanol extracts, total polyphenols, total tanshinones, and three phenolic compounds against toxicity mediated by Aβ(25-35) were tested with PC-12 cells. The results showed that Aβ(25-35) induced cytotoxicity was reversed by RSM aqueous/ethanol extracts and total polyphenols, and that danshen (22) and SalB could protect PC-12 cells by blocking Aβ(25-35)-induced Ca²⁺ intake, lactate dehydrogenase release, cell viability decrease, and apoptosis. Durairajan et al. (2008) found that SalB inhibited fibril aggregation (IC₅₀: 1.54–5.37 μmol·L⁻¹), as well as destabilizing preformed Aβ fibril (IC₅₀: 5.00–5.19 μmol·L⁻¹) in a dose- and time-dependent manner. SalB is a better aggregation inhibitor than ferulic acid, but less active than curcumin in the inhibition of Aβ₁₋₄₀ aggregation. Cryptotanshinone (23) (CT), an active component of Salvia miltiorrhiza, has been shown to improve learning and memory in several pharmacological models of Alzheimer’s disease.

Compounds calebin-A, curcumin, demethoxycurcumin, bis-demethoxycurcumin, and 1, 7-bis(4-hydroxyphenyl)-1-heptene-3, 5-dione, isolated from Curcuma longa (Table 1) were found to protect PC12 cells against Aβ insult, with ED₅₀ values ranging between 0.5 and 10 μg·mL⁻¹. Compound 1-hydroxy-1, 7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptatrien-3-one, 5-dione showed relatively weak protection against Aβ₁₋₄₀ and Aβ₁₋₄₂ insult with ED₅₀ values of 30.7 and 44.3 μg·mL⁻¹, respectively. Four compounds, 1, 7-bis(4-hydroxy-3-methoxyphenyl)-1, 4, 6-heptatrien-3-one, 1, 7-bis(4-hydroxyphenyl)-1, 6-heptatrien-3-one, and 1, 5-bis(4-hydroxy-3-methoxyphenyl)-1, 4-pentadien-3-one were inactive. Gupta et al. (2009) examined the in vitro anti-amyloidogenic properties of garlic extract, Allium sativum. The effects of aqueous garlic extract (both fresh and boiled) on Aβ aggregation and defibrillation were studied by thioflavin-T based fluorescence assay. Chen et al. (2006) analyzed the effects of commercially available preparations of ginseng, as ethanol extracts of both American ginseng and Chinese ginseng, on the accumulation of the Alzheimer’s amyloid β peptide (Aβ) in a cell based assay. Several compounds were isolated from ginseng, and it was found that...
some certain ginsenosides lowered Aβ concentration in a dose-dependent manner with ginsenoside Rg3 having an approximate IC₅₀ of under 25 µmol·L⁻¹ against Aβ42. Furthermore, the study found that three of the isolates, ginsenoside Rgl (33), Rg3 (34), and RE (35), resulted in significant reductions in the amount of Aβ detected in the brains of animals after single oral doses of these agents [65]. Ginsenoside Rg1, a major active component of sanchi ginseng (Panax notoginseng), was shown to inhibit β-secretase activity in vitro, to protect PC12 cells against Aβ25-35 [66], and to exert neuroprotective effects [67]. It has to be noted that Wang and Du (2009) treated neuronal-like cells with excessive Aβ concentrations of 50 µmol·L⁻¹ for 48 h [66]. Ginsenoside Rg3, one of the major active components of sanchi ginseng significantly reduced the levels of Aβ1-40 and Aβ1-42 in SK-N-SH cells transfected with Swedish mutant beta-APP. Enhanced Aβ degradation is due to ginsenoside Rg3-induced neprilysin expression [68], which represents the rate limiting enzyme in Aβ degradation [69].

Hage et al. (2010) investigated the activity on Aβ peptide production of crude extracts of nine plant species. Hexane, dichloromethane, ethyl-acetate, and water extracts were prepared for each species tested, at non-toxic concentrations, on CHO cells overexpressing the human neuronal β amyloid peptide precursor (APP695) to measure variations of APP processing by Western-blotting [70]. The related mechanisms of luteolin (36), a 3', 4', 5, 7-tetrahydroxyflavone (Table 1) isolated from Elsholtzia rugulosa, was examined in an Alzheimer's disease using a human neuroblastoma SH-SY5Y cell model. Luteolin down-regulated the expression of AβPP and lowered the secretion of Aβ1-42 [71]. Liu et al., 2012 screened eleven plants traditionally used in Chinese medicine and four plants used in Ayurvedic medicine for their effects on APP modulation and Aβ production. Among the screened plant extracts, only the root of Polygonum multiflorum and the leaves of Convolvulus pluricaulis showed profound inhibition of Aβ production [72]. The standardized Ginkgo biloba leaf extract EGB761 (100 µg·mL⁻¹ of EGB761) inhibits Aβ aggregation by 82% ± 6% (decreased Aβ fibrillogensis) [73]. Bastianetto et al. (2000) reported that G. biloba extract (EGB 761) is a well-defined plant extract containing flavonoids and terpenoids. It is viewed as a polyvalent agent with a possible therapeutic use in the treatment of neurodegenerative diseases of multifactorial origin, e.g. AD [74].

Two compounds were isolated and characterized as vitisin A (37) and heyneanol A (38) from Vitis amurensis. Aggregation of Aβ was evaluated in vitro based on a thioflavin T fluorescence assay, vitisin A and heyneanol A showed significant reduction of Aβ aggregation. Vitisin A inhibited Aβ aggregation by more than 50% at 10 µmol·L⁻¹, while the inhibitory activity of heyneanol A was weaker than vitisin A (below 50% at 50 µmol·L⁻¹) [75].

The methanol extract from the leaf and stem of Vitis amurensis (Vitaceae) and amurensin G (39), r-2-viniferin (40) and trans- -viniferin (41) isolated from V. amurensis were studied for possible neuroprotective effects on neurotoxicity induced by Aβ25-35 in cultured rat cortical neurons and also for antidementia activity in mice. Memory loss induced by intracerebroventricular injection of ICR mice with 16 nmol Aβ25-35 was inhibited by chronic treatment with V. amurenensis extract (50 and 100 mg·kg⁻¹, p.o. for 7 d), as measured by a passive avoidance test [76].

Two of the studied nine pure compounds selected from Chinese herbs vitamin E (42), α-asarone (43), salidroside (44), baicolin (45), magnolol (46), gastrodin (47), bilobalide (48), honokiol (49), and β-asarone (50), namely honokiol and magnolol, significantly decreased Aβ-induced cell death [77]. The aqueous extract from the fruits of Lycium barbarum significantly protects neurons against Aβ peptide toxicity at micromgrometer level [78]. Seo et al. (2010) investigated Jangwonhwan, (a boiled extract of 12 medicinal plants/ mushroom, including Korean red ginseng) to determine whether Jangwonhwan has a beneficial effect on the brain of Alzheimer disease patients. LMK02-Jangwonhwan, a modified recipe of traditional Jangwonhwan, notably suppressed Aβ levels and plaque deposition in the brains of Tg-APPswe/PS1dE9 mice [79]. Silibinin (silybin) (51), a flavonoid derived from milk thistle (Silybum marianum), was investigated to determine whether silibinin prevents memory impairment in an Aβ25-35-injected animal model of AD. Silibinin prevented the memory impairment induced by Aβ25-35 in the Y-maze and novel object recognition tests [80]. Marapuama (Psychotetum olacoides), a “brain tonic” in the Amazon region shows a nootropic profile in rodents. The study was to verify if the ethanol extract is also effective against Aβ1-42-induced cognitive deficit in mice. The data showed that 14 days of oral treatment with the ethanol extract (800 mg·kg⁻¹) was effective in preventing Aβ-induced cognitive impairment [81].

Tenuigenin (52), extracted from Polygala tenuifolia, (Table 1) inhibited the secretion of Aβ and the C-terminal 99 amino acids of APP (C99) in SH-SY5Y APP695 cells, but did not change the Aβ and C99 levels in SH-SY5Y SFA4CT cells. Fluorescence Resonance Energy Transfer (FRET) assays showed that tenuigenin inhibited the proteolytic activities of BACE1 (beta-secretase) on its substrate in vitro [82]. Tenuifolin (53), a crude extract derived from P. tenuifolia was found to decrease Aβ secretion from transfected cells, probably due to inhibition of the beta-site APP cleaving enzyme [83-84]. With respect to AD, Uncaria rhynchophylla intensively inhibited Aβ aggregation and significantly destabilized preformed Aβ1-40 and Aβ1-42 fibrils [85].

The ethanol extract of Punica granatum (Table 1) protected PC12 cells from hydrogen peroxide-induced oxidative stress [86]. Rosa laevigata was also investigated against Aβ-induced oxidative damage, in in vitro assays and in vivo behavioral tests. R. laevigata showed cell protective effects against oxidative stress-induced cytotoxicity. Administration of R. laevigata extracts to mice significantly reversed the Aβ...
induced learning and memory impairment in in vivo behavioral tests [87].

The therapeutic effects of a polyphenol-rich grape seed extract (GSE) were investigated in Alzheimer’s diseased mice. Total phenolic content of GSE was 592.5 mg·g⁻¹ dry weight, including gallic acid (54), catechin (55), epicatechin (56) and proanthocyanidins (57). Long-term feeding of a GSE diet was well tolerated without fatigue, behavioral abnormality, changes in food consumption, body weight, or liver function. The Aβ levels in the brain and serum of the mice fed with GSE were reduced by 33% and 44%, respectively, compared with the Alzheimer’s mice fed with the control diet. Amyloid plaques and microglia in the brain of Alzheimer’s mice fed with GSE were also reduced by 49% and 70%, respectively. 

Curcumin also significantly reduced brain Aβ burden and microglia activation [88]. The effects of curcumin mix and its different curcuminoids, including curcumin, demethoxycurcumin, and bisdemethoxycurcumin on α-amyloid protein, β-amyloid precursor protein, and β-site amyloid precursor protein cleaving enzyme 1 in swAPP HEK293 cells were studied [89]. L-3-n-Butylphthalide (58) (L-NBP) was extracted as a pure component from seeds of Apium graveolens. L-NBP treatment significantly reduced total cerebral Aβ plaque deposition and lowered Aβ levels in brain homogenates, but had no effect on fibrillar Aβ plaques, suggesting preferential removal of diffuse Aβ deposits [90]. Yager et al. (2002) analyzed a proprietary library of natural product extracts for their ability to influence Aβ accumulation [91].

The effect of aqueous extract of lavender (Lavandula angustifolia) on spatial performance of AD rats was studied. 50, 100, and 200 mg·kg⁻¹ of the lavender extract were administered, only at the highest dose of 200 mg·kg⁻¹, as compared with their counterparts with vehicle treatment, did lavender extract significantly improved the performance of control and AD rats in the probe test [92]. Kwon et al. (2011) examined an aqueous extract of Eucommia ulmoides (EUE) with graded doses on its neuroprotective effects on Aβ(25-35) induced learning and memory impairments in mice. EUE significantly improved the Aβ(25-35)-induced memory deficit in the Y-maze test, and also increased step-through latency time in the passive avoidance test [93]. Oroxylin A (59) is a flavonoid that is found in the roots of Scutellaria baicalensis (Table 1). A single dose of oroxylin A (5 mg·kg⁻¹, p.o.) treated 1 h before behavioral tests was found to significantly reverse Aβ(25-35)-induced cognitive impairment based on passive avoidance and Y-maze task findings (P < 0.05) [94]. Baicalin (60) isolated from S. baicalensis (Huang Qui), prevents the production of hydrogen peroxide and oxidative stress induced by Aβ aggregation in SH-SY5Y cells [95]. A flavone baicalein, isolated from S. baicalensis root strongly inhibited aggregation of neuronal amyloidogenic proteins in vitro and induces dissolution of amyloid deposits [96].

Extracts of Siberian ginseng, the rhizome of Eleutherococcus senticosus, were shown to have protective effects on the regeneration of neurites and the reconstitution of synapses in cultured rat cortical neurons damaged by Aβ(25-35), and eleutheroside B was one of the active constituents. The ethyl acetate, n-butanol, and aqueous fractions from the methanol extract of Siberian ginseng showed protective effects against Aβ-induced neuritic atrophy. Twelve compounds were isolated from the active fractions and identified. Among them, eleutheroside B (61), eleutheroside E (62) and isofraxidin (63) showed obvious protective effects against Aβ(25-35)-induced atrophies of axons and dendrites at 1 and 10 µmol·L⁻¹ [97]. Tohda et al. (2008) investigated the effects of E. senticosus extracts on the regeneration of neurites and the reconstitution of synapses in rat cultured cortical neurons damaged by Aβ(25-35). Treatment with Aβ(25-35) (10 µmol·L⁻¹) induced axonal and dendritic atrophies and synaptic loss in cortical neurons. Subsequent treatment with the methanol extract and the aqueous extract of E. senticosus (10-1000 ng·mL⁻¹) resulted in significant axonal and dendritic regenerations and reconstitution of neuronal synapses. Co-application of the extract and Aβ(25-35) attenuated Aβ(25-35)-induced neuronal death. Eleutherosides B and E and isofraxidin, isolated from E. senticosus, of which eleutheroside B protected against Aβ(25-35)-induced dendritic and axonal atrophies, the activities of eleutheroside E and isofraxidin were less than that of eleutheroside B [98].

Fuzhisan (FZS), a Chinese plant complex prescription, attenuated Aβ(25-35)-induced neurotoxicity in a dose-dependent manner, and also FZS blocked Aβ(25-35)-induced calcium influx, calpain activation, and decreased cleavage of p35 to p25 [99]. Kihi-to, a Japanese-Chinese traditional medicine, for memory deficits and losses of neurites and synapses was examined using Alzheimer’s disease model mice. Improvements of Aβ(25-35)-induced neuritic atrophy by Kihi-to, and the mechanism was investigated in cultured cortical neurons. Immunohistochemical comparisons suggested that Kihi-to attenuated neuritic, synaptic, and myelin losses in the cerebral cortex, hippocampus, and striatum. Kihi-to also attenuated the calpain increase in the cerebral cortex and hippocampus [100]. To assess its effect as a possible treatment for AD patients, oral administration of the plant medicine Juizen-taiho-to (JTT) was investigated. Amyloid β protein precursor transgenic mice were used as a model of AD to clarify the effect of JTT. The immunohistochemical examination of brain sections stained with polyclonal anti-Aβ antibody showed reduced Aβ burden, and Aβ levels were also decreased in the insoluble fractions of brain homogenates [100].

Icariin (64), a main constituent in Epimedi Herb, administered for 8 days (p.o.) improved spatial memory impairment in 5xFAD mice. These novel findings suggest that icariin may improve memory dysfunction in AD and have a potential to extend neurites, even when Aβ-induced neurite atrophy has already occurred [102]. The effects of the phytoestrogen genistein (10 mg·kg⁻¹) on learning and memory impairment was assessed in intrahippocampal Aβ(1-40) injected...
rats. The estrogen receptor antagonist fulvestrant was injected intracerebroventricularly in a group of Aβ-lesioned rats. The Aβ-injected animals exhibited the following: lower spontaneous alternation score in Y-maze tasks, impaired retention and recall capability in the passive avoidance test, and fewer correct choices and more errors in the RAM task. Genistein, but not genistin and fulvestrant, significantly improved most of these parameters \cite{103}. A green tea extract was subjected to a simulated gastrointestinal digestion and a colon-available extract (CAGTE) prepared and assessed for its potential protective effects against H₂O₂ and Aβ₁−₄₂-induced cytotoxicity using differentiated PC12 cells (dPC12) as a model for neuronal cells. The deposition and aggregation of β-amyloid peptides (Aβ) in the brain play a significant role in the development and pathogenesis of Alzheimer’s disease \cite{104}.

Ipomoea batatas extract was fed to ICR mice that had been injected with Aβ to induce neuronal deficits, the extract of I. batatas significantly reversed Aβ-induced neurotoxicity as assessed by the passive avoidance test, a behavioral experiment \cite{105}. An ethanol extract of the aerial part of Aralia cordata was studied for possible neuroprotective effects on neurotoxicity induced by Aβ₁−₄₂ and Aβ₁−₃₅ in cultured rat cortical neurons and antidementia activity in mice. Memory loss induced by intracerebroventricular injection of ICR mice with 15 nmol Aβ₁−₃₅ was inhibited by chronic treatment with A. cordata (50 and 100 mg·kg⁻¹, p.o. for 7 days) as measured by a passive avoidance test, and corresponding reductions were observed in brain cholinesterase activity and neuronal death measured histologically in the hippocampal region. Oleanolic acid \cite{65} isolated from A. cordata also inhibited neuronal death \cite{106}.

Genistein and biochanin A isolated from Pueraria lobata (Table 1) exhibited potent neuroprotective effects with ED₅₀ values of 33.7 and 27.8 μmol·L⁻¹, respectively \cite{107}. Tricin isolated from Ertagrodisis ferruginea (Table 1) was found to have a neuroprotective effect with an ED₅₀ value of 20.3 μmol·L⁻¹ against Aβ induced toxicity in PC12 cells. Ageonoyflavone A, nectandrin B and 4-ketopinoresinol demonstrated moderate neuroprotective effects with ED₅₀ values of 58.7, 44.1, and 54.8 μmol·L⁻¹, respectively \cite{108}. All effects induced by Aβ₁−₃₅ were markedly reversed by schisandrin B and schisandrin C, isolated from Schisandra chinensis (Table 1), pretreatment and also reversed homocysteine-induced cytotoxicity. The results indicated that Schisandra B and Schisandra C protected PC12 cells against Aβ toxicity by attenuating ROS production \cite{109}. 4', 5-Dihydroxy-6, 8-dimethyl-7-methoxyflavanone isolated from Callistemon lanceolatus (Table 1) showed the most promising neuroprotective effect with an ED₅₀ value of 6.7 μmol·L⁻¹ in terms of decreasing Aβ-induced apoptotic cell death \cite{110}. Hibifolin \cite{87}, a plant-derived, flavonol glycoside, possessed a strong protective activity against cell death induced by aggregated Aβ. Application of hibifolin in primary cortical neurons prevented the Aβ-induced cell death in a dose-dependent manner \cite{111}. Effect of Poria cocos water extract (PCW) against Aβ₁−₄₂-induced cell death was investigated using rat pheochromocytoma (PC12) cells. Exposure of PC12 cells to Aβ₁−₄₂ (20 μmol·L⁻¹) for 48 h resulted in neuronal cell death, whereas pretreatment with PCW at the concentration range of 5–125 μg·mL⁻¹ reduced Aβ₁−₄₂-induced cell death \cite{112}.

Oral pretreatment of 4-O-methylhonokiol (1 mg·kg⁻¹) isolated from Magnolia officinalis (Table 1) dissolved in water for five weeks suppressed the intraventricular treatment of Aβ₁−₄₂ (0.5 μg per mouse, i.c.v.) induced memory impairments. 4-O-Methylhonokiol prevented the Aβ₁−₄₂-induced apoptotic cell death, as well as beta-secretase expression \cite{113}. Different extracts of aerial parts of Cynomorium songaricum on amyloid-beta peptide (Aβ) was studied. The methanolic extract of C. songaricum attenuated Aβ-induced cell death at concentrations of 100 and 10 μg·mL⁻¹, an even stronger effect was observed for the ethyl acetate fraction obtained from the crude methanolic extract \cite{114}. Talaumidin \cite{89}, veraguensin \cite{90}, galgravin \cite{91}, aristolignin \cite{92}, nectandrin A \cite{93}, isonectandrin B \cite{94}, and nectandrin B were isolated from a methanol extract of the root of Aristolochia arcuata. Compounds talaumidin, veraguensin, galgravin, aristolignin, nectandrin A, isonectandrin B, and nectandrin B protected hippocampal neurons against Aβ₁−₃₅-induced cytotoxicity, compounds aristolignin, nectandrin A, isonectandrin B, and nectandrin B showed protective effects against serum withdrawal-induced neuronal death in the dose range of 1–30 μmol·L⁻¹ \cite{115}. α-Mangostin \cite{95}, a novel polyphenolic xanthone isolated from the pericarp, bark and dried sap of (mangosteen) Garcinia mangostana attenuated the neurotoxicity induced by Aβ₁−₄₀ or Aβ₁−₄₂, oligomers at an EC₅₀ = 3.89 nmol·L⁻¹, 4.14 nmol·L⁻¹, respectively. This was observed by decreased cell viability and impaired neurite outgrowth in primary rat cerebral cortical neurons \cite{116}.

**Conclusion**

Plant drugs constitute a major part in all traditional systems of medicines. Plant-based medicine is a triumph of popular therapeutic diversity. In last two to three decades, it has been observed that the number of phytochemicals with therapeutic efficacy has increased. Pharmacological study is being performed to find out the different therapeutic properties of plant medicines. Recently there has been a tremendous increase in the use of plant-based health products in developing, as well as developed, countries resulting in an exponential growth of plant products globally. An upward trend has been observed in the research on plants. Several studies are currently being undertaken to isolate the active compounds by bioassay-guided fractionation from the species that show high biological activity during screening. The presently available allopathic drugs approved by Food and Drug Administration (FDA), USA have not offered satisfactory solutions for the treatment and complete cure of several disease and they produce various side effects. Moreover, these drugs are sympto-
matic and do not alter the course or progression of the underlying disease. Plant medicines have a strong traditional or conceptual base and the potential to be useful as drugs in terms of safety and effectiveness of treatment. Therefore different plants above all other agents have been used for medicine from time immemorial because they have fitted the immediate personal need, are easily accessible, and inexpensive. All the therapeutic properties mentioned in Ayurvedic and other classical medicines are being tested, and if they are found correct they are accepted, or otherwise discarded. Both Ayurveda and TCM have clear millennium-old concepts of age-related cognitive disorders and corresponding treatments with specific remedies, whereas folk medicines mostly lack this kind of foundation.

Drug discovery from medicinal plants has traditionally been lengthier and more complicated than other drug discovery methods. As a result, many pharmaceutical companies have reduced their efforts and funding for natural-product research. Molecular targets play an important role in drug discovery, and since the sequencing of the human genome, a lot new molecular targets have been identified as important and useful in various diseases. Current research in drug discovery from medicinal plants involves a multidisciplinary approach combining botanical, phytochemical, biological, and molecular techniques. There is a need to improve technology for the rapid isolation of active compounds in large quantities for evaluation with the scientific collection of plant material and maintenance of biodiversity. Sometimes new chemical structures are very difficult to find during drug discovery from medicinal plants, in such cases known compounds with new biological activity can provide important drug directions. Moreover, future directions should emphasize the trial of new plants that are potentially effective in treating the root of the disease.

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