Pharmacogn. Res., 2026; 18(4):1395-1404.
https://www.phcogres.com

Original Article

Multi-Target Neuroprotective Activity of the Traditional Thai Polyherbal Formula Sangvichai against Amyloid-β42-Induced Cellular Stress: An *in vitro* Study

Lueacha Tabtimmai[1,2], Pussadee Srathong[3], Sulak Talawat[4], Wanwipha Premsang[4], Kiattawee Choowongkomon[4,*]

[1]Department of Biotechnology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Bangkok, THAILAND.
[2]Food and Agro-Industrial Research Center, King Mongkut's University of Technology North Bangkok, Bangkok, THAILAND.
[3]Boromarajonani College of Nursing, Nonthaburi, Faculty of Nursing, Praboromarajchanok Institute, THAILAND.
[4]Department of Biochemistry, Faculty of Science, Kasetsart University, Bangkok, THAILAND.

ABSTRACT

Background: Sangvichai is a traditional Thai polyherbal formula composed of 40 botanical ingredients and has been used for neurological complaints and age-related cognitive decline. However, scientific evidence supporting its neuroprotective effects and underlying mechanisms remains limited. **Objectives:** This study aimed to investigate the biological activity of Sangvichai against amyloid-β42 (Aβ42)-induced neuronal cytotoxicity and to evaluate its multi-target effects in cellular models relevant to Alzheimer's Disease (AD). **Materials and Methods:** Sangvichai extract was evaluated using a panel of in vitro assays. Antioxidant activity was assessed by DPPH radical scavenging and intracellular reactive oxygen species (ROS) assays. Anti-inflammatory effects were examined via nitric oxide production in lipopolysaccharide-stimulated macrophages. Cholinergic modulation was determined by acetylcholinesterase inhibition, while melatonin MT2 receptor-mediated cAMP signaling was analyzed. Neuroprotective effects were assessed in SH-SY5Y neuronal cells exposed to aggregated Aβ42. **Results:** Sangvichai showed moderate DPPH radical scavenging activity (IC_{50} = 737.80 ± 3.03 µg/mL) but demonstrated stronger cellular antioxidant effects, significantly reducing intracellular ROS under inflammatory conditions (IC_{50} = 503.20 ± 37.40 µg/mL). The extract suppressed nitric oxide production by 31.48% and inhibited acetylcholinesterase activity in a concentration-dependent manner (IC_{50} = 1.21 ± 0.20 mg/mL). In addition, Sangvichai reduced MT2 receptor-mediated cAMP signaling by 43.73% and significantly attenuated Aβ42-induced neuronal cytotoxicity. **Conclusion:** Sangvichai exerts coordinated antioxidant, anti-inflammatory, neuromodulatory, and neuroprotective effects in vitro, supporting a multi-target mode of action relevant to AD-associated cellular pathology and providing a foundation for further preclinical investigation.

Keywords: Amyloid-β42, Inflammation, Neuroprotective activity, Oxidative stress, Polyherbal formula, Sangvichai.

Correspondence:
Dr. Kiattawee Choowongkomon
Department of Biochemistry, Faculty of Science, Kasetsart University, Bangkok 10900, THAILAND.
Email: fsciktc@ku.ac.th

Received: 06-01-2026;
Revised: 12-03-2026;
Accepted: 28-05-2026.

INTRODUCTION

Neurodegenerative disorders associated with aging, particularly Alzheimer's Disease (AD), represent a major global health challenge due to their progressive cognitive and behavioral impairments and the increasing burden on healthcare systems worldwide (Breijyeh and Karaman, 2020; Selkoe and Hardy, 2016). AD is characterized by complex and interrelated pathological features, including extracellular Amyloid-β (Aβ) plaque accumulation, intracellular tau hyperphosphorylation, synaptic dysfunction, and progressive neuronal loss (Jeremic *et al.*, 2021; Long and Holtzman, 2019; Soria Lopez *et al.*, 2019). Among these pathological hallmarks, aggregated Aβ has been widely implicated as a central contributor to neuronal injury through its ability to disrupt cellular homeostasis and initiate downstream neurotoxic cascades, including oxidative damage, mitochondrial dysfunction, and synaptic impairment (Butterfield and Boyd-Kimball, 2018).

Accumulating evidence indicates a bidirectional relationship between oxidative stress and Aβ pathology in AD. Aβ accumulation promotes oxidative stress through mechanisms such as mitochondrial dysfunction and activation of neuroinflammatory pathways, while sustained oxidative stress further enhances Aβ generation. This self-amplifying cycle increases neuronal

DOI: 10.5530/pres.20260013

vulnerability and contributes to synaptic dysfunction and neurodegeneration (Butterfield and Boyd-Kimball, 2018; Tamagno *et al.*, 2021). Importantly, oxidative stress and inflammatory alterations are detectable at early stages of AD, supporting the concept that prolonged oxidative imbalance plays a critical role in disease progression (Tamagno *et al.*, 2021).

Current pharmacological treatments for AD, including Acetylcholinesterase (AChE) inhibitors, provide only symptomatic relief and do not effectively halt disease progression or prevent neuronal loss, largely due to the multifactorial nature of AD pathophysiology (Vecchio *et al.*, 2021; Zuliani *et al.*, 2024). In addition, these agents are frequently associated with adverse effects that limit long-term use and patient adherence (Ruangritchankul *et al.*, 2021; Vecchio *et al.*, 2021). These limitations have highlighted the need for alternative strategies capable of modulating multiple interconnected pathological mechanisms rather than relying on single-target interventions (Jeremic *et al.*, 2021).

In this context, natural products and polyherbal formulations have received increasing attention as potential multi-target therapeutic agents. Polyherbal medicines consist of chemically diverse phytoconstituents and have been traditionally employed in the management of chronic and age-related disorders. Experimental studies suggest that herbal extracts and polyherbal preparations may exert biological effects through coordinated modulation of oxidative stress, inflammatory signaling, and neuronal stress responses, rather than through direct interaction with a single molecular target (Baranowska-Wójcik *et al.*, 2025; Chen *et al.*, 2020). However, for many traditional formulations, systematic pharmacological characterization at the cellular level remains incomplete.

Sangvichai is a traditional Thai polyherbal formula, comprising 40 botanical ingredients that has been used in Thai traditional medicine for conditions associated with neurological symptoms and age-related functional decline. Although several constituent herbs have individually been reported to possess antioxidant or anti-inflammatory properties, the biological activity of Sangvichai as a combined formulation, as well as its underlying cellular mechanisms relevant to AD-associated pathology, has not been systematically investigated. In particular, its potential role in modulating Aβ-induced neuronal stress within a multi-target pharmacological framework remains unclear.

In vitro neuronal cell models provide a controlled and reproducible experimental platform for evaluating early pathogenic events associated with Aβ-induced cytotoxicity and for screening the biological activity of complex herbal formulations. Primary neuronal cultures and neuroblastoma-derived cell lines have been widely used to assess Aβ-related neurotoxicity, oxidative stress, and cell viability under defined conditions (Ferreira *et al.*, 2011; Prajapat *et al.*, 2023). Systematic reviews further support the utility of *in vitro* AD models as essential tools for mechanistic investigation and preliminary pharmacological evaluation before *in vivo* studies (Prajapat *et al.*, 2023).

Therefore, the present study aimed to investigate the biological activity of the traditional Sangvichai polyherbal formula against amyloid-β42-induced cytotoxicity using in vitro cellular models. Particular emphasis was placed on evaluating whether the observed effects reflect coordinated modulation of multiple pathological pathways relevant to AD, thereby providing pharmacological and mechanistic insight to support the rational development and scientific validation of traditional polyherbal medicine.

MATERIALS AND METHODS

Preparation of Sangvichai Polyherbal Extract

Sangvichai powder, a traditional Thai polyherbal formulation, was obtained from Vetchakorn Osot (Bangkok, Thailand) (Table S1). The powder was extracted by maceration in ethanol at a solid-to-solvent ratio of 1:5 (w/v) at room temperature to allow efficient recovery of both polar and moderately non-polar phytoconstituents. After filtration, the combined filtrates were concentrated under reduced pressure and subsequently spray-dried to obtain a stable powdered extract suitable for *in vitro* evaluation. For biological assays, the extract was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution of 100 mg/mL, vortexed until complete solubilization, and sterile-filtered through a 0.22 µm nylon syringe filter (13 mm diameter, Whatman). Working solutions were freshly prepared by diluting in culture medium, with the final DMSO concentration maintained below cytotoxic levels.

Preliminary Cytotoxicity Assessment

Preliminary cytotoxicity screening was performed using the MTT assay to determine non-toxic concentration ranges of the Sangvichai extract. RAW 264.7 murine macrophage cells were selected as a representative immune-responsive cell model relevant to oxidative and inflammatory processes associated with neurodegenerative conditions. Cells were seeded in 96-well plates at a density of 1×10^5 cells/well and incubated for 24 hr at 37°C in a humidified atmosphere containing 5% CO_2. Cells were treated with the extract at concentrations ranging from 7.8 to 1,000 µg/mL for 24 hr. Following treatment, MTT solution was added and incubated for 3 hr to allow mitochondrial dehydrogenase-mediated reduction of MTT to formazan crystals. The formazan product was dissolved in DMSO, and absorbance was measured at 570/630 nm using a microplate reader. Untreated cells served as the negative control (100% viability), while cells treated with 10% DMSO served as the positive cytotoxic control. All experiments were conducted in triplicate. Cell viability was calculated according to Equation (1).

$$\text{Cell viability } (\%) = (A_0 / A_1) \times 100 \quad (1)$$

where A_0 represents the absorbance of the sample (treated cells), and A_1 represents the absorbance of the negative control (untreated control cells).

Antioxidant Activity

DPPH Radical Scavenging Assay

The direct free radical scavenging capacity of the Sangvichai extract was evaluated using the DPPH assay as a chemical antioxidant screening method. A DPPH working solution (0.1 mg/mL) was prepared in absolute ethanol. Test samples (0.5 and 1.0 mg/mL) were mixed with DPPH solution and incubated in the dark at room temperature for 30 min. L-ascorbic acid (0.01 mg/mL) was used as a reference antioxidant. Absorbance was measured at 517 nm, and radical scavenging activity was calculated using Equation (2). All measurements were performed in triplicate.

$$\text{DPPH radical scavenging (\%)} = (A_0\text{-}A_1) / A_0 \times 100 \quad (2)$$

where A_0 represents the absorbance value of the DPPH radical solution without the sample (control), and A_1 represents the absorbance of the sample.

Intracellular Reactive Oxygen Species (ROS) Inhibition Assay

To evaluate cellular antioxidant effects under inflammatory stress, intracellular reactive oxygen species (ROS) levels were measured in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells using the DCF-DA assay. Cells were seeded at 1×10^5 cells/well and pretreated with non-toxic concentrations of the extract for 1 hr before stimulation with LPS, 1 µg/mL for 24 hr. Following treatment, cells were incubated with 10 µM DCFH-DA for 30 min in the dark, and fluorescence intensity was measured at excitation/emission wavelengths of 485/528 nm. LPS-stimulated and unstimulated cells served as oxidative stress and basal controls, respectively. ROS inhibition was calculated using Equation (3).

$$\text{ROS Inhibition (\%)} = [(F_0 - F_1) / (F_0 - F_2)] \times 100 \quad (3)$$

where F_0, F_1, and F_2 represent fluorescence intensity of LPS-stimulated, extract-treated, and unstimulated control cells, respectively.

Anti-inflammatory Activity

The anti-inflammatory potential of the Sangvichai extract was assessed by measuring nitric oxide (NO) production in LPS-activated RAW 264.7 cells using the Griess reaction. Cells were seeded at 1×10^5 cells/well and pretreated with serial dilutions of the extract for 1 hr before stimulation with LPS (1 µg/mL) for 24 hr. Diclofanac sodium (75 µg/mL) was used as a reference anti-inflammatory agent. Culture supernatants were reacted sequentially with Griess reagents A and B to form a chromophoric azo compound, and absorbance was measured at 540 nm. Nitrite concentrations were quantified using a sodium nitrite standard curve. The percentage inhibition of NO production was calculated using Equation (4).

$$\text{Anti-inflammation (\%)} = [(N_0 - N_1) / (N_0 - N_2)] \times 100 \quad (4)$$

where N_0, N_1, and N_2 represent the nitrite concentration of LPS-stimulated control, sample-treated cells, and untreated cells (negative control), respectively.

Acetylcholinesterase (AChE) Inhibition Assay

AChE inhibitory activity was evaluated using the Amplex Red Acetylcholine/Acetylcholinesterase Assay Kit (A12217, Thermo Fisher Scientific, USA), providing a functional readout relevant to cholinergic dysfunction in neurodegenerative disorders. The Sangvichai extract was tested at concentrations ranging from 0.0625 to 4 mg/mL. Enzymatic reactions were monitored kinetically at 571 nm, and relative inhibition was calculated based on reaction slopes using Equation (5). IC_{50} values were derived from concentration-response curves.

$$\text{Relative Inhibition (\%)} = [(V_0 - V_1) / (V_0 - V_2)] \times 100 \quad (5)$$

where V_0 is the reaction rate of the enzyme control (AChE + substrate, no inhibitor), V_1 is the reaction rate in the presence of Sangvichai extract, and V_2 is the background reaction rate measured in the absence of AChE enzyme.

Melatonin MT2-Mediated cAMP Suppression Assay

To explore neuromodulatory signaling relevant to circadian and neuroprotective pathways, MT2 receptor-mediated suppression of intracellular cAMP was evaluated in HEK293T cells transiently expressing MTNR1B and a cAMP biosensor. Non-toxic concentrations of the extract were applied, and luminescence signals were recorded as relative luminescence units (RLU). Melatonin served as a reference agonist. Suppression of cAMP signaling was interpreted as functional activation of MT2-associated pathways. Each condition was tested in duplicate wells in three independent experiments. cAMP expression (%) was calculated relative to the untreated control using Equation (6). cAMP inhibition (%) was subsequently calculated from cAMP expression values using Equation (7).

$$\text{cAMP expression (\%)} = (RLU_0 / RLU_1) \times 100 \quad (6)$$

where RLU_0 represents the relative luminescence units of test sample-treated cells, and RLU_1 represents the luminescence intensity of the untreated group (defined as 100% cAMP expression).

$$\text{cAMP inhibition (\%)} = 100 - \text{cAMP expression (\%)} \quad (7)$$

Neuroprotective Activity Against Amyloid Beta-Induced Toxicity in SH-SY5Y Cells

Cell Cytotoxicity Evaluation

Human SH-SY5Y neuroblastoma cells were employed as a neuronal cell model relevant to Alzheimer's disease-associated toxicity. Cells were treated with the Sangvichai extract across a concentration range of 0-500 µg/mL, and cell viability was assessed by the MTT assay after 48 hr to establish non-toxic concentrations. Cytotoxicity was measured by using a microplate reader with wavelengths of 570 nm and calculating the percentage of cell survival rate using Equation (8).

$$\text{Cell viability } (\%) = (A_0 / A_1) \times 100 \quad (8)$$

where A_0 represents the absorbance of treated cells and A_1 represents the absorbance of untreated control cells.

Amyloid-β42 Preparation and Treatment Protocol

Synthetic amyloid-β42 peptide (AnaSpec, USA) was dissolved in sterile PBS and incubated at 37°C for 5 days to induce aggregation. SH-SY5Y cells were pretreated with Sangvichai (150 µg/mL) for 24 hr before exposure to Aβ42 (1 µM) for 48 hr. Experimental groups included untreated control, Aβ42 alone, Sangvichai alone, and combined treatment. After treatment, cells were washed with phosphate buffered saline (PBS) and collected for protein analysis (Supplementary Figure S1).

Western blot analysis

Protein expression related to amyloid processing, tau phosphorylation, and signaling pathways was analyzed by Western blotting. Total protein was extracted, separated by SDS-PAGE, transferred to PVDF membranes, and probed with specific primary antibodies against p-Tau181, total Tau, Rheb, APP, and vinculin (Cell Signaling Technology) as a

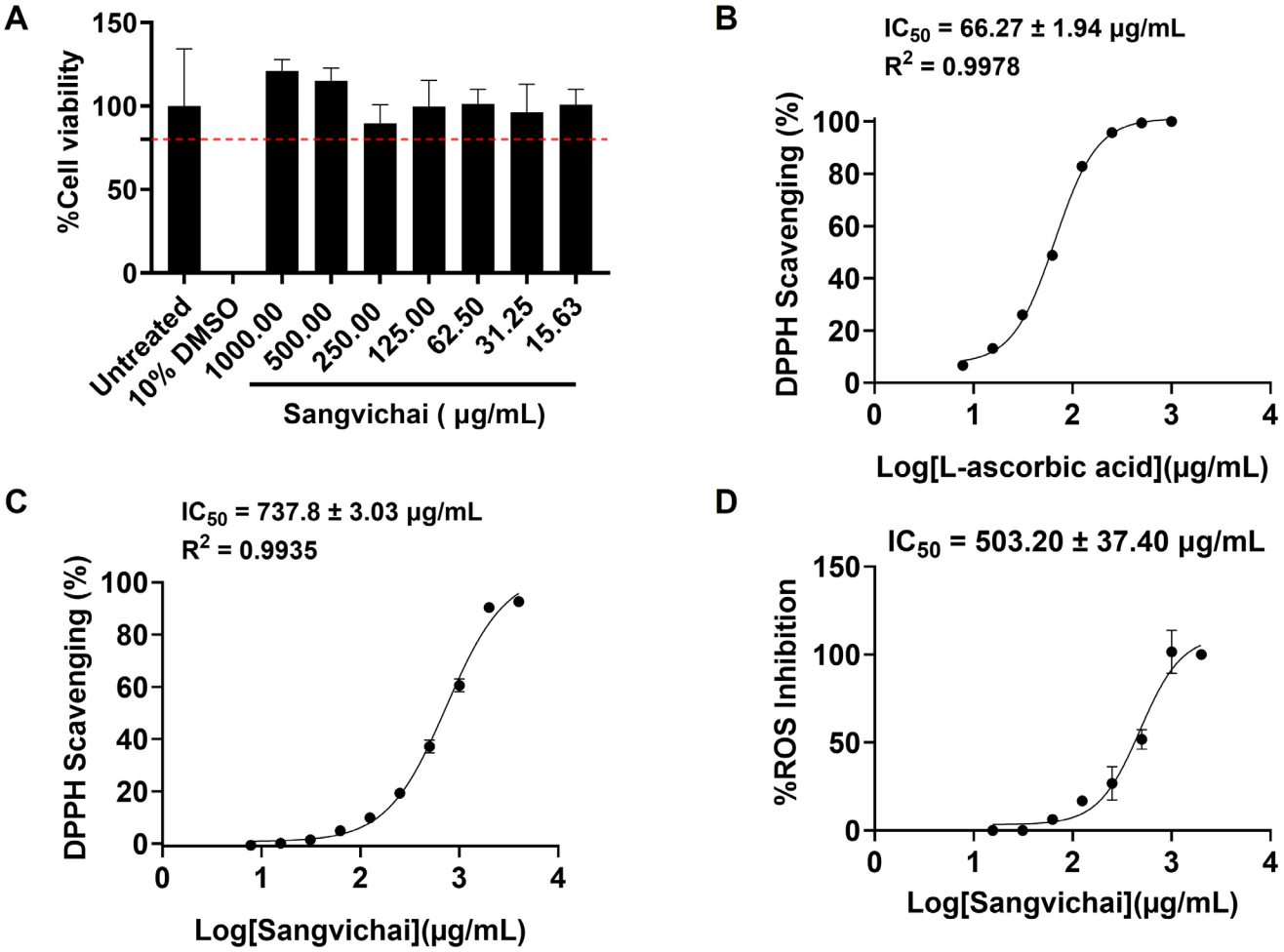

Figure 1: Cytotoxicity and antioxidant-related activities of Sangvichai extract in RAW 264.7 macrophages. (A) Cell viability determined by MTT assay after 24 hr treatment with Sangvichai extract (15.63-1,000 µg/mL). Results are expressed as percentage of viable cells relative to untreated control. (B) Dose-response curve of L-ascorbic acid in the DPPH radical scavenging assay (IC_{50} = 66.27 ± 1.94 µg/mL). (C) DPPH radical scavenging activity of Sangvichai extract (IC_{50} = 737.8 ± 3.03 µg/mL). (D) Effect of Sangvichai extract on intracellular ROS production in LPS-stimulated RAW 264.7 macrophages measured using the DCFH-DA assay (IC_{50} = 503.20 ± 37.40 µg/mL). Data are presented as mean ± SD (*n* = 3).

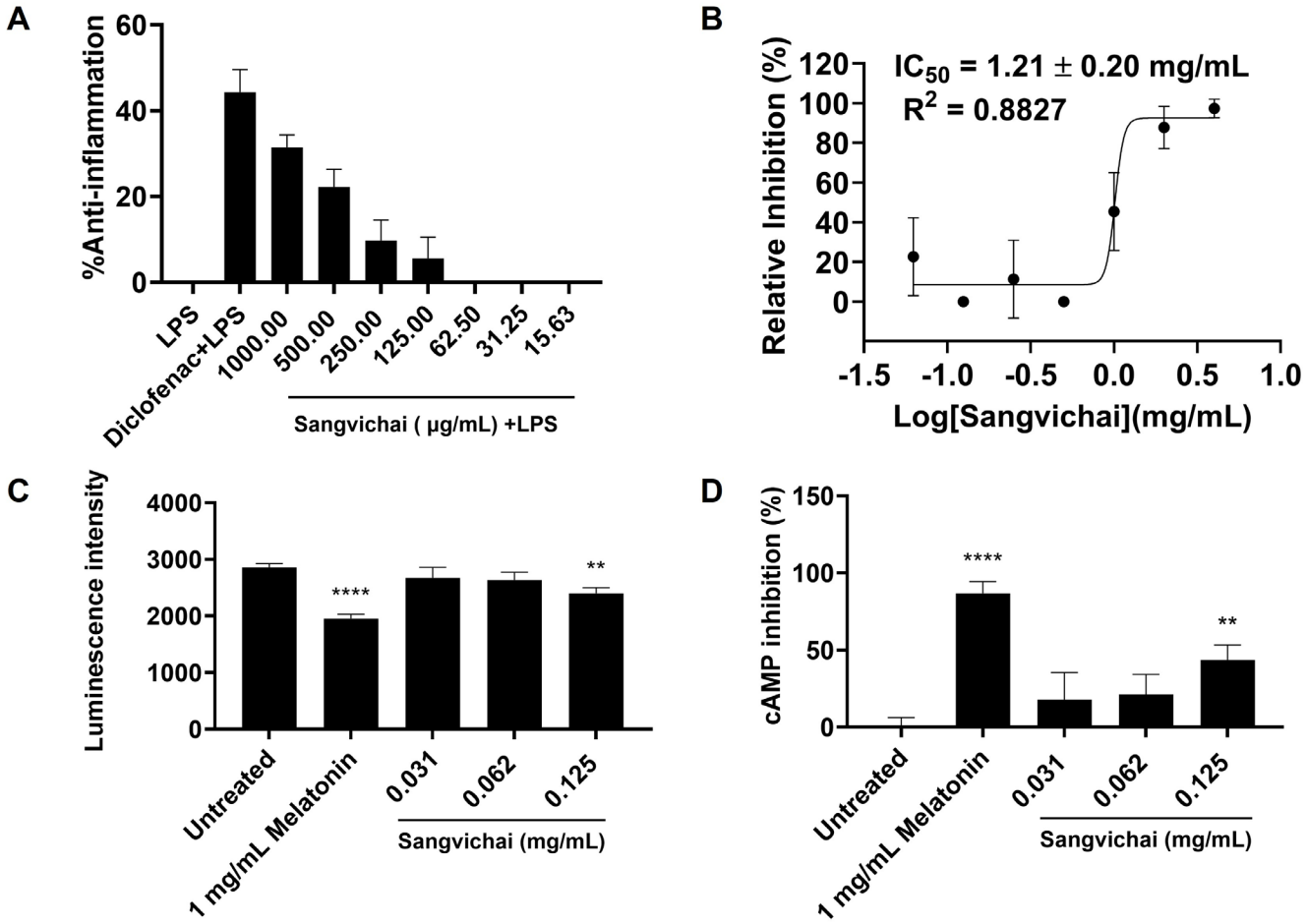

Figure 2: Anti-inflammatory, Acetylcholinesterase (AChE) inhibitory, and MT2 receptor-related cAMP inhibitory activities of Sangvichai extract. (A) Inhibition of nitric oxide production in LPS-stimulated RAW 264.7 macrophages measured by the Griess assay. Diclofenac sodium was used as reference drug. (B) Concentration-dependent inhibition of AChE activity determined by fluorescence assay. (C-D) Intracellular cAMP levels measured using the GloSensor assay and expressed as percentage inhibition relative to control. Melatonin (1 mg/mL) served as reference MT2 agonist. Data are presented as mean ± SD (n = 3). Statistical significance was determined versus control (p = 0.0037; **** p < 0.0001).

loading control. Immunoreactive bands were visualized using chemiluminescence.

Statistical Analysis

Data are presented as Mean ± SD or SEM. Statistical analyses were performed using GraphPad Prism (version 10.3.0). One-way ANOVA followed by appropriate post-hoc tests was used for multiple comparisons, with $p < 0.05$ considered statistically significant.

RESULTS

Cytotoxicity Profile of Sangvichai Extract in RAW 264.7

The cytotoxicity of Sangvichai extract was evaluated in RAW 264.7 macrophages using the MTT assay. Treatment with Sangvichai at concentrations ranging from 15.63 to 1,000 µg/mL for 24 hr did not induce significant cytotoxicity (Figure 1A). Cell viability remained above 80% across all tested concentrations, indicating that the extract was well tolerated within this range. Based on these findings, concentrations that maintained ≥80% cell viability were selected for subsequent cellular assays.

Antioxidant Activity

DPPH Radical Scavenging Activity

The antioxidant capacity of the Sangvichai extract was determined through the DPPH assay. The results revealed that both L-ascorbic acid and Sangvichai extract demonstrated dose-dependent DPPH radical scavenging activity. The scavenging effect increased with concentration, suggesting the presence of redox-active constituents with free radical scavenging activity in a cell-free system. The IC_{50} values of DPPH radical scavenging activity of L-ascorbic acid and Sangvichai extract were 66.27 ± 1.94 µg/mL and 737.80 ± 3.03 µg/mL, respectively (Figures 1B, 1C).

Intracellular Reactive Oxygen Species (ROS) Inhibition Activity

The intracellular antioxidant activity of Sangvichai was evaluated in LPS-stimulated RAW 264.7 macrophages using the DCFH-DA assay. LPS stimulation markedly increased intracellular ROS levels compared with unstimulated controls. Pretreatment with Sangvichai significantly reduced ROS production in a concentration-dependent manner (Figure 1D). The calculated IC_{50} value for intracellular ROS inhibition was 503.20 ± 37.40 µg/mL.

Anti-Inflammatory Activity via Suppression of Nitric Oxide Production

The anti-inflammatory activity of Sangvichai extract was assessed by measuring nitric oxide (NO) production in LPS-stimulated RAW 264.7 macrophages using the Griess assay. Sangvichai inhibited nitrite accumulation in a concentration-dependent manner (Figure 2A). At 1,000 µg/mL, the extract reduced NO production by 31.48% relative to LPS-stimulated control cells, while lower concentrations produced proportionally reduced inhibitory effects.

Acetylcholinesterase (AChE) Inhibition Activity

The inhibitory activity of Sangvichai extract against Acetylcholinesterase (AChE) was evaluated using a fluorescence-based enzymatic assay. Sangvichai exhibited concentration-dependent inhibition of AChE activity, with an IC_{50} value of 1.21 ± 0.20 mg/mL (Figure 2B).

Melatonin MT2-Mediated cAMP Suppression Activities

The melatonin-like activity of Sangvichai extract was evaluated by measuring MT2 receptor-mediated suppression of intracellular cAMP levels in transfected HEK293T cells using a GloSensor

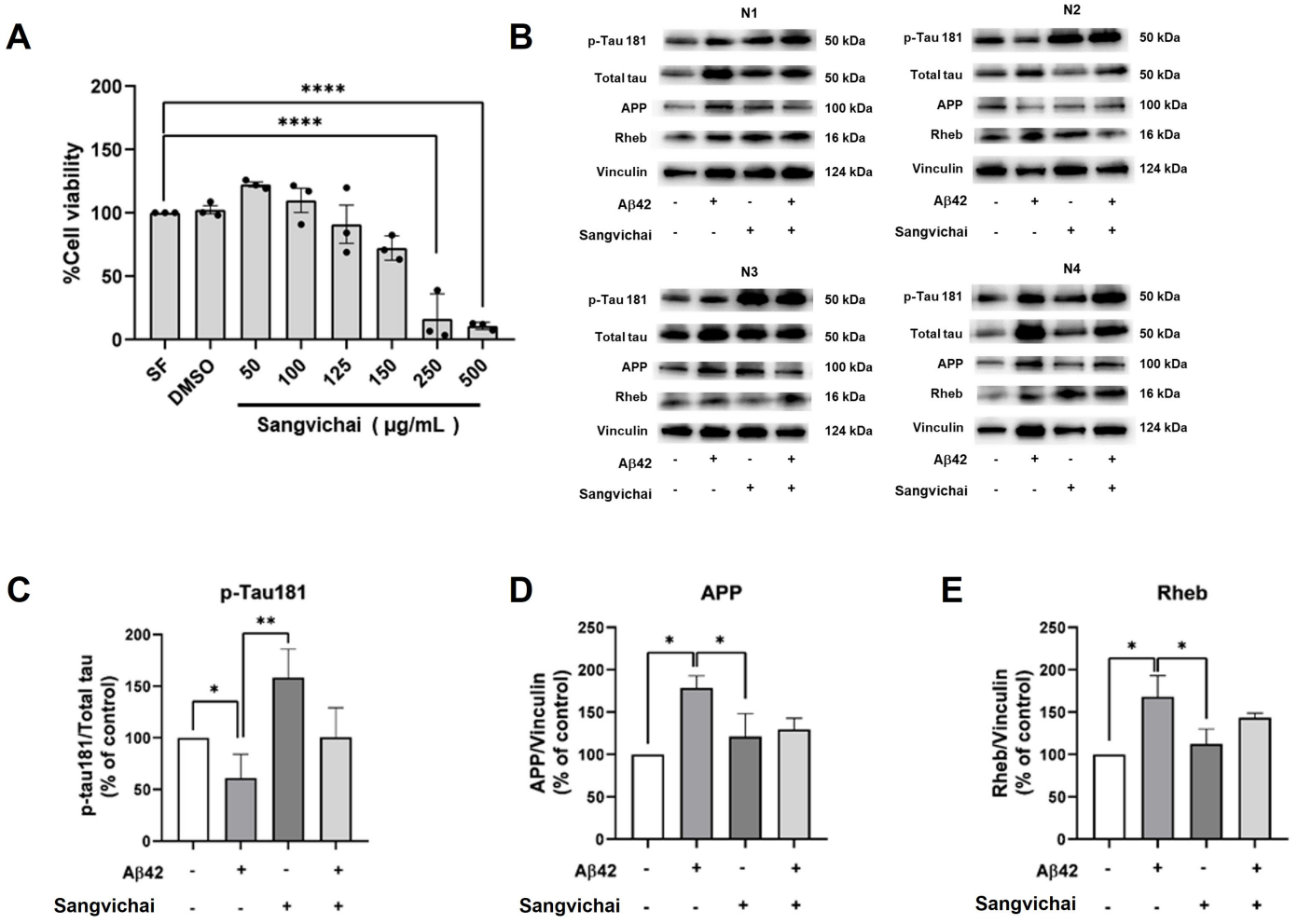

Figure 3: Cytotoxicity profile and effects of Sangvichai extract on Aβ42-induced molecular alterations in SH-SY5Y cells. (A) Cell viability assessed by MTT assay after 48 hr exposure to Sangvichai extract (50-500 µg/mL), expressed as percentage relative to untreated control cells. Data are presented as mean ± SEM (n = 3). Statistical significance was determined versus control (**** $p < 0.0001$). SH-SY5Y cells were treated with Aβ42 (1 µM) in the presence or absence of Sangvichai pretreatment (150 µg/mL). (B) Representative Western blot images of p-Tau181, total Tau, APP, and Rheb. (C) Quantification of p-Tau181/total Tau ratio. (D) Relative APP expression. (E) Relative Rheb expression. Protein levels were normalized to total Tau or Vinculin, as appropriate. Data represent four independent experiments. Statistical significance is indicated ($p < 0.05$; ** $p < 0.001$).

assay (Figure 2C). The untreated control group exhibited maximal luminescence (2,858.67 RLU), defined as 100% cAMP expression. Melatonin (1 mg/mL), used as a reference MT2 receptor agonist under assay-specific conditions, significantly reduced luminescence to 1,946.67 RLU, corresponding to 86.83% inhibition of cAMP production ($p < 0.0001$). Sangvichai extract induced a concentration-dependent reduction in intracellular cAMP levels (Figure 2D). At 0.125 mg/mL, Sangvichai significantly suppressed cAMP production by 43.73% compared with untreated controls ($p = 0.0037$), whereas lower concentrations (0.062 and 0.031 mg/mL) produced modest, non-significant inhibition ($p > 0.05$).

Neuroprotective Activity against Aβ42-Induced Toxicity in SH-SY5Y Cells

Cytotoxicity Profile in SH-SY5Y Cells

The cytotoxicity of Sangvichai extract in SH-SY5Y neuroblastoma cells was assessed using the MTT assay after 48 hr of treatment (Figure 3A). Sangvichai did not induce significant cytotoxicity at concentrations between 50 and 125 µg/mL, with cell viability ranging from 91% to 122% relative to untreated controls. At 150 µg/mL, cell viability decreased to approximately 72%, whereas higher concentrations (250 and 500 µg/mL) caused a marked reduction in viability to 11-16% ($p < 0.0001$). Based on these results, the concentration of 150 µg/mL was selected as a compromise between acceptable cell viability and sufficient biological activity for neuroprotection assessment.

Modulation of p-Tau181, APP, and Rheb Expression in Aβ42-Challenged SH-SY5Y Cells

The effects of Sangvichai on Aβ42-induced alterations in AD-related molecular markers were examined in SH-SY5Y cells (Figure 3B). Exposure to Aβ42 (1 µM) significantly altered the p-Tau181/total Tau ratio and increased the expression levels of APP and Rheb compared with untreated control cells (Figures 3B-3E). Pretreatment with Sangvichai extract (150 µg/mL) attenuated several Aβ42-induced molecular alterations by partially restoring the p-Tau181/total Tau ratio (Figures 3B, 3C) and significantly reducing APP and Rheb expression levels (Figures 3B-3E). Treatment with Sangvichai alone did not significantly affect the expression of these proteins compared with untreated control cells.

DISCUSSION

The present study investigated the biological activity of Sangvichai, a traditional Thai polyherbal formulation comprising 40 botanical ingredients, using a series of in vitro assays relevant to cellular processes implicated in AD. Rather than supporting a single dominant mechanism, the collective findings indicate that Sangvichai modulates multiple stress-responsive pathways, including oxidative stress, inflammatory signaling, cholinergic regulation, circadian-associated signaling, and Amyloid-β ($A\beta_{42}$)-induced neuronal cytotoxicity. This pattern of activity is consistent with the current understanding that AD pathogenesis arises from the convergence of multiple interrelated pathological processes rather than from a single molecular lesion (Hampel *et al.*, 2021; Tiwari *et al.*, 2019).

Oxidative stress is a central contributor to neuronal dysfunction in AD and is closely linked to amyloid pathology and neuroinflammatory processes (Butterfield and Boyd-Kimball, 2018). In the present study, Sangvichai exhibited measurable antioxidant activity in both acellular and cellular systems. Although its direct DPPH radical scavenging capacity was modest, a more pronounced effect was observed in cellular models, where the extract significantly attenuated intracellular ROS generation under inflammatory conditions. This divergence between chemical and cell-based assays highlights the limited biological translatability of acellular antioxidant tests and underscores the importance of cellular context when evaluating redox-modulating activity (Gulcin and Alwasel, 2023; Ngo and Duennwald, 2022). Importantly, the observed reduction in ROS does not necessarily indicate direct radical scavenging at the neuronal level but rather suggests modulation of intracellular oxidative stress responses under inflammatory stimulation, which may be relevant to neurodegenerative conditions characterized by sustained oxidative burden.

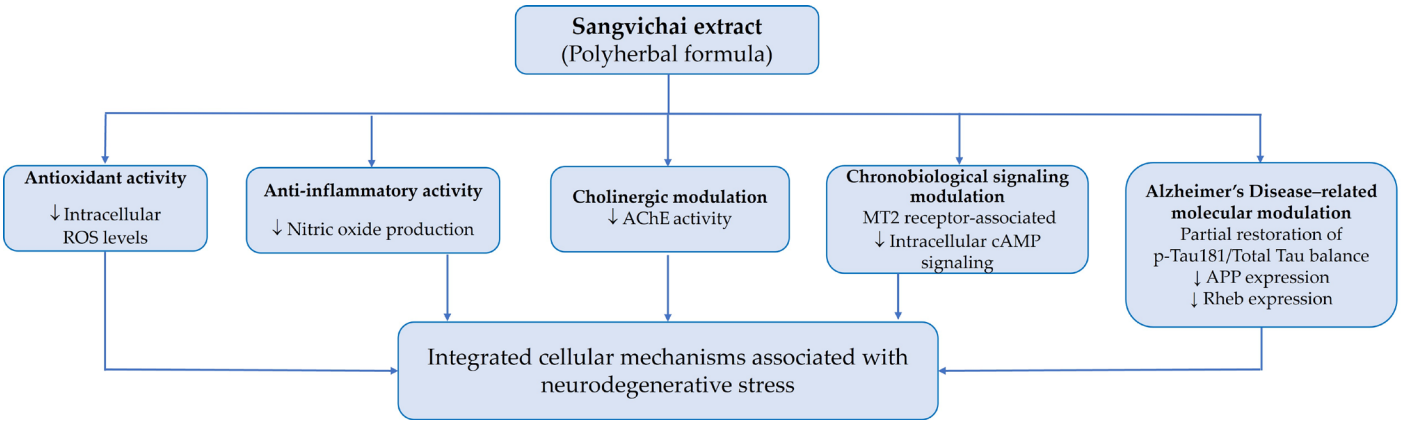

Figure 4: Proposed multi-target mechanistic framework of Sangvichai extract in Alzheimer's disease-relevant cellular models.

Given the close interplay between oxidative stress and neuroinflammation in AD, the anti-inflammatory activity of Sangvichai was further evaluated in LPS-stimulated macrophages. Chronic activation of innate immune responses and excessive production of nitric oxide and other pro-inflammatory mediators are known to contribute to neuronal injury and disease progression in AD (Fakorede *et al*., 2025; Kiraly *et al*., 2023). Sangvichai produced a concentration-dependent suppression of nitrite accumulation, indicating attenuation of inflammatory signaling in immune-responsive cells. Although macrophages do not fully recapitulate microglial biology, these findings provide supportive evidence that Sangvichai can modulate inflammatory pathways relevant to neurodegenerative contexts.

Consistent with this observation, several botanical constituents commonly reported in traditional formulations, such as *Centella asiatica, Curcuma longa, Bacopa monnieri*, *Ganoderma lucidum*, and *Zingiber officinale*, have been shown to exert anti-inflammatory and immunomodulatory effects, primarily through regulation of NF-κB-dependent signaling and cytokine production (Arcusa *et al*., 2022; Chakraborty *et al*., 2022; Cör Andrejč *et al*., 2022; Oh *et al*., 2025). Within the limitations of the present model, the observed anti-inflammatory activity of Sangvichai may therefore contribute indirectly to its overall neuroprotective profile.

Cholinergic dysfunction, partly driven by increased Acetylcholinesterase (AChE) activity, is a hallmark feature of AD and remains a principal target for symptomatic treatment. Sangvichai exhibited concentration-dependent inhibition of AChE, with moderate potency relative to synthetic inhibitors. Although the magnitude of inhibition observed is unlikely to confer stand-alone therapeutic efficacy, it is consistent with the expected pharmacological behavior of complex botanical mixtures and suggests a supportive role in maintaining cholinergic signaling. Previous experimental and computational studies have reported that extracts or bioactive constituents derived from *B. monnieri* and *C. asiatica* can modulate AChE activity while simultaneously influencing oxidative and inflammatory pathways (Chiroma *et al*., 2019; Khairinisa *et al*., 2025; Shoukat *et al*., 2023). In this context, the modest AChE inhibition observed for Sangvichai may contribute to an integrated cellular response rather than acting as a primary pharmacological driver.

An additional aspect explored in this study was the effect of Sangvichai on MT2 receptor-associated cyclic Adenosine Monophosphate (cAMP) signaling. Increasing evidence suggests that disruption of circadian and melatonin-related signaling contributes to AD pathophysiology by influencing oxidative stress, neuroinflammation, and synaptic homeostasis (Ahmad *et al*., 2022; Kołodziejska *et al*., 2025; Xin *et al*., 2025). The observed suppression of MT2-mediated cAMP signaling indicates functional interaction with melatonin-responsive pathways; however, this finding should be interpreted cautiously. The present data do not establish a direct causal relationship between MT2 modulation and neuroprotection, nor do they demonstrate circadian regulation at the organismal level. Instead, these results suggest a potential intersection between Sangvichai and chronobiological signaling pathways that warrants further investigation in more physiologically relevant systems.

At the neuronal level, Sangvichai displayed a concentration-dependent response profile in SH-SY5Y cells, characterized by preserved viability within a defined non-toxic range and reduced viability at higher concentrations. Such biphasic effects are frequently observed with polyherbal formulations and likely reflect the balance between adaptive cellular responses and metabolic stress at supraphysiological exposures. Within the non-toxic range, Sangvichai significantly attenuated $A\beta_{42}$-induced cytotoxicity and partially normalized several molecular alterations associated with amyloid-related stress, including changes in tau phosphorylation status and the expression of APP- and mTOR-related signaling components. These observations align with current models of AD pathogenesis, in which amyloid toxicity, tau dysregulation, oxidative stress, and metabolic signaling are tightly interconnected rather than functioning as isolated pathways (Butterfield and Boyd-Kimball, 2018; Hampel *et al*., 2021).

Collectively, the findings of this study support the view that Sangvichai exerts its biological effects through coordinated modulation of multiple cellular processes relevant to AD, rather than through direct interference with a single pathogenic target. A conceptual multi-target framework is therefore proposed (Figure 4), illustrating how antioxidant, anti-inflammatory, cholinergic, circadian-associated, and Aβ-related protective activities may converge at the cellular level. Although the present work is limited to in vitro systems, it provides mechanistic support for the traditional use of Sangvichai and establishes a rational foundation for subsequent investigations. In particular, future studies incorporating systematic phytochemical characterization will be essential to identify and quantify the bioactive constituents responsible for the observed cellular effects, alongside pharmacokinetic assessment and in vivo validation to clarify translational relevance.

CONCLUSION

This study demonstrates that the traditional Thai polyherbal formula Sangvichai exerts coordinated antioxidant, anti-inflammatory, cholinergic-modulating, circadian-associated, and neuroprotective activity in in vitro cellular models relevant to Alzheimer's disease pathology. Rather than acting through a single molecular target, Sangvichai modulates multiple interconnected stress-responsive pathways, including oxidative imbalance, inflammatory signaling, and amyloid-β42-associated neuronal toxicity. Although limited to cellular systems, these findings provide mechanistic support for the traditional

use of Sangvichai and establish a rational foundation for further investigation. Future studies incorporating systematic phytochemical characterization will be essential to identify and standardize the bioactive constituents underlying these effects, together with advanced neuronal models and in vivo studies to clarify the translational relevance of Sangvichai.

ACKNOWLEDGEMENT

The authors extend their gratitude to Kasetsart University for providing the necessary equipment and facilities for this research. Additionally, the authors would like to acknowledge technical support from the members of the Laboratory of Protein Engineering and Protein Bioinformatics, Department of Biochemistry, Kasetsart University.

ABBREVIATIONS

Aβ42: Amyloid-beta 42; **AChE:** Acetylcholinesterase; **AD:** Alzheimer's Disease; **ANOVA:** Analysis of Variance; **APP:** Amyloid Precursor Protein; **cAMP:** Cyclic Adenosine Monophosphate; **DCF-DA:** 2',7'-Dichlorofluorescin Diacetate; **DMSO:** Dimethyl Sulfoxide; **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **LPS:** Lipopolysaccharide; **MTT:** 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; **MT2:** Melatonin Receptor 2; **NO:** Nitric Oxide; **PBS:** Phosphate-Buffered Saline; **PVDF:** Polyvinylidene Difluoride; **Rheb:** Ras Homolog Enriched in Brain; **ROS:** Reactive Oxygen Species; **SD:** Standard Deviation; **SDS-PAGE:** Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis; **SEM:** Standard Error of the Mean.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FINANCIAL SUPPORT AND SPONSORSHIP

This research was supported by the Kasetsart University Research and Development Institute (KURDI), Bangkok, Thailand [Grant No. SRU69 FF(KU)54.69].

AUTHOR CONTRIBUTIONS

LT- Concept, Data acquisition, Data analysis, Manuscript preparation, PS- Design, Definition of intellectual content, ST- Manuscript editing, Manuscript review, WP- Experimental studies, Data analysis, KT- Concept, Design, Definition of intellectual content, Statistical analysis, Manuscript review.

SUMMARY

Sangvichai, a traditional Thai polyherbal formula, was evaluated for its potential neuroprotective effects against Alzheimer's Disease (AD) pathology. In vitro assays demonstrated that Sangvichai exhibits antioxidant activity by scavenging radicals and reducing intracellular ROS. It also showed anti-inflammatory effects by suppressing nitric oxide production and modulated cholinergic function via acetylcholinesterase inhibition. Furthermore, the extract activated Melatonin Receptor (MT2) signaling and protected neuronal cells from Amyloid-β42-induced toxicity. These findings suggest Sangvichai operates through a multi-target mechanism relevant to AD treatment.

REFERENCES

Ahmad, F., Sachdeva, P., Sarkar, J., and Izhaar, R. (2022). Circadian dysfunction and Alzheimer's disease: An updated review. Aging Medicine, 6(1), 71-81. https://doi.org/10.1002/agm2.12221

Arcusa, R., Villaño, D., Marhuenda, J., Cano, M., Cerdà, B., and Zafrilla, P. (2022). Potential role of ginger (Zingiber officinale Roscoe) in the prevention of neurodegenerative diseases. Frontiers in Nutrition, 9, 809621. https://doi.org/10.3389/fnut.2022.809621

Baranowska-Wójcik, E., Gajowniczek-Ałasa, D., Pawlikowska-Pawlęga, B., and Szwajgier, D. (2025). The potential role of phytochemicals in Alzheimer's disease. Nutrients, 17(4), 653. https://doi.org/10.3390/nu17040653

Breijyeh, Z., and Karaman, R. (2020). Comprehensive review on Alzheimer's disease: Causes and treatment. Molecules, 25(24), 5789. https://doi.org/10.3390/molecules25245789

Butterfield, D. A., and Boyd-Kimball, D. (2018). Oxidative stress, amyloid-β peptide, and altered key molecular pathways in the pathogenesis and progression of Alzheimer's disease. Journal of Alzheimer's Disease, 62(3), 1345-1367. https://doi.org/10.3233/JAD-170543

Chakraborty, B., Mukerjee, N., Maitra, S., Zehravi, M., Mukherjee, D., Ghosh, A., Massoud, E. E. S., and Rahman, M. H. (2022). Therapeutic potential of different natural products for the treatment of Alzheimer's disease. Oxidative Medicine and Cellular Longevity, 2022, 6873874. https://doi.org/10.1155/2022/6873874

Chen, S. Y., Gao, Y., Sun, J. Y., Meng, X. L., Yang, D., Fan, L. H., Xiang, L., and Wang, P. (2020). Traditional Chinese medicine: Role in reducing β-amyloid, apoptosis, autophagy, neuroinflammation, oxidative stress, and mitochondrial dysfunction of Alzheimer's disease. Frontiers in Pharmacology, 11, 497. https://doi.org/10.3389/fphar.2020.00497

Chiroma, S. M., Baharuldin, M. T. H., Mat Taib, C. N., Amom, Z., Jagadeesan, S., Ilham Adenan, M., Mahdi, O., and Moklas, M. A. M. (2019). Protective effects of Centella asiatica on cognitive deficits induced by D-gal/$AlCl_3$ via inhibition of oxidative stress and attenuation of acetylcholinesterase level. Toxics, 7(2), 19. https://doi.org/10.3390/toxics7020019

Cör Andrejč, D., Knez, Ž., and Knez Marevci, M. (2022). Antioxidant, antibacterial, antitumor, antifungal, antiviral, anti-inflammatory, and neuroprotective activity of Ganoderma lucidum: An overview. Frontiers in Pharmacology, 13, 934982. https://doi.org/10.3389/fphar.2022.934982

Fakorede, S., Lateef, O. M., Garuba, W. A., Akosile, P. O., Okon, D. A., and Aborode, A. T. (2025). Dual impact of neuroinflammation on cognitive and motor impairments in Alzheimer's disease. Journal of Alzheimer's Disease Reports, 9, 25424823251341870. https://doi.org/10.1177/25424823251341870

Ferreira, A., Sinjoanu, R. C., Nicholson, A., and Kleinschmidt, S. (2011). Aβ toxicity in primary cultured neurons. Methods in Molecular Biology, 670, 141-153. https://doi.org/10.1007/978-1-60761-744-0_11

Gulcin, İ., and Alwasel, S. H. (2023). DPPH radical scavenging assay. Processes, 11(8), 2248. https://doi.org/10.3390/pr11082248

Hampel, H., Hardy, J., Blennow, K., Chen, C., Perry, G., Kim, S. H., Villemagne, V. L., Aisen, P., Vendruscolo, M., Iwatsubo, T., Masters, C. L., Cho, M., Lannfelt, L., Cummings, J. L., and Vergallo, A. (2021). The amyloid-β pathway in Alzheimer's disease. Molecular Psychiatry, 26(10), 5481-5503. https://doi.org/10.1038/s41380-021-01249-0

Jeremic, D., Jiménez-Díaz, L., and Navarro-López, J. D. (2021). Past, present and future of therapeutic strategies against amyloid-β peptides in Alzheimer's disease: A systematic review. Ageing Research Reviews, 72, 101496. https://doi.org/10.1016/j.arr.2021.101496

Khairinisa, M. A., Fakih, T. M., and Ramadhan, D. S. F. (2025). Bioactive potential of Centella asiatica leaf extract in acetylcholinesterase (AChE) inhibition: Insights from multiple ligand mapping. Computers in Biology and Medicine, 197(Pt A), 110970. https://doi.org/10.1016/j.compbiomed.2025.110970

Kiraly, M., Foss, J. F., and Giordano, T. (2023). Neuroinflammation, its role in Alzheimer's disease and therapeutic strategies. Journal of Prevention of Alzheimer's Disease, 10(4), 686-698. https://doi.org/10.14283/jpad.2023.109

Kołodziejska, R., Woźniak, A., Bilski, R., Wesołowski, R., Kupczyk, D., Porzych, M., Wróblewska, W., and Pawluk, H. (2025). Melatonin—A powerful antioxidant in neurodegenerative diseases. Antioxidants, 14(7), 819. https://doi.org/10.3390/antiox14070819

Long, J. M., and Holtzman, D. M. (2019). Alzheimer disease: An update on pathobiology and treatment strategies. Cell, 179(2), 312-339. https://doi.org/10.1016/j.cell.2019.09.001

Ngo, V., and Duennwald, M. L. (2022). Nrf2 and oxidative stress: A general overview of mechanisms and implications in human disease. Antioxidants, 11(12), 2345. https://doi.org/10.3390/antiox11122345

Oh, K. N., Bae, D., Oh, D. R., Hong, J. A., Kim, Y., Kim, E., Son, S. A., Lee, K. Y., and Kim, S. (2025). TLR4/NF-κB-mediated anti-inflammatory and cognitive protective actions

of Curcuma longa and Ar-turmerone in Alzheimer's disease models. Journal of Microbiology and Biotechnology, 35, e2506044. https://doi.org/10.4014/jmb.2506.06044

Prajapat, M., Kaur, G., Choudhary, G., Pahwa, P., Bansal, S., Joshi, R., Batra, G., Mishra, A., Singla, R., Kaur, H., Prabha, P. K., Patel, A. P., and Medhi, B. (2023). A systematic review for the development of Alzheimer's disease in in vitro models: A focus on different inducing agents. Frontiers in Aging Neuroscience, 15, 1296919. https://doi.org/10.3389/fnagi.2023.1296919

Ruangritchankul, S., Chantharit, P., Srisuma, S., and Gray, L. C. (2021). Adverse drug reactions of acetylcholinesterase inhibitors in older people living with dementia: A comprehensive literature review. Therapeutics and Clinical Risk Management, 17, 927-949. https://doi.org/10.2147/TCRM.S323387

Selkoe, D. J., and Hardy, J. (2016). The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Molecular Medicine, 8(6), 595-608. https://doi.org/10.15252/emmm.201606210

Shoukat, S., Zia, M. A., Uzair, M., Attia, K. A., Abushady, A. M., Fiaz, S., Ali, S., Yang, S. H., and Ali, G. M. (2023). Bacopa monnieri: A promising herbal approach for neurodegenerative disease treatment supported by in silico and in vitro research. Heliyon, 9(11), e21161. https://doi.org/10.1016/j.heliyon.2023.e21161

Soria Lopez, J. A., González, H. M., and Léger, G. C. (2019). Alzheimer's disease. Handbook of Clinical Neurology, 167, 231-255. https://doi.org/10.1016/B978-0-12-804766-8.00013-3

Tamagno, E., Guglielmotto, M., Vasciaveo, V., and Tabaton, M. (2021). Oxidative stress and beta amyloid in Alzheimer's disease: Which comes first—the chicken or the egg? Antioxidants, 10(9), 1479. https://doi.org/10.3390/antiox10091479

Tiwari, S., Atluri, V., Kaushik, A., Yndart, A., and Nair, M. (2019). Alzheimer's disease: Pathogenesis, diagnostics, and therapeutics. International Journal of Nanomedicine, 14, 5541-5554. https://doi.org/10.2147/IJN.S200490

Vecchio, I., Sorrentino, L., Paoletti, A., Marra, R., and Arbitrio, M. (2021). The state of the art on acetylcholinesterase inhibitors in the treatment of Alzheimer's disease. Journal of Central Nervous System Disease, 13, 11795735211029113. https://doi.org/10.1177/11795735211029113

Xin, M., Bi, F., Wang, C., Huang, Y., Xu, Y., Liang, S., Cai, T., Xu, X., Dong, L., Li, T., Wang, X., Fang, Y., Xu, Z., Wang, M., Song, X., Zheng, Y., Sun, W., and Li, L. (2025). The circadian rhythm: A new target of natural products that can protect against diseases of the metabolic system, cardiovascular system, and nervous system. Journal of Advanced Research, 69, 495-514. https://doi.org/10.1016/j.jare.2024.04.005

Zuliani, G., Zuin, M., Romagnoli, T., Scarpa, C., and Volpato, S. (2024). Acetyl-cholinesterase inhibitors reconsidered: A narrative review of post-marketing studies on Alzheimer's disease. Aging Clinical and Experimental Research, 36(1), 23. https://doi.org/10.1007/s40520-023-02675-6

Cite this article: Tabtimmai L, Srathong P, Talawat S, Premsang W, Choowongkomon K. Multi-Target Neuroprotective Activity of the Traditional Thai Polyherbal Formula Sangvichai against Amyloid-β42-Induced Cellular Stress: An *in vitro* Study. Pharmacog Res. 2026;18(4):1395-404.

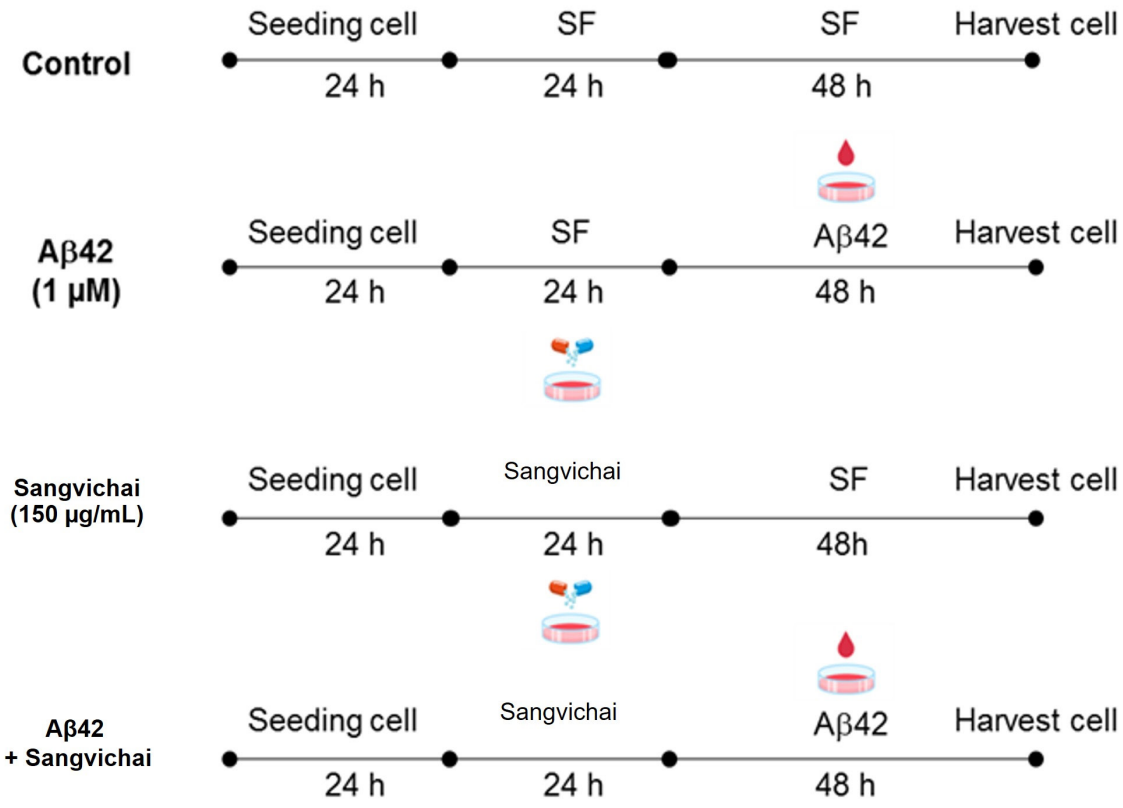

Figure S1: Experimental design for evaluation of neuroprotective activity against Aβ42-induced cytotoxicity. (SF, serum-free medium)

Table S1: List of 40 herbal ingredients of the Sangvichai formula.

Sl. No.	Ingredients	Scientific Name	Quantity
1.	Oat beta glucan	*Avena sativa*	0.03 mg
2.	Cordyceps powder	*Cordyceps sinensis*	0.03 mg
3.	Licorice powder	*Glycyrrhiza glabra*	0.03 mg
4.	Garlic powder	*Allium sativum*	0.03 mg
5.	Finger root powder	*Boesenbergia rotunda*	0.03 mg
6.	Ginger powder	*Zingiber officinale*	0.03 mg
7.	Black galingale powder	*Kaempferia parviflora*	0.03 mg
8.	Galangal powder	*Alpinia galanga*	0.03 mg
9.	Coriander powder	*Coriandrum sativum*	0.03 mg
10.	Green tea powder	*Camellia sinensis*	0.03 mg
11.	Celery powder	*Apium graveolens*	0.03 mg
12.	Jujube powder	*Ziziphus jujuba*	0.03 mg
13.	Pennywort powder	*Centella asiatica*	0.03 mg
14.	Cinnamon powder	*Cinnamomum verum*	0.03 mg
15.	Bael fruit powder	*Aegle marmelos*	0.03 mg

16.	Curcumin powder	*Curcuma longa*	0.03 mg
17.	Indian gooseberry powder	*Phyllanthus emblica*	0.03 mg
18.	San qi powder	*Panax notoginseng*	0.03 mg
19.	Kitchen mint powder	*Mentha cordifolia*	0.03 mg
20.	Neem leaf powder	*Azadirachta indica*	0.03 mg
21.	Maitake powder	*Grifola frondosa*	0.02 mg
22.	Shiitake mushroom powder	*Lentinula edodes*	0.02 mg
23.	Reishi mushroom powder	*Ganoderma lucidum*	0.02 mg
24.	Gac powder	*Momordica cochinchinensis*	0.02 mg
25.	Chinese olive powder	*Canarium album*	0.02 mg
26.	Dong quai powder	*Angelica sinensis*	0.02 mg
27.	Spirulina powder	*Arthrospira platensis*	0.02 mg
28.	Ear of rock powder	*Auricularia heimuer*	0.02 mg
29.	Astragalus powder	*Astragalus membranaceus*	0.02 mg
30.	Brahmi powder	*Bacopa monnieri*	0.02 mg
31.	Lime powder	*Citrus aurantifolia*	0.02 mg
32.	Wakame powder	*Undaria pinnatifida*	0.02 mg
33.	Terminalia powder	*Terminalia chebula*	0.02 mg
34.	Dang shen powder	*Codonopsis pilosula*	0.02 mg
35.	Gymnema powder	*Gymnema sylvestre*	0.02 mg
36.	Lotus powder	*Nelumbo nucifera*	0.02 mg
37.	Safflower powder	*Carthamus tinctorius*	0.02 mg
38.	Saffron powder	*Crocus sativus*	0.02 mg
39.	Korean ginseng powder	*Panax ginseng*	0.02 mg
40.	American ginseng powder	*Panax quinquefolius*	0.02 mg