In silico study on evaluation of corosolic acid of *Lagerstroemia speciosa* against Alzheimer's disease

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Abstract

Purpose – Alzheimer's disease (AD), the most common cause of dementia, is a neurodegenerative disorder caused by the aggregation of amyloid-beta (Aβ) at outside of neuron cells and also due to tau aggregation inside the cell. Corosolic acid is aimed to be selected as a main active constituent of *Lagerstroemia speciosa* for the study.

Design/methodology/approach – In the present study, molecular docking of corosolic acid and tau protein was examined using PyRx-v.0.8 software. Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties were described and a molecular dynamics study of the bound complex was performed using Desmond.

Findings – The docking score and interactions suggested that the corosolic acid (CID:6918774) could bind to tau protein to prevent the fibrillar network, to prevent AD. During simulation corosolic acid-bound protein root mean square deviation (RMSD) values showed more stability when compared to the Apo form of protein. Molecular dynamics study of tau protein and corosolic acid complex gave the insights to develop a drug-like candidate against AD.

Originality/value – The use of corosolic acid of *Lagerstroemia speciosa* to prevent AD is supported by preliminary analysis on a computational basis. This compound should explore in terms of experimental strategies for the further drug development process. However, *in vitro* and *in vivo* evaluation studies are required to suggest the use of corosolic acid against AD.

Keywords Alzheimer's disease, Molecular docking, Dynamics simulation, Corosolic acid

Paper type Research paper

Introduction

Neurodegenerative diseases like Alzheimer's and Parkinson's diseases go-up exponentially at the age of 75 years. Alzheimer's disease (AD) is the most prevalent of all the neurodegenerative diseases (Hampel *et al.*, 2017; Fan *et al.*, 2020; Casey, Antimisiaris, &

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O'Brien, 2010). The plaques contained aggregated fragments of a larger protein, A-beta, called as Amyloid precursor protein (APP) (Cheignon *et al.*, 2018). Within the brain of these patients such as Parkinson's disease, Frontal-temporal dementia, Huntington's disease and so forth, inside the dying neurons, tangles of tau protein associated to microtubules and similar aggregates of different proteins are also found (Irvine, El-Agnaf, Shankar, & Walsh, 2008). Even though the proteins and regions of the brain differ from disease to disease, but the common characteristic feature is always turned to be a protein misfolding disease in which a normal protein forms a peculiar beta-sheet structure called amyloid instead of forming a tightly compact functional structure leading to a variety of other structures such as oligomers, fibrils or the dense tangles of fibrils (Shukla, Shukla, Sonkar, Pandey, & Tripathi, 2018). The genetics of the Alzheimer's kind disease is etiologically sporadic and etiopathic as in many cases the causes remained unknown. Ten percent of Alzheimer's cases are genetic and are inherited in a family in an autosomal dominant pattern (Muralidar, Ambi, Sekaran, Thirumalai, & Palaniappan, 2020). Researchers are targeting amyloid β-tau protein phosphorylation which is characterized by senile plaques and the deposition of neurofibrillary tangles. Senile plaques are a neurocytotoxic Aβ protein (mainly Aβ42) deposited outside the nerve cell, and neurofibrillary tangles are phosphorylated tau proteins accumulated in the nerve cell. Although the etiology of AD is still unclear, but targeting the tau protein is considered as promising strategy for developing tau based therapeutics for managing Alzheimer's (Rad *et al.*, 2018). Corosolic acid, a direct natural derivative of ursolic acid known for its insulin-regulating mechanism in diabetes patients by increasing insulin sensitivity seems to have preventive action towards the formation of Aβ element and tau protein aggregation. Several epidemiological studies have found that the systemic insulin resistance condition of type 2 diabetes is a substantial risk factor for age-related cognitive decline, dementia, AD and progression from moderate cognitive impairment (MCI) to AD (Vangone *et al.*, 2019). Keeping this in view, the present study is focused on targeting the tau protein aggregates to identify the binding affinity between the tau protein monomer and corosolic acid molecule using *in silico* methods.

Methods

Molecular docking

The protein tau (Protein Data Bank (PDB) ID: 5O3L) was downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB) database (http://rcsb.org/pdb). Chain A monomer was retained and other chains of tau decamer were deleted followed by the addition of hydrogen atoms, assignment of atomic charges, deletion of water molecules that are not involved in ligand binding, and assignment of Histidine (HIS) protonation states based on the pKa value was done using protein preparation wizard of Schrodinger maestro. Molecular docking was performed using PyRx-v.0.8 open-source software. The ligand molecule corosolic acid (Pubchem: CID:6918774) from *Lagerstroemia speciosa* was subjected to energy minimization using the universal force field in PyRx-v.0.8 software.

ADMET and Lipinski descriptor analysis

The pkCSM and swissADME servers (http://www.swissadme.ch/index.php) were used to investigate the physicochemical characteristics of the corosolic acid. Lipinski`s rule of five is a significant rule that is effectively used to evaluate drugs. This rule dispenses with the molecular mass, high lipophilicity, hydrogen bond donors and acceptors, and molecular refractivity of the compounds (Lipinski, Lombardo, Dominy, & Feeney, 1997).

Binding free energy calculation

The PRODIGY-LIG (PROtein binDIng enerGY prediction- Ligand) service (https://bio.tools/PRODIGY-LIG) estimates the binding free energy (ΔG) based on an examination of intermolecular atomic contact electrostatic energy between the receptor molecule and ligand complexes within a 10.5 distance cut-off range. The expected binding free energy was represented as ΔG. By exporting 100 snapshots from 90 ns to 100 ns molecular dynamic (MD) simulations, the binding free energy was computed (Vangone *et al.*, 2019).

Molecular dynamic simulations

Desmond 2020.1 version [D.E shaw] was used for molecular dynamics simulation. For 500 ns, the Apo form of tau and tau protein complex with corosolic acid were investigated. The SPC (single point charge) water model was utilized to solve the system using the forcefield OPLS (Optimized Potentials for Liquid Simulations)-3e. The orthorhombic box with the size 10 × 10 × 10 was chosen. The counter ions (Na+ and Cl-) were supplied while the salt concentration remained at 0.15 M. To decrease the energy used by each system, the steepest descent approach was used for 50,000 steps. The system was heated to 310 K with 0.1 ns of NVT (Amount of substance (N), Volume (V) and Temperature (T)) steps on applying the constraint with a force constant of 100 kcal/mol to the protein backbone, followed by applying the restraint with a force constant of 100 kcal/mol NPT (Amount of substance (N), Pressure (P) and Temperature (T)) equilibration for 0.5 ns on protein backbone Temperature and pressure were maintained using thermostat and barostat. The coulombic and Vander Waals interactions were set at a cut-off value of 10.0 Å. The NPT ensemble with the parameters of the temperature of 310 K and 1.03 bar pressure was selected to mimic the physiological conditions and run over 500 ns with a 2 fs time step. The root mean square deviation (RMSD) and root mean square fluctuation (RMSF) graphs were generated and analyzed.

Results and discussion

Molecular docking

The tau protein was selected as the main drug target to prevent the fibrillar network so as to prevent AD. Molecular docking revealed the best binding pose of the corosolic acid in the binding pocket of the tau protein monomer. The stable docking complex of tau monomer with the top binding pose of corosolic acid was selected for the dynamic simulation study. Docking results showed a binding energy of −7.3 kcal/mol for the tau and corosolic acid complex. The 2-dimensional (2D) interaction diagram of corosolic acid showed the hydrogen bond interactions of Leucine (LEU) 344 and Isoleucine (ILE) 354 with tau monomer protein (Figure 1a and b). The $CaCO_2$ permeability of the docked compound was computed for absorption prediction and was found to be 0.641 cm/s. The steady-state volume distribution (Vss) was used to determine the distribution since it is regarded a trustworthy measure of drug distribution in the body; the result was −1.282 L/kg. The metabolism was calculated by taking into account the drug's Cytochrome P450 (CYP) inhibition factors, and the excretion was measured based on the total clearance, which when combined with Vss would help to understand the half-life and dosage of the drug, it resulted in 0.093 ml/min/kg, the higher the values, the faster the drug was eliminated from plasma. To test for toxicity of the ligand, minnow toxicity was assessed and value found to be 0.276 corresponding to minimal acute toxicity. The compound has a bioavailability of 0.56, suggesting that the compound is appropriate for oral administration (Table 1). The compound has a log P lipophilicity score of 6.0603. The chosen compound's topological polar surface area (Å) was 206 and 148. The number of rotatable bonds was one, which represents the ligand's flexibility characteristic and showed good binding interactions with the target receptor. For corosolic acid, the number of hydrogen bond acceptors and donors were of 3. The refractions of ligands have been

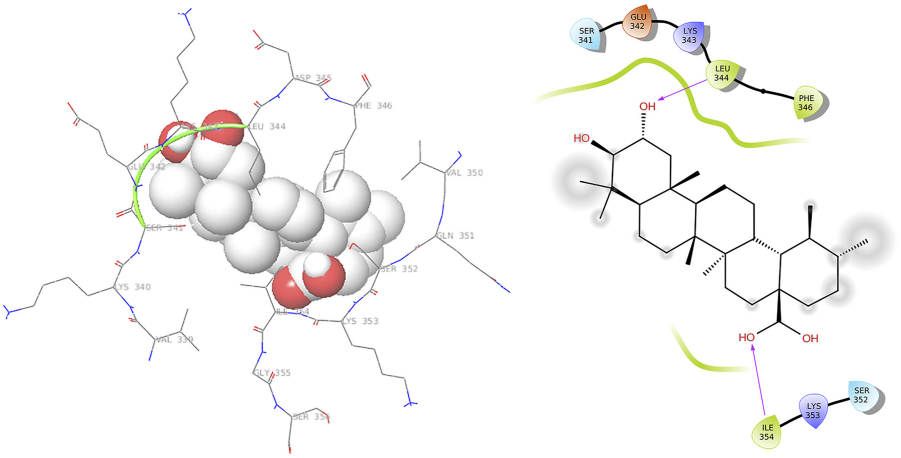

Figure 1.
(a) 3D-docked pose of corosolic acid within the binding pocket of tau monomer
(b) 2D-docked pose of ligand along with the interacting amino acids

Table 1.
ADMET properties of corosolic acid analyzed with pkCSM

S.No	Compound	CID of the compound	Minnow toxicity	$CaCO_2$ permeability	Bioavailability	VDss	CYP inhibition	Excretion (total clearance)
1	corosolic acid	CID87014	0.276	0.641	0.56	−1.282	1A2:N 2C19:N 2C9:N 2D6:N 3A4:N	0.093

Table 2.
Lipinski properties of corosolic acid analyzed with SwissADME

S.No	Compound	CID of the compound	Molecular weight	Log *P*	Rotatable bonds	Acceptors	Donors	Surface area
1	Corosolic acid	CID87014	472.71	6.0603	1	3	3	206.148

represented by molecular refractivity. The compound's molecular refractivity was found to be 138.08. According to the above predictions, the molecule has one violation Moriguchi octanol-water partition coefficient (MLOGP) >4.15 (Table 2).

Root mean square deviation (RMSD)

RMSD is a measurement of the difference between the backbones of a protein structure as while it transits from its initial structural conformation to its final location. The stability of the protein structure in relation to its conformation may be assessed using the variations caused during simulation (Kufareva & Abagyan, 2011). Figure 2a and b, depicts the RMSD development of a protein (left *Y*-axis). All protein frames are first aligned on the reference frame backbone, and then the RMSD based on atom selection is determined. The RMSD of the ligand (right *Y*-axis) revealed how stable the ligand is in relation to the protein and its binding pocket. If the simulation has equilibrated, the RMSD analysis showed that the fluctuations at the conclusion of the simulation were centered on some thermal average structure. Changes in

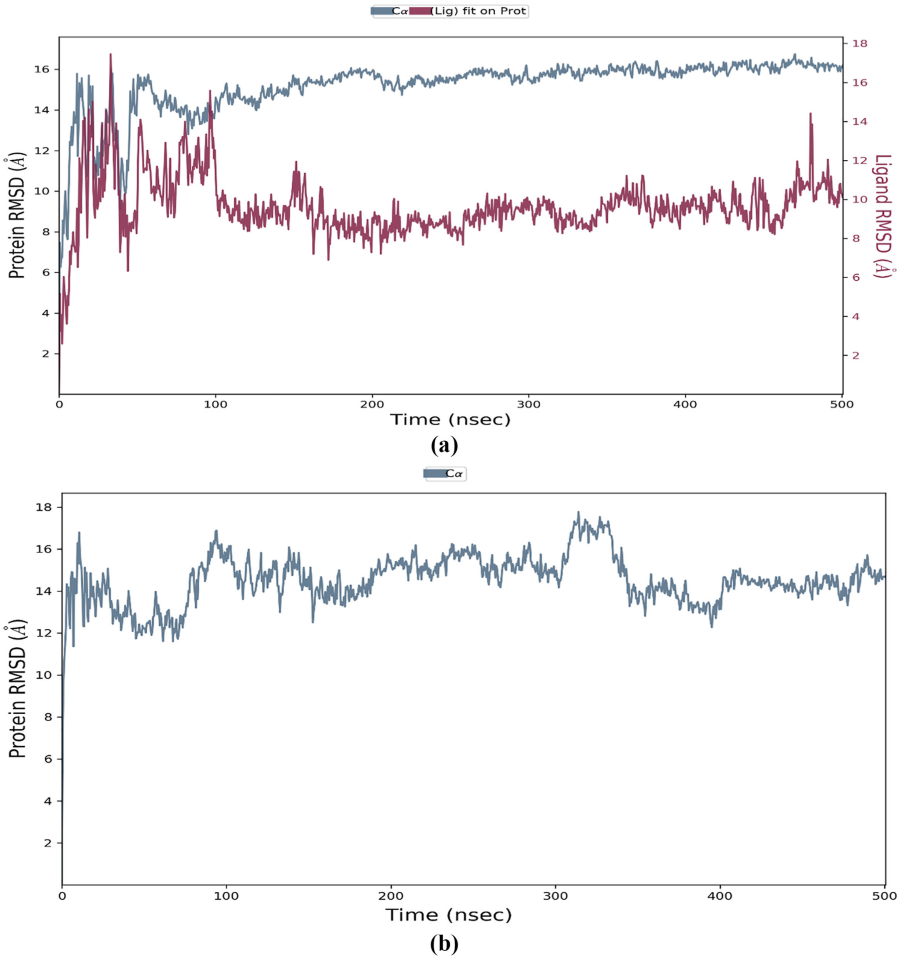

Figure 2.
RMSD of Cα atoms (a) Tau protein bound with corosolic acid and (b) apo protein

the order of 8-16 showed that the protein underwent a significant conformational shift throughout the simulation; the simulation converges and the RMSD values settle after 100 ns and remain steady until 500 ns. When compared to the Apo form of protein, the corosolic acid bound protein was more stable.

Root mean square fluctuation (RMSF)

RMSF was employed for identifying local alterations in the protein chain. When compared to the bound complex form with ligands, the RMSF of tau protein's C atoms fluctuated virtually identically in Apo form (Figure 3a and b). The stacked bar charts were normalized throughout the duration of the trajectory: for example, a value of 0.7 indicated that the specific contact was maintained 70% of the time during the simulation. Values greater than 1.0 were achievable because certain protein residues bound to form several interactions of the same subtype with the ligand (Figure 4). The top panel showed the total number of specific contacts the protein makes with the ligand over the course of the trajectory (Figure 5). The bottom panel showed which residues interact with the ligand in each trajectory frame. Some residues

made more than one specific contact with the ligand, which is represented by a darker shade of orange, according to the scale to the right of the plot (Figure 5). The plot above reported secondary structure elements (SSE) distribution by residue index throughout the protein structure. The plot (Figure 6) summarized the SSE composition for each trajectory frame over the course of the simulation, and the plot at the bottom monitors each residue and its SSE assignment over time (Figure 6). During the course of simulation, the hydrogen bond interactions of Leu 344 and ILE 354 were disappeared and found the interaction with

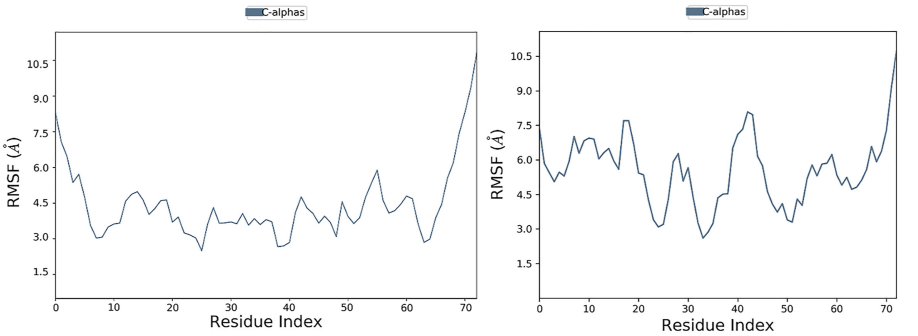

Figure 3.
RMSF of Cα atoms showing structural dynamics of tau protein as a function of time (a) tau protein bound with corosolic acid and (b) apo-protein throughout the simulation, protein-ligand interactions can be observed

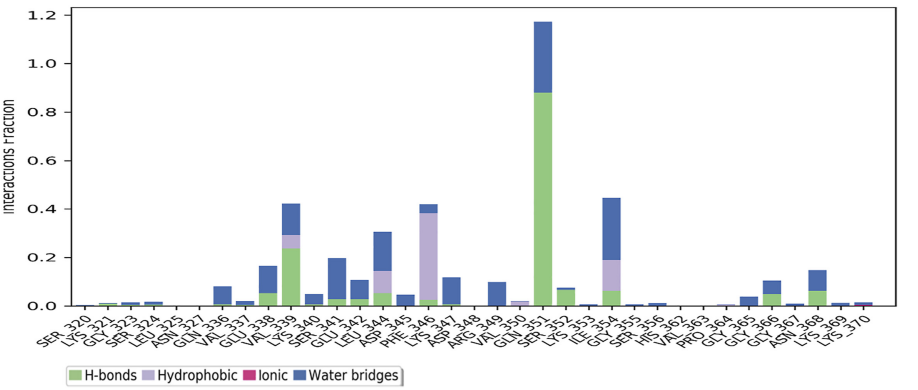

Figure 4.
Total number of contacts of tau protein bound with corosolic acid

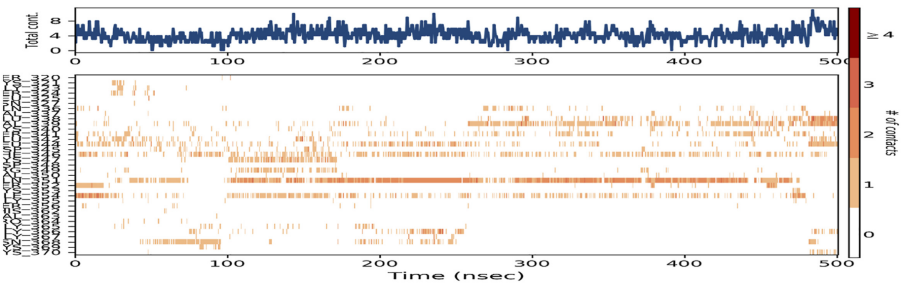

Figure 5.
A timeline representation of the interactions and contacts (H-bonds, Hydrophobic, Ionic, Water bridges)

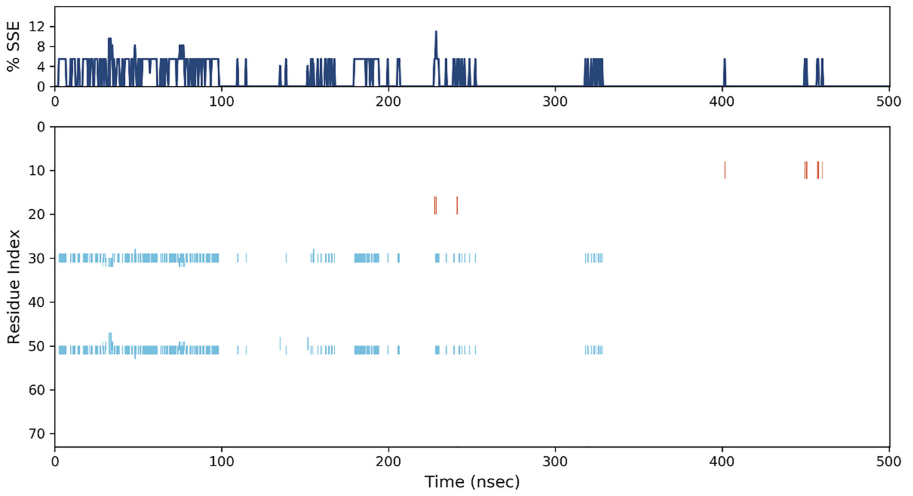

Figure 6.
Protein secondary structure elements (SSE) like alpha-helices (orange-red) and beta-strands (blue)

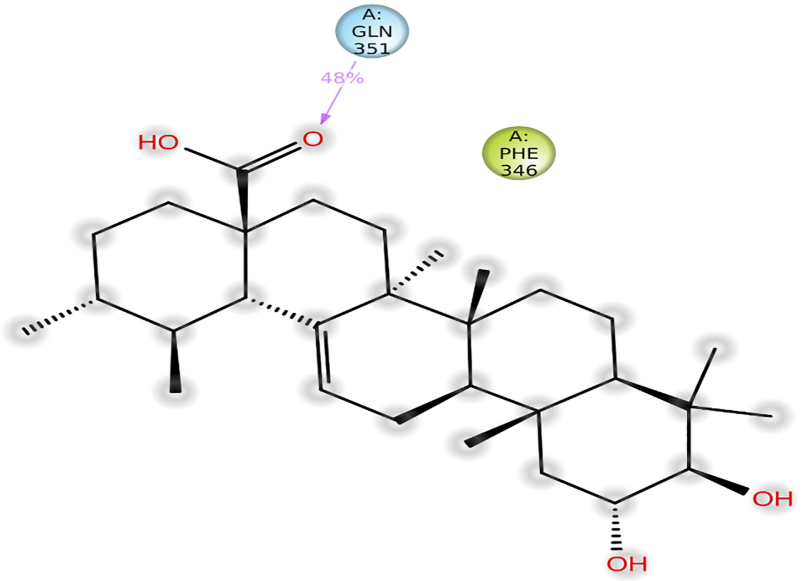

Figure 7.
The 2D schematic diagram of corosolic acid and interacting with tau monomer protein during the course of molecular dynamic (MD) simulations

Glutamine (GLN) 351 with 48.0% of the total simulation time (Figure 7). GLN351 amino acid forming H-bond with carboxyl group of the ligand. The amino acid Phenylalanine (PHE) 346 showed a hydrophobic interaction.

Conclusion

Corosolic acid compound is the main ingredient of *Lagerstroemia speciosa,* it showed good docking relation with the tau monomer, and good interactions in favorable conformation. The top docking pose was selected based on the highest binding affinity. The Apo form of tau and

tau protein complex with corosolic acid was studied for 500 ns. Based on the *in-silico* analysis of the study, it was concluded that the corosolic acid can control the protein aggregation and self-assembly which would possibly to prevent AD. Therefore, this compound is to be explored for *in vitro* and *in vivo* experimental strategies for further drug development.

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