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ORIGINAL RESEARCH

Molecular docking and dynamics simulation approach of *Camellia sinensis* leaf extract derived compounds as potential cholinesterase inhibitors

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Abstract

The tea plant (*Camellia sinensis*) belongs to the family Theaceae and contains many phytochemicals that are effective against various diseases, including neurodegenerative disorders. In this study, we aimed to characterize the phytochemicals present in the methanolic and n-hexane leaf extracts of *C. sinensis* using GC–MS, FTIR, and UV–visible analysis. We detected a total of 19 compounds of different chemical classes. We also performed molecular docking studies using the GC–MS detected phytochemicals, targeting acetylcholinesterase (AChE, PBD ID: 4BDT) and butyrylcholinesterase (BChE, PDB ID: 6QAB), which are responsible for the breakdown of the neurotransmitter acetylcholine (ACh). This breakdown leads to dementia and cognitive decline in Alzheimer's patients. The compounds Ergosta-7,22-dien-3-ol, (3.beta.,5.alpha.,22E)- and Benzene, 1,3-bis(1,1-dimethylethyl) showed better binding affinity against AChE, while dl-.alpha.-Tocopherol and Ergosta-7,22-dien-3-ol, (3.beta.,5.alpha.,22E)- showed better binding affinity against BChE. We determined the stability and rigidity of these best docked complexes through molecular dynamics simulation for a period of 100 ns. All complexes showed stability in terms of SASA, Rg, and hydrogen bonds, but some variations were found in the RMSD values. Our ADMET analysis revealed that all lead compounds are non-toxic. Therefore, these compounds could be potential inhibitors of AChE and BChE.

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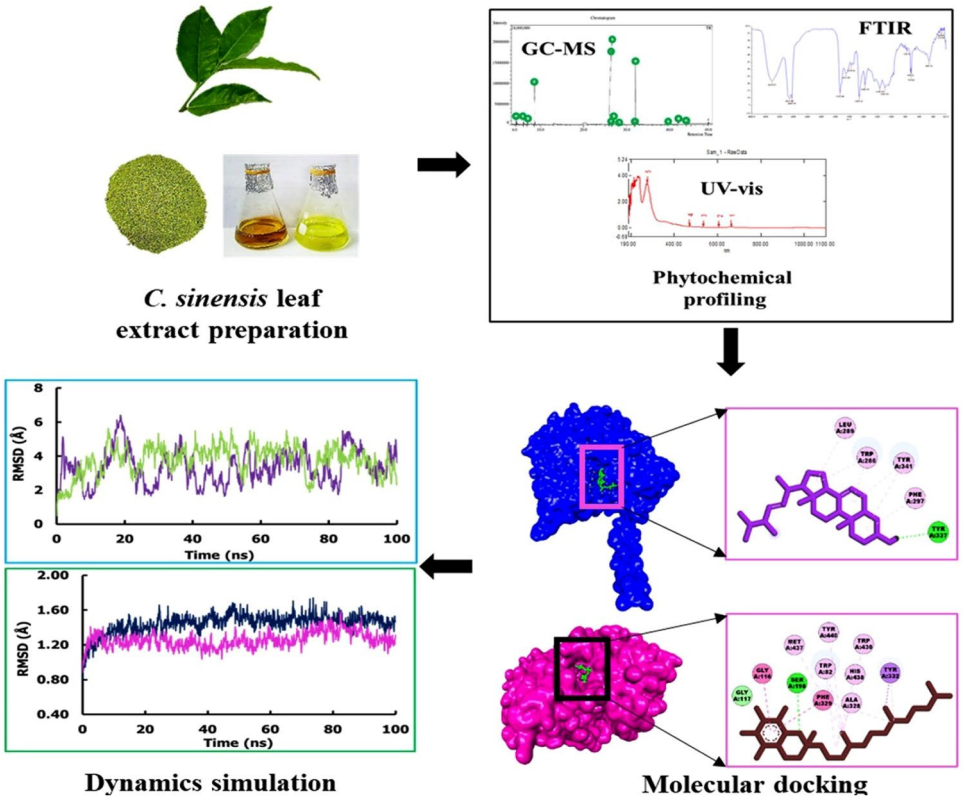
Graphical abstract



Keywords *Camellia sinensis* · Alzheimer's disease · Acetylcholinesterase · Butyrylcholinesterase · Molecular docking · Molecular dynamics

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that affects millions of people worldwide. According to the Alzheimer's Association, an estimated 6.2 million Americans aged 65 and older have AD, and this number is projected to increase to 13.8 million by 2050 (Tampi 2023). AD is characterized by the accumulation of amyloid beta (Aβ) plaques and tau tangles in the brain, leading to neuronal death and cognitive decline (Luo et al. 2021b). Currently, there is no cure for AD, but symptomatic treatments are available, such as cholinesterase inhibitors, which are designed to improve cognitive function by increasing the availability of acetylcholine in the brain (Marucci et al. 2021).

Cholinesterase inhibitors, such as donepezil, rivastigmine, and galantamine, have been shown to provide modest benefits in cognitive and functional measures for patients with mild to moderate AD (Haake et al. 2020). However, their clinical efficacy is limited, and they do not address the underlying pathological processes of the disease. Moreover, cholinesterase inhibitors are associated with several adverse effects, including nausea, vomiting, diarrhea, and bradycardia, which can limit their use (Schneider 2022). Therefore, there is a need to develop new drugs that target different aspects of the disease and have fewer adverse effects.

Natural products have long been used in traditional medicine for the treatment of various diseases, including neurodegenerative disorders. In recent years, there has been growing interest in natural products as potential sources of cholinesterase inhibitors (Lai Shi Min et al. 2022). Natural products, such as alkaloids, flavonoids, terpenoids, and polyphenols, have been reported to have cholinesterase inhibitory activity (Tamfu et al. 2021). For example, huperzine A, an alkaloid derived from the Chinese herb Huperzia serrata, has been shown to be a potent and selective inhibitor of acetylcholinesterase (Wen-Xia et al. 2020). Moreover, natural products have been reported to have neuroprotective and antioxidant properties, which could be beneficial in the treatment of AD (Chen et al. 2021).

Camellia sinensis, commonly known as tea, is a widely consumed beverage worldwide. It is rich in bioactive

compounds, such as catechins, theaflavins, and caffeine, which have been reported to have various health benefits, including antioxidant, anti-inflammatory, and neuroprotective properties (Samanta 2022). Recent studies have suggested that *C. sinensis* and its bioactive compounds could have potential in the treatment of AD (Devkota et al. 2021). For example, epigallocatechin gallate (EGCG), a catechin found in green tea, has been shown to have cholinesterase inhibitory activity and to reduce Aβ aggregation and neuroinflammation in animal models of AD (Lange et al. 2022). Moreover, studies have suggested that *C. sinensis* consumption may reduce the risk of cognitive decline and AD in humans (Howes et al. 2020).

Although *C. sinensis* and its bioactive compounds have shown promise in the treatment of AD, the underlying mechanisms of their cholinesterase inhibitory activity and their potential as therapeutic agents for AD remain unclear. Therefore, the objective of this study is to investigate the cholinesterase inhibitory activity of *C. sinensis* leaf extract and its bioactive compounds using a molecular docking and dynamics simulation approach. The study aims to identify potential candidates for further preclinical and clinical evaluation as therapeutic agents for AD.

Materials and methods

Plant material collection and preparation

Fresh, disease-free leaves of *C. sinensis* were collected from the BTRI located in Sreemangal, Sylhet, Bangladesh. The location was chosen based on the availability of the plant species and its suitable growth conditions in that particular region. The leaves were collected in the early morning hours of a sunny day in the month of May, which is the peak growing season for *C. sinensis*. The temperature was 31 °C during the time of collection. The leaves were washed thoroughly with distilled water to remove any dust or debris. Then, they were washed with 1% Triton X-100 solution to remove any surface contaminants or pesticides. Finally, they were rinsed with distilled water and air-dried for 2 days under shade. The dried leaves were pulverized into a fine powder using a commercial grade hammer mill with a 1.0 mm mesh sieve. A 50 g of powdered sample was extracted separately with 150 ml of 100% methanolic and 100% N-hexane solvent using a Soxhlet extractor for 8 h at 80 °C. The extracts were concentrated under reduced pressure and dried at 45 °C using a rotary evaporator. The concentrated extracts were allowed to macerate on an orbital shaker at 180 rpm for 48 h at 25 °C. Then they were filtered through Whatman No.1 filter paper and concentrated under reduced pressure. The extracts were weighed and stored individually in amber-colored glass vials at 4 °C in a dark place (Lefebvre et al. 2021).

Gas chromatography-mass spectroscopy analysis (GC–MS)

The purified extracts were then analyzed by GC–MS using a Shimadzu GCMS-QP2010 SE system equipped with a DB-5MS column. The sample was injected into the system in split mode, with a split ratio of 1:50. The GC was run using a temperature program, starting at 50 °C and ramping up to 250 °C at a rate of 10 °C/min. The MS detector was operated in electron ionization mode with an ionization energy of 70 eV. The data obtained from the GC–MS analysis was analyzed using a mass spectral library and a retention index database to identify the individual components in the extracts. The relative percentage of each component was calculated using the peak area normalization method. The results were reported as a list of identified components with their respective retention times, retention indices, and mass spectra (Song et al. 2021).

Fourier transform infrared spectroscopy (FTIR)

FTIR is a non-destructive analytical technique used to identify functional groups in a sample based on the absorption or transmission of infrared radiation by the sample. In the case of *C. sinensis* leaf extracts, FTIR was used to identify the characteristic functional groups present in the extracts. To perform FTIR, a very small amount of both extracts (methanolic and n-hexane) was mixed separately with dry potassium bromide (KBr) and thoroughly mixed in a mortar and pestle. KBr is used as a diluent to form a solid disk that is transparent to IR radiation, making it suitable for IR analysis. The extracts were then directly placed on the crystal plate center of the FTIR spectroscope and measured in a range of 225–4000 cm^{-1}. The resulting spectrum shows the different peaks, which correspond to the vibration frequencies of various chemical bonds in the sample. The peaks value of FTIR were recorded and used for subsequent analysis (Göl et al. 2020).

UV–Vis spectrophotometry

UV–Vis spectrophotometry is a technique that measures the absorbance or transmittance of light by a sample in the ultraviolet and visible regions of the electromagnetic spectrum. In the case of *C. sinensis* leaf extracts, UV–Vis spectrophotometry was used to detect the UV–Vis spectrum profile of methanolic and n-hexane leaf extracts of *C. sinensis*. This allowed for the detection of characteristic peaks, which can provide information about the composition of the extracts. To perform UV–Vis spectrophotometry, both extracts were

scanned in the wavelength ranging from 190 to 1100 nm by using a UV spectrophotometer. The resulting spectrum shows the absorbance or transmittance of light at different wavelengths. By analyzing the peaks present in the spectrum, information about the composition of the extracts can be obtained (Göl et al. 2020).

Preparation of ligand

The selected GC–MS detected phytochemicals from methanolic and n-hexane leaf extracts of *C. sinensis* were used as ligands for docking study. First, the 3D structures of these selected compounds were retrieved from the PubChem database as sdf format and were then prepared using Avogadro software (versions: 1.2.0). The mmff94 force field was used to construct and optimize the structures of the ligands. This involved removing any undesirable groups, adding hydrogen atoms, and optimizing the energy and geometry of the structures (Majumder and Mandal 2022).

Preparation of protein

AChE (PDB ID: 4BDT) and BChE (PDB ID: 6QAB) were selected as the target proteins for docking study through literature review. The 3 dimensional (3D) structures as X-ray diffraction model of these proteins were retrieved from the Protein Data Bank (PDB) database in sdf format. Before docking, the heteroatoms, water molecules and other additional molecules except target were removed from the protein structures using Discovery Studio (versions: 4.5) and Swiss-spdb viewer software (versions: 4.1). The GROMACS96 force field was then used to minimize the energy of the protein structures. The minimized protein structures were then saved in pdb format and used for docking study with the prepared ligands (Martiz et al. 2022).

Molecular docking

Molecular docking was performed to explore the potential of phytochemicals from *C. sinensis* as therapeutic agents for Alzheimer's disease. It was carried out using the PyRx software tool (versions: 0.8) (Luo et al. 2021a). First, the ligands were imputed with the help of OpenBabel and were then converted to PDBQT format. Second, the target proteins were also imputed in PyRx and make the proteins to macromolecule as PDBQT format. The center and grid box size for 4bdt were set as (X: 0.4990 Å, Y: − 40.504 Å, Z: − 58.7517 Å) and (X: 61.4969 Å, Y: 87.4343 Å, Z: 70.2044 Å) and for 6qab it was (X: 31.8498 Å, Y: 16.5821 Å, Z: 89.5222 Å) and (X: 56.6963 Å, Y: 58.9158 Å, Z: 74.5426 Å) respectively. The docking calculations were done with the PyRx program, while the analysis of the binding interactions was done with the Discovery Studio. For the docking investigation, the lowest binding scores were used, and energy was estimated in kcal/mole.

Molecular dynamics

To determine the stability and rigidity of a complex, molecular dynamics study was performed using YASARA (Yet Another Scientific Artificial Reality Application) Dynamics software (version 19.12.4) with the aid of Assisted Model Building with Energy Refinement (AMBER)14 force field (Hu et al. 2022). The docked complexes were initially cleaned and optimized, and the hydrogen bond network was also optimized as well. To minimize the protein complexes using a TIP3P water solvation model (0.997 g/L1, 25 c, 1 atm), the steepest gradient techniques were employed using a simulated annealing method (Yang et al. 2020). The physiological conditions were set at 0.9% NaCl, 310 K and pH 7.4 (Kim et al. 2021) to neutralized the simulated system, and time simulation time step was set at 1.25 frames per second. The particle mesh Ewald (PME) method with a cutoff radius of 8.0 Å was used to calculate the long-range electrostatic interaction (Pederson and McDaniel 2022). The simulation trajectories were saved after every 1.25 ps and the final simulation run was conducted for 100 ns (Koehler et al. 2021), and the root-mean-square deviation (RMSD), the radius of gyration (Rg), the solvent accessible surface area (SASA) and the hydrogen bonding were calculated using this simulation trajectories (Baildya et al. 2021; Islam et al. 2021).

ADMET analysis

To predict the pharmacokinetic properties of a compound, it is necessary to evaluate the absorption, distribution, metabolism, excretion and toxicity (ADMET) profile of this compound. The phytocompounds that passed the docking study were subjected to ADMET analysis to identify the presence of desirable properties to be considered as a lead molecule. Therefore, the pkcsm (2022 version) and SwissADME (2022 version) web servers were employed to analysis ADMET profiles and to calculate molecules' adherence to Lipinski's rule of five respectively (Attarroshan et al. 2022).

Results and discussion

GC–MS analysis

The GC–MS analysis of the methanolic and n-hexane leaf extracts of *C. sinensis* carried out in this study identified a total of 19 compounds, with 14 compounds detected in the methanolic extract extract (Table 1, Fig. 1a) and 5 compounds in the n-hexane extract (Table 2, Fig. 1b). The

Table 1 Compounds detected through GC–MS analysis from methanol leaf extract of *C. sinensis*

Serial no.	Compounds name	RT	Con. (%)
1	1,2,5,6-Tetrahydropyridin-2-one, 5-methyl	4.370	2.684
2	3,4-Dimethyl-3-pyrrolin-2-one	6.054	2.825
3	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	6.957	1.499
4	2-Hydroxymethyl-2-methyl-pyrrolidine-1-carboxaldehyde	8.649	26.028
5	Caffeine	26.456	41.048
6	Theobromine	26.922	5.265
7	Hexadecanoic acid, methyl ester	28.162	0.661
8	9,12,15-Octadecatrienoic acid, methyl ester	31.845	0.221
9	Phytol	32.105	15.118
10	Diisooctyl phthalate	39.590	0.604
11	Ergosta-7,22-dien-3-ol, (3.beta.,5.alpha.,22E)-	41.939	1.393
12	Squalene	43.906	0.643
13	Cholest-8-en-3.beta.-ol, acetate	44.549	0.075
14	dl-.alpha.-Tocopherol	49.037	1.929

RT retention time, *Con.* concentration

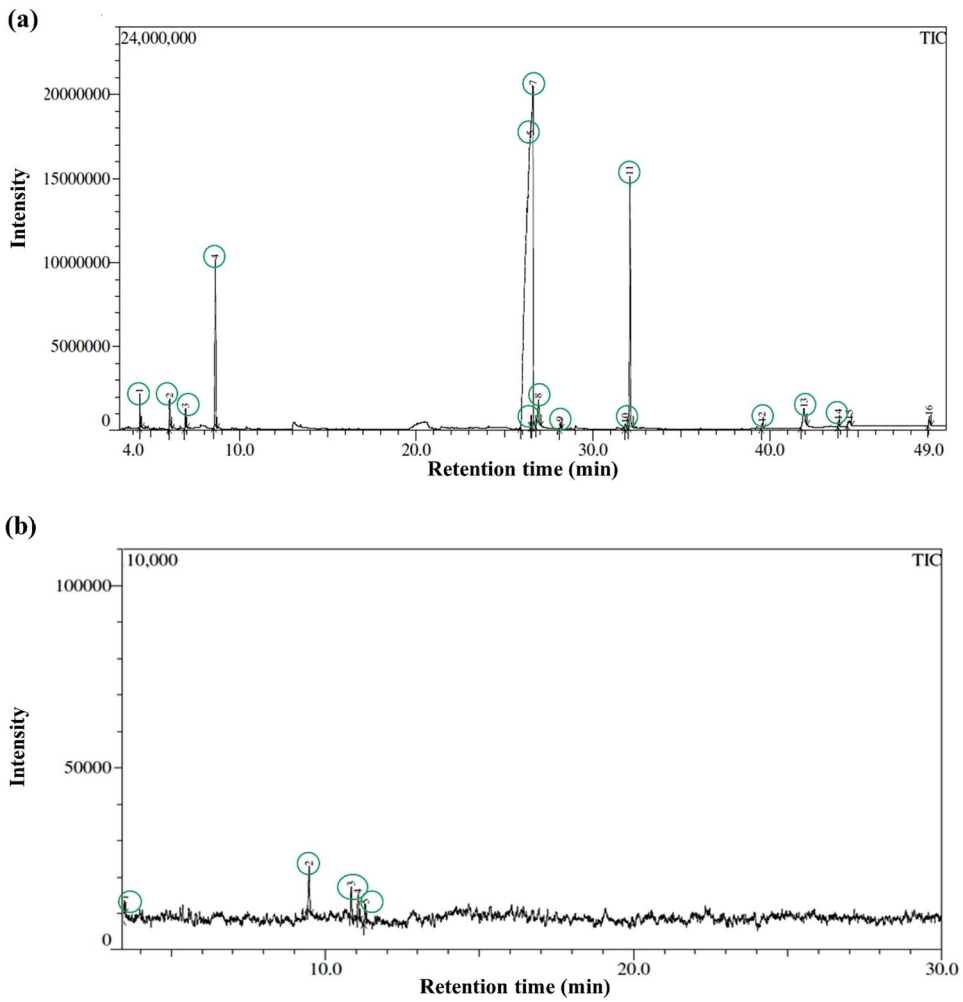


Fig. 1 GC–MS chromatography of *C. sinensis* methanolic (**a**) and n-hexane (**b**) leaf extracts

Table 2 Compounds detected through GC–MS analysis from n-hexane leaf extract of *C. sinensis*

Serial no	Compounds name	RT	Con. (%)
1	Hexanoic acid	3.477	13.469
2	Benzene, 1,3-bis(1,1-dimethylethyl)	9.472	43.327
3	1-Undecene, 7-methyl	10.842	16.837
4	3-Tridecene	11.068	16.598
5	n-Tridecan-1-ol	11.288	9.767

RT retention time, *Con.* concentration

predominant compounds in the methanolic extract were caffeine (41.048%) and Benzene, 1,3-bis(1,1-dimethylethyl) (43.327%), while the n-hexane extract contained mostly nonadecane (48.065%) and hexadecanoic acid (28.903%). These findings are consistent with a previous study by Mohammed et al. (2023), which identified 21 compounds from the *C. sinensis* extract, including caffeine and Benzene, 1,3-bis(1,1-dimethylethyl) (Mohammed et al. 2023). However, it should be noted that the most prevalent compounds found in *C. sinensis* can vary depending on the location, as evidenced by the study by Kumari and Kumar (2022) which identified different predominant compounds in *C. sinensis*, such as 2′,6′dihydroxyacetophenone, bis(trimethylsilyl) and N(trifuoracetyl)O,O′,O″tris(trimethylsilyl) epinephrine (Kumari and Kumar 2022). This suggests that the compounds and their concentrations in *C. sinensis* can be influenced by various factors such as environmental conditions, season, and soil type.

In a study published in 2020, Zhang et al. investigated the chemical composition of ethanolic extracts of *C. sinensis* and their neuroprotective effects in vitro. The authors identified 30 compounds, including flavonoids, phenolics, and alkaloids, that showed potential for neuroprotection and inhibition of amyloid beta aggregation, a hallmark of Alzheimer's disease (Zhang et al. 2020a). Another study published in 2022 by Kshirsagar et al. explored the neuroprotective effects of *C. sinensis* extract in a mouse model of Alzheimer's disease. The authors found that treatment with *C. sinensis* extract significantly improved cognitive function and reduced oxidative stress and neuroinflammation in the brain (Kshirsagar et al. 2022).

FTIR

The functional groups present in methanolic and n-hexane leaf extracts of *C. sinensis* were identified using FTIR analysis and the FTIR spectrum are shown in Fig. 2. The FTIR analysis of methanolic leaf extract showed 16 peaks (Fig. 2a) with the presence of mainly alcohol, alkane, esters, lipids and amine groups (Table 3). On the other hand, five peaks were found in n-hexane extract (Fig. 2b) with two different functional groups including aliphatic primary amine (N–H stretching) at 3344.12 cm^{-1} and alkane (C=C stretching) at 1628 cm^{-1} (Table 4).

The use of FTIR analysis to identify functional groups in *C. sinensis* extracts is not uncommon in current research. For example, a study by Gaur et al. (2023) used FTIR analysis to identify the functional groups present in extracts of *C. sinensis* leaves and found the presence of various functional groups, including carboxylic acid, alcohol, and amine groups (Gaur et al. 2023). In another study, Lee et al. (2021) used FTIR analysis to investigate the changes in the functional groups present in amyloid beta (Aβ) fibrils when treated with a *C. sinensis* extract (Lee et al. 2021). The authors found that the extract led to changes in the Aβ fibril structure and the appearance of additional functional groups, suggesting that *C. sinensis* may have potential for inhibiting Aβ aggregation, a hallmark of Alzheimer's disease. In addition to FTIR analysis, other techniques have been employed to investigate the potential role of *C. sinensis* in Alzheimer's disease. For example, a study by Iqubal et al. (2021) used molecular docking studies to investigate the potential of compounds found in *C. sinensis* to inhibit acetylcholinesterase (AChE), an enzyme that plays a role in the pathogenesis of Alzheimer's disease (Iqubal et al. 2021). The authors found that several compounds, including caffeine and theophylline, showed strong binding affinity to AChE, suggesting that *C. sinensis* may have potential as a natural source of AChE inhibitors.

UV–Vis spectrophotometry

UV–Vis analysis of methanolic and n-hexane leaf extracts of *C. sinensis* was performed at the wavelength between 190 to 1100 nm and the results are shown in Fig. 3. The UV–Vis profile of methanolic leaf extract of *C. sinensis* showed the five peaks (Fig. 3a) and the highest absorption (3.42) was found at 276 nm (Table 5). However, n-hexane extract showed eight peaks (Fig. 3b) with maximum absorption (0.449) at 410 nm (Table 5).

Several studies have employed UV–Vis spectrophotometry to investigate the presence of phenolic compounds in *C. sinensis* and their potential role in Alzheimer's disease. For example, a study by de Moura et al. (2022) used UV–Vis spectrophotometry to measure the total phenolic content and antioxidant activity of *C. sinensis* leaf extracts and found a positive correlation between the two. The authors suggested that the phenolic compounds in *C. sinensis* may have potential for preventing or slowing down the progression of Alzheimer's disease by reducing oxidative stress (de Moura et al. 2022). Similarly, a study by Sánchez et al. (2020) used UV–Vis spectrophotometry to measure the total phenolic content and flavonoid content of *C. sinensis* extract and

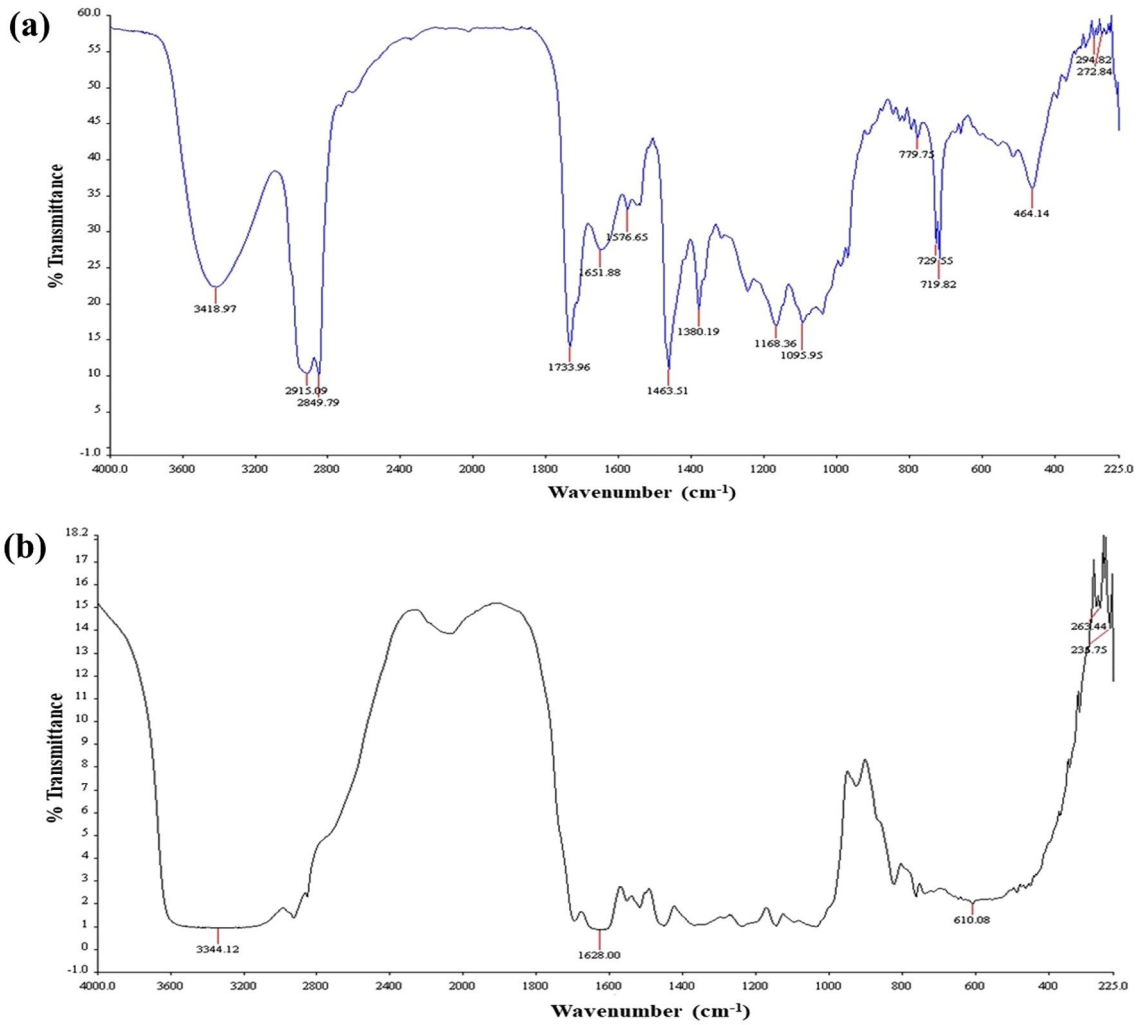


Fig. 2 Fourier transform infrared spectra (FTIR) of *C. sinensis* methanolic (**a**) and n-hexane (**b**) leaf extracts

found that it had antioxidant activity and protected against oxidative damage in neuronal cells. The authors suggested that these findings support the potential use of *C. sinensis* as a natural source of antioxidants for the prevention and treatment of Alzheimer's disease (Sánchez et al. 2020). Another study by Afzal et al. (2022) used UV–Vis spectrophotometry to investigate the effect of *C. sinensis* extract on the aggregation of amyloid beta (Aβ), a hallmark of Alzheimer's disease. The authors found that the extract had a dose-dependent inhibitory effect on Aβ aggregation and suggested that the phenolic compounds in *C. sinensis* may have potential for inhibiting Aβ aggregation and preventing the progression of Alzheimer's disease (Afzal et al. 2022).

Molecular docking

In this present investigation, total 19 phytochemicals from *C. sinensis* leaf extracts that were detected through GC–MS technique, were used to perform molecular docking targeting AChE and BChE of AD respectively. Top two compounds from each group were selected on the basis of lower binding energy. Against AChE, compounds CID: 5283669 and CID: 71343282 showed significant level of inhibitory potentiality with binding energy − 10.0 kcal/mol and − 8.9 kcal/mol respectively. Whereas, both compounds were showed better binding affinity than control drug Donepezil (− 8.1 kcal/mol). The interaction between AChE and CID: 5283669 revealed one hydrogen bond at Tyr:337 (2.51 Å), three alkyl bond at Leu:289 (5.18 Å), Phe:297 (5.13 Å) and Tyr:341 (5.22 Å) and one pi-alkyl bond at Trp:286 (4.26 Å) (Table 6, Fig. 4a). Similarly, the complex AChE + CID: 71343282 was stabilized with one pi-alkyl bond at Tyr:72 (5.20 Å), two pi-sigma bond at Tyr:341 (3.83 Å) and Trp:286 (3.74 Å) and one pi-pi sigma bond at Leu:289 (4.58 Å) (Table 6, Fig. 4b). In addition, the control drug donepezil was steadied with AChE

Table 3 Functional groups present in the methanolic leaf extract of *C. sinensis*

Wavenumber (cm^{-1})	Group	Compounds class
3418.97	O–H stretching	Alcohol
2915.09	C–H stretching	Alkane
2849.79	C–H stretching	Alkane
1733.96	C=O stretching	Esters
1651.88	C=C stretching	Alkene
1576.65	C=C stretching	Cyclic alkene
1463.51	CH_3 bend	Lipids
1380.19	CH_3 bend	Lipids
1168.36	C–O stretching	Ester
1095.95	C–N stretching	Amine
779.75	C–Cl	Alkyl halides
729.55	C–Br	Alkyl halides
719.82	C–Br	Alkyl halides
464.14	C–H bending vibrations	–
294.82	C–H bending vibrations	–
272.84	C–H bending vibrations	–

Table 4 Functional groups present in the n-hexane leaf extract of *C. sinensis*

Wavenumber (cm^{-1})	Group	Compound class
3344.12	N–H stretching	Aliphatic primary amine
1628.00	C=C stretching	Alkane
610.08	C–Br stretch	Alkyl halides
263.44	C–I	Alkyl halides
235.75	C–I	Alkyl halides

by one hydrogen, three carbon-hydrogen, one pi-alkyl and one pi-sigma bond (Table 6, Fig. 4c).

In case of BChE, compound CID: 2116 showed better binding energy compared to the control donepezil with − 11.6 kcal/mol and − 10.5 kcal/mol, whereas compound CID: 5,283,669 showed slightly lower inhibitory activity than the control (Table 7). The phytochemical CID: 2116 interacted with 10 amino acid residues of BChE with one hydrogen, five alkyls, one pi-alkyl, one pi-sigma, one pi-pi T-shaped and one amide Pi-stacked bonds (Table 7, Fig. 5a). Similarly compound CID: 5,283,669 interacted with BChE by a hydrogen bond at Tyr:332 (4.03 Å), alkyl bond at Ala:328 (3.71 Å) and pi-sigma bond at Trp:82 (3.64 Å) (Table 7, Fig. 5b). However, donepezil interacted with BChE by maximum 13 amino acid residues with six different type of bonds (Table 7, Fig. 5c). Interestingly, in our study compound CID: 5283669 showed the inhibitory activity against both AChE and BChE protein.

Recent studies have also utilized molecular docking to investigate the potential of phytochemicals from *C. sinensis* as inhibitors of AChE and BChE in Alzheimer's disease. For example, a study by Iqubal et al. (2021) used molecular docking to investigate the potential of *C. sinensis* leaf extracts to inhibit AChE and found that the extracts had a higher binding affinity than the standard drug, donepezil. The authors suggested that *C. sinensis* may have potential as a natural source of AChE inhibitors for the treatment of Alzheimer's disease (Iqubal et al. 2021). Similarly, a study by Lee et al. (2021) used molecular docking to investigate the potential of EGCG, a phenolic compound found in *C. sinensis*, as an inhibitor of AChE and BChE in Alzheimer's disease. The authors found that EGCG had a higher binding affinity than donepezil and suggested that it may have potential for the prevention and treatment of Alzheimer's disease (Lee et al. 2021). Another study by Luo et al. (2021a) used molecular docking to investigate the potential of theaflavin, a flavonoid compound found in *C. sinensis*, as an inhibitor of AChE and BChE in Alzheimer's disease. The authors found that theaflavin had a higher binding affinity than donepezil and suggested that it may have potential as a natural source of AChE and BChE inhibitors for the treatment of Alzheimer's disease (Luo et al. 2021a).

Molecular dynamics

In this study, only top two complexes showed best binding affinity against both target proteins AChE and BChE in molecular docking study. These two complexes were subjected to perform molecular dynamics (MD) simulation at 100 ns to determine the stability of protein–ligand complexes in a specific and artificial environment. The MD simulation results were analyzed based on the RMSD, Rg, SASA and intramolecular hydrogen bonds.

MD simulation against AChE

The MD simulation results of complexes C1 (CID: 5283669 + AChE) and C2 (CID: 71343282 + AChE) are shown in Fig. 6. The average RMSD value of C1 and C2 complexes were 3.353 Å and 3.779 Å where the C1 complex did not show stability during the simulation. However, after few ns C1 showed the increasing trend and maintain the stability during the simulation period although having some flocculation (Fig. 6a). Similarly, the Rg value of C2 complex had a stable profile during the simulation period with average Rg value 25.55 while initially it was 24.857. Complex C1 showed the decreasing trend until 20 ns, thereafter it was dramatically increased within few ns and then maintained slight stability at the end of simulation, although at 48 ns, an instability was found as like the instability found at 20 ns (Fig. 6b). The SASA analysis of both complexes

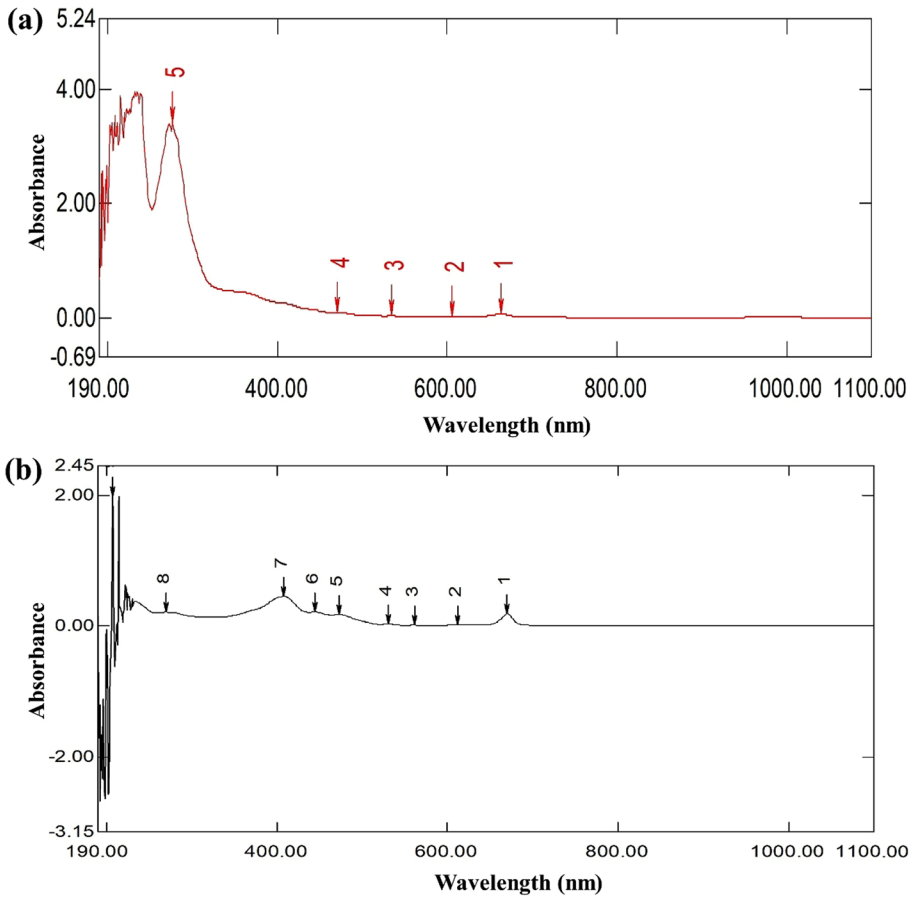


Fig. 3 UV–visible spectroscopy absorbance of methanolic (**a**) and n-hexane (**b**) leaf extracts of *C. sinensis*

Table 5 Absorbance of compounds present in the methanolic and n-hexane leaf extracts of *C. sinensis*

ID	Methanolic extract		N-hexane extract	
	Wavelength	Absorbance	Wavelength	Absorbance
1	669	0.05	673	0.165
2	615	0.016	622	0.014
3	526	0.025	573	0.006
4	480	0.078	537	0.019
5	276	3.42	478	0.166
6			451	0.199
7			410	0.449
8			276	0.2

had similar upward movement and showed the stability till approximately 85 ns. Subsequently it was dramatically showed a downward movement till the end of the simulation period (Fig. 6c). The hydrogen bond framework of the both C1 and C2 complexes showed the similar trend and stability during the MD simulation period (Fig. 6d).

Recent studies have also used molecular dynamics simulations to investigate the interactions between phytochemicals and AChE. For instance, a study by Wu et al. (2022) used MD simulation to explore the binding stability of the flavonoid, quercetin, with AChE. The study showed that the quercetin-AChE complex had a stable binding conformation during the simulation period (Wu et al. 2022). Similarly, another study by Almasi et al. (2022) performed MD simulation to explore the binding stability of the ginsenoside Rg3 with AChE. The results showed that ginsenoside Rg3 formed stable complexes with AChE and had inhibitory effects on the enzyme's activity (Almasi et al. 2022).

MD simulation against BChE

The results of MD simulation of complexes C3 (CID: 2116 + BChE) and C4 (CID: 5283669 + BChE) are shown in Fig. 7. The overall RMSD values of both C3 and C4 complexes were satisfactory whereas the average value was 1.447 Å and 1.257 Å respectively. Initially the RMSD values were shockingly increased within short period of time, afterward they were stable during the whole simulation time whereas C3 showed the uprising stability from C4 complexes (Fig. 7a). From the very beginning to end of simulation period the Rg profile of C3 complex had conserve stability whereas C4 maintained a stable profile with C3 until approximately 55 ns, subsequently it showed the

Table 6 Binding energy, interacting amino acid residues, bond types and their distance between AChE and the two best docked compounds

Complex	Binding energy, kcal/mol	Interacting residue	Bond type	Distance (Å)
AChE + CID: 5283669	− 10.0	Tyr:337	H	2.51
		Leu:289	A	5.18
		Phe:297	A	5.13
		Tyr:341	A	5.22
		Trp:286	PA	4.26
AChE + CID: 71343282	− 8.9	Tyr:72	PA	5.20
		Tyr:341	PS	3.83
		Trp:286	PS	3.74
		Leu:289	PPS	4.58
AChE + Donepezil (control)	− 8.1	Phe:295	H	3.10
		Trp:286	CH	4.46
		Ser:293	CH	4.36
		Arg:296	CH	3.25
		Trp:341	PA	3.66
		Leu:289	PS	5.22

Donepezil was used as a control drug

H hydrogen, *A* alkyl, *PA* Pi-alkyl, *PS* Pi-sigma, *PPS* Pi-pi sigma, *CH* conventional hydrogen bond

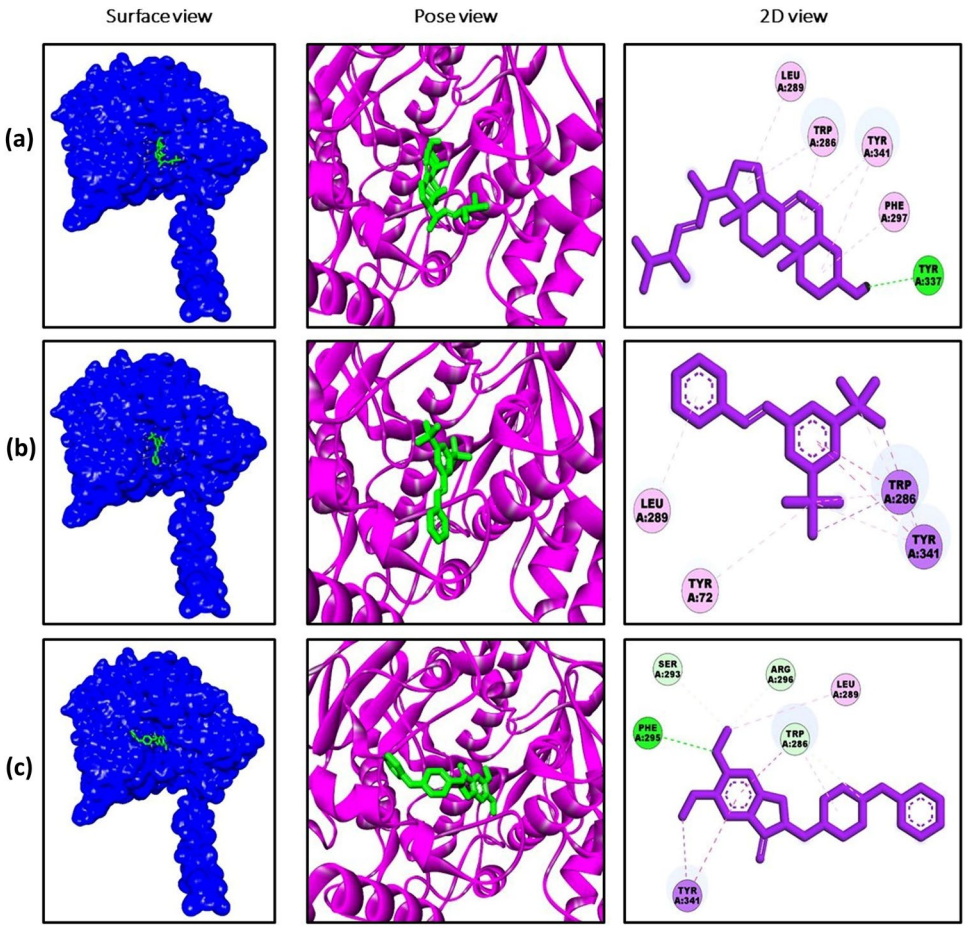


Fig. 4 Interactions of AChE with ligands CID: 5283669 (**a**), CID: 71343282 (**b**) and control drug donepezil (**c**)

Table 7 Binding energy, interacting amino acid residues, bond types and their distance between BChE and the two best docked compounds

Complex	Binding energy, kcal/mol	Interacting residue	Bond type	Distance (Å)
BChE + CID: 2116	− 11.6	Ser:198	H	2.41
		Tpr:82	A	4.36
		Ala:328	A	4.21
		Met:437	A	5.48
		His:438	A	5.38
		Tyr:440	A	5.37
		Trp:430	PA	5.81
		Tyr:332	PS	3.69
		Gly:116	PTS	4.17
		Phe:329	APS	5.39
BChE + CID: 5283669	− 9.3	Tyr:332	H	4.03
		Ala:328	A	3.71
		Trp:82	PS	3.64
BChE + Donepezil (control)	− 10.5	Tyr:332	H	2.97
		Glu:197	CH	3.55
		Ser:287	CH	3.76
		Pro:285	CH	3.46
		Ala:328	A	3.89
		Trp:430	A	5.87
		Met:437	A	5.00
		His:438	A	4.81
		Leu:286	PA	5.27
		Trp:82	PS	3.75
		Gly:116	PTS	5.06
		Trp:231	PTS	6.09
		Phe:329	PTS	4.92

Donepezil was used as a control drug

H hydrogen, *A* alkyl, *PA* Pi-alkyl, *PS* Pi-sigma, *PPS* Pi-pi sigma, *PTS* pi-pi T-shaped, *APS* amide Pi-stacked

upward movement till 80 ns and a downward trend at the end of the simulation period (Fig. 7b). Both complexes showed a stable SASA profile till 65 ns and then a decreasing movement till the end (Fig. 7c). Hydrogen bond profile of both complexes had a strong stable trend although initially it had some fluctuation (Fig. 7d).

Several studies have shown that the phytochemicals from *C. sinensis* have neuroprotective effects and can inhibit the activity of BChE, a key enzyme involved in the degradation of acetylcholine, which is essential for cognitive function. A recent study by Turumtay et al. (2022) used molecular docking and MD simulation to investigate the binding modes and stability of various catechins and theaflavins from *C. sinensis* with BChE. The study found that several compounds, including epicatechin gallate and theaflavin-3,3′-digallate, showed strong binding affinity and stability with BChE, indicating their potential as BChE inhibitors for Alzheimer's disease treatment (Turumtay et al. 2022). Another study by Zhang et al. (2020b) investigated the neuroprotective effects of epigallocatechin-3-gallate (EGCG), a major phytochemical in *C. sinensis*, in a mouse model of Alzheimer's disease. The study found that EGCG treatment reduced cognitive impairment and neuroinflammation in the mice, indicating its potential as a therapeutic agent for Alzheimer's disease (Zhang et al. 2020b).

ADMET analysis

The ADMET profiles of top three ligands were analyzed to filter a lead compound for Alzheimer's disease treatment. The Lipinski rule of drug-likeness was followed by all three compounds, and they were found to be physiologically active with good human intestinal absorption and positive results in the human blood–brain barrier permeability test. Additionally, no toxicity was found in hepatotoxicity and AMES test, and all screened compounds showed positive results in CYP2D6 substrate and hERG I inhibitor test (Table 8).

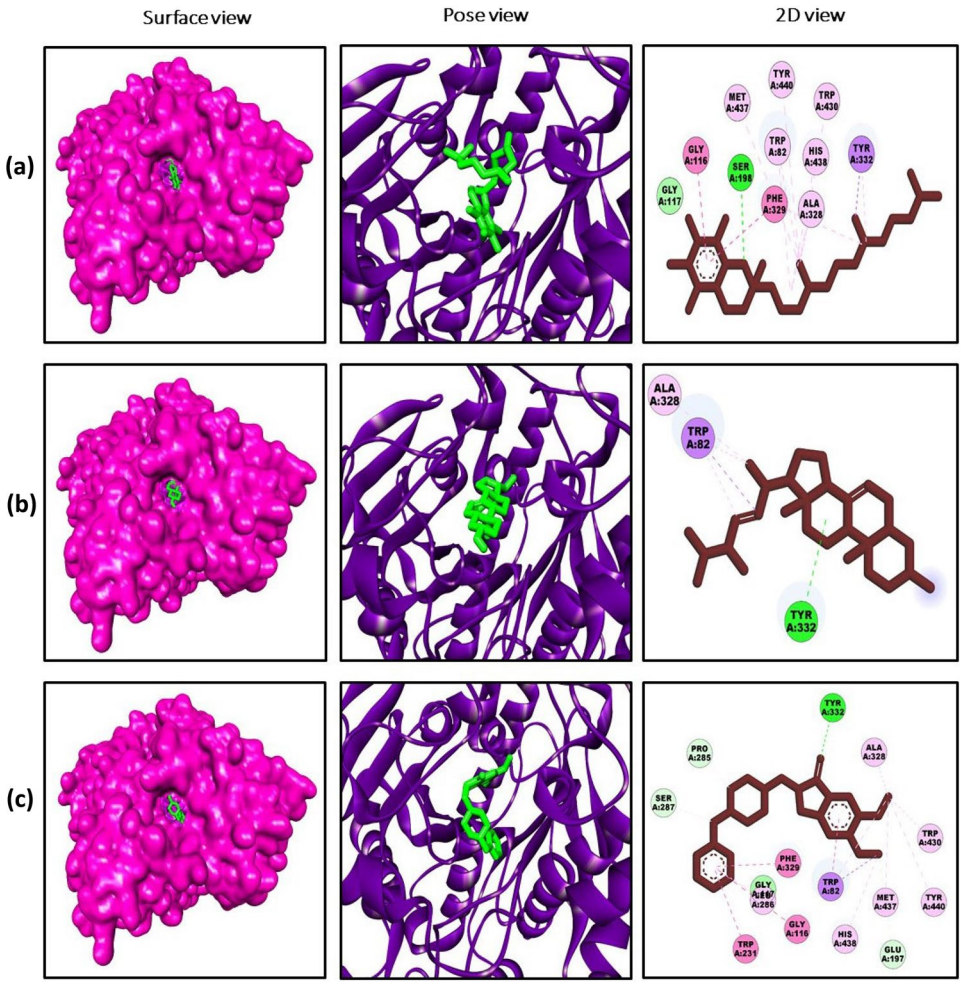


Fig. 5 Interactions of BChE with ligands CID: 2116 (**a**), CID: 5283669 (**b**) and control drug donepezil (**c**)

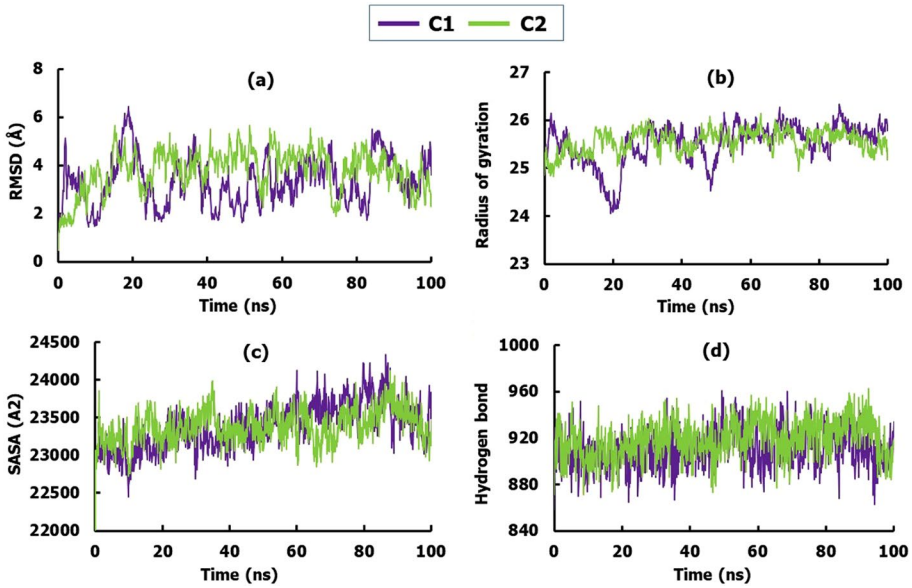


Fig. 6 The molecular dynamics simulation of complexes C1 (AChE + CID: 5283669) and C2 (AChE + CID: 71343282) with RMSD (**a**), Rg (**b**), SASA (**c**) and hydrogen bonding (**d**) values

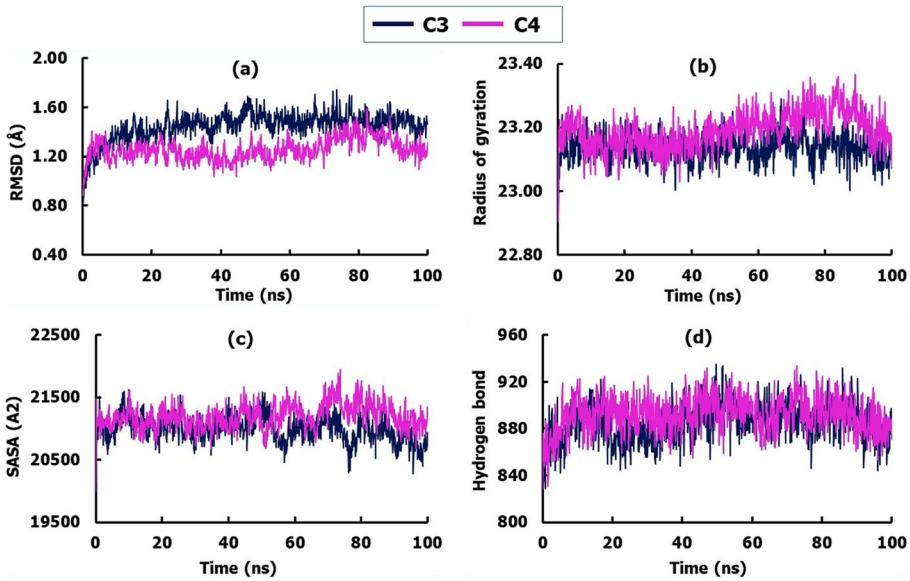


Fig. 7 The molecular dynamics simulation of complexes C3 (BChE + CID: 2116) and C4 (BChE + CID: 5283669) with RMSD (**a**), Rg (**b**), SASA (**c**) and hydrogen bonding (**d**) values

Table 8 The ADMET profiles of the top three screened compounds

Parameters	CID: 5283669	CID:71343282	CID: 2116
Molecular weight	398.66	292.46	430.71
Molecular formula	C28H46O	$C_{22}H_{28}$	$C_{29}H_{50}O_2$
Hydrogen bond donor	1	0	1
Hydrogen bond acceptor	1	0	2
Rotatable bonds	4	2	12
LogP	7.4107	6.452	8.840
Surface area	179.984	135.448	192.727
Bioavailability score	0.55	0.55	0.55
Water solubility	− 6.974	− 6.54	− 8.60
Lipinski	Yes; 1 violation	Yes; 1 Violation	Yes; 1 Violation
Human intestinal absorption	95.05	93.833	89.782
Blood brain barrier	0.764	0.791	0.876
CNS permeability	− 1.705	− 7.94	− 1.669
P-Glycoprotein 1 inhibitor	Yes	No	No
CaCo2 permeability	1.235	1.56	1.345
CYP2D6 substrate	No	No	No
Oral rat acute toxicity (LD50)	2.057	2.712	2.072
AMES Toxicity	No	No	No
Hepatotoxicity	No	No	No
hERG 1 Inhibitor	No	No	No

The ADMET profiles were explored from Swissadme and pKCSM webserver

Recent studies have also investigated the potential of phytochemicals from *C. sinensis* in the treatment of Alzheimer's disease. For example, a study by Youn et al. (2022) found that a compound called (−)-epigallocatechin-3-gallate (EGCG) could improve cognitive function and reduce amyloid beta plaque formation in a mouse model of Alzheimer's disease (Youn et al. 2022). Another study by Li et al. (2023) found that a compound called theaflavin-3,3′-digallate (TF3) could protect against cognitive impairment and neuroinflammation in a mouse model of Alzheimer's disease by regulating the gut-brain axis (Li et al. 2023). They also investigated the neuroprotective effects of a compound called theaflavin-3′-*O*-gallate (TF2B) in a mouse model of Alzheimer's disease and found that TF2B could improve cognitive function and reduce neuroinflammation by modulating the gut microbiota. In terms of ADMET profiles, a recent study by Okello and Mather (2020) investigated the pharmacokinetic profiles of EGCG and its metabolites in

rats and found that EGCG and its metabolites could cross the blood–brain barrier and have good oral bioavailability, indicating their potential as therapeutic agents for Alzheimer's disease (Okello and Mather 2020). Overall, these studies support the potential of phytochemicals from *C. sinensis* as therapeutic agents for Alzheimer's disease and highlight the importance of investigating their ADMET profiles to identify lead compounds.

Conclusion

The findings of this research article suggest that phytochemicals derived from *C. sinensis*, specifically Ergosta-7,22-dien-3-ol, (3.beta.,5.alpha.,22E)-, Benzene, 1,3-bis(1,1-dimethylethyl), and dl-.alpha.-Tocopherol, have the potential to serve as lead compounds for the treatment of Alzheimer's disease. The in silico molecular docking, dynamics, and ADMET analysis indicated that these compounds possess high binding affinity, stability, and nontoxicity. These results suggest that these compounds could be developed as potential cholinesterase inhibitors to mitigate the symptoms of Alzheimer's disease. The findings of this research article and recent studies support the potential of *C. sinensis*-derived phytochemicals as therapeutic agents for the treatment of Alzheimer's disease. Further research is necessary to develop these compounds into effective drugs for the treatment of Alzheimer's disease.

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Author contributions MEH and MSR were equally involved in the conception and design of the experiments. MEH and MSR conducted the experiments. MEH and MSR were supervised by RZ, and MOF, MK, MAI and UKA provided critical facilities for the experiments. MEH, MSR, RZ and MOF analyzed the data. MEH, MSR and RZ contributed to drafting the article. RZ, MK, MAI and UKA contributed to revising it critically. All authors approved the final version of this manuscript.

Availability of data All relevant data are within the manuscript.

Declarations

Conflict of interest On behalf of all authors, the corresponding author declares that there is no conflict of interest.

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