Supplementary information for the article entitled Exploring the Therapeutic Potential of Spilanthol from *Acmella paniculata* (Wall ex DC.) R. K. Jansen in Attenuating Neurodegenerative Diseases: A Multi-Faceted Approach Integrating *In-Silico* and *In-Vitro* Methodologies

Sanith Sri Jayashan [1,2], Nitchakan Darai [3], Thanyada Rungrotmongkol [4,5] , Peththa Wadu Dasuni Wasana[6], San Yoon Nwe [1,2], Wisuwat Thongphichai [1,2], Gunasekaran Suriyakala [1,2], Pasarapa Towiwat [7,8] and Suchada Sukrong [1,2]*

[1] Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand
[2] Center of Excellence in DNA Barcoding of Thai Medicinal Plants, Chulalongkorn University, Bangkok 10330, Thailand
[3] Futuristic Science Research Center, School of Science, Walailak University, Nakhon Si Thammarat, 80160, Thailand
[4] Center of Excellence in Biocatalyst and Sustainable Biotechnology, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand
[5] Program in Bioinformatics and Computational Biology, Graduate School, Chulalongkorn University, Bangkok, Thailand
[6] Department of Pharmacy, Faculty of Allied Health Sciences, University of Ruhuna, Galle 80000, Sri Lanka
[7] Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand
[8] Animal Models of Chronic Inflammation-associated Diseases for Drug Discovery Research Unit, Chulalongkorn University, Bangkok 10330, Thailand
* Correspondence: Suchada.su@chula.ac.th, Tel.: (66)218-8363

Table S1. *In-Silico* ADMET Prediction of spilanthol using pkCSM

Properties	**Model name (Unit)**	**Result**
Absorption	Water solubility (log mol/L)	-4.374
	Caco2 permeability (log Papp in 10− 6 cm/s)	1.368
	Intestinal absorption (human) (% absorbed)	92.81
	Skin Permeability (log Kp)	-1.923
	P-glycoprotein substrate	No
	P-glycoprotein I inhibitor	No
	P-glycoprotein II inhibitor	No
Distribution	VDss (human) (log L/kg)	0.096
	BBB permeability (log BB)	0.645
	CNS permeability (log PS)	-1.554
Metabolism	CYP2D6 substrate	No
	CYP3A4 substrate	No
	CYP2D6 inhibitor	No
	CYP3A4 inhibitor	No
Excretion	Total Clearance (log ml/min/kg)	0.583
	Renal OCT2 substrate	No

Table S2. Drug likeness prediction of spilanthol

Drug Likeness					
Lipinski	**Ghose**	**Veber**	**Egan**	**Muegge**	**Bioavailability Score**
Yes 0 violation	Yes	Yes	Yes	Yes	0.55

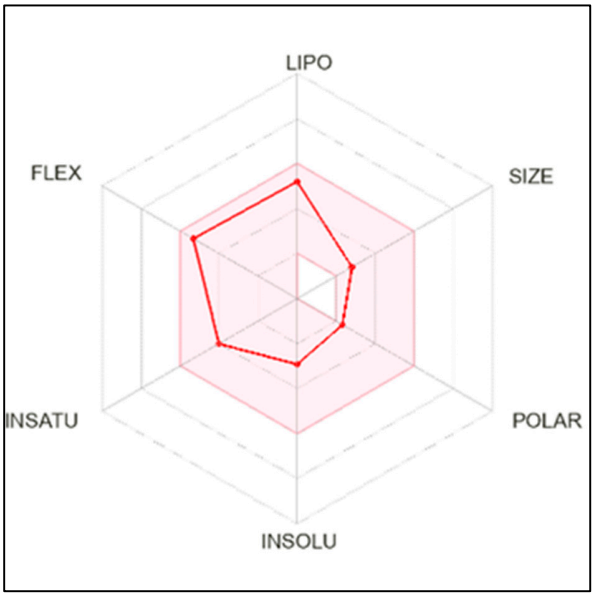

Figure S1. Bioavailability radar of the spilanthol

Table S3 *In-silico* Toxicity Prediction of Spilanthol using protox-II

Classification	**Target**	**Prediction**	**Probability**
Organ Toxicity	Hepatotoxicity	Inactive	0.86
Toxicity endpoint	Carcinogenicity	Inactive	0.61
	Immunotoxicity	Inactive	0.98
	Mutagenicity	Inactive	0.8
	Cytotoxicity	Inactive	0.75
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	Inactive	0.97
	Androgen Receptor (AR)	Inactive	0.99
	Androgen Receptor Ligand Binding Domain (AR-LBD)	Inactive	0.99
	Aromatase	Inactive	0.98
	Estrogen Receptor Alpha (ER)	Inactive	0.93
	Estrogen Receptor Ligand Binding Domain (ER-LBD)	Inactive	0.99

	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	Inactive	0.99
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	Inactive	0.98
	Heat shock factor response element (HSE)	Inactive	0.98
	Mitochondrial Membrane Potential (MMP)	Inactive	0.97
	Phosphoprotein (Tumor Supressor) p53	Inactive	0.99
	ATPase family AAA domain-containing protein 5 (ATAD5)	Inactive	0.99

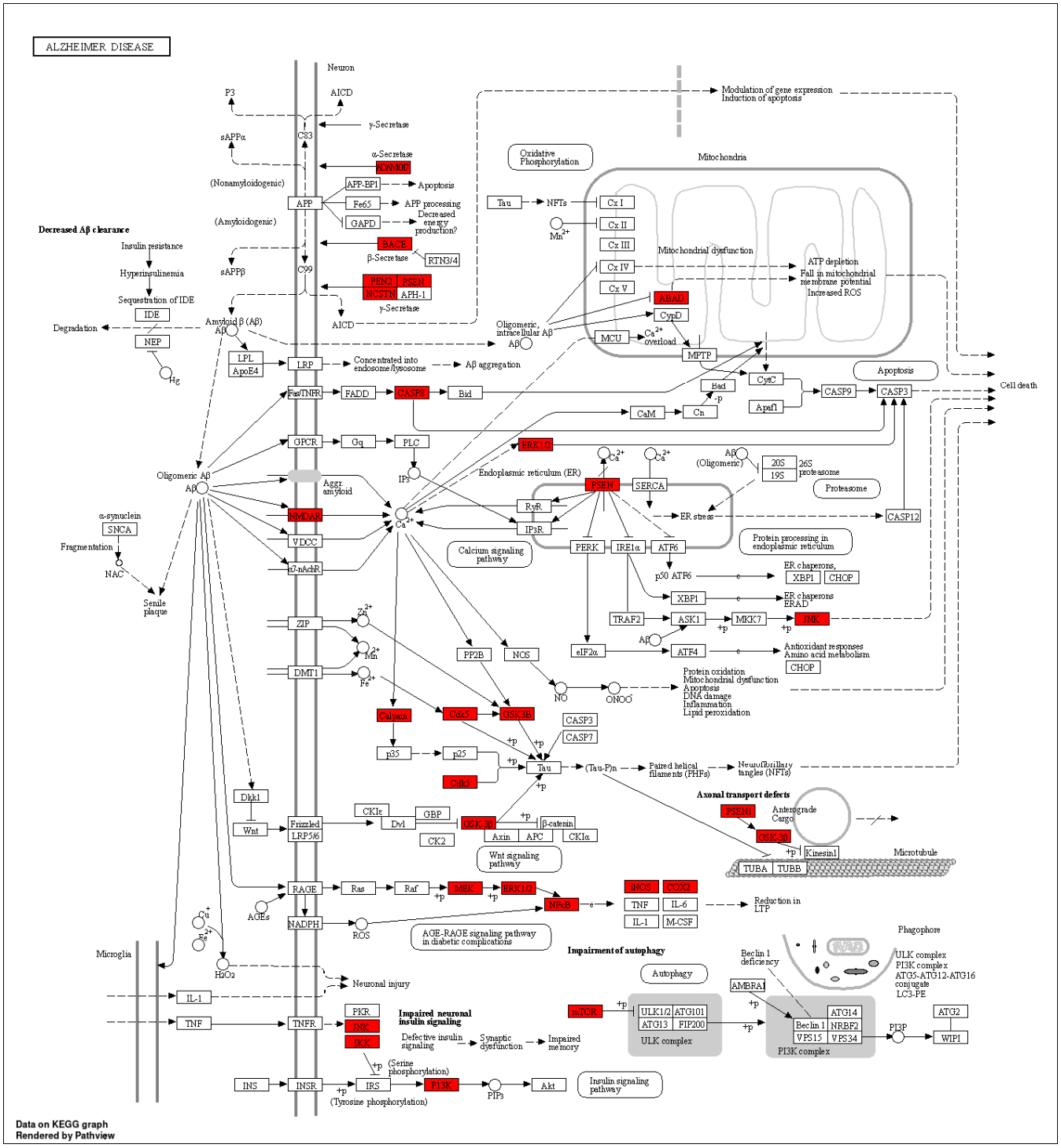

Figure S2. The distribution of genes in the Alzheimer's disease pathway. ⟶ represents the activation effect, T arrows represent the inhibition effect, and dashed lines represent activation or inhibition effects. The intersection genes between spilanthol and Alzheimer's disease pathway are highlighted in red.

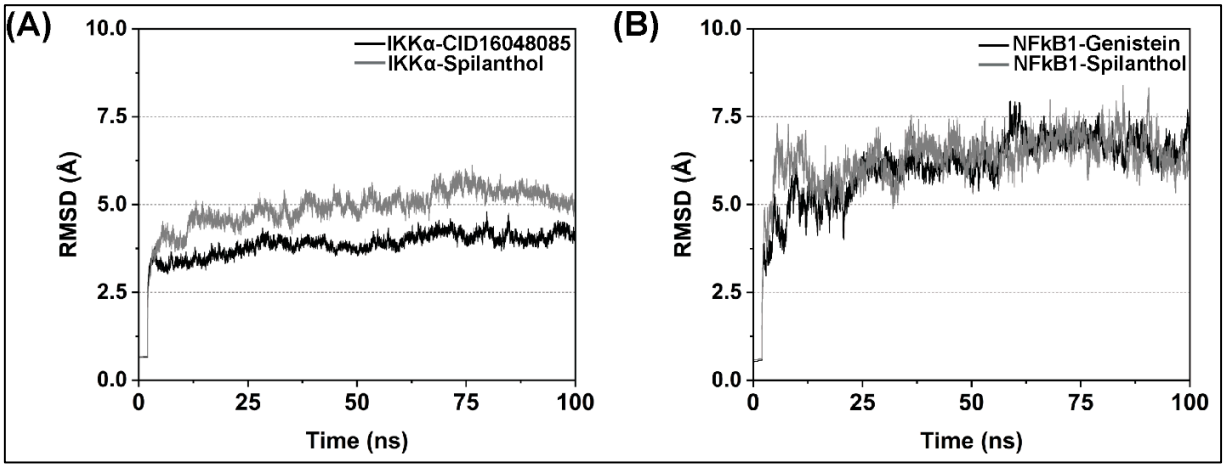

Figure S3. RMSD plots of all-atom residues for assessing structural stability and conformational changes in the molecular systems: IKKα and NFkB1

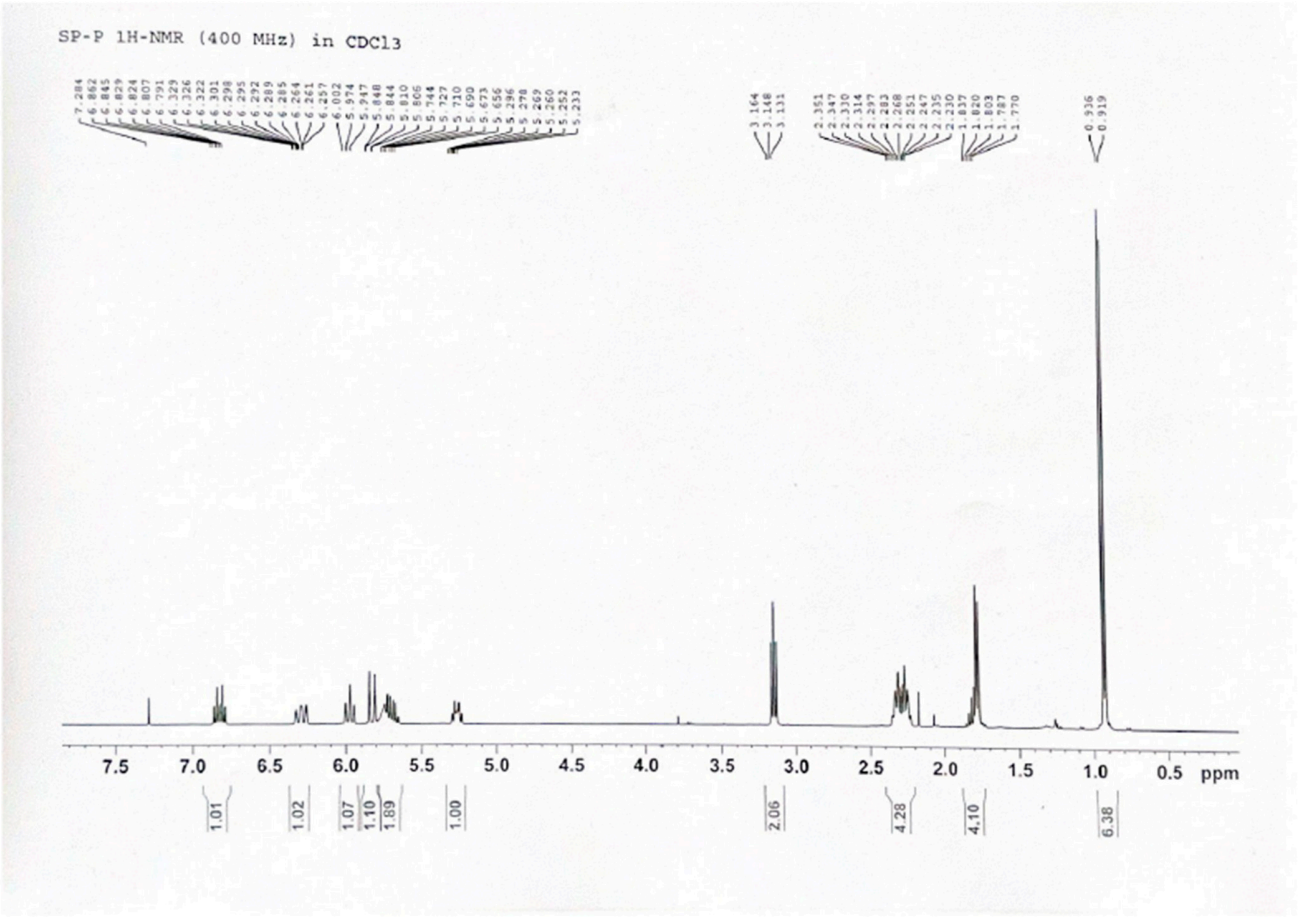

Figure S4. ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of spilanthol (ranging from 0.5 – 7.5)

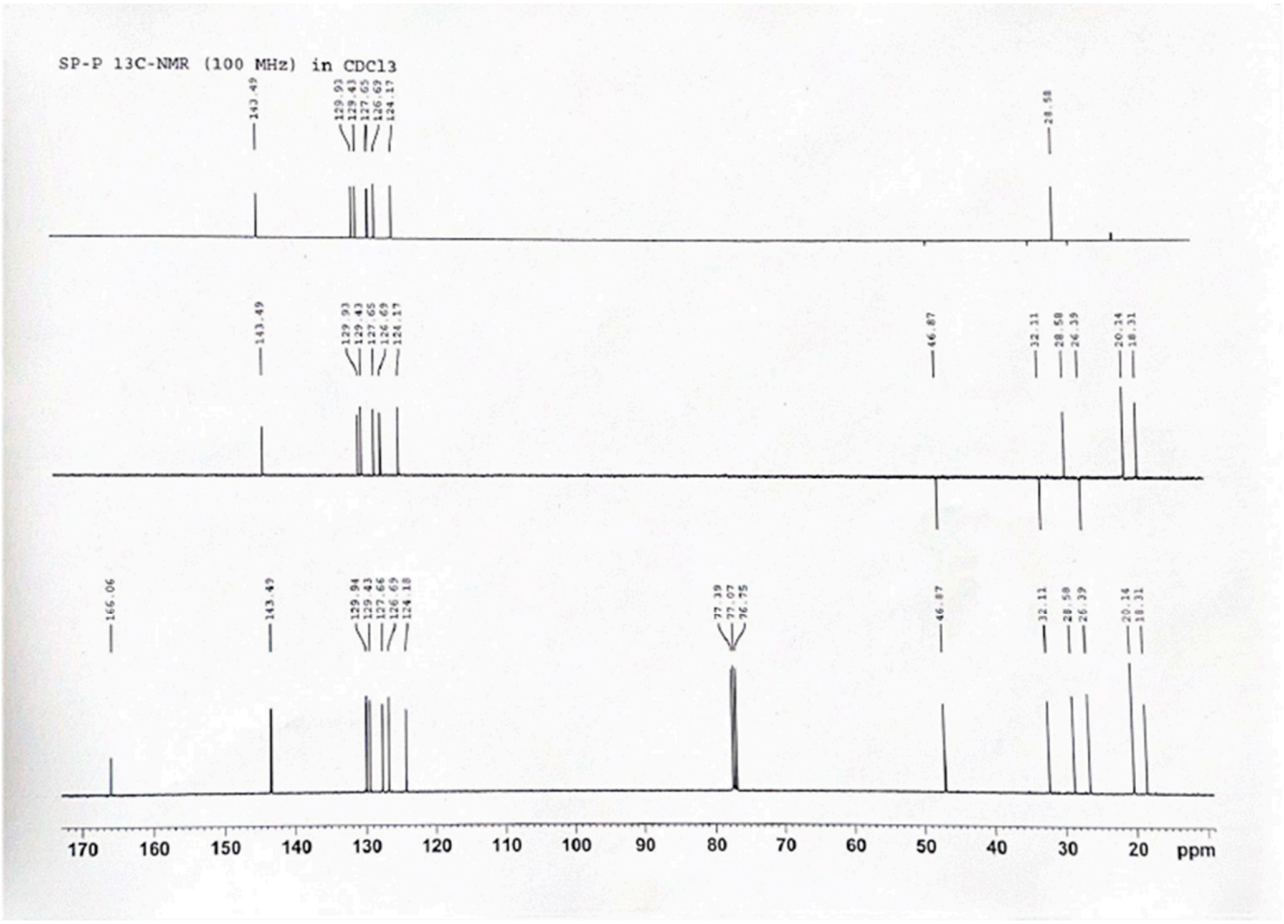

Figure S5. ^{13}C-NMR (125 MHz, CDCl3) spectrum of spilanthol