

Mechanisms of Action of Phytochemicals from Medicinal Herbs in the Treatment of Alzheimer's Disease

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Key words

- Alzheimer's disease
- phytochemical
- medicinal herbs
- drug development

Abstract

Alzheimer's disease is a chronic neurodegenerative disorder characterized by progressive dementia and deterioration of cognitive function. Although several drugs currently used for the treatment of Alzheimer's disease delay its onset and slow its progression, still there is no drug with profound disease-modifying effects. Studies aiming the treatment of this neurodegenerative disorder explore various disease mechanisms. Since antiquity, medicinal herbs have been used in traditional medicine. Recent studies suggest that the neurobiological effects of phytochemicals from medicinal herbs may contribute to clinical benefits in *in vitro* and *in vivo* models of Alzheimer's disease. This review focuses on five phytochemicals, berberine, curcumin, ginsenoside Rg1, puerarin, and silibinin, which have been mostly investigated to treat the development and progression of this neurodegenerative disorder.

Abbreviations

AChE:	acetylcholinesterase
AD:	Alzheimer's disease
Akt:	protein kinase B
A β :	β -amyloid
APP:	amyloid precursor protein
BACE:	β -secretase or beta-site amyloid precursor protein cleaving enzyme
Bax:	Bcl-2-associated X protein
Bcl-2:	B-cell leukemia protein
BDNF:	brain-derived neurotrophic factor
CAT:	catalase
Cox:	cyclooxygenase
CTF:	C terminal fragment
Cyt c:	cytochrome c

ELISA:	enzyme-linked immunosorbent assay
FDA:	Food and Drug Administration
γ -GCS:	γ -glutamylcysteine
GFAP:	glial fibrillary acidic protein
GRg1:	ginsenoside Rg1
GSH-Px:	glutathione peroxidase
GSK3 β :	glycogen synthase kinase
Iba-1:	ionized calcium binding adaptor molecule-1
IL:	interleukin
i-Nos:	inducible nitric oxide synthase
JNK:	c-Jun N-terminal kinase
LDH:	lactate dehydrogenase
MAP-2:	microtubule-associated protein (MAP)-2
MAPK:	mitogen-activated protein kinase signaling
MCP-1:	monocyte chemoattractant protein-1
MDA:	malondialdehyde
MWM task:	Morris water maze task
NF- κ B:	nuclear factor- κ B
NICE:	National Institute for Health and Clinical Excellence
NO:	nitric oxide
p-ERK1/2:	extracellular signal-regulated kinase 1 and 2
PI3K:	phosphoinositide 3-kinase
PS1:	presenilin 1
PTEN:	phosphatase and tensin homolog
RAWM:	radial arm water maze
RNS:	reactive nitrogen species
ROS:	reactive oxygen species
SYN:	synaptophysin
TEM:	transmission electron microscopy
ThT:	thioflavin T
(TNF)- α :	tumor necrosis factor- α
TUNEL:	terminal deoxynucleotidyl transferase dUTP nick end labeling

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Introduction

AD, a progressive irreversible neurodegenerative disorder characterized by the loss of certain cognitive functions, behavioral disturbances, and daily living difficulties, is the most common form of dementia found among elderly individuals [1]. This disease currently affects 27 million people all over the world [2]. The prevalence rate for AD has become the third greatest threat to the elderly, only behind cardiovascular disease and cancer [3]. Although neither a consensus concerning the pathogenesis of AD nor a perfect therapy for its treatment is available, it is now well accepted that multiple factors, including apoptosis, oxidative stress, excitotoxicity, and the disturbance of energy metabolism homeostasis, contribute to the progression of AD [4]. Especially, the aggregation and accumulation of extracellular and intracellular $A\beta$ play a critical role in the pathogenesis of AD and result in impaired synaptic plasticity and memory [5]. As a key rate-limiting enzyme, α -, β -, and γ -site amyloid precursor protein cleaving enzyme (α -, β -, and γ -secretase) initiates the formation of $A\beta$ by producing the peptide from APP [6]. APP can be cleaved in two ways, with formation of non-toxic $A\beta_{17-42}$ by α - and γ -secretases and the generation of toxic $A\beta_{1-42}$ by β - and γ -secretases [7]. Therefore, inhibiting the activity of β -secretases is important for treating AD due to their role in the direct cleavage of the $A\beta$ domain at the N-terminus in APP [8]. These changes are closely associated with tau phosphorylation and PI3K/Akt/GSK3 β signaling pathway along with MAPK signaling. The Akt-mediated control of GSK3 β activity is involved in tau hyperphosphorylation [9]. $A\beta$ generated by the cleavage of APP triggers immune responses and activates microglial cells that engulf $A\beta$ filaments, with secretion of various pro-inflammatory factors [9, 10]. In addition, neuron-related apoptosis and oxidative stress lead to neuronal damage [11].

Numerous *in vitro* and *in vivo* models have been used for studying AD. Various types of cells were stimulated with neurodegenerative exposures such as to subtypes of $A\beta$ and acrolein. $A\beta$ deposition causes synaptic plasticity and memory impairment and has been detected in AD patients at early stage [12]. Acrolein is not only a marker of lipid peroxidation but also an inducer of oxidative stress and an effector of tissue damage [13]. With growing evidence of the implication of acrolein in AD, the development of strategies to reduce its toxic effect is of great importance [12].

Cognitive impairments in animal models are analyzed with behavioral tests, such as MWM task and Y-maze, RAWM, novel object recognition as well as cued and contextual fear-conditioning tests, which are known to be sensitive to hippocampal-dependent learning and memory deficits. Specifically, TgCRND8 mice expressing human APP695 with the Swedish (K670N/M671L) and Indiana (V717F) mutations under regulatory control of the PrP gene promoter (heterozygous with respect to the transgene) on a C57BL/6 F3 background are used to breed the colony of experimental animals [14], while transgenic mouse overexpressing of APP/ $A\beta$ (Tg mAPP) is a well-used mouse model of AD [15, 16]. Despite great effort to discover a remedy for AD, no currently available drug can stop or cure this neurodegenerative disorder, and current treatments offer only small symptomatic benefits. The most commonly prescribed drugs are cholinesterase inhibitors such as donepezil, rivastigmine, and galantamine, and NMDA antagonists such as memantine. Though approved by the FDA, these drugs are not recommended by the NICE in England and Wales due to "limited and largely inconclusive" evidence

concerning their efficacy [17]. Nootropic agents such as piracetam are commonly prescribed in Europe for the treatment of dementia, including dementia associated with AD, but there are insufficient evidences to verify the efficacy of such drugs [18]. Historically, a number of medicinal herbs have been used to treat neurodegenerative diseases and cognitive disorders in traditional European, Ayurvedic, and Oriental medicines. Medicinal herbs consist primarily of multiple compounds and may influence multiple mechanisms with multi-target functions. This review concerns five phytochemicals, berberine, curcumin, GRg1, puerarin, and silibinin, that have been recently and mostly investigated as alternative treatments for AD and will discuss the advances in our knowledge about these phytochemicals derived from medicinal herbs based on available literature facts collected from books and scientific papers by electronic search (PubMed, ScienceDirect, and Google Scholar) (● Fig. 1).

Therapeutic Approaches to Alzheimer's Disease

Berberine from the cortex of *Coptis chinensis*

Berberine, an isoquinoline alkaloid, is one of the major components of Cortex Phellodendri and Rhizoma Coptidis (*Coptis chinensis*, Franch.; Ranunculaceae). Berberine has been used in herbal medicine for liver disease, skin inflammation, diarrhea, and other disorders due to its anti-diarrheal, anti-microbial, and anti-inflammatory effects [8, 19–21]. Specifically, several studies have reported that berberine possesses multiple pharmacological effects that culminate in neuroprotective action against cerebral ischemia, psychological depression, schizophrenia, anxiety, and AD [22, 23]. Asai et al. reported that berberine reduces extracellular $A\beta$ production and BACE activity without changes on release of LDH in H4 neuroglioma (APP_{NL}-H4) cells [8]. The production of both $A\beta$ and β -secretase are inhibited by berberine compared with the control in human embryonic kidney 293 (HEK293) cells. Berberine increased the expression level of p-ERK1/2, demonstrating that it may inhibit the production of $A\beta$ via an activation of ERK1/2-induced BACE activity in HEK293 [24].

In another study, the effect of berberine on $A\beta$ -induced neuroinflammation was examined in primary and BV2 microglial cells. Pretreatment with berberine reduces $A\beta$ -induced IL-6 production and MCP-1 release, as well as expression of Cox-2 and inducible i-Nos. Subsequently, NF- κ B and the phosphorylation of I κ B- α , Akt, p38 kinase, and ERK1/2, but not JNK, stimulated by $A\beta$, are regulated following treatment with berberine [25]. Moreover, pretreatment with berberine reduced $A\beta$ -stimulated activities of LDH and TNF- α with down-regulation of TNF receptor 1 in SK-N-SH neuroblastoma cells [26].

In APP transgenic mice, berberine treatment improved cognitive impairment as reflected in reductions in errors in the MWM task with respect to both conventional reference memory and memory retention (probe trial). $A\beta$ plaque immunostaining in TgCRND8 mice was associated with reductions in both the number and the area of coronal sections of the cortex and hippocampus following treatment with berberine. Moreover, consistent with the indication that berberine treatment reduces the accumulation of total $A\beta$ peptides in the brain, a significant reduction was found in the degree of microgliosis by Iba-1 burden and astrocytosis by GFAP burden. The mechanisms underlying the benefits of berberine on cognitive function and $A\beta$ neuropathology in TgCRND8 mice were expressed for CTF, APP, and tau phosphorylation. An inhibition of PI3K/Akt/GSK3 β activities in the TgCRND8 mouse brain

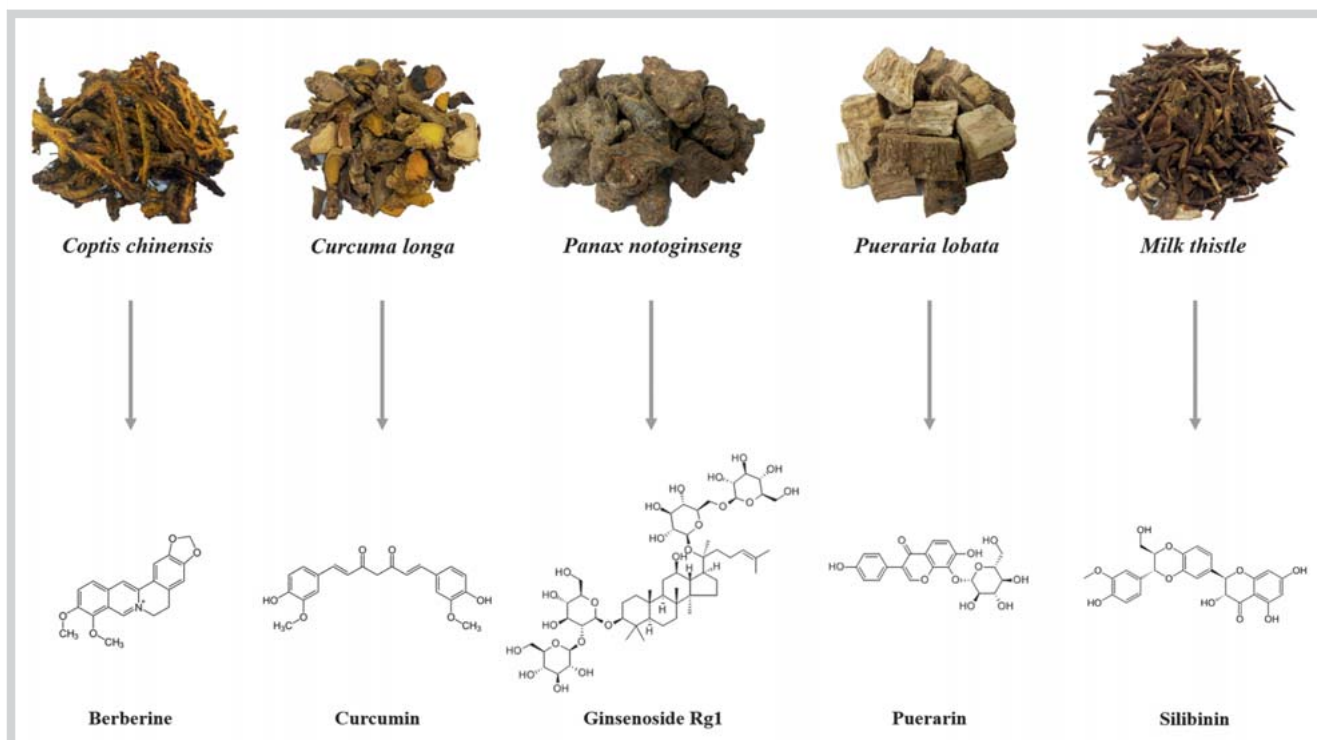


Fig. 1 Chemical structures of phytochemicals from medicinal herbs. (Color figure available online only.)

Table 1 Summary of findings on berberine as alternative treatment for Alzheimer's disease.

First author, year [ref]	Study design	Treatments	Results
Asai et al., 2006 [17]	APP _{NL} -H4 cells (H4 neuroglioma)	10, 20, 30, 40, 50, 100 μ M berberine for 48 h	\leftrightarrow release of LDH \downarrow β -secretase activity
Zhu et al., 2011 [23]	HEK293 cells (human embryonic kidney 293)	1, 5, 10, 20 μ M berberine for 24 h	\leftrightarrow cell viability \leftrightarrow release of LDH \downarrow extracellular A β _{40/42} levels \downarrow BACE activity \uparrow phosphorylation of ERK1/2
Jia et al., 2012 [24]	Murine primary cells and microglia cells and cultured BV2 cells (murine microglia)	Pretreatment of 1, 2.5, 5 μ M berberine for 30 min and 20 μ M A β ₂₅₋₃₅ for 24 h	\downarrow IL-6 production and MCP-1 release \downarrow Cox-2 and i-Nos expressions \downarrow NF- κ B \downarrow phosphorylation of I κ B- α , Akt, p38 kinase and ERK1/2 \leftrightarrow phosphorylation of JNK
Xu et al., 2013 [25]	SK-N-SH cell (neuroblastoma)	Pretreatment of 10 μ M berberine for 2 h and incubation of 5 μ M A β ₂₅₋₃₅ for 24 h	\downarrow LDH activity \downarrow level of TNF- α \downarrow TNFR1 gene and protein expressions
Durairajan et al., 2012 [26]	TgCRND8 mice	Orally administered by gavage once daily with a 25 mg/kg and 100 mg/kg dose of berberine for 6 months	\downarrow MWM task \downarrow number and area of A β plaque \downarrow Iba-1 and GFAP burden \downarrow CTF- α and - β expression \downarrow levels of p-APP \uparrow phosphorylation of Akt at Ser 473 and GSK3 β at Ser21 and Ser9

\uparrow Increase; \downarrow decrease; \leftrightarrow no difference

influenced CTF and p-APP levels and led to a blockage of A β accumulation [27] (Table 1).

Curcumin from *Curcuma longa*

Curcumin, a hydrophobic polyphenol, is isolated from the rhizome of the herb *Curcuma longa* L. (Zingiberaceae). It is one of the active components of turmeric involved in antioxidant, anti-inflammatory, metal chelators, anti-amyloid, anti-tau, and neu-

roprotective activities [13]. In addition, curcumin has been reported to bind to A β and prevent aggregation [28] and protect against cell death, as indicated by increased cell viability and decreased TUNEL-positive cells in rat primary neuron cells infected by adeno-5 virus packaged with iA β ₁₋₄₂. Curcumin treatment decreased ROS level and protected from intracellular A β toxicity [12]. It also inhibited the expression of ROS in acrolein-induced toxicity on SK-N-SH human neuroblastoma cell with increasing

Table 2 Summary of findings on curcumin as alternative treatment for Alzheimer's disease.

First author, year [ref]	Study design	Treatments	Results
Ye et al., 2012 [10]	Rat primary neurons from new born Sprague-Dawley rat hippocampus	Infection of by adeno-5 virus packaged with $iA\beta_{1-42}$ and treatment with 30 μ M curcumin for 24 h	<ul style="list-style-type: none"> ↑ LDH release ↓ TUNEL⁺ cells ↓ stained ROS levels
Doggui et al., 2013 [11]	SK-N-SH cell (neuroblastoma)	Cotreatment with 5, 10, or 20 μ M curcumin and acrolein for 24 h and 30 min	<ul style="list-style-type: none"> ↑ LDH release ↑ cell survival ↓ ROS levels ↓ γ-GCS expression ↓ RNS level ↔ GSH ↓ Nrf2 ↓ NF-κB ↓ Sirt1 ↑ phosphorylation of Akt
Huang et al., 2013 [28]	SH-SY5Y cell (human neuroblastoma)	Treatment with cultured with 1, 5, or 10 mM curcumin for 4 h and then continued incubating with 10 mM $A\beta_{1-40}$ for 24 h	<ul style="list-style-type: none"> ↓ tau phosphorylation ↑ phosphorylation of GSK3β at Ser9 ↑ phosphorylations of Akt at Thr308 and Ser473 ↓ up-regulation of PTEN
Xiong et al., 2011 [29]	pAPPswe-transfected SH-SY5Y cell	Treatment with curcumin at 0, 1.25, 5, or 20 μ M for 24 h, or at 5.0 μ M for 0, 12, 24, or 48 h	<ul style="list-style-type: none"> ↓ extracellular $A\beta_{40/42}$ levels ↓ PS1 mRNA and protein levels ↓ GSK3β mRNA and protein levels
Hoppe et al., 2013 [30]	Wistar rat	Intracerebroventricular injection of $A\beta_{1-42}$ and after 2 weeks, administration of 50 mg/kg curcumin intraperitoneally for 10 days	<ul style="list-style-type: none"> ↑ spontaneous alternation in the Y-maze ↑ novel object recognition memory task ↑ SYN levels ↓ tau phosphorylation ↓ TNF-α and IL-1β ↓ GFAP+ immunocent levels ↑ BDNF ↑ phosphorylations of Akt ↑ phosphorylation GSK3β

↑ Increase; ↓ decrease; ↔ no difference

LDH release. Lipid peroxidation and oxidative stress by acrolein were protected by curcumin treatment. In addition, the expressions of γ -GCS synthetase and RNS levels, except GSH, were restored by treatment of curcumin. Other oxidative damage marker expressions, such as those of Nrf2, NF- κ B, Sirt1, and Akt, were regulated under the presence of curcumin [13].

In other type of human neuroblastoma (SH-SY5Y cells), curcumin treatment decreased $A\beta$ -induced tau phosphorylation at Thr231 and Ser396. In addition, it increased the expression of GSK3 β and the phosphorylations of Akt at Thr308 and Ser473 sites and suppressed up-regulation of PTEN [29]. These results were supported by a previous report about the effect of curcumin on production of $A\beta$ in pAPPswe-transfected SH-SY5Y cells. Xiong et al. reported that the production of $A\beta_{40}$ and $A\beta_{42}$ was decreased by treatment with curcumin [29]. Curcumin reduced the activation of PS1 in both mRNA and protein levels in APP-overexpressing cells. This change was accompanied with decreased GSK3 β mRNA and protein levels [30].

Furthermore, curcumin has shown beneficial effects through hippocampal-dependent memory improvement. Treatment with curcumin improved the spontaneous alternation behavior as well as the recognition memory task in $A\beta$ -infused rats. Concerning protection of cognitive impairment, a decrease in SYN levels was blocked and the phosphorylation of tau protein in rat hippocampus was decreased under the presence of curcumin. The production of TNF- α and IL-1 β and activation of GFAP immunocent levels were decreased in curcumin-treated hippocampus compared with non-treated samples. Additionally, BDNF concentration and the phosphorylation of Akt and GSK3 β revealed an increase after curcumin treatment [31] (● **Table 2**).

Ginsenoside Rg1 from *Panax notoginseng*

GRg1, a major active component of *Panax notoginseng* (Burk.) F.H. Chen (Araliaceae), is used to treat central nervous system dysfunctions, especially those involving cognitive abilities such as learning and memory [32]. The activity of β -secretase was inhibited in a concentration-dependent manner and a dose-dependent reversal of $A\beta$ -induced decreased cell viability was observed following treatment with GRg1 in PC12 cells. GRg1 appeared to prevent oxidative damage through inhibition of LDH efflux, NO production, ROS induction, and lipid peroxidation. Additionally, treatment with GRg1 blocked this $A\beta$ -induced calcium increase. To confirm the influence of GRg1 on apoptosis, caspase-3 activity was investigated by measuring the proteolytic cleavage of the fluorogenic substrate Ac-DEVD-AMC. The $A\beta$ -induced increase in caspase-3 activity was inhibited by treatment with GRg1 [33]. In addition, Li et al. reported that GRg1 displayed anti- $A\beta$ neurotoxicity via p38 pathway activation in SK-N-SH neuroblastoma cells induced by $A\beta$ -stimulated THP-1 supernatant. Pretreatment with GRg1 markedly decreased LDH leakage, indicating that $A\beta$ -induced neuronal injury can be blocked by GRg1. Along with decreased tau phosphorylation, GRg1 treatment resulted in elevated IL-1 β and decreased SYN, number of MAP-2 positive cells, and activation of p38 MAPK, which is associated with elevations of phosphorylated tau [34]. In terms of apoptosis, some reports also found that increased cytokine release, including IL-1 β , IL-8, and TNF- α , was inhibited by GRg1 pretreatment in conjunction with an up-regulation of Bcl-2 and a down-regulation of Bax. This, in turn, resulted in an increase in the Bcl-2/Bax ratio as well as a reduction in the activation of caspase-3 [35].

Table 3 Summary of findings on ginsenoside Rg1 as alternative treatment for Alzheimer's disease.

First author, year [ref]	Study design	Treatments	Results
Wang and Du, 2009 [32]	PC12 cells (rat pheochromocytoma)	Pretreatment with 0.1, 1, 10 μM GRg1 for 1 h and incubation with 50 μM $\text{A}\beta_{25-35}$ for 48 h	<ul style="list-style-type: none"> ↓ activity of β-secretase ↓ cell survival ↓ excessive LDH efflux ↓ excessive generation of NO level ↓ ROS generation ↓ MDA level ↓ rise of $[\text{Ca}^{2+}]$ ↓ caspase-3 activity
Li et al., 2012a [33]	SK-N-SH neuroblastoma cells induced by $\text{A}\beta$ -stimulated THP-1 supernatant	Pretreatment with 50, 100 and 150 μM GRg1 for 30 min and then incubation with 125 nM $\text{A}\beta_{1-40}$	<ul style="list-style-type: none"> ↓ LDH leakage ↓ tau-phosphorylation ↓ content of IL-1β release ↑ MAP-2 expression ↑ SYN expression ↓ phosphorylation of p38
Li et al., 2012b [34]	SK-N-SH neuroblastoma cells induced by $\text{A}\beta$ -stimulated THP-1 supernatant	Pretreatment with 50, 100, and 150 μM GRg1 for 30 min and then incubation with 125 nM $\text{A}\beta_{1-40}$ for 2 h	<ul style="list-style-type: none"> ↓ LDH release ↓ levels of IL-1β, IL-8, and TNF-α ↓ TUNEL-positive cells ↓ annexin V-FITC ↑ ratio of Bcl-2/Bax ↓ caspase-3 expression
Huang et al., 2012 [35]	Cortical neurons from C57BL/6 mouse fetuses	Pretreatment with GRg1 for 24 h, and then incubation with 5 μM $\text{A}\beta_{1-42}$ for 48 h	<ul style="list-style-type: none"> ↓ Cyt c release from mitochondria ↓ TUNEL⁺ neurons ↓ activity of caspase-3
Wu et al., 2012 [36]	Cortical neurons from embryonic (E18d) rat fetus	Pretreatment with 1, 10, 20 μM GRg1 for 24 h and incubation with 10 with 10 μM $\text{A}\beta_{25-35}$ for 72 h	<ul style="list-style-type: none"> ↑ cell viability ↑ Bcl-2/Bax ratio ↓ release of Cyt c ↓ caspase-9 ↓ caspase-3
Gong et al., 2011 [37]	Primary cultured hippocampal neurons from embryonic brains of Wistar rats	Pretreatment with 1 mg/mL $\text{A}\beta_{25-35}$ for 7 days and incubation with GRg1 0.1, 1, 10 μM for 72 h	<ul style="list-style-type: none"> ↑ cell viability ↓ LDH release ↓ annexin V⁺/PI⁻ fraction ↑ Bcl-2/Bax ratio ↓ caspase-3
Fang et al., 2012 [38]	Tg mAPP (transgenic mice with neuronal overexpression of a mutant human form of amyloid precursor protein)	Four experimental groups: (1) non Tg with vehicle-treated group (2) non Tg with 1 mg/kg GRg1-treated group (3) Tg mAPP with vehicle-treated group (4) Tg mAPP with 1 mg/kg GRg1-treated group	<ul style="list-style-type: none"> ↓ levels of $\text{A}\beta_{1-40}$ and $\text{A}\beta_{1-42}$ ↓ $\text{A}\beta$ plaque loads ↓ γ-secretase activity ↔ levels of AChE-positive neuritis ↑ radial arm water maze

↑ Increase; ↓ decrease; ↔ no difference

In addition, GRg1-treated cortical neurons from C57BL/6 mouse fetuses with $\text{A}\beta_{1-42}$ inhibited the expression of Cyt c in cytosolic fraction, whereas Cyt c levels in mitochondrial fractions were increased. Marked decrease in caspase-3 activity and TUNEL-positive apoptotic neurons were shown in GRg1-treated groups [36]. In cortical neurons from embryonic rat fetus, GRg1 treatment protected $\text{A}\beta_{25-35}$ -induced cell death. Bcl-2/Bax ratio was increased with reduced caspase-9 and -3 activities by GRg1 treatment. Release Cyt c from mitochondria was blocked with GRg1 treatment [37]. Similarly, increased LDH release was suppressed against cytotoxicity of $\text{A}\beta_{25-35}$ under the presence of GRg1. GRg1 reduced the numbers of apoptotic cells, as shown in decreased annexin V⁺/PI⁻ fraction. The ratio of Bcl-2/Bax was up-regulated and activation of caspase-3 was down-regulated by GRg1 treatment [38].

In an animal study, Fang et al. demonstrated the neuroprotective effects of GRg1 in transgenic AD mice. ELISA and immunostaining with an $\text{A}\beta$ antibody revealed that $\text{A}\beta$ plaque loads were lower in the cerebral cortex and hippocampus of GRg1-treated mAPP mice than in vehicle-treated mice. This lower accumulation of $\text{A}\beta$ in the cortices of GRg1-treated mice stemmed from the inhibition of γ -secretase activity and did not affect levels of AChE-

positive neurites in the entorhinal cortex. Compared with vehicle-treated mAPP mice, GRg1-treated mice were shown to improve their performance in RAWM task [39] (● Table 3).

Puerarin from the radix of *Pueraria lobata*

Previous research has demonstrated that puerarin (from *Pueraria lobata* (Willd.) Ohwi.; Fabaceae), which has been widely used in traditional medicine for thousands of years, exhibits anti-oxidant, anti-myocardial, anti-ischemic retinopathy, and anti-hyperglycemic effects [40,41]. Zou et al. reported that puerarin have a protective effect against $\text{A}\beta$ -induced neurotoxicity in rat hippocampal neurons via various mechanisms. Pretreatment with puerarin decreased $\text{A}\beta$ -induced cell death in cultured rat hippocampal neurons by inhibiting GSK-3 β via activation of Akt [42]. Furthermore, puerarin treatment resulted in an increase in GSH-Px and CAT activities and a decrease in the production of ROS [43].

In addition, treatment with puerarin prevented $\text{A}\beta_{25-35}$ -induced apoptosis, as a result of decrease of TUNEL-positive cells and annexin V⁺/PI⁻ fraction. Puerarin-treated cells inhibited the expression of caspase-3 with increased expression of Bcl-2 and decreased expression of Bax [44]. Similarly, Zhang et al. demon-

Table 4 Summary of findings on puerarin as alternative treatment for Alzheimer's disease.

First author, year [ref]	Study design	Treatments	Results
Zou et al., 2013 [41]	Primary cultured hippocampal neurons from Sprague – Dawley rats	Pretreatment with 3, 10, 30, 100 μ M puerarin for 2 hours and addition with 20 μ M $A\beta_{25-35}$ for 24 h	<ul style="list-style-type: none"> ↑ cell viability ↓ ROS levels ↑ phosphorylation of GSK3β at Ser9
Lin et al., 2012 [42]	Primary cultured hippocampal neurons from Sprague – Dawley rats	Pretreatment with 3, 10, 30, 100 μ M puerarin for 2 hours and addition with 20 μ M $A\beta_{25-35}$ for 24 h	<ul style="list-style-type: none"> ↑ cell viability ↓ GSK3β activity ↑ phosphorylation of Akt ↑ level of GSH-Px ↑ level of CAT activity
Zhang et al., 2008 [43]	PC12 cells (rat pheochromocytoma)	Preincubation with 0.1, 1, and 10 μ M puerarin for 30 min and addition with 50 μ M $A\beta_{25-35}$ for 24 h	<ul style="list-style-type: none"> ↑ cell viability ↓ TUNEL-positive cells ↓ annexin V⁺/PI⁻ fraction ↑ expression of Bcl-2 ↓ expression of Bax ↓ caspase-3
Zhang et al., 2011 [44]	mitochondrial transgenic neuronal cell used Rho ^o SH-SY5Y cells lacking mtDNA	Treatment with 0.1, 1, and 10 μ M puerarin for 24 hours	<ul style="list-style-type: none"> ↑ cell viability of SAD cybrids ↓ annexin V⁺/PI⁻ fraction ↓ intracellular ROS generation ↑ expression of Bcl-2 ↓ expression of Bax ↓ phosphorylation of JNK and p38 ↓ caspase-3 activity
Li et al., 2010 [45]	Male Sprague–Dawley rats	Intracerebroventricular injection of 10 μ g/ μ L $A\beta_{1-42}$ and after 3 days, orally administration by gavage with puerarin for 28 days; Six experimental groups: (1) sham-operated group (2) saline-treated $A\beta_{1-42}$ group (3) donepezil-treated $A\beta_{1-42}$ group (4) 25 mg/kg puerarin-treated $A\beta_{1-42}$ group (5) 50 mg/kg puerarin-treated $A\beta_{1-42}$ group (6) 25 mg/kg puerarin-treated $A\beta_{1-42}$ group	<ul style="list-style-type: none"> ↓ MWM task ↑ number of crossings over a platform position ↓ TUNEL-positive cells ↑ phosphorylation of Akt-ser473 and -Bad levels ↔ unchanged on total Akt and Bad levels ↓ caspase-9 protein and mRNA levels

↑ Increase; ↓ decrease; ↔ no difference

strated that puerarin protected against cybrid viability loss in mitochondrial transgenic neuronal cells. Annexin V⁺/PI⁻ fraction and intracellular ROS levels were attenuated by puerarin treatment. In addition, the expression of Bax, Bcl-2, phosphorylation of JNK and p38 were regulated by treatment of puerarin [45]. Another study found that puerarin treatment resulted in a significant enhancement of MWM task against $A\beta$ injection. Treatment with puerarin decreased $A\beta$ -induced cell death in rat hippocampus via activation of Akt. Furthermore, the phosphorylation of Bad was increased, while the production of caspase-9 was decreased in puerarin-treated group [46] (● Table 4).

Silibinin from the herb milk thistle (*Silybum marianum*)

Silibinin is a flavonoid derived from the herb milk thistle (*Silybum marianum* (L.) Gaertn.; Asteraceae) and is known to have anti-oxidative and anti-inflammatory properties [47]. Various studies have indicated that silibinin confers protection against oxidative stress in hepatocytes by decreasing lipid peroxidation, a sensitive marker of oxidative lipids and scavenging of free radicals [48,49]. Silibinin is protective against $A\beta$ -induced oxidative stress in SH-SY5Y human neuroblastoma cells. The aggregation of $A\beta$ was markedly inhibited following treatment with silibinin in ThT binding assay and TEM imaging assay. The presence of silibinin with $A\beta$ increased cell viability, whereas its absence decreased it. Additionally, the level of H₂O₂ in $A\beta$ -treated cells decreased following incubation with silibinin [50].

In animal experiments, silibinin ameliorates $A\beta$ -induced short-term memory impairment as measured by a spontaneous alternation behavior task (Y-maze test) although no significant differ-

ences in the number of spontaneous locomotor activities were observed. Recognition memory in novel object recognition tests was improved by silibinin in $A\beta$ -injected mice, while the amounts of time spent exploring objects in training and retention sessions did not differ. To further explore the effects of silibinin on $A\beta$ -induced oxidative stress, levels of MDA and GSH were analyzed. Treatment with silibinin appeared to decrease MDA levels and increase GSH levels in the hippocampus [51]. The cued freezing response and contextual freezing response were attenuated in silibinin-treated animals, indicating an impairment in associative memory. However, silibinin did not change the level of response to electric foot shock (flinching, vocalization, and jumping) in either group. A significant increase in nitrotyrosine, TNF- α , and i-Nos levels in the hippocampus and amygdala were reduced following treatment with silibinin compared with distilled water [52] (● Table 5).

Discussion

▼
The aggregation and accumulation of extracellular and intracellular $A\beta$ induce impaired synaptic plasticity and memory [4]. Three proteases, such as α -, β -, and γ -secretases, cleave APP and generate the physiological peptide $A\beta$ along with increased production of CTFs [53–55]. These soluble peptides spontaneously aggregate to form $A\beta$ oligomers and fibrils that are subsequently deposited within the brain to form amyloid plaques [56]. The intracerebroventricular administration of $A\beta$ peptide induces histological and biochemical changes, memory deficits, oxidative

Table 5 Summary of findings on silibinin as alternative treatment for Alzheimer's disease.

First author, year [ref]	Study design	Treatments	Results
Yin et al., 2010 [49]	SH-SY5Y cells (human neuroblastoma)	Pretreatment of 0, 2.5, 5, 10, 20, 50 μM silibinin and addition with 100 μM $\text{A}\beta_{1-42}$ for 24 h	<ul style="list-style-type: none"> ↓ ThT binding assay ↓ $\text{A}\beta_{1-42}$ aggregation ↓ cytotoxicity of $\text{A}\beta_{1-42}$ ↓ level of H_2O_2
Lu et al., 2009a [51]	Male ICR mice	Intracerebroventricular injection of 3 nmol/mouse $\text{A}\beta_{25-35}$ and after 4 days, orally administration by gavage with silibinin for 11 days; Six experimental groups: <ol style="list-style-type: none"> (1) vehicle-treated group (2) vehicle-treated $\text{A}\beta_{25-35}$ group (3) 2 mg/kg silibinin-treated $\text{A}\beta_{25-35}$ group (4) 20 mg/kg silibinin-treated $\text{A}\beta_{25-35}$ group (5) 200 mg/kg silibinin-treated $\text{A}\beta_{25-35}$ group (6) 200 mg/kg silibinin-treated group 	<ul style="list-style-type: none"> ↔ no significant differences in the time course and total of locomotor activity ↑ impairment of spontaneous alternation behaviour ↑ level of exploration preference in the retention session ↓ level of MDA ↑ level of GSH
Lu et al., 2009b [52]	Male ICR mice	Intracerebroventricular injection of 3 nmol/mouse $\text{A}\beta_{25-35}$ and after 4 days, orally administration by gavage with silibinin for 8 days; Eight experimental groups: <ol style="list-style-type: none"> (1) vehicle-treated group (2) vehicle-treated $\text{A}\beta_{25-35}$ group (3) 2 mg/kg silibinin-treated $\text{A}\beta_{25-35}$ group (4) 20 mg/kg silibinin-treated $\text{A}\beta_{25-35}$ group (5) 200 mg/kg silibinin-treated $\text{A}\beta_{25-35}$ group (6) 2 mg/kg silibinin-treated group (7) 20 mg/kg silibinin-treated group (8) 200 mg/kg silibinin-treated group 	<ul style="list-style-type: none"> ↑ cued and contextual freezing responses ↔ no differences on the levels of electric current required to elicit flinching, vocalization, and jumping ↑ nitrotyrosine levels in the hippocampus and amygdala ↓ i-Nos and TNF-α mRNA expression

↑ Increase; ↓ decrease; ↔ no difference

damage, and inflammatory responses [4, 5]. Based on our review, berberine, GRg1, and silibinin appear to block the formation of $\text{A}\beta$ via an inhibition of β -secretase activity. In addition, GRg1 suppressed γ -secretase activity.

The PI3K/Akt/GSK3 β signaling pathway is associated with various aspects of AD pathology. PI3K regulates the trafficking of intracellular APP-CTFs, whereas GSK modulates APP processing and thereby influences the production of $\text{A}\beta$ in neurons. Inhibition of PI3K contributes to tau phosphorylation and impairment in spatial memory. Suppressing GSK3 activity, which is induced by phosphorylation at specific serine residues (Ser 9 and Ser21) by Akt, has been demonstrated to contribute to the accumulation of $\text{A}\beta$ in APP mice and the hyperphosphorylation of tau protein [57–62]. Berberine, curcumin, and puerarin regulated the PI3K/Akt/GSK3 β signaling pathway. Berberine increased the phosphorylation of Akt and GSK3 β , resulting in decreases of APP-CTFs levels. *In vitro* and *in vivo* data demonstrated that curcumin exhibits significant increases in the phosphorylation of Akt and GSK3 β . Puerarin also upregulated the phosphorylation of GSK3 β and Akt. The activation of glial cells and the expression of inflammatory mediators in conjunction with the presence of neurotoxic free radicals lead to neuroinflammation. Activated microglial cells induced by $\text{A}\beta$ are able to engulf filament $\text{A}\beta$; thus, the decrease in the Iba-1 burden correlates with a reduced plaque burden [63–65]. Release of proinflammatory cytokines, such as IL-6, IL-1 β , and TNF- α , along with chemokines is responsible for the initiation and progression of inflammatory responses [66–68]. Several studies have shown that IL-6 induces the processing of APP and regulates $\text{A}\beta$ production [69]. Microglia and macrophages are a major source of TNF- α although TNF- α is also expressed to a lesser extent by GFAP-positive astrocytes, and IL-1 β is expressed by neurons and astrocytes in response to AD-like pathology [66, 67]. High level of MCP-1 promotes the migration and recruitment of inflammatory cells [70, 71]. Furthermore, $\text{A}\beta$ -induced MCP-1

mRNA expression is associated with the activation of the PI3K/Akt signaling pathway [72]. Additionally, NF- κB , and its key inhibitor, I κB - α , primary regulator of inflammatory processes in almost all cell types including neurons, are expressed in microglial cells that express i-Nos and Cox-2 [73, 74]. Previous studies have shown that the MAPK signaling pathways play an important role in the regulation of chemokine and pro-inflammatory cytokine production in microglia [75, 76]. Berberine, curcumin, and GRg1 suppressed the activation of microglial cells and neuroinflammation by blocking the production of proinflammatory cytokines. Berberine, GRg1, and puerarin inhibited NF- κB and MAPK signaling pathways. In particular, berberine decreased the Iba-1 burden, indicating a reduction in the degree of microgliosis. In addition, treatment with berberine reduced IL-6 production and MCP-1 release in microglial cells, and suppressed NF- κB translocation into the nucleus as well as the phosphorylation of I κB - α , ERK1/2, and p38. Curcumin treatment showed a significant decrease in activated microglia along with reductions in IL-1 β and TNF- α . In case of GRg1, the content of IL-1 β , IL-8, and TNF- α were reduced with the phosphorylation of p38. And puerarin attenuated the phosphorylation of JNK and p38, resulting in the protection against neuronal cell death.

Concerning neuron cell survival, the loss of neuron-related apoptosis is regulated by several intracellular signaling events [77]. During early apoptosis stages, the Bcl-2 protein family which includes anti-apoptotic molecules such as Bcl-2 and Bcl- x_L and pro-apoptotic molecules such as Bax, Bak, Bid, and Bad, is related to the formation of channels in mitochondrial membranes. The Bcl-2/Bax ratio, in particular, is crucial for initiating apoptosis, and its translocation to the mitochondrial membrane may lead to the loss of mitochondrial membrane potential and increased mitochondrial permeability [78] which results in the release of Cyt c from mitochondria and the subsequent activation of procaspase-3 to caspase-3, eventually leading to apoptosis [79]. GRg1 and

puerarin attenuated apoptosis by regulating anti-apoptotic and pro-apoptotic molecules. GRg1 regulated the ratio of Bcl-2/Bax, resulting in the release of Cyt c from mitochondria and the reduction of caspase-9 and caspase-3 activities. Puerarin protected cell death via up-regulating the ratio of Bcl-2/Bax and down-regulating the caspase-9 and -3 activities which were induced by the phosphorylation of Bad.

Furthermore, it is well established that oxidative stress is involved in apoptosis in that excessive production of ROS can lead to neuronal apoptosis in AD [11, 80]. GSH and MDA are important intracellular anti-oxidants necessary for the formation of peroxynitrite and responsible for removing oxygen-free radicals [51, 81]. In addition, the activations of NF- κ B, Sirt1, and Nrf2 pathways are related to protection of cells against oxidant damage as stress sensor molecules [82]. Curcumin, GRg1, puerarin, and silibinin exhibited protection against oxidative stress. Specifically, curcumin regulated γ -GCS expression and RNS level. And treatment with GRg1 inhibited the excessive generation of NO and MDA levels as well as ROS generation. Puerarin also decreased intracellular ROS generation while increasing GSH-Px and CAT activities in various neuronal cells. Silibinin decreased the levels of MDA, whereas GSH was increased in *in vivo* study.

Apart from the pathology, memory enhancement is one of major issues of AD. Loss of neuroblasts and markers, characteristic of striatal medium spiny neurons, belong to the process of neuronal cell death [83]. In terms of neurotrophic factors, BDNF promotes the survival of new hippocampal neurons [84]. Synaptic proteins take part in the development of nerve synapses and adjust their plasticity. SYN, one of the synaptic proteins, is a marker of synaptic distribution and synaptic density [85]. GRg1 increased SYN expression, and curcumin contributed to the formation of new neurons by regulating the synaptic proteins SYN and BDNF.

To date, evidence from recent studies suggests that commonly used medicinal herbs and their phytochemicals could potentially be used to treat AD. Although these studies focus on the efficacy of inhibiting AD development, and research on humans is limited, numerous findings demonstrate the possibilities of the use of medicinal herbs for the treatment of AD. The approach to investigate the potential treatment of AD may support drug development from herbal medicine.

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Conflict of Interest

▼ The authors state no conflict of interest.

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