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ORIGINAL RESEARCH ARTICLE

Molecular docking and dynamics simulation studies on sakuranetin a phytomolecule targeting AChE receptor against Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) is a common type of dementia, mainly characterized by neurodegeneration and memory loss with other cognitive impairments in the brain. AD is one of the fastest emerging irrecoverable diseases in the aging population. Moreover, the degeneration of central cholinergic neurons damages memory and leads to cholinergic dysfunction. Several natural phytomolecules and semisynthetic analogs have been used to treat AD for years. However, no preventive treatment is available to date because most of the available drugs/compounds have failed in clinical trials. AChE is one of the most promising therapeutic targets for symptomatic improvement in AD patients. The current study was done to screen several natural phytomolecules derived from the Verbenaceae family's Lippia graveolens (Mexican Oregano). Based on the in silico results, Sakuranetin was the best potential AChE antagonist (-8.24 kcal/mol), and the primary amino acid residues, i.e., Tyr124, Tyr341, Leu289, and Phe295, were involved in the active binding site of AChE. The compound has shown a good fit and can cross the blood–brain barrier (BBB) along with high levels of intestinal absorption. Furthermore, Sakuranetin and AChE complex was found to be stable as analyzed by 10 ns molecular dynamics simulations. Based on the free binding energy and stability, it may be concluded that Sakuranetin is a potential hit compound for treating Alzheimer's disease.

INTRODUCTION

Alzheimer's disease (AD) is a type of dementia and a significant concern that is continuously increasing worldwide (Weller and Budson, 2018). AD is messiness, an irreversible and progressive global health challenge that causes deterioration of neurons in brain function by atrophy in thinking and declension. AD is considered a conglomerate disease affecting >46 million worldwide (Jan *et al.,* 2017). AD is a pernicious neurodegenerative disorder that is not understood well to date, and no preventive treatment is currently available. In the United States of America, the pervasiveness of AD is expected to rise three-fold by 2050 (Hebert *et al.,* 2013). However, in 2017, a total number of 121,404 mortality rates was observed, while in 2018, the estimated global cost of caring was $1 trillion for AD patients. Presently, the etiology of the disease is still not understood well, and no safe, targeted drug is available. The significant symptoms of AD are mainly associated with the structural modifications in the cholinergic synapses and loss/alterations of acetylcholine (ACh) linked receptors.

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Plants are the primary source of unlimited natural molecules that exhibit several therapeutic and medicinal properties. Numerous natural and semisynthetic molecules have shown efficacies in treating AD (Mangialasche *et al.,* 2010). According to The Indian Ayurvedic system, several plant-derived drugs and remedies are currently being used to control Alzheimer's disease and its symptoms (Rao *et al.,* 2012). Phytochemicals or bioactive compounds from *Lippia graveolens* (Maxican Oregano, Verbenaceae family) showed potent efficacies against AD (Lin *et al.,* 2007; Torre *et al.,* 2022; Bautista-Hernanadez *et al.,* 2021). The essential oil obtained from Mexican Oregano exhibited anti-viral (Pilau *et al.,* 2011), anti-bacterial, antipyretic, antioxidant (Pascal *et al.,* 2001) as well as anxiolytic property (Gonzalaz-Trujano *et al.,* 2017). These findings suggest scientific evidence which may be exploited for more effective treatment of Alzheimer's disease.

Sakuranetin is a natural dihydro flavonoid compound obtained from several plants. It shows several physiological properties and may exert potent neuroprotective effects on brain cells through an anti-oxidation mechanism as well as anti-inflammatory activity against several inflammatory mediators such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) involved in brain cells AD (Liang and Maher, 2022; Prathap *et al.*, 2021; Li *et al.*, 2019). As a prelude to published literature, we examined the activity of Sakuranetin and other structurally similar flavanoids from *L. graveolens* against AD (AChE) receptors to understand the mechanism of action based on key chemical functional group contributing to the beneficial neuroprotective effects of the compound. Additionally, the phytoconstituents from Mexican Oregano have not been well studied against neurological disorders. These potent bioactivities suggest that Sakuranetin and several other phytoconstituents from *Lippia graveolens* need further examination in AD.

Recent advancements in structural and computational biology approaches provide possibilities for reconditioning natural phytomolecules and benefiting from their structures to produce novel drugs (Thomford *et al.,* 2018). The pharmacotherapeutic approach to enhance the synaptic levels of acetylcholine (ACh) in cholinergic synapses is to find some potential cholinesterase inhibitors (Perry *et al.,* Bales *et al.,* 2006). Several Cholinesterase inhibitors (ChEIs) are approved and are currently used for symptomatic treatment (Han *et al.,* 2019, Spitzer, 2012). In addition, Tacrine is the first ChEI drug used to treat AD (Horak*et al.,* 2017, Summers, 2006) and was approved by Food and Drug Administration (FDA). But unfortunately, Tacrine as several side effects, such as gastrointestinal and hepatotoxicity problems in the long run. Therefore, the present study is aimed to screen the phytochemicals of the Maxican Oregano plant for anti-Alzheimer efficacy using an *in-silico* approach.

MATERIALS AND METHODS

Data collection and library preparation

The data was collected from various online published research paper reviews and online databases such as Pubmed (http://www.ncbi.nlm.nih.gov/pubmed) and ChEMBL (https://www.ebi.ac.uk/chembl/). The molecular structures of all compounds (α-Copaene, Scutellarin, Sakuranetin, α-Cadinine, Galangin, Hispidulin, and Tacrine) were retrieved from the PubChem database. One hundred structurally similar ligands were downloaded and processed for further docking study.

Drug-likeliness property analysis

The ligand library was tested first for drug-relevant properties using Lipinski's Rule of 5 to evaluate drug-likeness properties. The set criteria for drug-likeness properties were molecular mass (≤ 500 Dalton), partitioning coefficient for lipophilicity (LogP ≤ 5), H-bond donors (≤ 5), and H-bond acceptors (≤ 10). Afterward, those compounds that do not violate any rules were filtered and evaluated for their pharmacokinetic properties *viz;* absorption, distribution, metabolism, and absorption (ADME) using the SWISS-ADME online tool (http://www.swissadme.ch/).

Molecular docking and dynamics simulation

All compounds that did not violate the Lipinski's rule and satisfied all the above-stated ADMET properties were further subjected to docking analysis. The crystal structure of recombinant human Acetylcholinesterase (AChE) was obtained from the Protein Data Bank (PDB ID 4EY5 with resolution 2.30 Å). The energy minimization and preparation process was done through Discovery studio. All ligands/compounds were docked

against AChE protein target using Pyrx (https://pyrx.sourceforge.io/) (Trott and Olson, 2010).

For assessing the stability and dynamics of ligand-protein complex, the best-predicted hit (Sakuranetin) against AChE protein target was selected to perform for 10000 ps all-atoms molecular dynamics simulation using GROMACS package (v5.1.4) (Abraham *et al.,* 2015). To generate the molecular topology files for the protein-ligand complex, we used the CHARMM General Force Field (CGenFF) program server (Vanommeslaeghe *et al.,* 2010). The force field was CHARMM36 all-atom force field Feb2021, while the water molecule type was SPC, with a size of 19.44810 × 19.44810 × 13.75188 nm3, and the distance between the complex and the box was 1 nm. The neutralization was done by adding 16Na+ ions in the solvent system containing 8e- 8Cl− ions. A minimum number of 10000 steps were used to minimize the energy. The first phase of equilibration was conducted under an *NVT* ensemble. From the plot, it was clear that the system's temperature quickly reached the target value (300 K) (Parrinello& Rahman, 1981) and remained stable over the remainder of the equilibration. In the second phase, pressure and density were equilibrated under an *NPT* ensemble, wherein the number of particles, pressure, and temperature were all constant. The ensemble is also called the "isothermal-isobaric" ensemble and most closely resembles experimental conditions. Finally, the related calculation file of root-mean-square deviation (RMSD), root mean square fluctuation (RMSF), and the radius of gyration (Rg), solvent accessible surface area (SASA) and interaction energy were obtained by removing the periodic boundary conditions. The graph plot analysis was done in MS excel and QtGrace software.

RESULTS AND DISCUSSION

Lippia graveolens (Oregano) is an important medicinal plant belonging to the family Verbenaceae. It is one of the major commercially traded culinary herbs used globally as a flavoring and aromatic agent. Several kinds of literature and sources validate its potential and benefits as an anti-inflammatory, antimicrobial, antioxidant, antifungal, and skin defensive mechanism related to its rich polyphenolic fraction (Gutierrez-Grijalva *et al.,* 2019; Ortega-Ramirez *et al.,* 2016). Additionally, Oregano has gained much recognition for its bioactive phytochemicals, which may work wonders in disease prevention (Torres-Leon *et al.,* 2017; Cui *et al.,* 2019; Turek*et al.,* 2013). The current demand/supply for bioactive phytoconstituents has increased significantly. The activity of phytochemicals of Mexican oregano in the neurological disorder needs further exploration. Based on the literature survey, a dataset consisting of 100 bioactive naturally occurring phytochemicals from *Lippia graveolens* (Mexican Oregano) was collected. The 3D structures of all compounds were retrieved from Pubchem and ChEMBL online databases.

After the initial assessment, only six compounds, namely α-copaene, Scutellarin, Sakuranetin, α-cadinine, Galangin, and Hispidulin, showed a good fit and better binding affinity than the standard drug Tacrine towards the target protein AChE receptor. The analysis was done by analyzing the binding conformation, mode of interaction, and binding pocket/site of the ligand within the target protein. Therefore, molecular docking was used to check the receptor's and ligands' interaction. Based on these results, as determined by molecular docking analysis, Sakuranetin was selected with the overall lowest binding energy and higher free energy of interaction with the AChE receptor. Further, the best predicted hit Sakuranetin was screened for *in silico* pharmacokinetics (ADME) analysis, toxicity risk assessment, and molecular dynamics simulations.

Molecular docking and dynamics simulations

The Molecular docking study was done to find the potential candidates against AD. The ligands, α-copaene, Scutellarin, Sakuranetin, α-cadinine, Galangin, and Hispidulin showed good affinity against Human Acetylcholinesterase (AChE) target protein. Tacrine was taken as reference standard positive control. The observed docking scores against the AChE target were -8.5, −8.2, -8.2, -8.2, -8.1, and −7.3 kcal/mol for α-copaene, Scutellarin, Sakuranetin, α-cadinine, Galangin, and Hispidulin respectively. In contrast, the docking score of Tacrine (reference drug) was -7.7 kcal/mol.

The best predicted hit Sakuranetin has interacted within the active site (hydrophobic cavity) of the protein target AChE. The interaction between Sakuranetin and AChE involved two H bonds, i.e., PHE295 and TYR337, at a distance of 1.98 Å and 2.191 Å, respectively, providing stability to

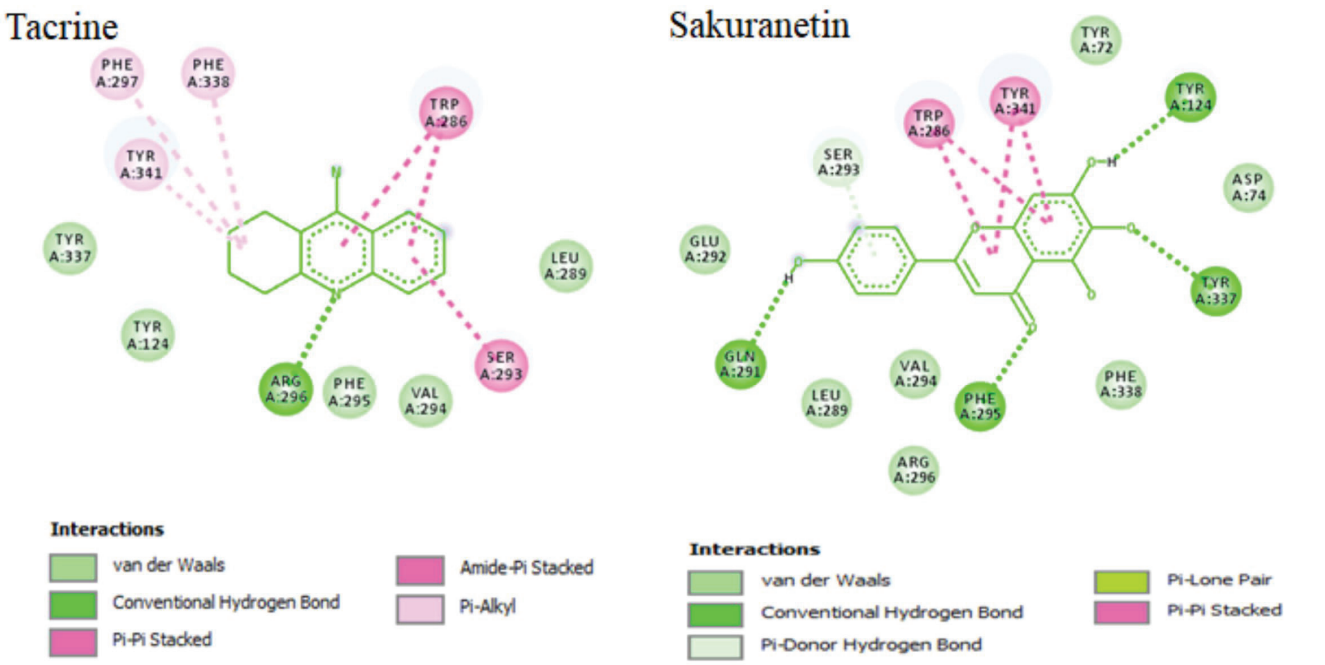

Figure 1: Molecular docking study: 2D pose representing crucial amino acid residues involved within the active site of the Acetylcholinesterase interacted with Tacrine (left), and Sakuranetin (right).

the complex. While in the case of Tacrine (reference drug), only a single H-bond was observed. Other amino acid residues involved during interaction were TYR124, TYR341, TYR337, LEU289, SER293, PHE295, GLU292, SER293, VAL294 and GLN291. The pictorial representation of the binding is shown in Fig.1. In addition, several other significant interactions, such as pi-pi T-shaped, pi-alkyl, pi-donor hydrogen bond, van der Waals, were also involved in the formation of stable Sakuranetin–AChE complex. For example, Tyr341 and Trp285 of Acetylcholinesterase showed pi-pi interactions with Sakuranetin and played an essential role in the binding. The interaction of amino acid residues (tyrosine, phenylalanine, and tryptophan) is involved in forming peripheral anionic sites influencing the conformational binding of ACh to the binding pocket/site (Stanton *et al.*, 2017). Results showed that the binding of Sakuranetin at the active site of Acetylcholinesterase was chiefly driven by H-bonding and hydrophobic forces, as shown in Fig.2. Additionally, an increase in the percentage of α-helix and β-turn, and a decrease in the rate of β-sheet and random coil resulted in the structural compactness of the complex. The interactions showed a good docking score, as summarized in Table 1.

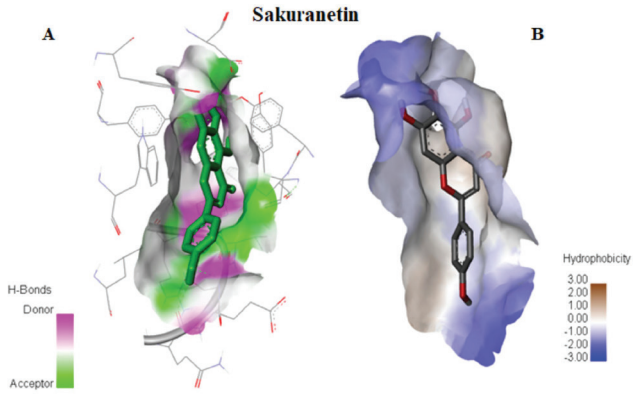

Figure 2: Docking result of Acetylcholinesterase (A): Most possible binding site of Sakuranetin in Acetylcholinesterase with hydrogen bond donor and acceptor surface (B): Hydrophobic surface around Sakuranetin and Acetylcholinesterase complex.

Pharmacokinetics and ADME analysis

Currently, computational-assisted drug discovery methodologies have become valuable for scrutinizing particular drugs/compounds against a disease. The drug/compound must show good bioavailability, i.e., quickly reach the action site and perform its potential response (activity) within the protein target. The selected compound Sakuranetin was assessed for its drug-relevant properties based on Lipinski's Rule of five, pharmacokinetic

Table 1: Binding pattern of selected inhibitors against AChE

SN	Compound	Binding Energy (ÄG) (kcal/mol)	H-Bond	Residues involved
1	Tacrine (Standard Drug)	-7.7	ARG296	PHE295, PHE338, PHE297, ARG296, SER293, LEU289, TYR341, TYR124, TRP286
2	α-copaene	-8.5	-	TYR337, TYR341, TYR124, TRP286, PHE338, PHE295, VAL294, SER293, PHE297, ARG296
3	Scutellarein	-8.2	TYR72, ARG296	ASP74, TYR72, LEU76, TRP286, TYR341, SER293, LEU289, ARG296
4	Sakuranetin	-8.2	PHE295, TYR337	TYR124, TYR341, TYR337, LEU289, SER293, PHE295, GLU292, SER293, VAL294, GLN291
5	α-Cadinine	-8.2	-	ARG296, VAL294, PHE338, TRP338, TYR337, TRP286, PHE295, PHE297, SER293, TRP286, TYR123, TYR341, TYR72
6	Galangin	-8.1	ARG296	ARG296, PHE295, SER293, LEU289, TYR341, TRP286, ASP74, TYR72
7	Hispidulin	-7.3	TYR124, ARG296	TYR124, TYR341, PHE338, TRP286, PHE338, PHE295, SER293, VAL294, ARG296

parameters, including absorption, distribution, metabolism, excretion (ADME), and Toxicity risk assessment. The pharmacokinetics properties of Tacrine and Sakuranetin were calculated using SwissADME online software. Table 2 depicts all parameters relevant to Lipinski's Rule of 5 and drug-like properties. Both the compounds showed moderate deviation and good bioavailability, represented as a radar plot in Fig. 3.

Molecular dynamics Simulation analysis

Molecular dynamics simulation was done to evaluate the stability and flexibility of ligand Sakuranetin and AChE complex. The fluctuations and deviations of amino acid residues were assessed through 10 ns simulation run. Root mean square deviation (RMSD) tells about the changes in the stability of the complex structure. The Sakuranetin-AChE complex had a stable backbone structure after 4000 ps, compared with the acetylcholinesterase peptide. RMSD values decreased significantly, revealing that the complex had a more stable structure, as shown in Fig. 4A. Also, root mean square fluctuation (RMSF) of each amino acid residue showed the flexible nature of specific regions within the protein-ligand complex. RMSF values of the corresponding amino acid in the complex were smaller than that of Acetylcholinesterase, indicating that the complex had lower mobility, forming a more stable enzyme-inhibitor complex. RMSFs of each amino acid in both systems have been shown in Fig. 4B.

Further, the radius of gyration (Rg) provides knowledge regarding the compactness of the protein-ligand complex. However, the protein's compactness and stability generally are inversely correlated. The Rg value of the Sakuranetin-AChE complex was low, indicating that the protein became denser after forming the complex. Furthermore, the strength of molecular interactions and nonbonded interactions between the Sakuranetin-Acetylcholinesterase complex was computed through GROMACS. The CHARMM force field was parameterized to specifically target quantum mechanical interaction energies with water to intrinsically balance within the simulation system. The energy was extracted through the energy module of GROMACS using Coul- SR: Protein-Ligand and LJ-SR: Protein-ligand terms. The average short-range Coulombic interaction energy was -60.4764 kJ/mol, and the short-range Lennard- Jones energy was -135.334kJ/mol. Total interaction energy was -195.8104 kJ/mol after forming the complex. The plots of RMSD, RMSF, Rg, solvent-accessible surface area (SASA), and H-bond for Sakuranetin-Acetylcholinesterase complex for 10 ns at 300K are shown in Fig. 4 (A, B, C, D,&E).

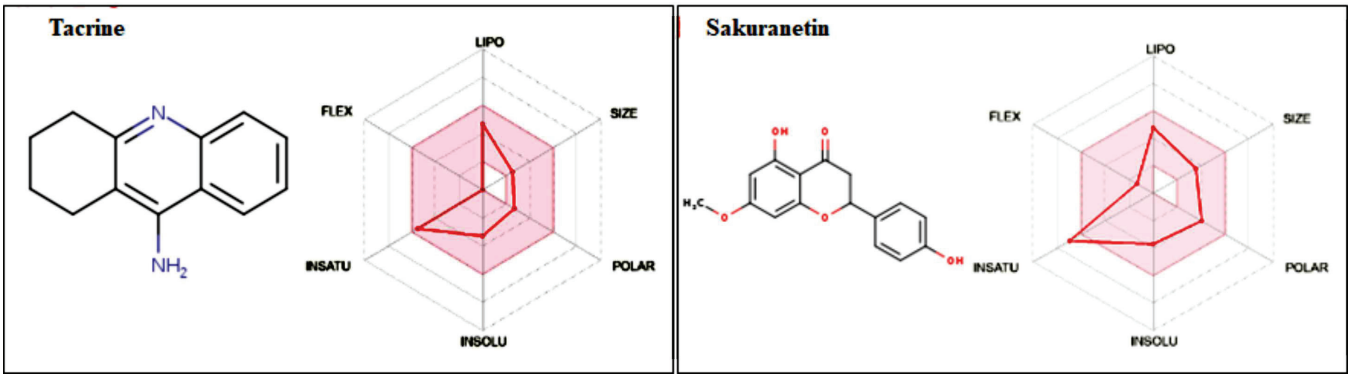

Figure 3: Bioavailability radar plot of Tacrine and Sakuranetin

Footnote: Six physiochemical properties are taken into account: lipophilicity, size, polarity, solubility, flexibility, and saturation. The pink area represents the optimal range for each property (lipophilicity: XLOGP3 between − 0.7 and + 5.0, size: MW between 150 and 500 g/mol, polarity: TPSA between 20 and 130 A2, solubility: log S not higher than 6, saturation: fraction of carbons in the sp3 hybridization not less than 0.25, and flexibility: no more than nine rotatable bonds. In this example, the compound is predicted moderately orally bioavailable, it deviates from bioavailability radar due to many rotable bonds)

CONCLUSION

Several phytomolecules have already been investigated for their efficient activity against Acetylcholinesterase (AChE), but, to date, none have been approved by FDA. This is due to toxicity, failure to cross the BBB, and several other shortcomings. The current *in silico* study predicted that Sakuranetin binds strongly to the active site residues of the AChE target. Also, Sakuranetin does not violate oral bioavailability parameters and follows other drug-like properties. In this study, the compound Sakuranetin, a potent phytochemical, showed best binding mode with Acetylcholinesterase. The proposed hit (Sakuranetin) may act as a cholinesterase inhibitor and could be used as a potential symptomatic drug lead in AD. Additionally, based on the present study, the compound may accelerate cognitive function by elevating the level of acetylcholine in the neuron and could efficiently treat AD. Furthermore, the availability of Sakuranetin from several important medicinal and aromatic plants may also be hypothesized as a nutraceutical component that can help in AD.

Table 2: Various druggability parameters of tacrine and sakuranetin

Parameters	Properties	Tacrine	Sakuranetin
Physicochemical Properties	Molecular formulae	C13H14N2	C16H14O5
	M. Weight (≤ 500)	198.26	286.28
	Rotatable bonds (≤ 10)	0	2
	H-bond acceptors (≤ 10)	1	5
	H-bond donors (≤ 5)	1	2
	Molar refractivity (40 to 130)	63.58	76.04
	SP3 hybridisation fraction (≥ 5)	0.31	0.19
	TPSA (30 Å to 140 Å)	38.91	75.99
Lipophilicity	Log Po/w (≤ 5)	2.55	2.25
Water Solubility	Log S	-3.97	-3.7
	Class	Soluble	Soluble
Pharmacokinetics	GI absorption	High	High
	BBB permeability	Yes	Yes
	P-gp substrate	No	No
	CYP1A2 inhibitor	Yes	Yes
	CYP2C19 inhibitor	Yes	Yes
	CYP2C9 inhibitor	No	No
	CYP2D6 inhibitor	No	No
	CYP3A4 inhibitor	Yes	Yes
Drug likeness	Lipinski rule of five	Yes (0 violation)	Yes; (0 violation)
	Bioavailability Score	0.55	0.55
Medicinal Chemistry	PAINS	0 alert	0 alert

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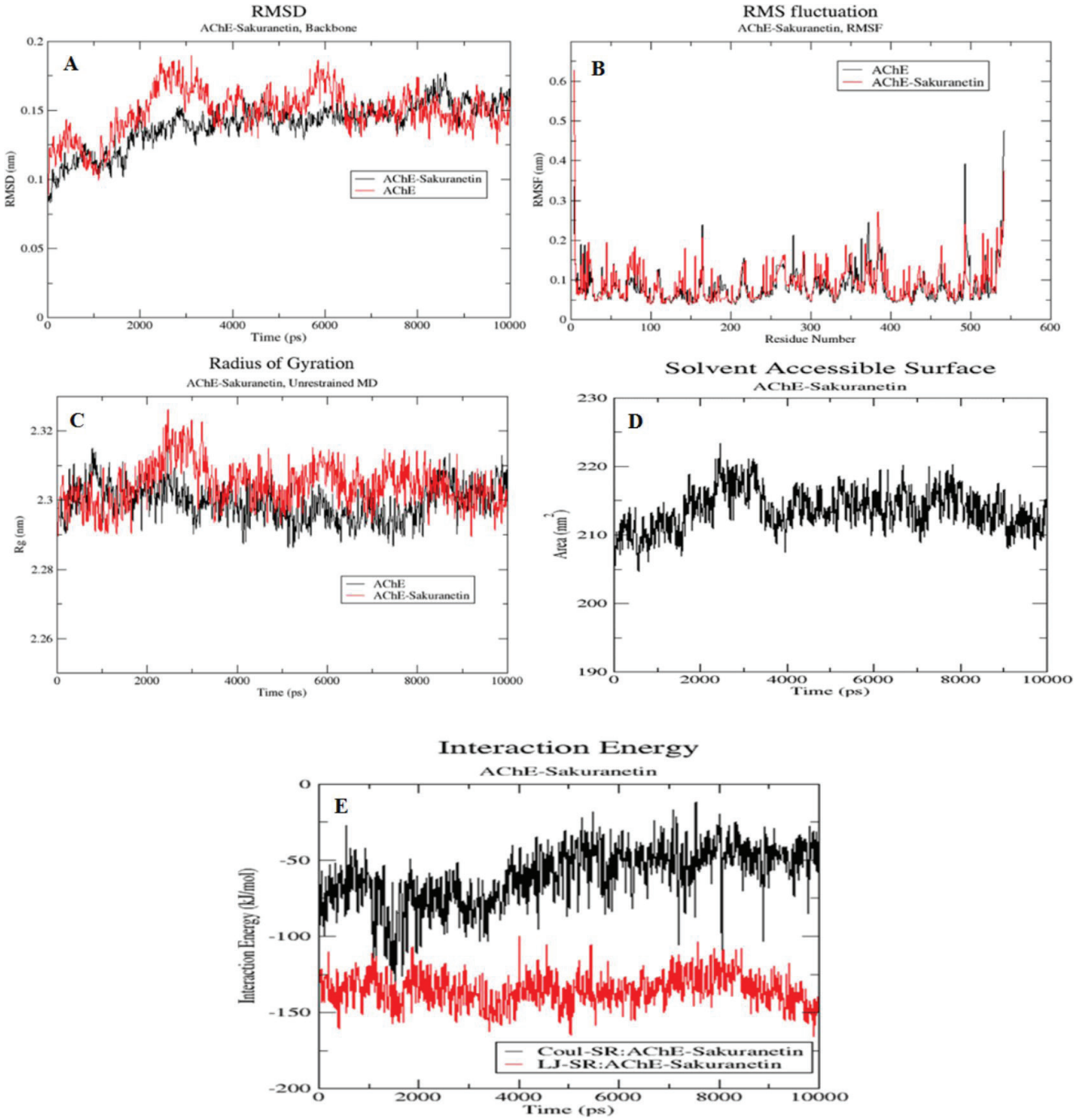

Figure 4: The results of molecular dynamics simulation of Sakuranetin and Acetylcholinesterase (AChE) complex: **(A)** RMSD **(B)** RMSF **(C)** Rg **(D)** SASA **(E)** Total interaction energy.

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