Natural Resources for Human Health

Regulating Effect of Phenolic Contents-abundant Plants on Acetylcholinesterase Enzyme Activity in Undifferentiated and Differentiated Human Neuroblastoma Cell Line (SHSY5Y)

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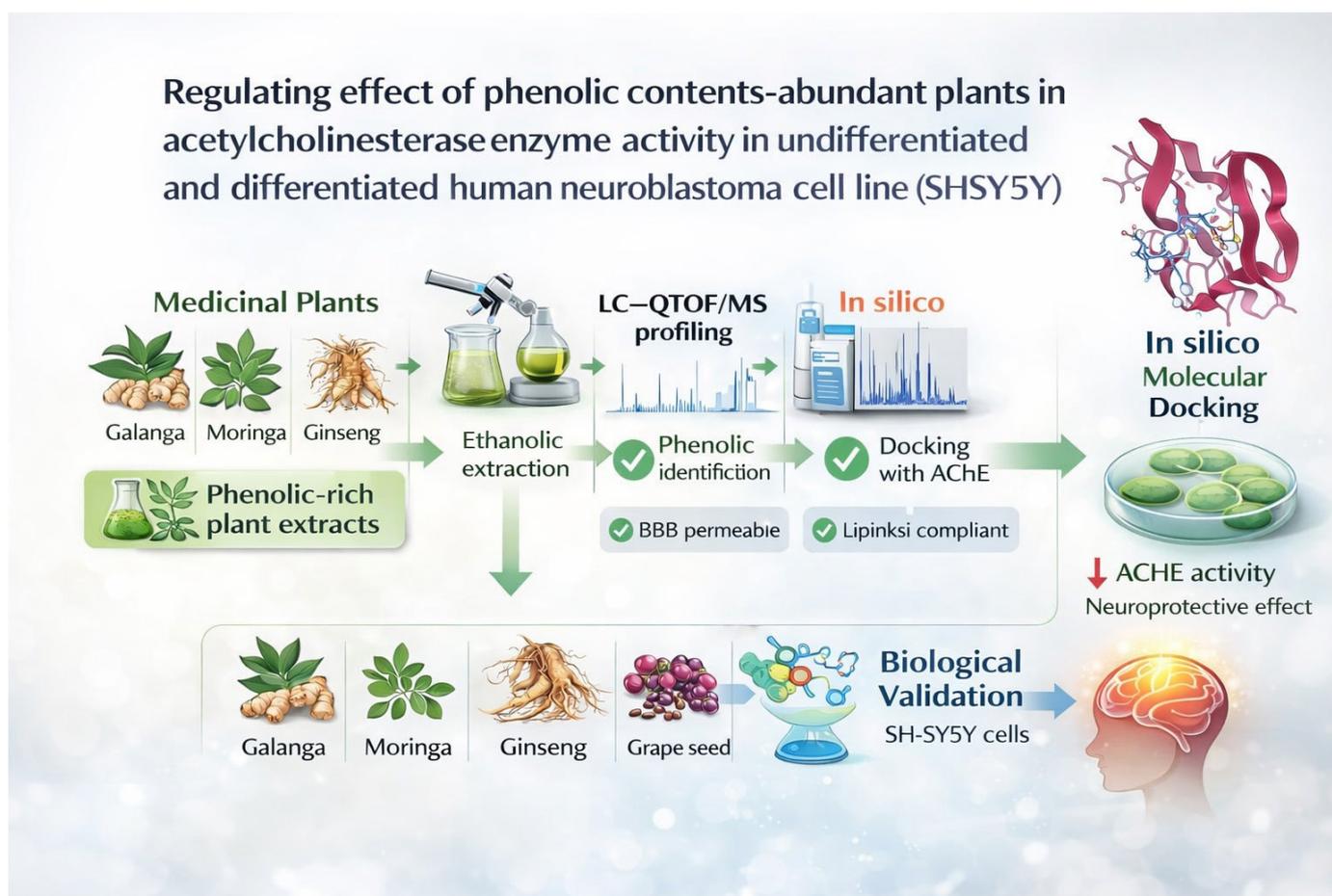[7]Biotechnology, Khon Kaen University, Thailand

ABSTRACT: This study was aimed to detect the biological activity of *Alpinia galanga* rhizomes (ARE), *Moringa oleifera* leaves (MLE), *A. galanga* leaves (ALE), *Panax ginseng* leaves (PLE), *P. ginseng* rhizomes (PRE), and *Vitis vinifera* seeds (VSE) ethanolic extracts as acetylcholinesterase (AChE) inhibitors in both undifferentiated (undiff.) and differentiated (diff.) human neuroblastoma cell lines (SHSY5Y). AChE activity was estimated by Ellman's colorimetric method. The bioactive compounds involved in the extract associated with impact on AChE activity were revealed by liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC–QTOF/MS). As a result, AChE inhibition was determined by ARE [PYL] (0.040 ± 0.026 mg/mL for undiff. cells and 0.048 ± 0.030 mg/mL for diff. cells), followed by MLE (0.150 ± 0.08 mg/mL for undiff. cells and 0.339 ± 0.020 mg/mL for diff. cells) when treated by MLE. The phytochemical analysis by LC–QTOF/MS identified abundant phenolic compounds, namely dephospho-CoA, nimodipine, and embelin, among the 40 compounds present in MLE, which have beneficial effects on AChE enzyme activity in undifferentiated and differentiated SHSY5Y cells. Furthermore, the compounds were estimated for the physicochemical and pharmacokinetic properties as well as molecular docking of ligand molecules and protein target prediction by freely accessible web tools, such as the PubChem database, SwissADME, and SwissDock.

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GRAPHICAL ABSTRACT

Regulating effect of phenolic contents-abundant plants in acetylcholinesterase enzyme activity in undifferentiated and differentiated human neuroblastoma cell line (SHSY5Y)

1. INTRODUCTION

Neurotransmitters that facilitate neurotransmission are endogenous chemical messengers. These neurotransmitters carry signals across the synapse (between the neuro-muscular junctions and neurons). Neurotransmitters are commonly stored in the synaptic vesicles, under the membrane in the axon terminal, and are secreted into the synapse with the proper signal (Kandimalla and Reddy, 2017). Neurotransmitters are synthesized in the brain based on the availability of appropriate precursors to neurons. The consumption of tyrosine or choline, under appropriate conditions, leads to the production of the neurotransmitters dopamine (DA) and acetylcholine (ACh) (Fernstrom, 1981).

ACh is a neurotransmitter produced from choline by the catalytic activity of choline acetyltransferase enzyme in a single-step reaction (Fernstrom, 1981). This neurotransmitter is terminated by the activity of the acetylcholinesterase enzyme (AChE). AChE is the key enzyme responsible for the hydrolysis of acetylcholine (ACh) to choline and acetate. Consequently, decreased expression and activity of this enzyme, leading to the accumulation of ACh, can improve numerous neurological disorders, mainly Alzheimer's disease (AD) (Garcimartín et al., 2017).

Researchers found that although DA synthesis deficiency (i.e., TH enzyme inhibition) is the main neurochemical feature in PD, a significant increase in AChE activity has been described and is associated with Parkinsonism (Pagano et al., 2015). Moreover, focusing on treating both cholinergic and dopaminergic deficiencies is more precise than targeting the dopaminergic pathway alone (Falkenburger, 2017). Talented, naturally derived plants that target both dopaminergic and cholinergic systems have not yet been reported. The identification of eco-friendly natural sources of active compounds that can induce TH enzyme synthesis and activity, and inhibit AChE activity in a safe, routine manner, will provide promising, novel, and safe treatment for neurodegenerative disorders.

Some of the phenolic content-abundant plants that are regularly utilized by some societies, particularly Asian, in their daily meals were selected to assess their effects and benefits as regulators of dopamine and acetylcholine

neurotransmitter formation enzymes. Phytoactive compounds that occur naturally in most plants are considered a rich source of eco-friendly, reliable, economical, and safe neurotransmitter-related enzyme regulators for popular consumers. In this study, the extracts of a group of active compound-abundant plants, *Moringa oleifera* leaves (MLE), rhizomes and leaves of *Alpinia galanga* (ARE and ALE), *Vitis vinifera* seeds (VSE), and rhizomes and leaves of *Panax ginseng* (PRE and PLE), were selected for detection of their abilities to regulate TH and AChE enzymes on a human neuroblastoma cell line (SHSY5Y). Furthermore, the bioactive compounds in the MLE associated with the ability to alter enzyme activities responsible for the synthesis of DA and the degradation of ACh were revealed by liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC–QTOF/MS). This is the first report on the determination of the role of *M. oleifera* cultivated in Thailand in AChE inhibition, as well as profiling the valued phenolic contents, mainly flavonoids. Recorded data could shed light on the additional investigative capabilities of plant-derived compounds in controlling the formation of neurotransmitter-forming enzymes. In addition, the effects of a single compound on AChE activity that are significant in controlling body function, mainly those related to neurodegenerative diseases such as AD and PD by an imbalance in AChE. Moreover, this study offers insights into the potential use of natural effective compounds such as dephospho-CoA, nimodipine, and embelin in the treatment of neuroblastoma. It emphasizes the structural and physicochemical or pharmacokinetic properties that influence compound activity, which is essential for the development of new therapeutic agents for neuroblastoma.

2. MATERIALS AND METHODS

2.1. Plant materials collection

The plant parts, leaves and rhizomes of *A. galanga* and *P. ginseng*, leaves of *M. oleifera*, and seeds of *V. vinifera* (grape) were selected as abundant sources of phenolic contents. Parts of *A. galanga* and *P. ginseng* were collected from the local gardens of Khon Kaen, Thailand. At the same time, the grape seeds were obtained from a winemaking factory (Nikki) located in Pak Chong, Nakorn Rachasima, Thailand. Selected plant parts were cleaned, dried at 65 °C using a hot air oven (Model FD240, Binder, Frankfurt, Germany), and crushed using a Philips mill (Model 600W, Eindhoven, Netherlands). The obtained powder was stored in screw-cap containers at room temperature away from sunlight until further handling of the samples.

2.2. Preparation of plant extracts

Prepared plant parts were extracted using ethanol (EOH) solvent in a ratio of 1:4, using a magnetic stirrer for 8 h. Each extract was then filtered using Whatman No. 1 filter paper (Camlab, Cambridge, UK). *V. vinifera* seeds (Vayupharp and Laksanalamai, 2012; Li et al., 2008), *M. oleifera* leaves (Sinagawa-García et al., 2013; Hashim et al., 2017), and rhizomes and leaves parts (Shin et al., 2017; Hwang et al., 2017) of *P. ginseng* were subjected to extraction using 70% ethanol, while *A. galanga* rhizomes and leaves were extracted using 95% ethanol (Lo et al., 2013; Chan and Lim, 2007). Each obtained extract was concentrated using a rotary evaporator (Model Heidolph VV2000, Heidolph Instruments GmbH, Schwabach, Germany). The ARE part contained 2 layers, one of which was a pale yellowish upper layer (PYL) with oily features, while the other was a brown lower layer (BL). The obtained extracts were kept separately in screw-capped vials at 2 °C.

2.3. Human neuroblastoma cell (SHSY5Y) cultures

The SHSY5Y cell line (human neuroblastoma) was provided by the Institute of Biotechnology and Genetic Engineering, Chulalongkorn University, Bangkok, Thailand. The DMEM/F-12 medium, consisting of Dulbecco's modified Eagle's medium (DMEM) and Ham's F-12 Nutrient Mixture at a ratio of 1:1, was used for culturing the neuroblastoma cells (SHSY5Y). The cells were developed in DMEM/F-12 medium supplemented with 10% heat-activated fetal bovine serum (FBS) (Thermo fisher scientific, Massachusetts, USA) and containing 0.1 mM nonessential amino acid, 1 mM sodium pyruvate, 1.5 g/L sodium bicarbonate, 100 U/mL penicillin, and 100 μg/mL streptomycin and incubated at 37°C with 85% humidified atmosphere containing 5% CO_2.
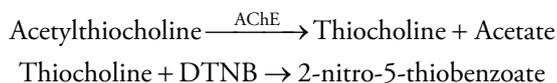
2.4. Differentiation of SHSY5Y cells

For the differentiation step, SHSY5Y cells were treated with 10 μM of retinoic acid (RA). The medium containing RA was changed daily for 5 days. The differentiated (diff. cells) and undifferentiated cells (undiff. cells) obtained were subjected to further analysis.

2.5. Determination of AChE activity

AChE activity was estimated by Ellman's colorimetric method (Ellman et al., 1961; Sun et al., 2017) based on the specific reaction of the Ellman's reagent (5, 5'-dithiobis

[2-nitrobenzoic acid] or DNTB) with the thiol groups in a sample (Thiocholine), according to the following reactions:

$$\text{Acetylthiocholine} \xrightarrow{\text{AChE}} \text{Thiocholine} + \text{Acetate}$$
$$\text{Thiocholine} + \text{DTNB} \rightarrow \text{2-nitro-5-thiobenzoate}$$

AChE is a serine hydrolase that breaks down and inactivates acetylthiocholine into acetate and thiocholine. Thiocholine is detected using a specific chemical (DNTB) that turns the 2-nitro-5-thiobenzoate (NTB) solution to yellow color. Hence, it could identify the absorbance of NTB and quantify the inhibition capability achieved by the plant extracts at different concentrations.

AChE activity was determined using a spectrophotometer in a 96-well microtiter plate. In this experiment, viable SHSY5Y cells were used as the AChE source. Undifferentiated and differentiated cells (2×10^4 cells/well) were cultured in a 96-well plate, incubated for 24 h, and then pretreated with plant extracts (0.05, 0.1, 0.25, 0.5, and 1 mg/mL) for 1 h. Supernatants of SHSY5Y cells in each well were eliminated and replaced by DMEM/F12 medium containing 1 mM H_2O_2 to induce AChE. The negative control consisted of cells incubated with medium only, while the positive control contained cells incubated with 1 mM H_2O_2 in medium. SHSY5Y cells were further incubated at 37 °C with 5% CO_2 conditions for 3 h, washed with ice-cold DPBS thrice, and incubated at room temperature with 0.2 mL of reaction mixture containing 0.5 mM DTNB and 1.0 mM acetylthiocholine. The optical density of the product TNB was recorded at a wavelength of 410 nm. The percentage inhibition of plant extract compared with the negative control considered as AChE activity 100%) was calculated using the following equation:

$$\text{Inhibition } \% = \frac{\text{Ab. of control cells} - \text{Ab. of treated cells}}{\text{Ab. of control cells}} \times 100$$

The per cent inhibition plot versus sample concentration. was applied to calculate the extract concentration that inhibit 50% of AChE (IC_{50}).

2.6. MLE analysis by Liquid Chromatography–Quadrupole Time-of-Flight Mass Spectrometer (LC–QTOF/MS)

The Moringa leaves extract (MLE) sample was prepared using 50% methanol in a concentration of 101.23 mg/mL, ultrasonicated for 20 min, and centrifuged at 10,000 rpm at 4 °C for 10 min. The supernatant was filtered using a 0.2 μm nylon membrane and subjected to analysis by LC– QTOF/MS (1290 Infinity II LC-6545 Quadrupole-TOF, Agilent Technologies, Santa Clara, CA, USA). The LC system used for analysis consisted of a dual pump and an online vacuum degasser connected to a binary AJS ESI source mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Full-scan mode was used from m/z 100 to 1700. Zorbax Eclipse Plus column (Agilent Technologies, Santa Clara, CA, USA) (C18 2.1 × 150 mm, 1.8 μm) was used for the analysis. Formic acid 0.1% in distilled water (solvent A) and 100% acetonitrile (solvent B) were adopted as the mobile phase-gradient elution as follows: 98% A, 0–2 min; 90% A, 2–25 min; 85% A, 25–40 min; 80% A, 40–48 min; 75% A, 48–68 min; 70% A, 68–80 min; 50% A, 80–85 min; 0% A, 85–90 min; 98% A, 90–100 min. Peaks were identified at wavelengths 254 and 280 nm. The MS spectra were acquired in both positive and negative ion modes, auto MS/MS. The mass fragmentations were identified using the spectrum database for organic compounds in the METLIN database (a cost-free, accessible, web-based data source applied to ease identification of a wide range of metabolite compounds through mass detection).

2.7. Molecular docking and in silico study of bioactive compounds

This investigation predicted and confirmed the efficacy of selected compounds, such as dephospho-CoA, nimodipine, and embelin, which are ligand molecules, obtained from MLE, against Alzheimer's disease. Their different SMILES structures are shown in Table 1. In addition, this study used the protein active site targets, including amyloid-ß (Aß) proteins of (1–40) fibril derived from Alzheimer's disease cortical tissue (PDB ID: 6W0O), amyloid precursor protein (APP) from crystal structure of amyloid precursor-like protein 2 (APLP2) E2 domain (PDB ID: 5TPT), and phosphorylated tau (P-tau) proteins from crystal structure of human anti-tau antibody CBTAU-24.1 in complex with its phosphorylated tau peptide (PDB ID: 5ZIA). Molecular docking prediction was performed by ligand molecule binding with the active site on protein target structures using the SwissDock web server (https://www.swiss-dock.ch/) through AutoDock Vina platform (Bugnon et al., 2024 ; Phupaboon et al., 2025; Punyauppa-Path et al., 2025).

For in silico evaluation, the physicochemical and pharmacokinetic properties of dephospho-CoA, nimodipine, and embelin in SMILES structures were entered into SwissADME web tool (http://www.swissadme.ch/) for assessing the molecular weight (MW), lipophilicity (Consensus Log Po/w), water solubility (Log S), gastrointestinal absorption, BBB permeation, and drug likeness by Lipinski (Phupaboon et al., 2025).

2.8. Statistical analysis

In this study, tests were conducted in triplicate (n = 3), while data were obtained as mean ± SD values. Analysis of variance

(ANOVA) was applied to assess the differences among the treatments by Duncan's multiple range tests (DMRTs) with a p-value of 0.05 using SPSS Statistics Base version 19 program for Windows (IBM Corp, Chicago, IL, USA).

3. RESULTS AND DISCUSSION

3.1. Detection of the effect of plant extracts on AChE activity

Using AChE inhibitors is one of the main strategies for controlling the range of acetylcholine in the brain depleted in AD. In the present study, four different plants were assessed as natural AChE inhibitors. Enzyme activity determination was achieved based on the specific reaction of Ellman's reagent. Plant extracts were investigated for AChE inhibition activity in SHSY5Y (both undiff. and diff. cells), and the results are provided in Figure 1. The results revealed that ARE (PYL) gave the best IC50 (0.040 ± 0.026 mg/mL for undiff. cells and 0.048 ± 0.03 mg/mL for diff. cells), followed by MLE (0.150 ± 0.08 mg/mL for undiff. cells and 0.339 ± 0.02 mg/mL for diff. cells). The findings regarding the inhibition activity of ARE (PYL) were consistent with the results obtained by Adewusi et al. (Adewusi, Moodley and Steenkamp, 2010). The AChE inhibition potency of ARE may be described by the presence of kaempferol-3-O-L-rhamnoside, which was reported as a treatment for AD (Murata et al., 2017; Khairullah et al., 2020). Anti-AChE activity of PRE (0.281 ± 0.04 for undiff. cells and 0.444±0.10 for diff. cells) showed that the inhibitory effect of PRE in diff. cells is significantly lower than their effect on undifferentiated cells. Previous studies reported that the complex metabolism of ginsenosides (main compounds in the ginseng plant), when administered orally, causes low bioavailability, which is considered the main limitation of using ginseng extracts as AChE inhibitors (Kawamoto, Takeshita and Murata, 2019). Furthermore, the study by Ong et al., 2015 in the streptozotocin-induced memory-damaged rat brain models found that the loss of brain choline was significantly reduced by the reduction of AChE activity after intake of the ginseng plant.

The AChE inhibitory activities of MLE significantly correlated with the richness of phenolic compounds (Nwidu et al., 2018); meanwhile, our previous study showed that MLE has a neuroprotective effect against oxidative stress in SHSY5Y cells (Hashim et al., 2021). Phytoconstituent analysis by LC–QTOF/MS represented 40 bioactive compounds out of 1080 compounds, previously tested by different researchers and reported as active agents against Alzheimer's disease. Taken together, MLE is interesting as a potential inhibitor agent with further applications due to its low cytotoxicity and high anti-AChE activities. LC–QTOF/MS analysis (Figure 2, Table 2) of MLE, in the current study, was confirmed by the fragmentation MS spectra (see Supplementary Materials Figure S1).

It is also possible that these plant extracts contain several anti-AChE or the same inhibitor compound(s) but at different concentrations, which leads to different activities. We found that differentiated SHSY5Y cells are more resistant to the anti-AChE effect of plant extracts than undifferentiated cells, as shown in the levels of IC50 of plant extracts.
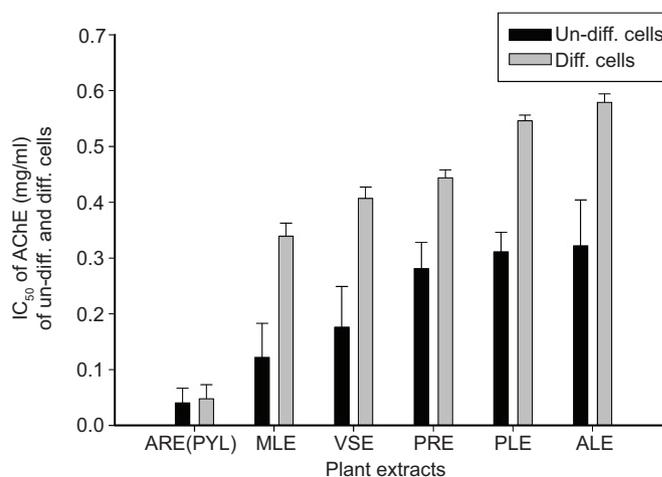

Figure 1. IC_{50} of various plant extracts (MLE, VSE, PLE, PRE ALE, and ARE [PYL]) on AchE in SHSY5Y (both un-diff. and diff.) cells

Table 1

The formula and SMILES structures of dephospho-CoA, nimodipine, and embelin obtained from the PubChem database.

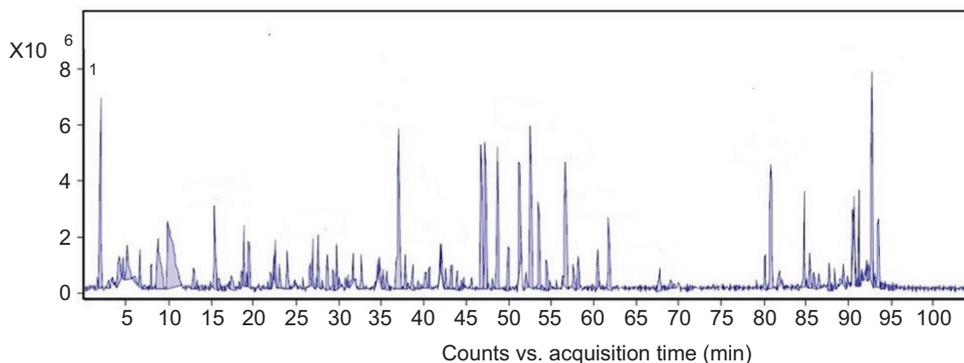| Compounds	Formula	SMILES structures
Dephospho-CoA	$C_{21}H_{35}N_7O_{13}P_2S$	CC(C)(COP(=O)(O)OP(=O)(O)OC[C@@H]1[C@H]([C@H]([C@@H](O$_1$)N$_2$C=NC$_3$=C(N=CN=C$_{32}$)N)O)O)[C@H](C(=O)NCCC(=O)NCCS)O
Nimodipine	$C_{21}H_{26}N_2O_7$	CC1=C(C(C(=C(N1)C)C(=O)OC(C)C)C$_2$=CC(=CC=C2)[N+](=O)[O-])C(=O)OCCOC
Embelin	$C_{17}H_{26}O_4$	CCCCCCCCCCCC$_1$=C(C(=O)C=C(C$_1$=O)O)O

Figure 2. LC–QTOF/MS chromatogram of *M. oleifera* ethanol extract (MLE).

Table 2

List of phenolic composition identified in MLE by LC–QTOF/MS analysis.

Compound	Molecular formula	Retention time (min)	Candidate mass	Effect	References
Isorhamnetin	$C_{16}H_{12}O_7$	83.499	315.05	• inhibition of AchE activity	(Olennikov et al., 2017)
Quercetin	$C_{15}H_{10}O_7$	48.551	303.04	• inhibition of AchE	(Ademosun et al., 2016)
Apigenin	$C_{15}H_{10}O_5$	79.602	269.04	• inhibition of AchE activity • decreasing of β-Amyloid fibrils formation • reduction of AChE- Amyloid interaction	(Álvarez-Berbel et al., 2022)
Luteolin 7-(6‴-acetyl allosyl-(1->2)-glucoside)	$C_{29}H_{32}O_{17}$	37.044	651.16	• inhibition of AChE activity	(Sezen Karaoğlan et al., 2023)
Hesperetin	$C_{16}H_{14}O_6$	79.820	301.07	• inhibition of AChE activity • inhibition of β-Amyloid aggregation	(Li et al., 2017)
Hydroxy tyrosol 1-O-glucoside (HT)	$C_{14}H_{20}O_8$	13.423	315.10	• inhibition of β-Amyloid aggregation	(Romero-Márquez et al., 2022)
Kaempferol	$C_{15}H_{10}O_6$	35.262	287.05	• inhibits AChE activity	(Shi et al., 2023)
hydroquinone	$C_6H_6O_2$	14.005	110.0368	• inhibits AChE activity	(Scozzafava et al., 2015)
Chlorogenic Acid	$C_{16}H_{18}O_9$	18.834	354.0949	• suppression of Aβ aggregation caused cellular injury in SHSY5Y cells • inhibits Aβ1–42 self-induced aggregation in PC12 cells	(Nguyen et al., 2024)
Ferulic Acid	$C_{10}H_{10}O_4$	27.61	194.057	• inhibition of AChE activity • reducing amyloid beta plaque formation	(Mugundhan et al., 2024)
Geniposide	$C_{17}H_{24}O_{10}$	36.056	387.129	• Geniposide cerium oxide • Nanoparticles decrease the severity of Aβ1–42 fibril formation	(Pérez Gutiérrez et al., 2024)
Inosine	$C_{10}H_{12}N_4O_5$	8.017	268.080	• suppress the rise of acetylcholinesterase activity	(Basile, Bramanti and Mazzon, 2022)
Quinic acid	$C_7H_{12}O_6$	18.884	192.063	• AChE inhibitory activities	(Baysal et al., 2019)
Rutin	$C_{27}H_{30}O_{16}$	35.299	610.153	• reduce the AChE in all parts of the rat brain	(Naddafi, Mohammadi and Karami, 2021)
L-Threonate	$C_4H_8O_5$	2.05	135.036	• inhibition of AChE activities	(Gangoda et al., 2023)
Tirandamycin	$C_{22}H_{27}NO_7$	18.508	417.1804	• decreasing the cytotoxicity of Aβ	(Duangupama et al., 2023)
Salidroside	$C_{14}H_{20}O_7$	20.98	300.120	• regulate neurotransmission • AChE inhibitory activities	(Zhong et al., 2018)
Folic acid	$C_{19}H_{19}N_7O_6$	64.017	441.140	• reduces the AChE activity in the heart and brain cells of rats without affecting the AChE levels in the blood.	(Čolović, 2009)
Caffeoylquinic acid	$C_{16}H_{18}O_9$	19.106	354.094	• AChE inhibitory activities	(Alcázar Magaña et al., 2021)
Piperic acid	$C_{12}H_{10}O_4$	46.987	218.057	• inhibition of AChE activity	(Azam et al., 2022)

(*continues*)

Table 2
Continued.

Compound	Molecular formula	Retention time (min)	Candidate mass	Effect	References
Lobeline	$C_{22}H_{27}NO_2$	84.935	337.204	• inhibition of AChE activity	(Remya et al., 2023)
Granisetron	$C_{18}H_{24}N_4O$	30.764	312.195	• reduced amyloid-β (Aβ) pathology and enhanced memory	(Al Rihani, Lan and Kaddoumi, 2019)
Ramipril glucuronide	$C_{29}H_{40}N_2O_{11}$	37.755	592.263	• reduction of reduced amyloid-β (Aβ)	(Wharton et al., 2012)
Crocin 3	$C_{32}H_{44}O_{14}$	59.614	652.273	• decrease the amyloid-β (Aβ) • decrease in AChE activity	(Yuan et al., 2020)
Cilostazol	$C_{20}H_{27}N_5O_2$	29.338	369.217	• promote Aβ clearance, inhibit Aβ oligomerization, and suppress Aβ-induced neurotoxicity	(Ono and Tsuji, 2019)
Aucubin	$C_{15}H_{22}O_9$	16.334	346.126	• deposition of Aβ plaques	(Wang et al., 2024)
3- Hydroxycapric	$C_{10}H_{20}O_3$	26.432	188.141	• decrease in AChE activity	(Budryn et al., 2022)
Astragalin	$C_{21}H_{20}O_{11}$	52.464	448.1008	• decrease in AChE activity	(Athipornchai, Ketpoo and Saeeng, 2020)
Ceanothine E	$C_{34}H_{40}N_4O_4$	45.690	568.307	• decrease in AChE activity	(Pastene-Burgos et al., 2024)
Salicylanilide	$C_{13}H_{11}NO_2$	64.054	213.078	• decrease in AChE activity	(Imramovsky et al., 2012)
Succinoadenosine	$C_{14}H_{17}N_5O_8$	17.058	383.107	• decrease in AChE activity	(Grodner et al., 2024)
naringenin-7-O-glucuronide	$C_{21}H_{20}O_{11}$	52.426	448.101	• significantly recovered A_-stimulated axonal atrophy in cultured cortical neurons	(Nouri et al., 2019)
Isovitexin	$C_{21}H_{20}O_{10}$	47.171	432.106	• decrease in AChE activity	(Hassan et al., 2021)
dephospho-CoA	$C_{21}H_{35}N_7O_{13}P_2S$	13.365	687.149	• precursors for synthesis of acetylcholine	(Banns, Hebb and Mann, 1977)
Nimodipine	$C_{21}H_{26}N_2O_7$	41.972	418.173	• induces acetylcholine synthesis and improves memory	(Levy et al., 1991)
Idebenone	$C_{15}H_{20}O_6$	35.069	296.125	• increase the level of acetylcholine (ACh) in rats	(Kakihana, Yamazaki and Nagaoka, 1989)
Aesculin	$C_{15}H_{16}O_9$	21.798	340.079	• has protective effects against Aβ-induced neurotoxicity	(Wang et al., 2022)
Cinnamic acid	$C_9H_8O_2$	3.619	148.052	• decrease in AChE activity	(Gießel et al., 2019)
Penicillamine disulfide	$C_{10}H_{20}N_2O_4$	13.03	341.08	• inhibition or reversal of Aβ aggregation by chelation of metal ions (as nanoparticles)	(Hadavi and Poot, 2016)
Embelin	$C_{17}H_{26}O_4$	88.746	294.183	• inhibition of AChE activity by binding to the active site of the enzyme	(Bhuvanendran et al., 2019)

The differences in AChE reduction between undifferentiated and differentiated cells may be explained in terms of AChE gene expression; differentiated cells with RA upregulated AChE levels compared to undiff. cells, which resulted in different amounts of enzyme exposure to the tested inhibitor agents (Filograna et al., 2015).

3.2. Molecular docking profiles of selected phenolic compounds

Based on the results in Figure 1, MLE had the highest IC_{50} values on AChE in SHSY5Y (both undiff. and diff.) cells, and a total of 40 compounds were identified by LC-MS/MS. The most striking result is that the three selected compounds of ligand molecules, such as dephospho-CoA (PubChem ID:

444485), nimodipine (PubChem ID: 4497), and embelin (PubChem ID: 3218), were evaluated for molecular docking with Alzheimer's protein targets: amyloid-ß (Aß) proteins (PDB ID: 6W0O), amyloid precursor protein (APP) proteins (PDB ID: 5TPT), and phosphorylated tau (P-tau) proteins (PDB ID: 5ZIA) (Table 3). The present findings indicate that the computed affinity scores for three ligands range from –1.837 to –38.782 kcal/moL when docked with three distinct Alzheimer's protein targets. In particular, the interaction of dephospho-CoA with phosphorylated tau (P-tau) proteins exhibited a notable affinity score of –38.782 kcal/moL. However, this result has not been described previously. Furthermore, the 3D molecular binding structures generated using the SwissDock tool, utilizing the AutoDock Vina platform, as illustrated in Figures 3A–3I, engage through

Table 3
Molecular docking analysis results of selected ligands in various active sites (protein PDB) of amyloid-ß (Aß) proteins, amyloid precursor protein (APP) proteins, and phosphorylated tau (P-tau) proteins.

| PubChem ID of ligands	Ligand names	Protein PDB ID	Protein targets	Calculated affinity (kcal/moL)
444485	Dephospho-CoA	6W0O	Amyloid-ß (Aß) proteins	–4.561
444485	Dephospho-CoA	5TPT	Amyloid precursor protein (APP) proteins	–5.535
444485	Dephospho-CoA	5ZIA	Phosphorylated tau (P-tau) proteins	–38.782
4497	Nimodipine	6W0O	Amyloid-ß (Aß) proteins	–4.038
4497	Nimodipine	5TPT	Amyloid precursor protein (APP) proteins	–5.469
4497	Nimodipine	5ZIA	Phosphorylated tau (P-tau) proteins	–1.837
3218	Embelin	6W0O	Amyloid-ß (Aß) proteins	–3.229
3218	Embelin	5TPT	Amyloid precursor protein (APP) proteins	–4.644
3218	Embelin	5ZIA	Phosphorylated tau (P-tau) proteins	–3.478
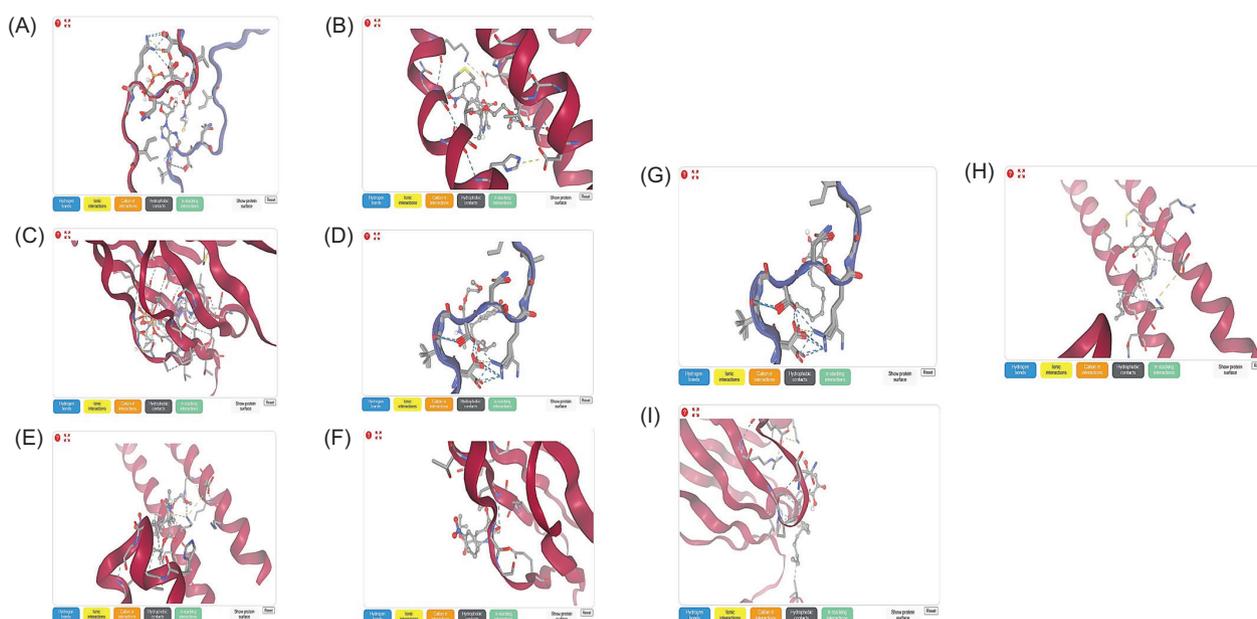
Figure 3. The 3D Molecular docking structures of selected ligands binding with various protein active sites, (A) dephospho-CoA + amyloid-ß (Aß) proteins; (B) dephospho-CoA + Amyloid precursor protein (APP) proteins; (C) dephospho-CoA + phosphorylated tau (P-tau) proteins; (D) nimodipine + amyloid-ß (Aß) proteins; (E) nimodipine + Amyloid precursor protein (APP) proteins; (F) nimodipine + phosphorylated tau (P-tau) proteins; (G) embelin + amyloid-ß (Aß) proteins; (H) embelin + Amyloid precursor protein (APP) proteins; (I) embelin + phosphorylated tau (P-tau) proteins.

hydrogen bonds, ionic bonds, cation contacts, hydrophobic interactions, and stacking interactions.

3.3. In silico properties of selected phenolic compounds

This study presents in silico, based on the physicochemical and pharmacokinetic properties obtained from the SwissADME server for three ligands—dephospho-CoA,

nimodipine, and embelin—focused on anti-Alzheimer's protein targets, emphasizing their physicochemical, pharmacokinetic, and drug-likeness properties (Table 4). The results indicate a molecular weight range of 294.39–687.55 g/moL, with high lipophilicity (consensus Log $P_{o/w}$) between 2.27 and 3.68, and water solubility (Log S) from –3.84 to –6.13. Furthermore, three compounds demonstrated significant gastrointestinal absorption, influencing blood–brain barrier (BBB) permeability and adhering to Lipinski's drug-likeness

Table 4

In silico profiles using the Swiss ADME server of three ligands based on anti-Alzheimer's protein targets related to physicochemical, pharmacokinetics, and drug-likeness properties.

Entry	ADME properties of compounds		
	Dephospho-CoA	**Nimodipine**	**Embelin**
MW (g/moL)	687.55	418.44	294.39
Lipophilicity (Consensus Log $P_{o/w}$)	2.67	2.27	3.68
Water solubility (Log S)	−6.13	−3.84	-4.42
Gastrointestinal absorption	High	High	High
BBB permeation	Yes	Yes	Yes
Lipinski	Yes	Yes	Yes

criteria. These findings align with other studies and indicate that the molecular weights of the selected compounds, ranging from 344.3 to 402.4 g/mol, exhibit a high likelihood of lipophilicity (consensus Log Po/w), water solubility, gastrointestinal absorption, and their placement in the yellow region (yolk) denotes a significant probability of brain penetration and access to the BBB (Rosa et al., 2025).

4. CONCLUSION

High phenolic content plant extracts are promising tools for novel treatment strategies targeting AChE in both undifferentiated and differentiated SHYSY5Y cells. In addition, they help in understanding the phytoextract-containing bioactive compounds of MLE to obtain an effective, reliable, and cheap drug for controlling neurodegenerative disorders, particularly Alzheimer's disease, as confirmed by molecular docking and physicochemical or pharmacokinetic properties. These motives may drive future studies involving dopaminergic neurons of SHSY5Y treated with *M. oleifera* purified fractions.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Farah J. Hashim: Writing and final approval of the artice; Srisan Phupaboon: Collection and/or assembly of data, analysis, and interpretation; Sukrita - Punyauppa-Path and Pongpat Kiatprasert: Data analysis and interpretation; Trai Wongsiri: Collection and/or assembly of data and writing of the article; Unchalee Thonsri: Critical revision of the article; Nattawadee Kanpipit: Collection and/or assembly of data; Sukanda Vichitphan and Kanit Vichitphan: Final approval of the article.

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