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Acetylcholinesterase Inhibition Activity of Ethanolic Extract of *Sesbania javanica* Miq. Flower

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Abstract

Cholinergic signaling impairment in Alzheimer's disease (AD) is associated with a low level of acetylcholine as a result of acetylcholinesterase hydrolysis. Acetylcholinesterase inhibitors are found in numerous plants and phytoconstituents. This study aims to investigate the effect of *Sesbania javanica* Miq. flower extracts on anti-acetylcholinesterase activity and identify its potential compounds. The ethanolic extracts of *Sesbania javanica* Miq. flower showed a relatively high percentage of acetylcholinesterase inhibitory activity in a dose-dependent manner (IC_{50} = 18.56±1.67 mg/mL). However, physostigmine (reference standard) shows the highest potency of acetylcholinesterase inhibition compared to the extracts, IC_{50} < 0.005 mg/mL. Gas chromatography-mass spectrometry-based analysis revealed that the ethanolic extract of the flower contained major phytochemicals including thiophene and 1-(5-Bicyclo[2.2.1]heptyl)ethylamine, which are predicted to penetrate across the blood-brain barrier. Thiophene was found to be the most abundant compound of the extract. The molecular docking demonstrated that the active phytochemical 1-(5-Bicyclo[2.2.1]heptyl) ethylamine forms hydrogen bonds with the active site of acetylcholinesterase, exhibiting the same orientation as physostigmine, thereby conferring anti-cholinesterase activity. Our findings suggest that the flower extract of *Sesbania javanica* Miq. (Fabaceae) may provide new phytochemicals for potential drug discovery as inhibitors of acetylcholinesterase activity.

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Introduction

Acetylcholinesterase (AChE) is a key enzyme in the cholinergic system (Boison, 2007). AChE plays an important role in the terminating nerve impulses at the cholinergic synapse by rapidly hydrolyzing acetylcholine, a neurotransmitter, into acetic acid and choline (McHardy et al., 2017). The rapidity of catalysis of acetylcholine is required in the nerve and skeletal neuromuscular junction to initiate subsequent impulse and trigger an excitatory postsynaptic potential (Taylor et al., 2009). Low levels of acetylcholine contribute to transmission inefficiency on afferent synaptic potentials, subsequently affecting memory impairment (Hasselmo, 2006). Dysfunction of cholinergic neurons results in transmission abnormalities leading to substantial cognitive decline in early-stage Alzheimer's disease (AD) patients (Terry & Buccafusco, 2003). The progression of neurodegeneration and dementia is associated with the aging process (Wyss-Coray, 2016). The presence of hallmark, i.e., amyloid-β and tau proteins in an AD brain leads to massive neuronal death and preceding synapse loss (Bloom, 2014). The pathogenic amyloid-β aggregates were also positively correlated with aged human brain tissue (Lesné et al., 2013). It has been reported that the accumulation of amyloid-β and tau protein in human brain causes synaptic dysfunction, memory loss and cognitive impairment (Gulisano et al., 2018). The current therapeutic approach for AD is acetylcholinesterase inhibitors (AChEIs) including donepezil, galantamine and rivastigmine (Cummings et al., 2019; Yiannopoulou & Papageorgiou, 2020). The role of AchEIs in improving cholinergic function has been reported and reviewed in this era (Mukherjee et al., 2007). Nowadays, the use of AchEIs from phytochemicals in the treatment of neurodegenerative diseases such as Alzheimer's disease (AD) is in the spotlight. *Sesbania javanica* Miq. (*S. javanica*) is classified in the Fabaceae family. The Thai names of *Sesbania javanica* Miq. are "Sano kin dok" or "Sano hin" (Pooma & Suddee, 2014). Its flower is a Thai local edible flower that dominates in a bright yellow color. It is also generally known as a tropical annual flower, which blooms from September to October of the year. This flower contains high levels of vitamin C, phytate, tannin and flavonoids (Bunma & Balslev, 2019). Recently, it has been reported that ethanolic extracts of *Sesbania javanica* Mig. flowers showed relatively powerful antioxidant properties (Lekdee et al., 2023). Acetylcholinesterase inhibitor is discovered in abundant flowering plants; however, phytoconstituents of *S. javanica* flower involved in the acetylcholine system has not been elucidated. Exploring an active compound of the *Sesbania javanic*a Miq. flower extract on the acetylcholinesterase system might reveal a novel substance for beneficial treatment of AD, a neurodegenerative disease. Therefore, this study aimed to investigate the active compounds of *S. javanica* flower ethanolic extract regarding acetylcholinesterase and elucidate a possible inhibitory activity on the acetylcholinesterase mechanism.

Materials and methods

This study was exempted by Institutional Review Board (IRB of Valaya Alongkorn Rajabhat University under the Royal Patronage (0045/2566)).

1. Chemicals and reagents

Acetylcholinesterase (Type-VI-S, EC 3.1.1.7) obtained from *Electrophorus electricus* (electric eel) was purchased from Sigma-Aldrich (MO, USA). All of the reagents and solvents used were analytical grades. Acetylthiocholine iodide (ATCI ≥ 99%), physostigmine hemisulfate salt, 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB ≥ 98%) and dimethyl sulfoxide were obtained from Sigma-Aldrich (MO, USA).

2. Identification of plant specimens

A complete specimen of *Sebania javanica* Miq, including flower, was collected from the arena of Valaya Alongkorn Rajabhat University under the Royal Patronage Pathum Thani Province, Thailand (14.0631° N, 100.6084° E, Altitudes 2.3 Nautical miles) during November 2022 – January 2023. Taxonomy identification was confirmed by Sirinthon Plant Herbarium Museum, Kasetsart University, Bangkok, Thailand and the voucher specimen (BK 085644) was deposited at the Department of Nutrition and Dietetics, Faculty of Science and Technology, Valaya Alongkorn Rajabhat University under the Royal Patronage Pathum Thani Province, Thailand.

3. Extract preparation of *Sesbania javanica* Miq. flower

A dried sample of *Sesbania javanica* Miq. flower was used for the extraction. One hundred grams of dried flower sample was extracted with 70% (*v/v*) ethanol (Merck, Germany) by soaking and stirring for 24 hr at room temperature, followed by filtration through Whatman No.1 filter paper. The ethanolic extract was de-fatted with 1:1 *v/v* with *n*-hexane (Merck, Germany)

and solvents removal was by rotary evaporator (Buchi Labortechnik AG, Switzerland). Then, the ethanolic extract was lyophilized and stored at -30°C in a dark area until used.

4. Identification of active volatile compounds by using GC-MS

Identification of candidate active compounds was analyzed by using gas chromatography-mass spectrometry (GC-MS) (Honour, 2006). The system comprised a gas chromatograph and a mass selective detector (GCMS-TQ8050NCI NX, Shimadzu, Japan) using a silica capillary column (30 x 0.25 mm ID x 1EM df, composed of 100% Dimethylpoly siloxane, and 99.999% helium) with electron ionization (70 eV) and an ion source operated at a temperature of 280°C. The GC oven temperature was set as follows: commencing at 40°C and held for 1 min, then raised (10°C/min) to 200°C and then to final temperature, 280°C (10°C/min) which was maintained for 29 min. The temperature of the injector was held at 200°C; the flow rate of the carrier gas was maintained at 1.0 mL/min; 10:1 split ratio. About 0.5 µL of the sample was injected into the GC-MS using a micro syringe and the scanning was done for 50 min. The spectrum data obtained were compared to the database of National Institute of Standard and Technology (NIST).

5. Acetylcholinesterase inhibition activity assay

The inhibitory effect of *Sesbania javanica* flower extract on acetylcholinesterase was performed by using a slightly modified procedure of Ellman's method (Worek et al., 2012). Briefly, the 14 mM acetylthiocholine iodide solution was prepared by initially dissolving acetylthiocholine iodide in deionized water. The acetylcholinesterase solution was prepared to a final concentration of 0.25 units/mL in phosphate buffer saline pH 7.

Pre-incubation of extracts and enzyme solution mixture in Tris HCl buffer was adjusted to pH 8.0 at 25°C for 20 min. The reaction was initiated by adding 100 µL of acetylthiocholine solution. Colorimetry was measured at 412 nm using a spectrophotometer (Shimadzu UV-1280, Japan) after adding substrate ATCI to mixture solution. Physostigmine was used as a standard inhibitor. The result was expressed as percentages of acetylcholinesterase inhibitory activity. To calculate the percentages of inhibition, equation (1) below was adopted (Mangmool et al., 2021). The absorbance of the background was subtracted from the absorbance of samples and control group. The formula for calculation is shown as follows:

$$\% \text{ inhibition} = [(Ao - A1)/Ao] \times 100 \quad (1)$$

Ao was the absorbance of the control (without the extracts), A1 = absorbance of the assay with the extracts. IC_{50} was calculated using linear regression analysis.

6. Computational analysis of active volatile compounds came from GC-MS chromatogram

All known structures identified from the NIST database of *Sesbania javanica* chromatogram were constructed and the energy of freely rotatable bonds of all active constituents was minimized by using Chem3D Professional 10.0 (CambridgeSoft Inc., Cambridge, USA). The canonical SMILES format of all known structures identified from GC-MS chromatogram was retrieved from the PubChem compound database and then submitted to SwissADME (http://www.swissadme.ch/index.php) to compute their physicochemical and pharmacokinetic properties (Daina et al., 2017; Kim et al., 2021). *In silico* bioactivity prediction of these compounds in SMILES format was evaluated through PASSonline software, which selected potential activities as acetylcholine agonist, acetylcholine antagonist, acetylcholine neuromuscular blocking agent, acetylcholine release stimulant, acetylcholinesterase stimulant, acetylesterase inhibitor and butyrylcholinesterase inhibitor (Filimonov et al., 2014). The crystal structure of human acetylcholinesterase (PDB ID: 4EY5) (Cheung et al., 2012) was prepared by removing all water molecules, solvents and ligands, using the ViewerLite program (Accelrys, San Diego, USA). The estimated binding free energy of compounds from *S. javanica* was docked and analyzed using AutoDock 4.2.6 software (Morris et al., 2009). Each energy-minimized compound from *S. javanica* and physostigmine (as a reference compound with acetylcholinesterase inhibitor) were submitted to the well-prepared targets with default docking parameters. The molecular docking protocol was derived from the active site of human acetylcholinesterase with a molecular grid at 0.375 Å grid spacing. The grid was located at x = -6.750, y = -6.250 and z = 3.389 Å. Docking results of all compounds were evaluated using the best predicted binding free energy (BE, kcal/mol) and inhibitory constant from all clusters of each conformational structure. The best results were virtually analyzed using UCSF Chimera (Pettersen et al., 2004). Furthermore, the compounds isolated from *S. javanica* flowers, as reported in previous literature, were also analyzed for binding free energy and pharmacokinetic parameters (Kijparkorn et al., 2010; Loedsaksaesakul,

2007; Mohammed et al., 2013; Tangvarasittichai et al., 2005).

7. Statistical analysis

The percentage inhibition of acetylcholinesterase activity for each tested concentration was the mean± standard deviation (SD) from three replicated experiments. Statistical difference compared with control group was calculated according to one-way analysis of variance (ANOVA), followed by Dunnett's post-hoc test ($p < 0.05$) by using GraphPad Prism software version 8.0.

Results and discussion

1. Identification of active volatile compounds by using GC-MS

The screening of candidate bioactive compounds for the ethanolic extract was obtained by using GC-MS. By their retention times, 30 peaks were distinguished in the chromatogram. However, 13 compounds eluting at retention times of 8.565, 15.955, 16.24, 17.28, 17.53, 18.765, 18.855, 18.91, 19.045, 19.145, 19.245, 19.72 and 19.89 min could not be identified; they appeared as unknown compounds. Nevertheless, the contents of these unknown compounds ranged from 0.031% to 0.836% of the area under the curve. As such, these compounds could be considered negligible. Among the 17 identified compounds in the ethanolic extract of *Sesbania javanica* flower, the most abundant compound was thiophene (compound 7), accounting for 85.57% of all the composition based on the GC-MS chromatogram (Table 1).

2. Acetylcholinesterase inhibition activity assay

The inhibitory effect on acetylcholinesterase activity of *Sesbania javanica* flower ethanolic extract was tested in concentrations ranging from 2.5 to 20 mg/mL. The *S. javanica* flower crude extract at concentrations of 2.5, 5, 7.5, 10, 12.5, 15 and 20 mg/mL showed the results in 14.81%, 15.51%, 23.64%, 30.08%, 40.65%, 41.79% and 52.62% inhibition, respectively (Fig. 1). At the concentration from 12.5 to 20 mg/mL, the extracts significantly inhibited acetylcholinesterase activity compared with the control group ($p < 0.05$). Among the tested concentrations, the highest concentration of extracts (20 mg/mL) showed the highest percentage of inhibition (52.62%). Furthermore, the percentage of inhibition was also found to be dose-dependent manner. The extract showed an inhibitory effect of 50% (IC_{50} value) at a concentration of 18.56±1.67 mg/mL. Whereas the positive control of physostigmine exhibited the most potent acetylcholinesterase inhibitory effect, at 5

Table 1 GC-MS chromatogram of ethanolic extract of *Sesbania javanica* Miq. flower

Peak	RT [min]	Area [%]	Name	Note
1	1.275	2.47	Propyne	Compound 1
2	1.485	0.535	Propanamide, 2-hydroxy-	Compound 2
3	1.8	0.264	Glycine, N-(dithiocarboxy)-N-methyl-	Compound 3
4	1.95	0.22	Propanedioic acid	Compound 4
5	2.175	1.103	3-Butynoic acid	Compound 5
6	2.705	0.701	Benzene	Compound 6
7	2.835	85.572	Thiophene	Compound 7
8	3.2	0.131	Azetidin-2-one 3,3-dimethyl-4-(1-aminoethyl)-	Compound 8
9	3.31	0.137	1-(5-Bicyclo[2.2.1]heptyl) ethylamine	Compound 9
10	4.245	0.122	2-Formylhistamine	Compound 10
11	4.4	0.155	2-Aminononadecane	Compound 11
12	8.565	0.057	Unknown	
13	14.755	0.428	Imidazole, 2-amino-5-[(2-carboxy) vinyl]-	Compound 12
14	15.955	0.157	Unknown	
15	16.24	0.128	Unknown	
16	17.165	1.01	1-Decanol, 2-octyl-	Compound 13
17	17.28	0.099	Unknown	
18	17.405	0.926	2-Methyltetracosane	Compound 14
19	17.53	0.836	Unknown	
20	18.065	1.676	Carbonic acid, eicosyl vinyl ester	Compound 15
21	18.765	0.214	Unknown	
22	18.855	0.015	Unknown	
23	18.91	0.386	Unknown	
24	19.045	0.129	Unknown	
25	19.145	0.16	Unknown	
26	19.245	0.031	Unknown	
27	19.585	1.82	2-Methylhexacosane	Compound 16
28	19.72	0.039	Unknown	
29	19.89	0.063	Unknown	
30	21.27	0.42	Hexacosane	Compound 17

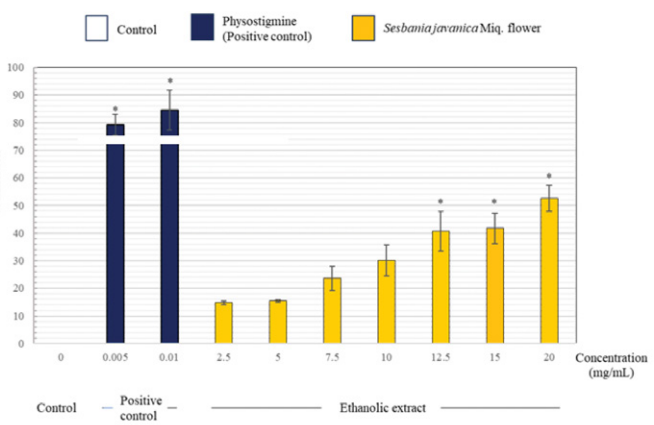

Fig. 1 The inhibitory effect of *Sesbania javanica* Miq. flower extract and physostigmine on acetylcholinesterase activity

Remark: The IC_{50} value was at 18.56 ± 1.67 mg/mL for the crude extracts. Data represented as the mean±SD of the percentage of inhibition relative to the control group from three replicated experiments.
*indicates a significant difference compared to control group according to one-way ANOVA, followed by Dunnett's post-hoc test ($p < 0.05$)

and 10 µg/mL, showing a relatively high percentages of inhibition, 79.20 ± 3.70% and 84.58 ± 7.34%, respectively.

3. Computational analysis of compound obtained from ethanolic extract of *Sesbania javanica* Miq. flower

In our study, we utilized two online tools, SwissADME and PASSonline to predict and estimate the potential pharmacokinetic properties and bioactive activity profiles of the identified compounds from the GC-MS chromatogram. Most compounds are derived from *Sesbania javanica* flower extracts complied with Lipinski's rule of five and showed no effect on any pharmacokinetic parameters, indicating their potential as orally active drug candidates. However, compounds 14 to 17 are exceptions, as they have a log *P* value greater than 5 (Table 2). A log *P* value exceeding 5 suggests high lipophilicity, which may impact a compound's water solubility and membrane permeability, potentially affecting its pharmacokinetic properties. Physostigmine, a known effective drug for the symptomatic treatment of Alzheimer's disease, has been confirmed to have the ability to permeate the blood-brain

Table 2 Physiochemical properties and molecular docking analysis of compounds identified by GC–MS analysis from ethanolic extract of *Sesbania javanica* Miq. flower toward acetylcholinesterase

	Phytochemical name	Predicted binding energy (kcal/mol)[a]	Predicted inhibitory constant[a]	MW[b]	HBA[c]	HBD[c]	*c*Log*P*[c]	BBB permeant
1	Propyne	-2.30	20.62 mM	40.06	0	0	1.48	No
2	Propanamide, 2-hydroxy-	-3.68	2.00 mM	89.09	2	2	0.50	No
3	Glycine, N-(dithiocarboxy)-N-methyl-	-3.27	4.00 mM	165.23	2	1	1.18	No
4	Propanedioic acid	-1.49	80.49 mM	104.06	4	2	-0.08	No
5	3-Butynoic acid	-2.77	9.32 mM	84.07	2	1	0.93	No
6	Benzene	-4.44	559.65 µM	78.11	0	0	1.58	No
7	*Thiophene*	-3.49	2.78 mM	84.14	0	0	0	Yes
8	Azetidin-2-one 3,3-dimethyl-4-(1-aminoethyl)-	-7.34	4.17 µM	142.20	2	2	1.26	No
9	*1-(5-Bicyclo[2.2.1]heptyl)ethylamine*	-7.75	2.08 µM	139.24	1	1	2.25	Yes
10	2-Formylhistamine	-6.67	12.84 µM	139.16	3	2	0.06	No
11	2-Aminononadecane	-8.95	273.35 nM	283.54	1	1	5.13	No
12	Imidazole, 2-amino-5-[(2-carboxy)vinyl]-	-6.99	7.52 µM	153.14	3	3	0.15	No
13	1-Decanol, 2-octyl-	-6.23	27.03 µM	270.49	1	1	4.86	No
14	2-Methyltetracosane	-8.12	1.12 µM	352.68	0	0	6.78	No
15	Carbonic acid, eicosyl vinyl ester	-5.81	54.81 µM	368.59	3	0	6.28	No
16	2-Methylhexacosane	-6.88	9.09 µM	380.73	0	0	7.22	No
17	Hexacosane	-6.17	30.25 µM	366.71	0	0	7.08	No
18	Quercetin 3-2G-rhamnosylrutinoside	+8.60	NA	756.66	20	12	-2.27	No
19	4-hydroxycinnamic acid	-5.54	86.79 µM	164.16	3	2	1.26	Yes
20	Beta-sitosterol	-5.50	92.26 µM	414.71	1	1	7.24	No
21	Beta-sitosterol-3-O-beta-D-glucopyranoside	-6.48	17.71 µM	576.85	6	4	5.55	No
22	Prunetin	-8.44	649.10 nM	284.26	5	2	2.43	No
23	Genistein	-8.75	388.13 nM	270.24	5	3	2.04	No
24	Beta-Carotene	+242.61	NA	536.87	0	0	7.06	No
25	Beta-Cryptoxanthin	+219.91	NA	552.87	1	1	7.06	No
26	cis-Lutein	+183.89	NA	568.87	2	2	6.92	No
27	trans-Lutein	+30.71	NA	568.87	2	2	6.98	No
28	Epoxide Lutein	+53.83	NA	584.87	3	2	6.92	No
29	trans-Zeaxanthin	+74.97	NA	568.87	2	2	6.98	No
30	Alpha-amyrin	-7.28	4.59 µM	426.72	1	1	7.06	No
31	Campesterol	-6.94	8.17 µM	400.68	1	1	6.92	No
32	Lignoceric acid	-2.50	14.59 mM	368.64	2	1	8.10	No
33	Linoleic acid	-6.50	17.22 µM	280.45	2	1	8.10	No
34	Lupeol	-7.38	3.91 µM	426.72	1	1	7.27	No
35	Oleic acid	-5.18	160.09 µM	282.46	2	1	5.65	No
36	Palmitic acid	-5.61	77.03 µM	256.42	2	1	5.20	Yes
37	Stearic acid	-5.28	135.69 µM	284.48	2	1	5.93	No
38	Stigmasterol	-7.24	4.94 µM	412.69	1	1	6.98	No
Ref	Physostigmine	-9.67	82.27 nM	275.35	3	1	1.65	Yes

Remark: [a] Results were obtained from AutoDock 4.2.6 software

[b] Calculated using ChemBioDraw Ultra16.0. MW: molecular weight

[c] Calculated using SwissADME. NA: Not available; HBA: number of hydrogen acceptors; HBD: number of hydrogen donors; RB: number of rotatable bonds; *t*PSA: total polar surface area; *c*Log *P*: log octanol/water partition coefficient

barrier in SwissADME, validating the tool's reliability. Among the compounds identified from *S. javanica* flower, only compounds 7 and 9 demonstrate the ability to permeate the blood-brain barrier, indicating their potential as drug candidates for targeting the CNS (Table 2).

The PASS Online web-based tool was then utilized to analyze the potential bioactive activities of the identified compounds. The results are presented in Fig. 2, where "Pa" represents the probability of being active in specific sub-classes of bioactivities. However, we did not find a specific class of bioactivity for acetylcholinesterase, which is the main target of this study. Instead, the tool provided similar classes that encompass acetylcholine agonist, acetylcholine antagonist, acetylcholine neuromuscular blocking agent, acetylcholine release stimulant, acetylcholinesterase stimulant, acetylesterase inhibitor and butyrylcholinesterase inhibitor.

In this study, we conducted molecular docking of 17 compounds identified from the GC-MS chromatogram (compounds 1-17), 21 compounds from previous literature (compounds 18-38) and physostigmine with human acetylcholinesterase at the acetylcholine-binding active site. The binding energy of the reference compound, physostigmine, was -9.67 kcal/mol, with an estimated inhibition constant (Ki) of 82.27 nM. Among the compounds identified from the ethanolic extract of *S. javanica* flower, compound 11 (2-Aminononadecane) exhibited a high predicted binding energy with -8.95 kcal/mol. However, none of the compounds showed a predicted binding energy greater than physostigmine (Table 2).

Interestingly, compounds 7, 9, 19 and 36 demonstrated the ability to permeate the blood-brain barrier (BBB). Compound 7 (Thiophene) has a molecular weight of 84.14, no hydrogen-bond donors or acceptors and a low log *P* of 0, indicating moderate hydrophilicity. Compound 9 (1-(5-Bicyclo[2.2.1]heptyl)ethylamine) shows a binding energy of -7.75 kcal/mol and balanced hydrophobicity (log *P* 2.25), with one hydrogen-bond donor and acceptor each, aiding BBB permeation. Compound 19 (4-hydroxycinnamic acid) has a moderate binding energy of -5.54 kcal/mol, a molecular weight of 164.16, higher hydrogen bonding capacity (3 acceptors, 2 donors) and a moderate log *P* of 1.26, suggesting good permeability balanced with solubility. Lastly, compound 36 (Palmitic acid) with a binding energy of -5.61 kcal/mol and a higher molecular weight of 256.42, has a log *P* of 5.2, indicating significant hydrophobicity which

Compound	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Acetylcholine agonist	0.19	0	0	0	0	0.13	0.14	0	0.1	0	0	0	0	0	0	0	0.12
Acetylcholine antagonist	0.19	0	0	0	0.09	0.1	0.1	0	0	0	0	0	0	0	0.09	0	0.11
Acetylcholine M1 receptor agonist	0.27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.05
Acetylcholine M1 receptor antagonist	0.16	0	0	0	0	0.05	0.05	0	0	0	0	0	0	0	0	0	0.06
Acetylcholine M2 receptor agonist	0.22	0	0	0	0.13	0	0.11	0.14	0.15	0	0.11	0	0	0.11	0	0.11	0.11
Acetylcholine M2 receptor antagonist	0.13	0	0	0	0	0.05	0.06	0	0	0	0	0	0	0	0	0	0.06
Acetylcholine M3 receptor agonist	0.32	0.1	0	0.14	0.18	0.24	0.17	0.12	0.11	0	0.1	0	0.09	0.15	0	0.15	0.21
Acetylcholine M3 receptor antagonist	0.15	0	0	0	0.07	0.08	0.1	0	0	0	0	0	0	0	0	0	0.08
Acetylcholine M4 receptor agonist	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0.06	0	0.06	0.08
Acetylcholine M4 receptor antagonist	0.13	0	0	0	0	0.06	0.05	0	0	0	0	0	0	0.05	0.05	0.05	0.08
Acetylcholine M5 receptor agonist	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetylcholine M5 receptor antagonist	0.13	0	0	0	0.05	0.07	0.06	0	0	0	0	0	0	0.06	0	0.06	0.08
Acetylcholine muscarinic agonist	0.2	0	0	0	0.06	0.08	0.08	0	0.07	0	0	0	0.06	0	0	0.07	0.1
Acetylcholine muscarinic antagonist	0.18	0	0	0	0.08	0.09	0.09	0	0	0	0	0	0	0.07	0	0	0.09
Acetylcholine neuromuscular blocking agent	0.57	0.47	0.28	0.6	0.45	0.68	0.54	0.53	0.52	0.57	0.5	0	0.51	0.62	0.4	0.62	0.63
Acetylcholine nicotinic agonist	0	0	0	0	0	0.12	0.15	0	0	0	0	0	0	0	0	0	0
Acetylcholine nicotinic antagonist	0.14	0	0	0	0	0.15	0.1	0.08	0.11	0	0.11	0	0	0.1	0.09	0.1	0.15
Acetylcholine release stimulant	0.13	0.1	0	0.11	0	0.16	0.14	0	0	0	0.1	0	0.1	0.12	0	0.12	0.16
Acetylcholinesterase stimulant	0	0	0	0	0	0.02	0	0	0	0	0	0	0	0	0	0	0
Acetylesterase inhibitor	0.82	0.85	0.32	0.85	0.7	0.89	0.79	0.54	0.41	0.1	0.88	0.21	0.9	0.92	0.64	0.92	0.93
Butyrylcholinesterase inhibitor	0.1	0	0	0	0	0.08	0	0	0	0	0	0	0	0.06	0	0.06	0.09

Fig. 2 Bioactivity prediction of compounds identified by GC–MS chromatogram from ethanolic extract of *Sesbania javanica* Miq. flower. aPa value is probability "to be active", by using PASSonline software (green = high probability, yellow = intermediate probability, red = low probability)

facilitates BBB penetration despite having two hydrogen-bond acceptors and one donor (Table 2).

Among these, compound 9 demonstrated the best predicted binding energy and also showed the ability to permeate the BBB. This compound holds promise as a potential candidate for further drug development targeting CNS-related diseases. The binding mode analysis reveals that compound 9 interacted with acetylcholinesterase through H-bonding with Glu202 residues and hydrophobic interactions with Trp86, Gly121, Ser203, Tyr337 and His447 (Fig. 3). The favorable conformational position of this compound near the catalytic site of acetylcholinesterase is similar to the orientation of physostigmine.

Active phytochemicals have been reported to have acetylcholinesterase inhibitor properties in the present day. Natural-occurring compounds from plants such as aromatic alkaloids, terpenoids, polyphenol compounds and xanthones have been reported to possess a strong acetylcholinesterase activity (Murray et al., 2013). Our finding indicated that ethanolic extract of *Sesbania javanica* flower showed the inhibitory effects on acetylcholinesterase activity. This finding suggests that the ethanolic extract of *S. javanica* flower may contain phytochemicals capable of modulating acetylcholinesterase activity. Recently, it has been reported that *Sophora mollis* leaf extract; the plant in Fabaceae species, showed effectively inhibited acetylcholinesterase activity (IC_{50} = 75.96 µg/mL)

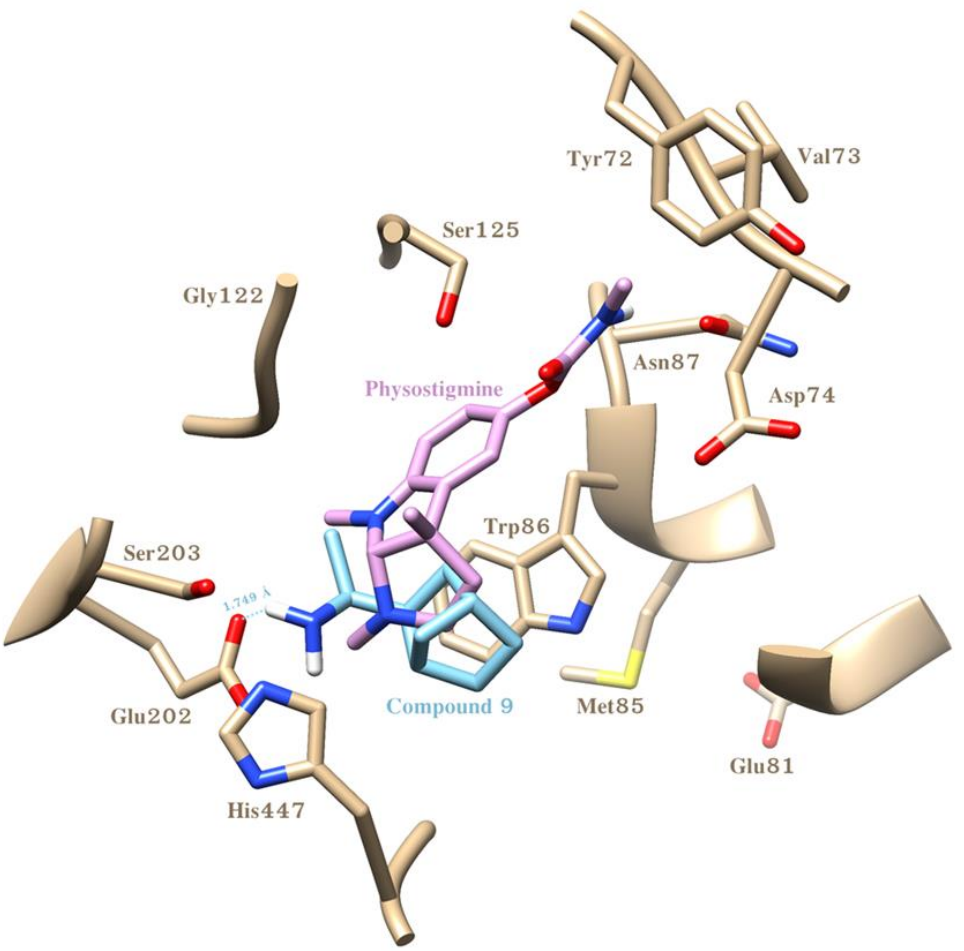

Fig. 3 The predicted binding mode of compound 9 and physostigmine to the active site of AChE (PDB ID: 4EY5). The structures of compounds are represented in a stick model with color (grey: carbon atom of amino acid, bright blue: carbon atom of compound 9, magenta: carbon atom of physostigmine, red: oxygen atom, dark blue: nitrogen atom and white: hydrogen atom). The blue line denoted the H-bonding interaction

(Fatima et al., 2023). However, the edible flower extract from the petal of *S. javanica* flower was reported to possess a strong antimutagenic effect (Tangvarasittichai et al., 2005). Here, we first report the efficacy of *S. javanica* flower in an aspect of acetylcholinesterase activity inhibition. Fabaceae plants, although the phytochemical compounds of *Sophora mollis* leaf extract showed higher efficacy of acetylcholinesterase inhibition than that of *S. javanica* flower, contain potential predicted compounds to penetrate across the blood-brain barrier.

Our GC/MS study investigates the potential of active volatile compounds derived from *S. javanica* flower as acetylcholinesterase inhibitors, which are crucial for managing conditions like Alzheimer's disease. Molecular docking techniques were employed to evaluate these compounds. Notably, compound 7, identified as thiophene, was found to be abundantly present in the flower extract. Thiophene is known for its diverse pharmacological activities and exhibits potent acetylcholinesterase inhibitory effects comparable to the established drug donepezil (Ismail et al., 2012). Despite the unfavorable estimated binding free energy of this compound, reports are suggesting that the tetrahydrobenzo [b]-thiophene ring within thiophene targets the peripheral anionic site of acetylcholinesterase (Zhou et al., 2008). This suggests its potential as a therapeutic agent for acetylcholinesterase inhibition.

Another compound of interest, identified as 1-(5-Bicyclo[2.2.1]heptyl)ethylamine (Compound 9), demonstrated promising attributes through computational analysis. This compound shows potential in penetrating the blood-brain barrier and exhibits inhibitory effects on acetylcholinesterase. Its structural similarity to FDA-approved drugs like rimantadine and amantadine hints at its potential for further drug development (Blanpied et al., 2005). Moreover, computational models suggest that this compound can interact with the active site of acetylcholinesterase through hydrogen bonding, further supporting its role as a lead compound for therapeutic development. However, compound 9 is a minor component in the ethanolic extracts of *S. javanica.* For future research, we recommend exploring the semisynthesis of this compound to enhance its availability, enabling more comprehensive evaluations of its potential as a therapeutic agent for CNS-related diseases. This approach would provide sufficient quantities for in-depth pharmacological studies and support the development of new drugs based on this promising compound.

The study emphasizes the utility of computational analysis in drug discovery, providing insights into compound bioactivities, blood-brain barrier penetration and enzyme interactions. However, experimental validation and pharmacokinetic studies are essential to fully assess the therapeutic potential of identified compounds. In addition, an *in vitro* model to test bioactivities, cytotoxicity and neuroprotection is required for further study in order to support the potential drug discovery for AD of *S. javanica* flower extract.

While GC-MS was employed to isolate compounds from *S. javanica* flower, this technique has inherent limitations. The compounds identified through GC-MS may face challenges in accurate identification and quantification, as well as limitations in detecting compounds present in low concentrations. Moreover, GC-MS may not provide comprehensive coverage of all compounds in the sample, particularly those with high molecular weights or low volatility. The technique's sensitivity to sample preparation methods and potential interference from co-eluting compounds can also affect the reliability of results (Medeiros, 2018). Nonetheless, the GC-MS chromatogram serves as a fingerprint of this extract for further analysis and characterization of the isolated compounds. However, the validation of the extraction method for the *S. javanica* flower is also important. Therefore, the variation of conditions, such as percentages of ethanol and periods of extraction, would enable us to identify the most effective extraction method for obtaining bioactive compounds from *S. javanica* flower, ultimately facilitating the development of potential therapeutic agents for various applications, including those related to central nervous system disorders.

Conclusion

Our finding suggests that phytochemicals, thiophene and 1-(5-Bicyclo[2.2.1]heptyl)ethylamine, contained in *Sesbania javanica* Miq. flower might be the major compound contributing to acetylcholinesterase activity inhibition. Predicting penetration across the blood-brain barrier of 1-(5-Bicyclo[2.2.1]heptyl) ethylamine is attributed to demonstrating H-bonding potential at the active site of acetylcholinesterase. Thus, *Sesbania javanica* Miq. flower provides a new valuable candidate as an acetylcholinesterase inhibitor. This discovery may elevate the local flowers, *Sesbania javanica* Miq. to medicinal plants for therapeutic purposes.

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