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Review Article

Potential role of phytochemicals in attenuating Alzheimer's disease - A comprehensive review

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Abstract

Modern pharmacotherapy encourages the use of phytochemicals for combating Alzheimer's Disease. The clinical findings and recent developments in using flavonoids, alkaloids, glycosides, and terpenoids against Alzheimer's have been summarized in this review. Quercetin contains flavonoids that inhibit BACE-1 enzyme activity that is responsible for the synthesis of Amyloid-B peptide. Quercetin also increases AMPK activity. Alkaloids inhibit AchE activity. Moreover, ginkgo biloba produces antioxidant effects by scavenging free peroxyl radicals. Phenylethanoid glycoside exerts neuroprotective properties by improving the impairment of neuronal apoptosis. All these claims have been supported through in-vitro and in-vivo studies. Furthermore, employing these therapies will only be feasible through delivery through a nanoparticle delivery system. Phytochemicals possess great potential for Alzheimer's Disease treatment. They contain antioxidants, anti-inflammatory, and certain neuroprotective constituents which make them ideal for Alzheimer's therapy. The delivery of these agents can be done through nanocomposites to make sure they bypass the blood-brain barrier to elicit a therapeutic response.

Keywords: Alzheimer's Disease, Phytochemicals, Modern Pharmacotherapy, Flavonoids, Alkaloids, Quercetin, Gingko Biloba, Naringin, Apigenin, Antioxidants, Neuroprotective Properties.

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Introduction

Alzheimer's is a type of dementia that is common in the world. It actively affects a significant number of populations, especially 65 years of age and older. From modest amnesia to severe decline in cognition, illness causes memory loss, confusion, behavioral changes, and personality changes. The disease pathology includes the buildup of tau protein tangles within neurons and deposits of amyloid-β (Aβ) plaques in surrounding brain tissue, which leads to neuronal damage and brain atrophy. According to current theories, a complex interaction of genes, environment, and lifestyle factors contributes to the onset and progression of the illness, with neuro inflammation being a key component. The central nervous system causes neuro

inflammation as a reaction to an infection or a disease. During this process, immune cells are recruited to trigger inflammatory mediators, which activate glial cells (microglia and astrocytes). Long-term or severe neuro inflammation, while initially helpful, can worsen neuronal injury, accumulate amyloid beta plaque and tau proteins, and impair cognitive function. Understanding the function of neuro inflammation in neurodegenerative diseases, such as Alzheimer's, is of increasing interest [1].

Epidemiology of Alzheimer's disease

According to a 2005 study employing the Delphi consensus methodology, projections indicated a global dementia prevalence of approximately 24.3 million individuals. Furthermore, the study highlighted a significant annual incidence of 4 to 6 million new cases. Notably, the data suggested an exponential growth trajectory, with a projected doubling of affected individuals every two decades, culminating in an estimated 81.1 million cases by 2040. A striking demographic feature revealed that 60% of dementia cases are concentrated within developing nations. Specifically, Asian countries, including China, India, Japan, and Indonesia, were identified as possessing some of the highest dementia prevalence rates globally [2].

The estimated global occurrence of dementia in adults 60 years of age and above was 3.9%. By regional estimations, the rates were 1.6% in Africa, 4.0% in China, and the Western Pacific, 4.6% in Latin America, 5.4% in Western Europe, and 6.4 % in North America.[3] According to estimates, there were 36.5 million dementia sufferers in 2010; 7.7 million additional cases are reported every year, or one every four seconds. The number of individuals with dementia is set to rise every 20 years. Most of them will reside in low- and middle-income nations [4].

For various reasons, including the serious presymptomatic neuronal harm due to amyloid β(Aβ) peptide accumulation and tau protein misfolding, deleterious side effects of drug candidates, and limited clinical trial design, recent conventional drug development targeting Alzheimer's have been unable to produce effective therapeutic agents. For identification and to address early pathogenic abnormalities, there is a crucial need to develop novel molecular targets, biomarkers, and diagnostic procedures, as well as non-pharmacological alternatives [5].

Pathophysiology of Alzheimer's disease

Alzheimer's disease (AD) stands out as a leading neurological disorder globally. It can be categorized into various stages according to the degree of cognitive decline and the level of disability that individuals experience. Alzheimer's is a multifactorial disease linked to a variety of known risk factors such as age, obesity, diabetes, family history of dementia, head injury and so on [6]. It is caused by the accumulation of abnormal of neuritic plaque, neurofibrillary tangles and the degeneration of cortical neurons. Oxidative stress, being a major contributing factor in AD pathogenesis, plays a huge role in the onset and advancement of said pathological changes. It leads to an imbalance between antioxidants and oxidants, which leads to excessive free radical production. Free radicals, particularly reactive oxygen species (ROS) are produced by reducing molecular oxygen into superoxide radicals, which further produce hydrogen peroxide. Progressive reduction of hydrogen peroxide forms highly reactive hydroxyl radicals that interact with lipids, proteins, and nucleic acids, causing structural and functional damage. The brain's high oxygen demand and high lipid content make it particularly more vulnerable to oxidative injury [7].

This oxidative stress induced changes contribute to the Altered amyloid precursor protein (APP) which leads to the generation of an amyloid β peptide(Aβ). Usually, APP

is cut by enzymes α-, β-, and γ-secretase. In normal individuals, first the amyloid precursor protein is cleaved by α and then by γ-secretase. Cleavage by α-secretase reduces the generation risk of an amyloid β peptide(Aβ). Patients with AD, the β-secretase enzyme act and cleave the APP into Aβ42. The elevated level of Aβ42 promotes the generation of oligomers, which have neurotoxic potential. These oligomers form clusters around the meningeal, cerebral clusters and gray matter. These deposits lead to the formation of miliary structures which are known as Plaques. Concerning neurofibrillary tangles, they are thread-like structures residing within neurons, made up of a protein called Tau. Medically, the key role of tau is to stabilize the microtubules [8].

Microtubules extend along the axons of neurons and play a crucial role in transporting materials within the cell. Tau protein also helps in maintaining the integrity of microtubules [6]. Tau protein is physiologically attached to microtubules and has a particular quantity of phosphate molecules bonded to it. The precise cause of the aberrant tau phosphorylation mechanism in AD patients is unknown. This change results in an excessive increase in phosphorylation, which causes Tau molecules to detach from the microtubules. Hyper-phosphorylated Tau proteins, once detached, tend to aggregate into filamentous structures. These filamentous are called paired helical filaments. These filaments then cluster together, forming insoluble neurofibrillary tangles [8].

They first develop in the trans entorhinal cortex, then move to the hippocampus, and then in the later stages spread to the cerebral cortex [8]. These NFTS causes abnormal communication between the neurons and signal processing and ultimately, this leads to apoptosis in neurons [9].

Another characteristic feature in Alzheimer's disease is granulovacuolar degeneration that is observed in the pyramidal cells of the hippocampus. This degeneration is believed to be associated with cognitive decline (memory formation, learning ability, spatial navigation). Changes in cognitive processes are related to a reduction in presynaptic terminals from pyramidal cells located in cortical layers III and IV. The reduction in synaptic terminals is likely connected to vascular degeneration. In fact, the risk of dementia becomes higher by four times with the presence of subcortical lesions. Additionally, cerebrovascular disease can aggravate both the severity of dementia and the speed at which it progresses. However, the exact underlying mechanisms are still not fully understood [8], as explained in Figure 1.

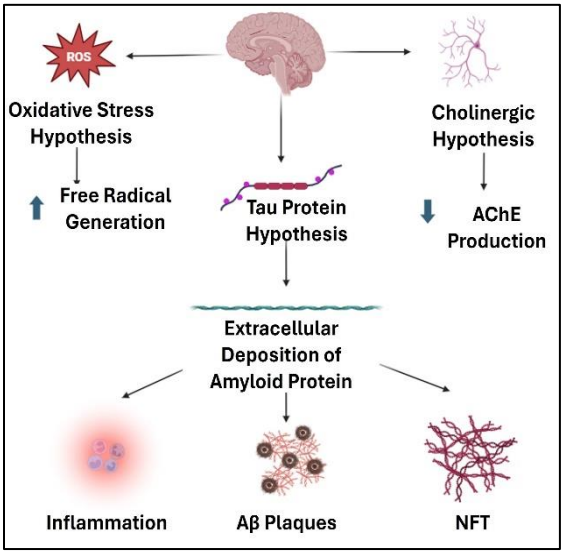

Figure 1: Pathophysiology of Alzheimer's disease.

The potential health benefits of phytochemicals

Their anti-inflammatory, neuroprotective, and antioxidant qualities are recognized. These substances, which include sulfides, terpenoids, flavonoids, and polyphenols, are essential for shielding plants from environmental stressors and pathogens. Phytochemicals have gathered interest in the setting of AD because of their ability to affect the activity of different cell types and

cytokines implicated responses to inflammation, hence regulating distinct biological pathways linked to neuro inflammation. Additionally, they influence signaling pathways that support neuronal survival, which helps them to perform a neuroprotective role. By avoiding and slowing the development of neuro inflammatory disorders, this regulation can offer protection against inflammation-mediated neurodegeneration. Phyto-chemicals modify the normal flora of gut, improve the integrity of intestines, and reduce the synthesis of harmful metabolic compounds and factors that induce inflammation. Targeting the resident microbes present in gut may be a therapeutic strategy for treating neuro inflammation and slowing the course of AD, given the function that GBA plays in bridging gut health and neurodegenerative diseases [10].

Effects of phytochemicals attenuating Alzheimer's disease

Flavonoids

Food plants contain many useful compounds, one of which is Quercetin, an antioxidant, and a flavonoid by structure [11]. It is a Diphenyl propane, having 15 Carbon atoms. It has two benzene rings and one heterocyclic pyran ring. Distinct variations exist among individual compounds just by substituting benzene rings of flavonoid structures [11 - 13]. An OH group may be found in quercetin at positions 3, 5, 7, 3′, and 4′ [11]. The plant groups Compositae, Passiflorae, Rhamnaceae, and Solanaceae are high in quercetin [11, 14]. Berries, apples, capers, red leaf lettuce, onions, asparagus, and strawberries all have comparatively high quercetin contents [15].

Quercetin

Many in vitro studies have shown that quercetin has a significant role in inhibiting beta-secretase-1 (BACE-1) enzyme, by forming hydrogen bonds. According to the manufacturer's instructions, BACE-1 test kit was used to perform the study. Each flavonoid's final measurement values were 0.01–0.03, 1.0–3.0, 5.0, 10.0–20.0, 30.0, and 50.0 µM. this equation determines the inhibition ratio : suppression (%) = [1−{(S−S0)/(C−C0)}] × 100, C being the control's (enzyme, assay buffer, and substrate) fluorescence after 150 minutes (about 5 hours) of incubation, C0 was the control's fluorescence, S was the florescence of flavonoid solution (enzyme, flavonoid solution, and substrate) about 5 hours after the incubation, and S0 was the fluorescence of tested sample . By adding the sample solution to reaction mixture C, the flavonoids' quenching effect was tested. Then the sample was examined and IC50 values were determined by the Graph Pad Prism [16].

The study used different doses of flavonoids to assess their inhibitory effects on the BACE-1 enzyme. The findings demonstrated that a higher quantity of flavonoids resulted in a higher inhibition of BACE-1 activity. GraphPad Prism served as an indicator of the effectiveness of each flavonoid as an enzyme inhibitor. Higher efficacy is indicated by lower IC50 values. These results imply that flavonoids may be useful as powerful BACE-1 inhibitors, which may be important for future studies regarding Alzheimer's disease. Inhibitory concentrations were given as follows in Table 1 [16].

Table 1: Inhibitory concentrations of quercetin used.

S/No	Compound	Concentration
1.	Quercetin	5.4 µM
2.	Kaempherol	14.7 µM
3.	Morin	21.7 µM
4.	Apigenin	38.5 µM

Quercetin also exerts neuroprotective effects, as many in vivo studies have supported. By protecting neuronal cells from oxidative stress [11, 15], it has been directly linked to improving memory, learning, and cognitive function [17] in vivo studies in mice have supported that it influences spatial memory tasks, by increasing the activity of AMPK [11, 18, 19].

This in vivo study, using an animal model with Alzheimer's disease, involves testing if taking quercetin has any effect on memory loss prevention, mitochondrial dysfunction and amyloid-related neuro-degeneration [20, 18]. Five groups of mice were created: one for minimal-dose quercetin (20 mg/kg/day), another for increased-dose quercetin (40 mg/kg/day), a group treated with donepezil(2 mg/kg/day), a group for transgenic control (no medication), and a group for wild-type control. There were the same number of men and women in each group. Before tissue collection and examinations of cognitive function, the therapies were given for 16 weeks [18].

The Novel Object Recognition (NOR) test was conducted to assess cognitive abilities. Three stages comprise of the test: training, habituation, and retention. One known object was swapped for a new one during the retention phase, and the recognition index (the amount of time spent investigating) was calculated. The brains of half of the mice were removed for the sake of behavioral testing. Sections of brain tissue were taken for histological examination. By using Western Blot Analysis, the amounts of total AMPK and phosphorylated energy-sensing kinase (p-AMPK) in brain tissue were measured. As loading control, protein levels were standardized to GAPDH.

AMPK a proline-directed serine/threonine kinase, is closely associated with Ab and mitochondrial function in AD. The activation of AMPK is indicated by the presence of phosphorylated AMPK Thr172 (pAMPK172). To evaluate its activation, Western blot analysis measures the levels of this marker. Quercetin treatment resulted in a 53.1% increase in pAMPK172 levels compared to control APPswe/PS1dE9 mice, whereas donepezil treatment did not influence its activity [18].

Alkaloids

Alkaloids have many known benefits to health, as they have many therapeutic properties like activity against inflammation, free radicals, and micro-organisms. It is an antinociceptive and antitumoral agent [21]. Even though it has all these positive effects, it is still not marketed as an active agent in drugs. The FDA has given its approval to Galantamine, one of the three anticholinesterase drugs, for treating AD, Galantamine is an alkaloid derived from Amaryllidaceae family [22]. It is a tetracycle with three asymmetric centers and a quaternary benzylic carbon and belongs to the isoquinoline alkaloid family [23 - 25].

Galantamine

In vitro studies have demonstrated that Amaryllidaceae alkaloids, particularly those from the galantamine and lycorine groups, exhibit significant acetyl-cholinesterase (AChE) inhibitory activity. Structural features, like having quaternary nitrogen atoms and hydroxyl groups, appear to enhance binding affinity and inhibitory potency. For instance, galantamine and its derivatives, including hydroxy galantamine, showed potent AChE inhibition, attributed to additional hydrogen bonding. In contrast, isomers like chlidanthine, with altered stereochemistry, displayed reduced activity. Lycorine-type alkaloids such as assoanine and ungeremine also exhibited strong AChE inhibition due to their aromatic rings and quaternary structures. Alkaloids with other skeleton

types, however, showed much weaker cholinesterase inhibition, highlighting the critical role of structural characteristics in determining efficacy [26].

The study demonstrated that a number of alkaloids, including galantamine, hydroxy galantamine, and ungeremine, efficiently inhibited AChE activity, by observing variable ranges of IC50 values. These results are consistent with the theory that molecular structures, such as the existence of aromatic rings and a quaternary nitrogen atom, significantly increase inhibitory efficacy. Because of stereochemical differences, the galantamine isomer chloridanthine was significantly less powerful, which validated the study's findings. Overall, the study's findings validate the validity of the findings by agreeing with hypothesized association between structural characteristics and AChE inhibition [26]. Table 2 presents the study's outcomes.

This study used diseased APdE9 mouse experimental model to assess the activity of galantamine in treating cognitive function and oxidative stress. Many tests like novel object recognition (NOR) and Morris water maze (MWM) were performed on mice of both sexes as a part of behavioral studies, but biochemical analysis and EPR imaging was only done on female mice. Oral dose of galantamine 5mg/kg was administered to APdE9 mice from either 3 months (pre-plaque phase) or 6 months until 9 months of life. Assessment of behavioral outcomes were performed after drug discontinuation, followed by EPR imaging to visualize oxidative stress levels in the brain. After completing the tests, their brain tissues were collected for immunohistochemical and biochemical analyses to assess Aβ plaque accumulation and inflammation markers [27].

A substantial decrease in platform latency and an increase in spatial bias in the probing test indicate that galantamine administration enhanced cognitive function in the APdE9 mice, especially in the MWM test. Mice administered with galantamine had improved identification of unfamiliar items in the NOR test, indicating improved memory retention. Mice treated with galantamine had lower levels of oxidative stress in their brains, especially in the cortex, according to EPR imaging. Galantamine therapy preserved synaptic integrity as seen by synaptophysin staining, but it also decreased Aβ plaque formation and inflammatory markers, including Iba1 and GFAP, according to immuno-histochemical research. These results imply that in this Alzheimer's disease model, galantamine reduces neuroinflammation, oxidative stress, and cognitive deterioration [27].

Table 2: Study's outcomes on Acetylcholinesterase (AChE) inhibitors activity.

Alkaloid	**Type**	**IC50 (µM)**	**Key Structural Features**	**Comments**
Galantamine	Galantamine	9.60	Hydroxyl group	Potent AChE inhibitor due to hydrogen bonding capabilities.
11a Hydroxy-galantamine	Galantamine	0.10	Additional hydroxyl group	More potent than galantamine due to stronger binding.
Ungeremine	Lycorine	0.35	Quaternary nitrogen atom, aromatic ring	Very potent inhibitor, similar to other isoquinoline alkaloids.

Naringin

Many citrus fruits but mostly grapefruit contains naringin, which is a flavonoid glycoside having many health advantages. It is made up of the disaccharide neohesperidose and contains many properties like antioxidant, anti-inflammatory, and anti-cancer, which reduces the chances of having chronic diseases. Naringin contains the backbone of a flavonoid with two rings of phenol and a heterocyclic pyran ring. The molar mass of naringin is approximately 580g/mole, and its chemical formula is C27H32O14 [28]. Many in vitro studies explore the activity of Naringin in treating Alzheimer's disease by formulating a Naringin nano-suspension (NNS) using biodegradable, FDA-approved PLGA polymer. They were synthesized by the precipitation method. The organic phase (PLGA and naringin in dimethyl sulfoxide) was added dropwise to the aqueous phase (PVA and Polysorbate 80 in water) under probe sonication, followed by high-pressure homogenization [29].

To assess the in vitro drug release, the study used a dialysis-based separation technique in phosphate buffer (pH 7.4) at 37°C, 50 rpm over 10 hours. Samples were withdrawn periodically and analyzed at 284 nm to determine cumulative drug release. Characterization of nanoparticles for particle size, polydispersity index (PDI), entrapment efficiency, release rate of drug, X-ray diffraction (XRD), and differential scanning calorimetry (DSC) was done. After confirming the desired physicochemical properties, the formulation underwent preclinical evaluation in rats with amnesia, which was induced by scopolamine. It is a broad-spectrum cholinergic antagonist and is known for disrupting the performance of spatial memory, mimicking AD-like cognitive impairment. The study assessed Naringin's impact on spatial learning, ROS, and inflammation of brain in SCP-treated mice, providing insights into its neuroprotective potential. This study highlighted the therapeutic promise of Naringin nano-formulations for AD management, emphasizing its ability to improve drug stability, bioavailability, and CNS targeting [29].

The investigation of the effects of naringin in an APPswe/PS1dE9 transgenic diseased mouse model was done through an in vivo study. Both male and female mice aged three months were randomly chosen to go for different treatment groups and fed a diet containing minimal-dose naringin (50 mg/kg/day), increased-dose naringin (100 mg/kg/day), donepezil (2 mg/kg/day), or without any agent for 16 weeks. Wild-type littermates were included as controls. Donepezil, an inhibitor of acetylcho-linesterase used for AD treatment, was administered at a dose equivalent to human therapeutic levels. Different studies were performed, and at the end results were in favor of naringin. This study demonstrates the role of naringin in the improvement of long-term memory in an AD transgenic animal model. The increase in the activity of CaMKII seems to be a central mechanism responsible for its cognitive benefits [30].

Terpenoids

Ginkgo biloba leaves EGb761 contain 24% flavonoid glycosides (which contain quercetin, kaempferol and isorhamnetin), 6% terpenoids (3.1% are ginkgolides A, B, C, and J, and 2.9% is bilobalide), and 5–10% organic acids [31]. The active constituents are flavonoids and terpenoids. Ginkgo biloba is believed to be a neuroprotective agent due to its ability to produce anti-oxidant, free radical scavenging, and membrane-stabilizing effects. It also inhibits platelet-activating factor via the terpene ginkgolide B. Other pharmacologic effects are blood vessel relaxation mediated by inhibition of 3',5'-cyclic guanosine monophosphate phospho-

diesterase; inhibition of age-related decline of certain brain receptors, i.e, muscarinic and alpha adrenergic; and stimulation of choline uptake in the hippocampus to support memory. Ginkgo also shows inhibition of beta-amyloid deposition.

In a previous study, we observed that primary cultures of hepatocytes from rats treated with EGb761 (50 mg/kg/day) for 8 days showed reduced cell viability and apoptosis when subjected to 2,2-azobis 2 amidinopropane (AAPH), a peroxyl radical generator, compared to hepatocytes from control rats. This suggests that EGb 761 may have induced changes in liver cells, making them more resistant to the damaging effects of oxidants. Based on these findings, the effects of EGb 761 were investigated on the viability and apoptosis of primary cultures of hippocampal nerve cells from rats treated with a dose of EGb 761 known to protect the hippocampus against ischemia [32]. Additionally, we tested ginkgolide B and bilobalide at concentrations or doses relative to their respective percentages in EGb 761 and their bioavailability was confirmed as pure substances after oral administration of the total extract in rat plasma [33].

Regarding the cells treated with test substances, they were sorted into four different population 24 hours after the preparation of the cultures and then incubated for 24 hours using the culture medium, containing either EGb 761 (0.5–50 µg/ml), ginkgolide B (0.1–1.0 µg/ml), or bilobalide (0.1–1.0 µg/ml). After the treatment, the cells were rinsed with fresh culture medium and then exposed to a 2-hour incubation in either AAPH-free culture medium or in medium containing AAPH (20 or 50 mM). Subsequently, the medium condition was changed & the cells were readied for assays of cell viability and apoptosis.

AAPH reduced cell viability, while EGb 761 improved it at concentrations of 5–20 µg/ml. Ginkgolide B also enhanced cell viability at lower doses and blocked glutamate-induced cell death, supporting neural stem cell growth in rats with cerebral ischemia. Bilobalide had no effect on cell viability except at high concentrations.

EGb 761 and ginkgolide B both were protected against AAPH-induced cell death, while bilobalide did not [34]. However, bilobalide promotes neuro-genesis in the hippocampus and increases alpha secretase activity in processing amyloid precursor protein, reducing beta-amyloid production. It also improves cognitive function in AD models, making it a potential neuroprotective agent.

Mitochondrial function, assessed through cytochrome c oxidase (COX) activity, mitochondrial ATP levels, and glutathione (GSH) content, was found to decline with age. In SAMP8 (one of the nine substrains of SAMP) mice, a model of accelerated aging, mitochondrial function in platelets and hippocampi was tested alongside SAMR1 1 (one of the three substrains of SAMR) mice, a normal aging control. Results showed that mitochondrial function deteriorates earlier in SAMP8 compared to SAMR1. Additionally, six-month-old SAMP8 mice exhibited relative insensitivity to beta-amyloid protein, the key component of amyloid plaques found in Alzheimer's disease brains [35].

EGb761 was administered orally to SAMP8 mice at 3 and 24 weeks (about 5 and a half months) for 12-week duration to study tests the preventive and restorative effects on age-related mitochondrial dysfunction. Moreover, the in vivo pharmacologic effect of ginko through comparison of effects of EGb761 on platelets and hippocampi was studied.

Mice were divided into two groups i.e control (fed with 4% sucrose) and EGb761 treated group (dose of 100mg/kg body weight). Mice blood (0.8-1.5ml) was

collected by cardiac puncture and centrifuged. Platelet mitochondria were isolated and suspended in a hypotonic medium (10 mM Tris–Cl, pH 7.6) and centrifuged again. The brain was removed and hippocampi were quickly dissected on a cold plate at 20C. Half of the mitochondrial plates were used to measure mitochondrial GSF content and other half were used for measuring COX activity and ATP content [36]. Table 3 summarizes the key findings.

EGb761 demonstrated protective effects in both young and old mice, proposing it may have a peripheral role in combating age-related degeneration in Alzheimer's disease patients. However, in the hippocampus, these protective effects were only observed in older mice, possibly due to age-related increases in blood-brain barrier (BBB) permeability. BBB permeability rises with age in humans and becomes even more pronounced in neurodegenerative conditions like AD compared to normal aging. As the BBB becomes more permeable with age, EGb761 may have better brain penetration, enhancing its effects on the central nervous system.

Phenylethanoid glycoside

Phenylethanoid glycoside is a constituent of H. Cistanche. It is composed of five major components among which acteoside and echinacoside has proved to have a neuroprotective role in Alzheimer disease. It improves the impairment of neuronal cell death caused by Aβ25–35 via its antioxidant effects [37]. Aβ the major constituent of senile plaques promote the aggregation of reactive oxygen species which leads to protein and lipid oxidation and eventually DNA damage. Increased levels of H2O2 may lead to increased oxidation and trigger apoptosis of PC12 cells in AD patients. Studies have proved that acteoside and echinacoside improve cognitive decline caused by Aβ1–42 [38].

Table 1: Summary of the findings of the in vivo study.

Parameter	**Effect of Age (SAMP8 Mice)**	**Effect of 12-Week EGb761 Treatment**
COX Activity in Platelet and hippocampi of mice	Decrease with age	**Young Mice:** Completely prevented decrease. **Old Mice:** Rescued decrease.
Mitochondrial ATP content in both (platelet and hippocampi)	Gradual decrease, significant after 40 weeks	**Young Mice:** No significant effect. **Old Mice:** Protected against decrease.
Mitochondrial GSH Content in Platelets	Decreased with age	**Young Mice:** Completely prevented reduces. **Old Mice:** Rescued decrease.
Mitochondrial GSH Content in hippocampi	Decrease with age	**Young Mice:** Failed to prevent decrease. **Old Mice:** Rescued decrease.

The best conditions for initiating the AD model were determined by testing various incubation intervals (24, 48, 72 or 96 h) and concentrations (0, 0.5, 5, 25 or 50 µg/ml). PC12 cells were treated to 0.5µM Aβ1-42 and hydrogen peroxide in the presence of phenylethanoid glycosides for 24 hours. Following the treatment cell viability was assessed using an MTT assay, and levels of lactate dehydrogenase release and malondialdehyde content were also assessed [39]. The most established optimal conditions for AD model involved treating PC12 cells with 0.5 µM Aβ1-42 for 48 hours or with 25 µM H2O2 dissolved in DMEM (Dulbecco's modified Eagle's medium) with PBS.

This study successfully developed an in vitro Alzheimer's disease (AD) model using Aβ1–42 and H2O2 to induce injury in PC12 cells. PhGs (phenylethanoid glycosides) demonstrated significant neuroprotective effects by increasing cell viability and reducing the release of lactate dehydrogenase (LDH) and malondi-aldehyde (MDA), markers of oxidative stress and cell damage. The results indicate that PhGs at 75, 100, 125, 150, 175 and 200 µg/ml significantly inhibited P12 cell growth ($P<0.05$, $P<0.01$), while cell viability remained above 80% at concentrations of 5, 25 and 50 µg/ml. The safe doses of PhGs (5, 25, and 50 µg/ml) were identified as shown in Figure 2. It showed nontoxic and no adverse effects on cell growth. The study concluded that PhGs offer strong protection against Aβ1–42- and H2O2-induced damage in PC12 cells, highlighting their therapeutic potential for AD [39].

Apigenin

Apigenin is a 4′,5,7-trihydroxyflavone, based on the skeleton of 2-phenylchromen-4-one (2-phenyl-1-benzopyran-4-one). In plants, apigenin is present in the form of aglycone and its C- and O-glycosides which include 6C and 8C glycoside, and 7O glycoside and glucuronides. One theory regarding the formation of apigenin suggests that free apigenin results from a degradation process occurring after harvest. Apigenin is last class of polyphenols [40]. In vivo studies of apigenin were observed on APP/PSI double transgenic mice. For this purpose, 4-month-old mice and littermate WT were divided into 4 females and 5 males' group. Apigenin was prepared by dissolving in distilled water containing 5% sodium carboxyl methyl cellulose (CMC-Na) at a concentration of 10 mg/mL. A dosage of 40mg/kg apigenin was administered and treatment was sustained for a period of 12 weeks. The study evaluated the impact of apigenin on oxidative enzyme, levels of neurotrophic molecule, Aβ burden and APP processing. The finding indicated that apigenin improve learning and memory. Additionally, it restored the ERK/ CREB/BDNF pathway in the cerebral cortex of APP/PS1 mice. Consequently, apigenin may serve as an alternative treatment for preventing Alzheimer's disease [41].

Invitro studies has shown a human induced pluripotent stem cell (iPSC) model of familial and sporadic AD, alongside healthy controls, was used to evaluate apigenin's neuroprotective potential. Results showed that iPSC-derived AD neurons exhibited increased calcium signaling, higher nitrite levels, increased cell death, reduced neurite length, and greater vulnerability to inflammation from activated murine microglia compared to controls. Apigenin demonstrated significant anti-inflammatory effects, protecting neurites and cell viability by decreasing cytokines and nitric oxide release. Furthermore, apigenin reduced spontaneous calcium signals and significantly decreased caspase-3/7 mediated apoptosis, underscoring its wide-ranging neuroprotective impact against AD pathology in a human model [41].

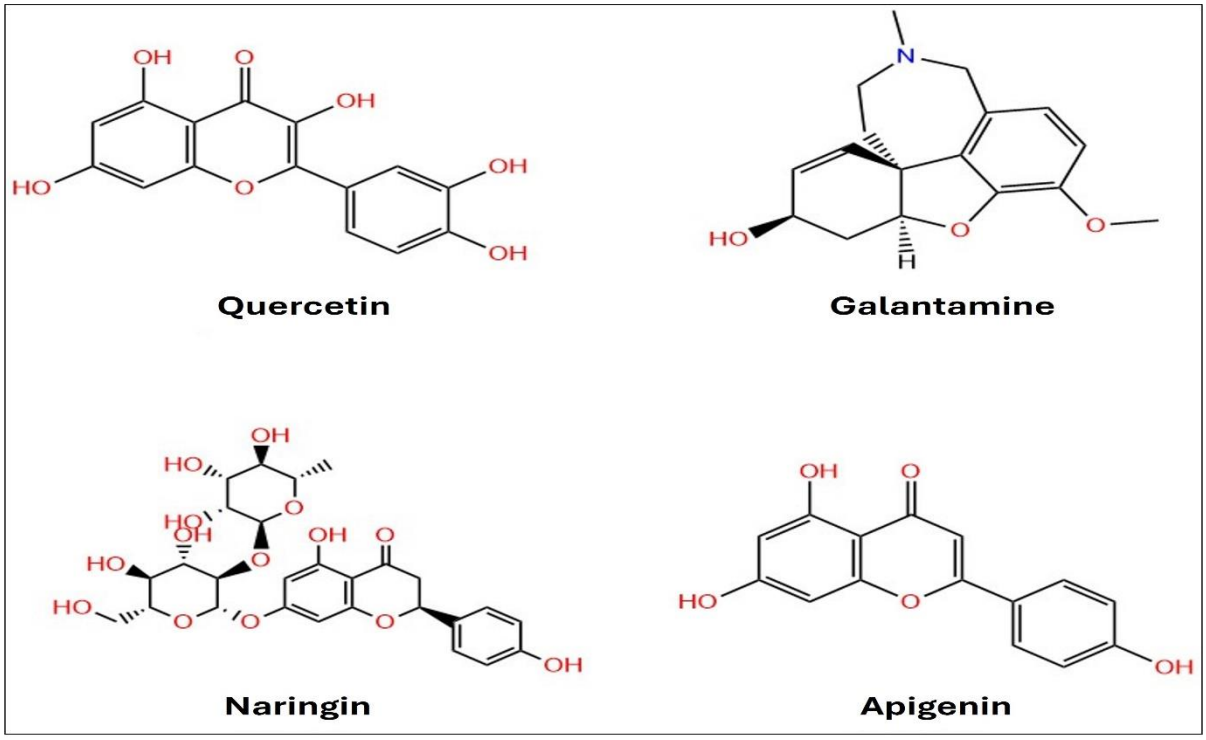

Figure 2: Structures of phytochemicals known for attenuating AD.

Opportunities and challenges in employing phytochemical therapy for Alzheimer's disease

The first and foremost challenge in translating phytochemicals into pharmaceuticals is determining and extrapolating the dose needed for humans to produce a therapeutic effect from the in-vivo and in-vitro therapeutic dose [42].

Secondly, Phytochemicals face many challenges in being bioavailable due to factors such as metabolism in gut by gut microflora, instability in colonic and gastric pH, absorption past the intestine wall, active efflux system, and first-pass metabolism [43]. However, if we introduce the phytochemical as a co-delivery system with an agent which can modulate the glucuronidation activity or hinder the clearance mechanism by CYP450, it's bioavailability could be significantly increased [43]. Nano therapy could also be employed to deliver these phytochemicals to their targets but unfortunately, they lack potential in clinical settings. Due to their oral administration route, these face challenges due to physiological elements such as degradation by enzymes, pH, target specificity, and adverse effects observed when high doses are administered [44]. Nevertheless, these challenges can be overcome by employing some modern techniques such as the developing 1-1000 nm sized nanostructured materials, such as nano-structured lipid-based carriers, liposomes, PLGA nanoparticles, micelles, emulsions, and solid lipid nanoparticles etc. for delivering the phytochemicals [44]. These nanosized particles can be modified as required. In particular, surface properties can be enhanced to determine the hydrophilicity, hydrophobicity, and physiological responses produced by the Nanoparticles, including immunologic responses, plasma protein interactions, cellular uptake, and their elimination [45]. To overcome the challenges of nano-delivery systems, lipid nanoparticles have been specially designed because they show good release patterns and greater stability [46]. Other techniques can also be employed, such as loading Phyto-bioactive compounds into nano-delivery devices, as it has been confirmed that they have the

ability to overcome inflammation and oxidative damage, that are known to be the main factors in neurological disorders [47].

Since delivering therapeutic drugs to the brain is challenging because of the blood-brain barrier, using nanocomposites which can cross and enter the BBB seems to be the solution for targeted delivery of CNS therapeutics [48]. Alzheimer's is a complex disorder defined by memory impairment, dementia, and cognitive dysfunction. It progresses due to abnormal deposits of Aβ peptides, extracellular plaques, intra-neurofibrillary and inter-neurofibrillary tangles consisting of tau proteins, and progressive neuronal loss. These pathological changes impair neuron communication, leading to dementia progression and cognitive impairment. This article covers various in-vitro and in-vivo investigations that have set forth the significant use of quercetin, a flavonoid, and galantamine, an Amaryllidaceae alkaloid, as phytochemicals with therapeutic uses in Alzheimer's disease due to their neuroprotective properties. They inhibit BACE-1 enzyme activity, acetylcholinesterase (AChE), and increase AMPK activity, which leads to a reduction in oxidative stress and neuroinflammation. Similarly, ginkgo biloba, a free radical scavenger and mitochondrial dysfunction in platelets, shows reduced cell viability and apoptosis when exposed to APPH in hepatocytes from rats and hippocampus in young and old mice. They have a role in combating age-related AD as BBB becomes more permeable with age. Phenylethanoid glycoside a constituent of Herba Cistanches improves the neuronal apoptosis impairment due to Aβ25–35 with its antioxidant properties in Alzheimer's disease. These therapeutic agents, hence, alleviate AD symptoms and improve cognitive function. Lastly, encapsulating these therapeutic agents in the form of nanocomposites proves to be the future of delivering drugs to the brain, as it can bypass the blood-brain barrier, produce therapeutic effects, and accommodate targeted therapy for Alzheimer's Disease.

Conflict of interest

The authors declare that they have no competing interests.

Data availability

All the raw data related to this study is available with the authors.

Author's contribution

The study was conceptualized by MU, literature was searched by MU, WI, JS, MA and EF. Graphics were made MU & JS. Initial draft was written by MU, WI, JS, MA and EF. Final draft was written by MU and MA.

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