Plants and their chemical compounds affecting β-amyloid and secretase activity as potential sources of neuroprotective herbal medicinal products. Part 1.

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S u m m a r y

Plant preparations, especially fractions of biologically active compounds may play an important role in improving the life quality of patients with diagnosed dementia as well as delaying the progress of neurodegenerative diseases through various mechanisms of pharmacological action. Recent years have brought a number of reports on the issue, nevertheless, it seems that there is still a lack of detailed, synthetical analysis.

So far, main biological markers of Alzheimer's disease pathogenesis which is currently the most common form of dementia, are the β-amyloid plaques deposits, neurofibrillary degeneration processes and atrophy of cholinergic neurons in the brain regions crucial for memory processes maintenance. At present, acetylcholinesterase inhibitors are the main drugs for the treatment of Alzheimer's disease. In our previous review article, we pointed out the interesting mechanisms of action such as inhibition of acetyl-, butyrylcholinesterases and the antioxidant activity of bio-compounds of selected medicinal plants from *Lamiaceae* family (including rosmarinic acid).

The aim of this paper is to systematize the knowledge about the influence of plant extracts and isolated natural compounds (e.g. cryptotanshinone, epigallocatechin gallate) on the pathway of β-amyloid formation and deposition in pharmacological models, especially by interacting with the brain enzyme, α- and β-, γ-secretase or on their genes expression. This is a long-established trend of research in search of new neuroprotective drugs from natural sources which raises new therapeutic hopes. *Salvia miltiorrhiza* and *Camellia sinensis,* medicinal plants from Asia, have interesting therapeutic potential in neurodegenerative disorders. In addition to them, there are known at least 10 Asian plants extensively researched in this area (e.g. *Aralia cordata, Magnolia officinalis, Perilla frutescens, Polygala tenuifolia, Punica granatum, Sophora flavescens*). However, due to the fact that many aspects of their phytochemical, neurochemical and pharmacological activities are not well known, further studies should be performed in this field.

Key words: *Salvia miltiorrhiza, Camelia sinensis, β-amyloid, secretase, Alzheimer disease, neuroprotection*

INTRODUCTION

In World Alzheimer Report published last year, Alzheimer's Disease International estimated that there are 35.6 million people living with dementia worldwide in 2010, increasing to 65.7 million by 2030 and 115.4 million by 2050 [1]. According to this World Alzheimer Report, the disappointing results from preventive intervention trials to data indicate that, despite much research, we still understand far too little about the environmental and lifestyle factors linked to dementia and Alzheimer's disease (AD). Numerous therapeutic strategies are currently being developed for AD [2]. It is necessary to stress that at the moment there are no treatments available that cure or even alter the progressive course of dementia, although, number of new therapies is being investigated in various stages of pharmacological and clinical trials using acetylcholinesterase inhibitors (see our previous review article [3]), β-, γ-secretase inhibitors, nicotine acetylcholine receptor agonists, NMDA-receptor antagonists, AMPA-receptor modulators, caspase inhibitors, antioxidants, and astrocyte-modulating, homocysteine-lowering and/or anti-inflammatory agents [4].

Alzheimer's disease or dementia of the Alzheimer type (DAT) is the most prevalent neurological disease worldwide, leading to nerve cell death and tissue loss throughout the brain. AD is characterized by progressive accumulation and aggregation of neurotoxic β-amyloid (amyloid- β peptides, Aβ) in the brain regions

crucial for memory and cognition processes maintenance [5-7]. Aβ is the major component of amyloid plaques and vascular deposits in Alzheimer's disease brains and believed to initiate the deadly amyloid cascade [8] including induction of neuronal cell death. This neurodegenerative pathological mechanism became the basis for Aβ cascade hypothesis in the AD development. The death of the neurons caused by the Aβ deposition is produced via a number of possible mechanisms including oxidative stress, excitotoxicity, energy depletion, inflammation and apoptosis. Moreover some genetic aspects play a role in progression of the disease [9].

Another major pathological hallmark of AD is the presence of intracellular neurofibrillary tangles (NFT), which consist mainly of the hyperphosphorylated tau protein (MAP-tau) [10]. Aβ and MAP-tau are the most extensively evaluated biological markers of AD pathogenesis [11-15]. Up to date, it 27 proteins which can form amyloid deposits were discovered and well-known [16, 17]. Amyloid and amyloid-like deposits are present in several known diseases including: Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Creutzfeldt-Jakob disease, senile systemic amyloidosis, familial amyloidotic polyneuropathy, also in the type 2 diabetes [18], and Down syndrome also [19].

In the Aβ formation an APP proteolysis process is fundamental. In recent years our understanding of the enzymes involved in APP proteolysis has increased dramatically but still remains not fully recognized. The APP metabolism consists mainly of two cleavage routes: the non-amyloidogenic and the amyloidogenic pathways in which a cleavage is processed by three types of proteases: α-, β- and γ-secretases. The APP proteolysis catalyzed by β- and γ-secretases yields in the production of $A\beta_{1-40}$ and $A\beta_{1-42}$ peptides and defines the amyloidogenic pathway, as opposed to the proteolytic cleavage of APP by α- and γ-secretase which produces noncytotoxic soluble fragments sAPPα [15; 20].

Recent studies allow to conclude that sAPPα shows various neuroprotective and neurotrophic activities [21] such as enhancing long-term potentiation (LTP) in hippocampal slices [22], enhancing memory in normal and amnesic mice [23], mediating synapse formation [24], and protecting hippocampal and cortical neurons against the toxic effects of glutamate and Aβ [25]. There is a belief that α-secretase upregulation may be a important target for prevention and more effective therapy of AD.

β-Secretase (transmembrane aspartic protease – β-site amyloid precursor protein cleaving enzyme 1; BACE1 or Asp2) has been identified as a rate limiting enzyme for Aβ production by cleaving the APP at the N terminus of Aβ (the β site) [26]. BACE1 is one of the only two known aspartyl proteases localized on the cell membrane due to the presence of a membrane anchoring domain. Although BACE1 and BACE2 share 59% homology and are of a similar structural organization, the second enzyme preferentially cleaves the APP within the Aβ region between F19 and F20 and between F20 and A21 [27]. Studies showed that BACE1 is the β-secretase while BACE2 cleaves at sites within the Aβ domain to limit Aβ secretion. Recent studies indicated that BACE1 might be a susceptibility factor to brain amyloidosis and an excellent therapeutic target in AD [28]. β-Secretase is ex-

pressed in all tissues but is higher in neurons of the brain. The evidence suggests that astrocytes may also express significant levels of BACE1 and contribute to Aβ production, at least under certain pro-inflammatory conditions [29].

Nowadays, newly discovered γ-secretase activating protein (GSAP) which drastically and selectively increases Aβ production is a therapeutic target for Alzheimer's disease [30]. This enzyme comprises a complex of integral membrane proteins that are present in most cells, but may have different functions depending on cell type [31]. On the other hand, results of Chow et al. [32] allowed to state that the novel anti-amyloid combination therapy that modestly targets both BACE1 (β-secretase) and γ-secretase represents a more effective treatment strategy to diminish Aβ amyloidosis in AD (fig. 1).
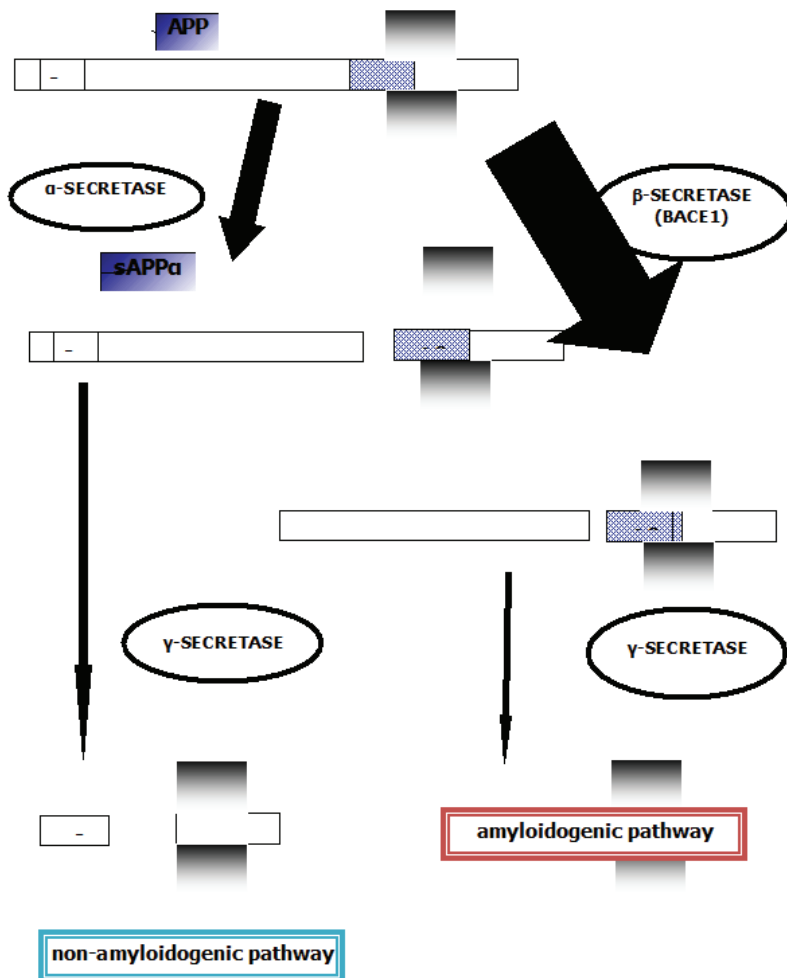

Figure 1. Graphic presentation of the APP proteolysis through non-amyloidogenic and amyloidogenic pathways

According to Wiśniewski and Sadowski (New York University School of Medicine) opinions [33], it is likely that, in the future, multimodal individual to patients therapies will be tailored based on the genotype, immune status, and pathological stage of the disease, but nowadays β-sheet breakers and pathological chaperone inhibitors are assumed to be among the most effective and safest strategies.

In order to develop a potent anti-dementive BACE1 inhibitor from plants as a source of biologically active compounds, many medicinal plants were investigated. It seems that secondary metabolites of plants with relatively low molecular weight and high lipophilicity might be good candidates for BACE1 inhibitors as well [34].

THE MOST POPULAR MEDICINAL NEUROACTIVE PLANTS FROM ASIA

Salvia miltiorrhiza Bunge

Salvia miltiorrhiza Bunge (*Lamiaceae*) is a plant well-known in traditional Chinese medicine. The lipophylic extract from the root of this plant (Danshen) contains more than 30 diterpenoid tanshinones: tanshinone I, IIA, B, cryptotanshinone, dihydrotanshinone I, methylenetanshinone, and isotanshinone IIA [35; 36]. Danshen products are regarded as an effective traditional Chinese medicine for prevention and treatment of cardiovascular diseases [39]. Extracts of *Salvia miltiorrhiza* root have demonstrated various biological activities such as antibacterial, antitumor, and antimutagenic effects [36]. It is known that Danshen extract includes also the most abundant phenolic compounds such as rosmarinic and salvianolic acids which are the major contributors to antioxidant activities of this plant [37]. It seems that, according to results of Matkowski et al. [38], the high content of tanshinones in *Salvia miltiorrhiza* roots is unlikely to contribute to the antioxidant activity.

Cryptotanshinone as a neuropharmacological compound

Cryptotanshinone (CTS) (fig. 2), one of the most well-known active compounds of the medicinal root of *Salvia miltiorrhiza*, has been shown to improve learning and memory in several pharmacological models of AD [40]. This compound produces well-documented antioxidative, anti-inflammatory effects by reducing the prostaglandin E2 synthesis and reactive oxygen species generation via COX-2 enzyme [41]. Moreover, Kim et al. [44] showed in animal model that CTS can cross the blood–brain barrier. It is known that CTS is able to inhibit the acetylcholinesterase activity [42; 28]. CTS protects primary rat cortical neurons from glutamate--induced neurotoxicity via the activation of the phosphatidylinositol 3-kinase [43]. In a recent study [44], not only CTS but also tanshinone I, tanshinone IIA, and 15, 16-dihydrotanshinone I were effective in reversing scopolamine-induced cognitive impairments using passive avoidance tasks.
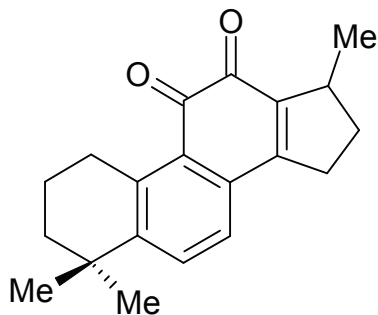
Figure 2. Chemical structure of cryptotanshinone (CTS)

Influence of cryptotanshinone on secretase activity

Results of recent pharmacological studies [45] have shown that CTS not only improve the cognitive ability in AD transgenic mice by improvement results in Morris water maze test but also promote APP metabolism in rat cortical neuronal cells toward the non-amyloidogenic products pathway. The effect of CTS on Aβ levels was confirmed by *in vitro* analysis (rat cortical neurons expressing APP cultured for 18 h). It was demonstrated that CTS treatment on a concentration-dependent manner (0–10 μM) caused a decrease the Aβ40 and Aβ42 fragments levels and increase the release of the N-terminal APP cleavage product (sAPP) [45]. During this experiment the 10 μM CTS concentration was the most effective one and Aβ42 levels were decreased by max 45%. In another *in vitro* assay it was shown that α-secretase activity was increased by CTS in a concentration dependent manner without changing the β-secretase or γ-secretase activity [45]. Mei et al. [45] has emphasized that CTS may serve as a neuroprotective agent and contribute to the treatment of AD patients through regulating α-secretase activity.

In another study performed by Mei et al. [46] it was shown that CTS dose dependently (0–10 μM) induced ADAM10 protein (disintegrin and metalloproteinase domain-containing protein 10) possessing an α-secretase – like activity and promoted non-amyloidogenic α-secretase processing of APP in cortical neurons. This α-secretase induced effect by CTS was partially dependent on phosphatidylinositol 3-kinase (PI3K). The abovementioned result seems to be interesting due to the fact that over-expression of ADAM10 in mice transgenic for human APP increased the release of the sAPPα, reduced the formation of Aβ40 and Aβ42 and prevented their deposition in plaques [46]. To summarize, CTS may be a promising compound in the new drug for AD therapy development.

Camelia sinensis (Green tea)

Camelia sinensis (L.) O. Kuntze (*Theaceae*) is an evergreen, heavily branched shrub, all varieties of tea are produced from this plant. Green tea has a longstanding worl-

dwide reputation for its health-promoting properties, i.e. chemopreventive, hepatoprotective, anti-inflammatory activities and possesses a beneficial effects on cardiovascular disorders and cancer [47, 48].

The identification of the phytochemical profile of green tea is complex due to the numerous compounds formed during the drying and another different ways of technological processing of the tea leaves, as well as climate changes and variations in the harvest season and horticultural practices [49, 50]. Nowadays, all tea phytochemicals are classified into two groups: volatile and non-volatile compounds [47]. The chemical composition of *Theae folium* contains many polyphenolic compounds (mainly six groups of compounds, up to 35%) with the predominance of catechins (10–20%) [47]. The main catechins present in green tea are: (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC), and (−)-epigallocatechin gallate (EGCG). Among them, EGCG (fig. 3) is the most active major polyphenol compound of green tea. In addition, EGCG has been demonstrated to display potent antioxidant properties [52]. Other chemicals were identified such as caffeine (2.5–4.5%), theophylline (0.02–0.05%), theobromine (0.05%) and flavonoid glycosides [51], and volatile oil [48].
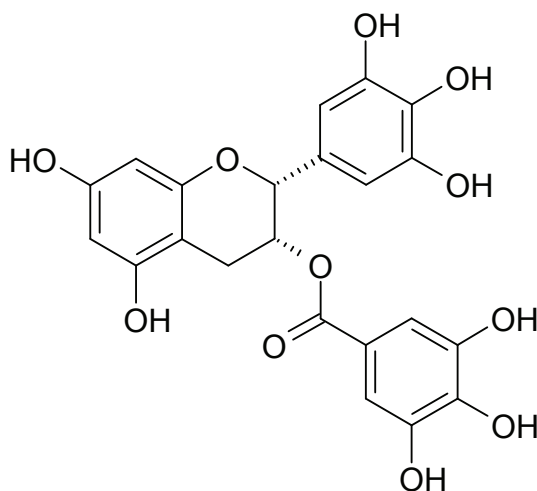

Figure 3.
Chemical structure of (−)-epigallocatechin gallate (EGCG)

In the study performed by Okello et al. [53] it was shown that infusions of both green and black tea exhibited concentration-dependent inhibition of acetylcholinesterase (AChE) activity *in vitro* (the colorimetric – Ellman's method) with IC_{50} of 0.03±0.004 mg/ml and 0.06±0.005 mg/ml, respectively. In the same conditions, two teas have inhibited the butyrylcholinesterase (BuChE) activity *in vitro* with IC_{50} values of 0.05±0.005 mg/ml and 0.05±0.007 mg/ml, respectively. It was also reported that green tea infusion has anti-β-secretase activity [53]. These results indicate that active compounds of green and black tea may play important role in neurodegenerative disease treatment.

The another interesting compound affecting the central nervous system – a γ-aminobutyric acid (GABA, human brain neurotransmitter) is present in new type tea. According to the findings of Tsushida et al. [54], the anaerobic fermentation of fresh tea leaves is a key step of manufacturing GABA-containing tea. Recently many new techniques for enhancement of GABA content in GABA-containing tea have been developed [50].

(−)-Epigallocatechin gallate as a neuropharmacological compound

Health beneficial effects of green tea are attributed to its principal neurobiological active compound – EGCG [55, 56]. The neuroprotective effects of EGCG have been investigated in several studies [57, 43]. Zhang et al., [43] have showed that EGCG strongly inhibited the AChE activity and enhanced the inhibitory effect of a sesquiterpene alkaloid - huperzine A on AChE. In a few *in vitro* studies the EGCG protected neuronal cells against a wide spectrum of neurotactive/neurotoxic agents, such as alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA), kainate, N-methyl-D-aspartate (NMDA), glutamate [58; 59], and lipopolysaccharide (LPS) [60]. He et al. [61] revealed the protective EGCG effects in aging mice induced by D-galactose. In another study EGCG was demonstrated to reduce Aβ levels both *in vitro* and *in vivo* by promoting APP non-amyloidogenic cleavage [62]. Lee et al. [63] demonstrated that the administration of EGCG attenuates the ischemia-induced increase of putrescine levels and has a neuroprotective effect against hippocampal neuronal damage in a gerbil model of global ischemia.

Moreover, results of Reznichenko et al. [64] research suggested that EGCG may have a positive impact on aging and neurodegenerative diseases by retarding or perhaps even reversing the accelerated rate of neuronal degeneration, because EGCG showed the induction of neurite outgrowth in PC12 cells and abolished the induced cell damage. Other studies have established that green tea extract and EGCG, given earlier than the parkinsonism-inducing neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), prevent striatal dopamine depletion and substantia nigra dopaminergic neuron loss caused by MPTP in animal model [65]. Moreover, EGCG significantly inhibited microglial activation induced by MPTP [66] and also reduced the dichlorodiphenyl-trichloroethane (DDT)-induced cell death in dopaminergic SHSY-5Y cells [67]. Levites et al. [68] also demonstrated in neuronal cell culture that EGCG attenuated 6-hydroxydopamine (neurotoxin)-induced cell death. An increased phosphorylated protein kinase C (PKC) activity due to EGCG treatment allow the researchers to conclude that PKC isoenzymes are involved in the neuroprotective action of EGCG against 6-hydroxydopamine Levites et al. [68] as well as Jang et al. [69] demonstrated that EGCG showed neuroprotective effect against quinolinic acid-induced excitotoxicity via PI3K pathway and NO inhibition. A recent *in vitro* study showed a significant reduction in the striatal 3-O-methyldopa level in EGCG-treated rats through inhibition of the central COMT-mediated L-DOPA methylation [59]. According to the conclusions of Kang's

research team [59] EGCG may have significant beneficial effects in Parkinson's patients treated with L-DOPA and carbidopa by exerting a modest inhibition of L-DOPA methylation plus a strong neuroprotection against oxidative damage and degeneration.

The systematic review showed an increasing evidence that green tea polyphenols, especially EGCG, possessing an antioxidant activity may protect neurons from neurotoxic chemicals but the mechanisms underlying the neuroprotective effect of EGCG in neurodegenerative diseases models are not fully explained.

The influence of (-)-epigallocatechin gallate and other tea compounds on secretase activity

The systematic review of pharmacological studies allow to underline the beneficial role of EGCG in the prevention and attenuation of different pathways in neurodegenerative diseases. This effects were shown not only in the mice model of Parkinson's disease but also in the transgenic model of Alzheimer's disease *in vivo* and other dementias. Most investigations have focused on the therapeutic potential of compounds that may promote the non-amyloidogenic pathway of APP [68], due to the fact that EGCG is known to enhance the processing of APP to sAPP-α *in vitro* [62].

Before the study on the neuronal activity of EGCG, the researchers founded that green tea infusion at a concentration of 0.03 mg/ml inhibited BACE1 activity by 27% after 5 min incubation, whereas after 60 min the inhibition reached 38% [53]. These results indicated a new scientific way in order to demonstrate the activity of chemical compounds of green tea extract on the Aβ and enzymes regulating its deposition in the brain.

In detail, an immunoenzymatic assay showed that EGCG could reduce intracellular Aβ generation (both Aβ1–40 and Aβ1–42 peptides) in a dose-dependent manner. MC65 cells treated with EGCG reduced Aβ generation by 20–30% at a concentration of $20\,\mu$M compared with untreated cells. EGCG showed also a dose-dependent effect on soluble APP (sAPP) release in culture medium. Also in transgenic mice model it was shown the reduced hippocampal level of Aβ after EGCG treatment was shown [55].

In another studies it was attempted to clarify the enzymatic mechanism of the action of EGCG. Results of neurochemical study with use of ELISA and Western blot methods allowed to state that that synthetic EGCG significantly elevated N-terminal APP cleavage producing soluble APP-α through α-secretase activity in murine neuron-like cells transfected with Swedish mutant form of APP (SweAPP N2a cells) as well as transgenic mice carrying Swedish mutant APP (Tg APPsw line 2576) [70]. It was shown that EGCG ($20\,\mu$M) diminished Aβ deposition in SweAPP N2a cells and Tg APPsw line by 61% and 38% compared with untreated cells, respectively. Moreover, epicatechin ($80\,\mu$M) inhibited Aβ generation by nearly 20–30% in both cell types, but other green tea compounds as (-)-gallocatechin and (-)-catechin (C)

showed the opposite effect as compared to the effects of EGCG. The results allowed to state that purified EGCG was more capable of reducing Aβ generation in cell cultures than when it was present in a mixture in whole green tea extract [70]. Besides, in transgenic mouse model of AD, it was shown that intraperitoneally (20 mg/kg daily for 60 days) and intracerebroventricularly (5 μl (10 μg)/mouse) administered EGCG to mice significantly elevated non-amyloidogenic level of APP fragment (sAPP-α) as well as reduced $A\beta_{1-40}$ and $A\beta_{1-42}$ fragments levels by 38–44%. It was found that 32–40% increase in α-secretase cleavage activity which was associated with reduction of total Aβ levels in the brain homogenate [70].

Other findings focused on the influence of EGCG on β-, and γ-secretase activity. Lee et al. [60] demonstrated that EGCG dose-dependently inhibited lipopolysaccharide (LPS)-induced elevation of Aβ level through attenuation of β- and γ-secretase activities. EGCG at doses of 1.5 and 3mg/kg was orally administered to mice in drinking water for 3 weeks. LPS was intracerebroventricularly injected at dose of 1 μg/mouse on day 22 from the start of the EGCG treatment. It was shown that treatment of EGCG dose-dependently lowered LPS-induced β- and γ-secretase activities both in the cortex and hippocampus [60]

In another model research using commercial BACE1 assay kit it has been shown that not only EGCG inhibited BACE1 activity *in vitro* (IC_{50}=1.6μM), but also other compounds of ethyl acetate extract of green tea [71]. Jeon et al. [71] proved that (-)-gallocatechin gallate showed inhibition of BACE1 activity with IC_{50} value of 1.8 μM and (-)-epicatechin (IC_{50}=2.3μM), (-)-epigallocatechin (IC_{50}=2.4μM), (-)-gallocatechin (IC_{50}=2.5μM), (+)-epicatechin (IC_{50}=2.8μM), (-)-catechin (IC_{50}=3.0μM), (+)-catechin (IC_{50}=3.5μM), (-)-epicatechin gallate (IC_{50}=4.5μM), (-)-catechin gallate (IC_{50}=6.0μM). It follows that the pyrogallol moiety in the catechin skeleton appeared to be essential for the stronger inhibitory activity [71].

Recently, Giunta et al. [72] showed in AD mouse model that enrich food with EGCG and fish oil (at a dose of 0.25 mg of fish oil plus 1.875 mg of EGCG or 0.25 mg of fish oil plus 0.375 mg of EGCG/mouse/day for 6 months) enhanced the production of sAPP-α, indicating the increased non-amyloidogenic processing. Moreover, it was shown that co-treatment of mice with EGCG and fish oil synergistically inhibited Aβ deposition in examined brain regions such as cingulate cortex, hippocampus, and entorhinal cortex. This has been observed that the low dose EGCG supplemented with fish oil was more effective in diminish of soluble and insoluble forms of $A\beta_{1-40, 42}$. Of course there is a need to perform clinical trials on the effectiveness of this complex natural product.

The study on molecular mechanisms of the action of EGCG on the level of neuronal cells were made by Obregon et al. [73]. The investigation demonstrated that EGCG in a dose-dependent manner increased mRNA expression of ADAM10 protein (disintegrin and metalloproteinase domain-containing protein 10) in both cultured neuronal and microglial cells. Protein ADAM10 was a key effector in EGCG promotion of non-amyloidogenic APP proteolysis. Moreover, it was observed that EGCG dose-dependently reduced of $A\beta_{1-40}$ and $A\beta_{1-42}$ concentrations and non-am-

yloidogenic APP processing was correlated with increased ADAM10 maturation after EGCG treatment. However, more studies concerning the role of direct and/ or indirect molecular interactions between EGCG and ADAM10 are needed to perform.

CONCLUSION

In conclusion, it can be stated that discovery and development of new drugs for neurodegenerative diseases (anti-dementia drugs) is one of the most intensively developed scientific areas due to the fact that a major risk factor for central neurodegeneration is brain aging [74-76]. Currently, four approaches for the prevention and modification of AD progress are applied, mainly: anti-amyloid deposition strategy (up-regulation of α-secretase and down-regulation of β- and γ-secretase), acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activity inhibition, neuroprotective treatment and neurorestorative/neuroregenerative therapies [4]. Recent studies are directed to the discovery of the new plant substances increasing the concentration and activity of α-secretase in brain and, therefore, lead to the cleavage of APP (amyloid precursor protein) within the Aβ sequence and releasing soluble N-terminal nonaggregating fragments (sAPP). Numerous studies have shown that stimulation of sAPP release is associated with diminished formation of amyloidogenic peptides [75]. There is a strong belief that α-secretase upregulation may be an important target for the prevention and more effective therapy of Alzheimer's disease, however, there are strong evidences pointing the role of β- and γ-secretases as a susceptibility factor of brain amyloidosis and as an excellent therapeutic target in AD [28]. Results of Chow et al. [77] allowed to state that the novel anti-amyloid combination therapy that modestly targets both β-secretase (BACE1) and γ-secretase represents a more effective treatment strategy diminishing Aβ and amyloidosis in AD. In this paper the authors, taking into account the abovementioned facts, the prevalence of plant materials and multidirectional nature of the pharmacological effects of plant origin preparations, made a literature study in order to point the new plant biologically active compounds influencing on the brain Aβ pathway induced in Alzheimer's disease via modulation of secretases concentration and activity in different laboratory research models. Our work demonstrates the worldwide growing investigations of action of plant substances in this field with the predominance of Asian plants. The analysis of scientific data allowed us to conclude that plant biologically active compounds such as (−)-epigallocatechin gallate from the leaf of *Camellia sinensis* and cryptotanshinone from root of *Salvia miltiorrhiza* may open a new promising therapeutic window for the prevention and treatment of degenerative diseases such as AD. However, there is a need to perform further studies and clinical trials in this area.

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REFERENCES

1. Wimo A, Prince M. Alzheimer's Disease International World Alzheimer Report 2010. The Global Economic Impact of Dementia. Alzheimer's Disease International, London. 2010:1-56.
2. Rafii MS, Aisen PS. Recent developments in Alzheimer's disease therapeutics. BMC Med 2009;7:7.
3. Ożarowski M, Mikołajczak PŁ, Bobkiewicz-Kozłowska T, Kujawski R, Mrozikiewicz PM. Neuroactive compounds from medicinal plants of the *Lamiaceae* family showing potentally beneficial activity in treatment of Alzheimer's disease. Herba Pol 2009; 55(4):148-63.
4. Salloway S, Mintzer J, Weiner MF, Cummings JL. Disease-modifying therapies in Alzheimer's disease. Alzheimer's & Dementia 2008; 4:65-79.
5. Selkoe DJ. Translating cell biology into therapeutic advances in Alzheimer's disease. Nature (Suppl.) 1999; 399:A23-A31.
6. Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. Physiological Rev 2002; 81(2):741-66.
7. Fraering PC. Structural and functional determinants of β-secretase, an intramembrane protease implicated in Alzheimer's disease. Curr Genom 2007; 8:531-49.
8. Willem M, Lammich S, Haass C. Function, regulation and therapeutic properties of β-secretase (BACE1) Semin. Cell Dev Biol 2009; 20:175-82.
9. Parihar MS, Hemnani T. Alzheimer's disease pathogenesis and therapeutic interventions. J Clin Neurosci 2004; 11(5):456-67.
10. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 2002; 297(5580):353-6.
11. Sobów T, Flirski M, Liberski PP. Amyloid-beta and tau proteins as biochemical markers of Alzheimer's disease. Acta Neurobiol Exp 2004; 64:53-70.
12. Ożarowski M, Kupsz J, Torlińska T. Biologiczne czynniki ryzyka choroby Alzheimera. Nowiny Lek 2006; 2(75):193-8.
13. Delacourte N, Sergeant N, Robitaille Y, Buee-Scherrer V, Buee L, Bussiere DT, Vermersch P, Hof PR, Gauvreau D, Wattez A. Pathological tau proteins are biochemical markers that differentiate several types of neurofibrillary degeneration. In: Iqbal K, Nishimura T, Takeda M, Wiśniewski HM. (eds.). Alzheimer's disease: biology, diagnosis and therapeutics. New York 1997:205-12.
14. Skovronsky DM, Lee VMY, Trojanowski JQ. Neurodegenerative diseases: new concepts of pathogenesis and their therapeutic implications. Annu Rev Pathol Mech Dis 2006; 1:151-70.
15. Tillement JP, Lecanu L, Papadopoulos V. Amyloidosis and neurodegenerative diseases: current treatments and new pharmacological options. Pharmacology 2010; 85:1-17.
16. Westermark P. et al. A primer of amyloid nomenclature. Amyloid 2007, 14(3):179-83.
17. Pezzini A, Padovani A. Cerebral amyloid angiopathy-related hemorrhages. Neurol Sci 2008; 29:S260-S263.
18. Chiti F, Dobson CM. Protein misfolding, functional amyloid, and human disease. Ann Rev Biochem 2006; 75:333-66.
19. Head E. Down syndrome and beta-amyloid deposition. Curr Opinion Neurol 2004; 17(2):95-100.
20. De Strooper B, Vassar R, Goldeet T. The secretases: enzymes with therapeutic potential in Alzheimer disease. Nat Rev Neurol 2010; 6:99-107.

21. Mattson MP. Cellular actions of beta-amyloid precursor protein and its soluble and fibrillogenic derivatives. Physiol Rev 1997; 77:1081-132.

22. Taylor CJ, Ireland DR, Ballagh I, Bourne K, Marechal NM, Turner PR, Bilkey DK, Tate WP, Abraham WC. Endogenous secreted amyloid precursor protein-alpha regulates hippocampal NMDA receptor function, long-term potentiation and spatial memory. Neurobiol Dis 2008; 31:250-60.

23. Meziane H, Dodart JC, Mathis C, Little S, Clemens J, Paul SM, Ungerer A. Memory-enhancing effects of secreted forms of the β-amyloid precursor protein in normal and amnestic mice. Proc Natl Acad Sci 1998; 95:12683-8.

24. Morimoto T, Ohsawa I, Takamura C, Ishiguro M, Nakamura Y, Kohsaka S. Novel domain-specific actions of amyloid precursor protein on developing synapses. J Neurosci 1998; 18: 9386-93.

25. Furukawa K, Barger SW, Blalock EM, Mattson MP. Activation of K+channels and suppression of neuronal activity by secreted beta-amyloid precursor protein. Nature 1996; 379:74-8.

26. Hussain I, Powell D, Howlett DR, Tew DG, Meek TD, Chapman C, Gloger IS, Murphy KE, Southan CD, Ryan DM, Smith TS, Simmons DL, Walsh FS, Dingwall C, Christie G. Identification of a novel aspartic protease (Asp2) as betasecretase. Mol Cell Neuosci 1999; 14:419-27.

27. Yan R, Munzner JB, Shuck ME, Bienkowski MJ. BACE2 functions as an alternative alphasecretase in cells. J Biol Chem 2001; 276:34019-27.

28. Wong P. Beta-amyloid modulation role of BACE1 and BACE2. Basic Research Overview. Johns Hopkins University School of Medicine. Alzheimer's Disease Research Center.http://www.alzresearch.org/basicresearch.cfm (02.12.2010)

29. Cole SL, Vassar R. The Alzheimer's disease β-secretase enzyme, BACE1. Mol Neurodegeneration 2007; 2(22):1-25.

30. He G, Luo W, Li P, Remmers C, Netzer WJ, Hendrick J, Bettayeb K, Flajolet M, Gorelick F, Wennogle LP, Greengard P. Gamma-secretase activating protein is a therapeutic target for Alzheimer's disease. Nature 2010; 46:95-8.

31. Steiner H, Fluhrer R, Haass C. Intramembrane proteolysis by gamma-secretase. J Biol Chem 2008; 283:29627-31.

32. Chow VW, Savonenko AV, Melnikova T, Kim H, Price DL, Li T, Wong PC. Modeling an anti-amyloid combination therapy for Alzheimer's disease. Sci Transl Med 2010; 2(13):13ra1.

33. Wiśniewski T, Sadowski M. Preventing β-amyloid fibrillization and deposition: β-sheet breakers and pathological chaperone inhibitors. BMC Neurosci 2008; 9(Suppl 2):S5.

34. Choi SH, Hur JM, Yang EJ, Jun M, Park HJ, Lee KB, Moon E, Song KS. Beta-secretase (BACE1) inhibitors from *Perilla frutescens* var. acuta. Arch Pharm Res 2008; 31(2):183-7.

35. Zhou L, Zuo Z, Chow MS. Danshen: an overview of its chemistry, pharmacology, pharmacokinetics, and clinical use. J Clin Pharmacol 2005; 45:1345–59.

36. Wu MH, Tsai WJ, Don MJ, Chen YC, Chen IS, Kuo YC. Tanshinlactone A from *Salvia miltiorrhiza* modulates interleukin-2and interferon-γ gene expression. J Ethnopharmacol 2007; 113:210-17.

37. Zhang Y, Li X, Wang Z. Antioxidant activities of leaf extract of *Salvia miltiorrhiza* Bunge and related phenolic constituents. Food Chem Toxicol 2010; 48(10):2656-62.

38. Matkowski A, Zielińska S, Oszmiański J, Lamer-Zarawska E. Antioxidant activity of extracts from leaves and roots of *Salvia miltiorrhiza* Bunge, *S. przewalskii* Maxim., and *S. verticillata* L. Bioresource Technol 2008; 99(16):7892-6.

39. Lin TH, Hsieh CL. Pharmacological effects of *Salvia miltiorrhiza* (Danshen) on cerebral infarction. Chin Med 2010; 5(22):1-6.

40. Yu XY, Lin SG, Chen X, Zhou ZW, Liang J, Duan W, Chowbay B, Wen JY, Chan E, Cao J, Li CG, Zhou SF. Transport of cryptotanshinone, a major active triterpenoid in Salvia miltiorrhiza Bunge widely used in the treatment of stroke and Alzheimer's disease, across the blood-brain barrier. Curr Drug Metab 2007; 8(4):365-78.

41. Jin DZ, Yin LL, Ji XQ, Zhu XZ. Cryptotanshinone inhibits cyclooxygenase-2 enzyme activity but not its expression. Eur J Pharmacol 2006; 549(1-3):166-72.

42. Ren Y, Houghton PJ, Hider RC, Howes MJ. Novel diterpenoid acetylcholinesterase inhibitors from *Salvia miltiorrhiza*. Planta Med 2004; 70(3):201-4.

43. Zhang L, Cao H, Wen J, Xu M. Green tea polyphenol (-)-epigallocatechin-3-gallate enhances the inhibitory effect of huperzine A on acetylcholinesterase by increasing the affinity with serum albumin. Nutr Neurosci 2009;12(4):142-8.

44. Kim DH, Jeon SJ, Jung JW, Lee S, Yoon BH, Shin BY,. Son KH, Cheong JH, Kim YS, Kang SS, Ko KH, Ryu JH. Tanshinone congeners improve memory impairments induced by scopolamine on passive avoidance tasks in mice, Eur J Pharmacol 2007; 574:140-7.

45. Mei Z, Zhang F, Tao L, Zheng W, Cao Y, Wang Z, Tang S, Le K, Chen S, Pi R, Liu P. Cryptotanshinone, a compound from Salvia miltiorrhiza modulates amyloid precursor protein metabolism and attenuates beta-amyloid deposition through upregulating alpha-secretase *in vivo* and *in vitro*. Neurosci Lett 2009; 452(2):90-5.

46. Mei Z, Situ B, Tan X, Zheng S, Zhang F, Pengke Y, Peiqing L. Cryptotanshinione upregulates α-secretase by activation PI3K pathway in cortical neurons. Brain Res 2010; 1348:165-73.

47. Zhen YS. (ed.). Tea. Bioactivity and Therapeutic Potential. Medicinal and Aromatic Plants–Industrial Profiles. Taylor & Francis e-Library, 2005.

48. Gruenwald J, Brendler T, Jaenicke C. PDR for Herbal Medicines. Fourth edition. New York 2007:414-22.

49. Blumberg J. Introduction to the proceedings of the third international scientific symposium on tea and human health: role of flavonoids in the diet. J Nutr 2003; 133:3244S-3246S.

50. Ho CT, Lin JK, Shahidi F. (eds.). Tea and tea products. chemistry and health-promoting properties. Nutraceutical Science and Technology. New York 2008:1-9.

51. Wagner H, Bladt S. Plant Drug Analysis. A Thin Layer Chromatography Atlas. Second edition. Berlin-Heidelberg 2009.

52. Guo Q, Zhao B, Li M, Shen S, Xin W. Studies on protective mechanisms of four components of green tea polyphenols against lipid peroxidation in synaptosomes, Biochim Biophys Acta 1996; 1304:210-22.

53. Okello EJ, Savelev SU, Perry EK. In vitro anti-beta-secretase and dual anti-cholinesterase activities of *Camellia sinensis* L. (tea) relevant to treatment of dementia. Phytother Res 2004; 18(8):624-7.

54. Tsushida T, Murai T, Omori M, Okamoto J.. Production of a new type tea containing a high level of γ-aminobutyric acid. *Nippon Nogeikagaku Kaishi* 1987; 6:817-22. In: Ho Chi-Tang, Lin Jen-Kun, Shahidi Fereidoon (eds.). Tea and tea products. Chemistry and health-promoting properties. Nutraceutical Science and Technology. New York 2008:1-9.

55. Lin CL, Chen TF, Chiu MJ, Way TD, Lin JK. Epigallocatechin gallate (EGCG) suppresses β-amyloid-induced neurotoxicity through inhibiting c-Abl/FE65 nuclear translocation and GSK3β activation. Neurobiol Aging 2009; 30:81-92.

56. Abbas S, Wink M. Epigallocatechin gallate inhibits beta amyloid oligomerization in Caenorhabditis elegans and affects the daf-2/insulin-like signaling pathway. Phytomed 2010; 17(11):902-9.

57. Mandel S, Reznichenko L, Amit T, Youdim MB. Green tea polyphenol (−)-epigallocatechin-3-gallate protects rat PC12 cells from apoptosis induced by serum withdrawal independent of P13-Akt pathway. Neurotox Res 2003; 5(6):419-24.

58. Lee JH, Song DK, Jung CH et al. (–)-epigallocatechin gallate attenuates glutamate-induced cytotoxicity via intracellular ca modulation in PC12 cells. Clin Exp Pharmacol Physiol 2004; 31:530-36.

59. Kang KS, Wen Y, Yamabe N, Fukui M, Bishop SC, Zhu BT. Dual beneficial effects of (-)-epigallocatechin-3-gallate on levodopa methylation and hippocampal neurodegeneration: *in vitro* and *in vivo* studies. PLoS ONE 2010;5(8): e11951. doi:10.1371/journal.pone.0011951.

60. Lee YK, Yuk DY, Lee JW, Lee SY, Ha TY, Oh KW, Yun YP, Hong JT. (−)-Epigallocatechin-3-gallate prevents lipopolysaccharide-induced elevation of beta-amyloid generation and memory deficiency. Brain Res 2009; 1250:164-74.

61. He M, Zhao L, Wei MJ, Yao WF, Zhao HS, Chen FJ. Neuroprotective effects of (-)-epigallocatechin-3-gallate on aging mice induced by D-galactose. Biol Pharm Bull 2009; 32(1):55-60.

62. Levites Y, Amit T, Mandel S, Youdim MB. Neuroprotection and neurorescue against beta toxicity and PKC-dependent release of nonamyloidogenic soluble precursor protein by green tea polyphenol (−)-epigallocatechin-3-gallate. FASEB J 2003; 17(8):952-4.

63. Lee SY, Kim CY, Lee JJ, Jung JG, Lee SR. Effects of delayed administration of (-)–epigallocatechin gallate, a green tea poliphenol on the chaanges in polyamine levels and neuronal damage after transient forebrain ischemia in gerbils. Brain Res Bull 2003; 61:399-406.

64. Reznichenko L, Amit T, Youdim MBH, Mandel S. Green tea polyphenol (–)-epigallocatechin-3-gallate induces neurorescue of long-term serum-deprived PC12 cells and promotes neurite out growth. J Neurochem 2005: 93:1157-67.

65. Levites Y, Weinreb O, Maor G, Youdim MB, Mandel S. Green tea polyphenol (–)-epigallocatechin-3-gallate prevents N-methyl-4-phenyl-1,2,3,6 tetrahydropyridine-induced dopaminergic neurodegeneration. J Neurochem 2001; 78:1073-82.

66. Li R, Peng N, Du F, Li XP, Le WD. Epigallocatechin gallate protects dopaminergic neurons against 1-methyl-4- phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity by inhibiting microglial cell activation. Nan Fang Yi Ke Da Xue Xue Bao 2006; 26(4):376-80.

67. Tai KK, Truong DD. (-)-Epigallocatechin-3-gallate (EGCG), a green tea polyphenol, reduces dichlorodiphenyl-trichloroethane (DDT)-induced cell death in dopaminergic SHSY-5Y cells. Neurosci Lett 2010; 482(3):183-7.

68. Levites Y, Tamar A, Moussa YBH, Silvia M. Involvement of protein kinase C activation and cell survival/ cell cycle genes in green tea polyphenol (−)-epigallocatechin 3-gallate neuroprotective action. J Biol Chem 2002; 277:30574-80.

69. Jang S, Jeong HS, Park JS, Kim YS, Jin CY, Seol MB, Kim BC, Lee MC. Neuroprotective effects of (-)-epigallocatechin-3-gallate against quinolinic acid-induced excitotoxity via PI3K pathway and NO inhibition. Brain Res 2010; 1313:25-33.

70. Rezai-Zadeh K, Shytle D, Sun N, Mori T, Hou H, Jeanniton D, Ehrhart J, Townsend K, Zeng J, Morgan D, Hardy J, Town T, Tan J. Green tea epigallocatechin-3-gallate (EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice. J Neurosci 2005; 25(38):8807-14.

71. Jeon SY, Bae KH, Seong YH, Song KS. Green tea catechins as a BACE1 (beta-secretase) inhibitor. Bioorg Med Chem Lett 2003; 13:3905-8.

72. Giunta B, Hou H, Zhu Y, Salemi J, Ruscin A, Shytle RD, Tan J. Fish oil enhances anti-amyloidogenic properties of green tea. EGCG in Tg2576 mice. Neurosci Lett 2010; 471:134-8.

73. Obregon DF, Rezai-Zadeh K, Bai Y, Sun N, Hou H, Ehrhart J, Zeng J, Mori T, Arendash GW, Shytle D, Town T, Tan J. ADAM10 activation is required for green tea (−)-epigallocatechin-3-gallate-induced alpha-secretase cleavage of amyloid precursor protein. J Biol Chem 2006; 281(24):16419-27.

74. Zhang Z-J. Therapeutic effects of herbal extracts and constituents in animal models of psychiatric disorders. Life Sci 2004; 75:1659-99.

75. Giacobini E. Cholinesterases: New roles in brain function and in Alzheimer's disease. Neurochem Res 2003; 28(3/4):515-22.

76. García-Palomero E, Muńoz P, Usan P, Garcia P, Delgado E, De Austria C, Valenzuela R, Rubio L, Medina M, Martínez A. Potent β -amyloid modulators. Neurodegenerative Dis 2008; 5:153-6.

77. Chow VW, Savonenko AV, Melnikova T, Kim H, Price DL, Li T, Wong PC. Modeling an anti-amyloid combination therapy for Alzheimer's disease. Sci Transl Med 2010; 2(13):13ra1.

ROŚLINY I ICH ZWIĄZKI CHEMICZNE WPŁYWAJĄCE NA β-AMYLOID I AKTYWNOŚĆ SEKRETAZ JAKO POTENCJALNE ŹRÓDŁA NEUROPROTEKCYJNYCH PRODUKTÓW ZIOŁOWYCH. CZĘŚĆ 1.

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S t r e s z c z e n i e

Przetwory roślinne, a szczególnie frakcje związków biologicznie czynnych mogą odgrywać doniosłą rolę w poprawianiu jakości życia pacjentów z otępieniem, a także opóźniać postępy chorób neurodegeneracyjnych na drodze różnych mechanizmów ich farmakologicznego działania. Ostatnie lata przyniosły szereg opracowań i naukowych badań, jednak wydaje się, że w dalszym ciągu brakuje szczegółowej, syntetycznej analizy na ten temat. Głównymi markerami biologicznymi procesu patogenetycznego choroby Alzheimera, będącej najczęstszą postacią otępienia są, jak dotąd, odkładające się płytki β-amyloidu, zwyrodnienie neurofibrylarne oraz zaniki neuronów cholinergicznych w kluczowych dla pamięci regionach mózgu. Obecnie w leczeniu choroby Alzheimera stosuje się głównie leki z grupy inhibitorów acetylocholinoesterazy. W naszym poprzednim artykule przeglądowym zwróciliśmy uwagę na interesujące mechanizmy działania biozwiązków wybranych roślin z rodziny *Lamiaceae*, np. hamowanie acetylo-, i butyrylocholinoesterazy czy ich działanie

antyoksydacyjne. Celem niniejszego artykułu przeglądowego jest analiza wyników dotyczących oddziaływania roślinnych ekstraktów oraz wyizolowanych roślinnych związków chemicznych (m.in. kryptotanszinonów, galusanu epigalokatechiny EGCG) na szlak powstawania i odkładania β-amyloidu w modelach farmakologicznych, między innymi poprzez interakcję z α-, β-, γ- sekretazą (zarówno na poziomie genomowym jak i białkowym). Jest to jeden z głównych nurtów poszukiwania nowych leków ze źródeł naturalnych o działaniu neuroprotekcyjnym. Wydaje się, że *Salvia miltiorrhiza* oraz *Camelia sinensis* są roślinami leczniczymi pochodzącymi z Azji posiadającymi odpowiedni potencjał terapeutyczny w tym względzie. Oprócz nich znanych jest co najmniej 10 azjatyckich roślin intensywnie badanych pod kątem prewencji chorób neurodegeneracyjnych (m.in. *Aralia cordata, Magnolia officinalis, Perilla frutescens, Polygala tenuifolia, Punica granatum, Sophora flavescens*). Jednak wiele aspektów ich działania jest niezbyt dobrze poznanych, dlatego istnieje potrzeba przeprowadzenia dalszych badań zarówno fitochemicznych, jak i farmakologicznych.

Słowa kluczowe: Salvia miltiorrhiza, Camelia sinensis, β-amyloid, choroba Alzheimera, neuroprotekcja