

RESEARCH ARTICLE

Allium sativum-Derived Alliin and Allicin Stably Bind to α -Synuclein and Prevent Its Cytotoxic Aggregation

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ABSTRACT

Neurodegenerative diseases such as Parkinson's disease are characterized by the pathological aggregation of α -synuclein. Targeting α -synuclein aggregation through natural bioactive compounds offers a promising therapeutic strategy. In this study, sulfur-containing compounds derived from *Allium sativum* were evaluated for their drug-likeness, pharmacokinetic properties, and ability to inhibit α -synuclein aggregation using a combination of in silico and in vitro approaches. ADMET profiling indicated high gastrointestinal absorption for nine compounds, supporting their drug-like properties. Six compounds were predicted to cross the blood–brain barrier, suggesting potential efficacy in the central nervous system. Molecular docking identified alliin, allicin, E-ajoene, and diallyl disulfide as top binders to α -synuclein, forming stable interactions with key aggregation-prone regions. Molecular dynamics simulations over 100 ns confirmed the structural stability of alliin- and allicin- α -synuclein complexes, with minimal residue fluctuations and persistent hydrogen bonding. MM-GBSA binding energy analysis corroborated these results, showing favorable binding free energies, particularly for alliin and E-ajoene. Principal component analysis (PCA) further supported the role of alliin in stabilizing α -synuclein dynamics. In vitro cellular assays further validated these computational findings. Using an SH-SY5Y cell-based α -synuclein aggregation model, treatment with alliin and allicin significantly reduced α -synuclein aggregation. Furthermore, MTT-based cytotoxicity assays in SH-SY5Y neuroblastoma cells overexpressing α -synuclein revealed that alliin and allicin conferred notable cytoprotective effects by reducing α -synuclein-induced toxicity. Taken together, these findings highlight alliin and allicin as potent lead compounds that not only bind and stabilize α -synuclein but also attenuate its aggregation and associated cytotoxicity.

1 | Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder affecting over 10 million people worldwide and is second only to Alzheimer's disease in prevalence among age-related

neurodegenerative conditions [1]. Clinically, PD is characterized by a constellation of motor symptoms such as resting tremors, bradykinesia, muscular rigidity, and postural instability, often accompanied by non-motor manifestations including cognitive impairment, mood disorders, sleep disturbances, and autonomic

dysfunction [2]. The hallmark neuropathological feature of PD is the selective degeneration of dopaminergic neurons in the substantia nigra pars compacta and the presence of intracellular inclusions known as Lewy bodies, primarily composed of aggregated α -synuclein [3].

α -Synuclein is a 140-amino acid presynaptic neuronal protein that exists in a dynamic equilibrium between soluble cytosolic and membrane-associated helical conformations under physiological conditions [4]. However, under pathological circumstances, α -synuclein misfolds and self-aggregates into β -sheet-rich oligomers and fibrils, which are neurotoxic and contribute to the formation of Lewy bodies [4]. The central non-amyloid component (NAC) domain of α -synuclein (residues 61–95) is critical for aggregation, while the N- and C-terminal regions modulate solubility and interaction with cellular components [5]. Furthermore, point mutations such as A53T, A30P, and E46K, as well as gene duplications or triplications, have been linked to familial PD and are known to enhance the propensity of α -synuclein to form toxic aggregates [6]. These misfolded species disrupt intracellular vesicle trafficking, mitochondrial function, proteasomal degradation, and calcium homeostasis, ultimately leading to neuronal dysfunction and death [7].

Given the central role of α -synuclein aggregation in PD pathogenesis, it represents a compelling therapeutic target. Inhibiting the initial nucleation of monomeric α -synuclein, destabilizing oligomeric intermediates, or promoting clearance of preformed fibrils are considered viable strategies to mitigate disease progression [8]. Recent advances have led to the development of small molecules, peptides, antibodies, and gene-silencing approaches aimed at reducing α -synuclein toxicity [9]. Structure-based drug design, molecular dynamics (MD) simulations, and high-throughput virtual screening have also enabled the identification of compounds that can bind to specific regions of α -synuclein and stabilize its native conformation, thereby preventing its pathological aggregation [10].

Among emerging therapeutic options, plant-derived phytochemicals have shown promise due to their antioxidant, anti-inflammatory, and neuroprotective properties. Numerous studies have demonstrated that natural compounds such as curcumin, resveratrol, epigallocatechin gallate (EGCG), and baicalein can inhibit α -synuclein fibrillation, disrupt preformed fibrils, and ameliorate motor symptoms in cellular and animal models of PD [11]. These polyphenols exert their protective effects by directly interacting with aggregation-prone regions of α -synuclein, modulating signaling pathways, or enhancing the cell's proteostatic capacity through autophagy and chaperone induction [12].

Among the most bioactive-rich plants, *Allium sativum* (garlic) has long been revered in both traditional and modern medicine for its broad-spectrum pharmacological properties. Garlic is a potent source of organosulfur compounds which exhibit antimicrobial, antioxidant, cardioprotective, anticancer, and hepatoprotective effects [13]. These compounds are also known to modulate immune responses, inhibit inflammation, and scavenge reactive oxygen species—mechanisms that are particularly relevant in neurodegenerative disorders [14]. In recent years, *A.*

sativum-derived phytochemicals have been reported to improve cognitive performance, enhance neurogenesis, and attenuate neuroinflammation in experimental models of AD and PD [15]. Despite these promising attributes, their direct role in modulating α -synuclein aggregation and toxicity remains underexplored.

In this study, we investigated the pharmacokinetic properties, binding potential, and neuroprotective effects of nine bioactive sulfur-containing compounds from *A. sativum* against α -synuclein. Using a combination of in silico approaches—including ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiling, molecular docking, MD simulations, and principal component analysis (PCA)—we identified key interactions that stabilize α -synuclein and prevent its misfolding. These computational results were validated through in vitro cellular assays, where the effects of alliin and allicin on α -synuclein aggregation and cytotoxicity were assessed in SH-SY5Y cells. Our findings provide a mechanistic basis for the anti-aggregation potential of *A. sativum*-derived compounds and support their further exploration as therapeutic agents in the treatment of synucleinopathies.

2 | Methods

2.1 | Curation of Organosulfur Compounds From *A. sativum*

To investigate the neuroprotective potential of organosulfur compounds derived from *A. sativum*, a systematic in silico curation approach was employed. Initially, an extensive literature review was conducted using scientific databases to identify bioactive sulfur-containing molecules previously reported in garlic. This was complemented by data mining from chemical repositories including PubChem, ChEMBL, and Dr. Duke's Phytochemical and Ethnobotanical Databases [16, 17]. Compounds known for their antioxidant, antimicrobial, or neuroprotective properties were prioritized [18]. The selection criteria required that each compound be a naturally occurring constituent of *A. sativum*, possess sulfur-containing functional groups (e.g., thiol, disulfide, sulfoxide, or thiosulfinate), and have potential biological activity relevant to neurodegenerative processes. Based on these criteria, nine compounds were curated: alliin, allicin, E-ajoene, Z-ajoene, diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), 2-vinyl-4H-1,3-dithiin, and allyl methyl sulfide (AMS). Canonical SMILES, InChIKeys, and 3D conformers for each compound were retrieved from the PubChem database [19]. Structures were optimized using Avogadro with the MMFF94 force field to obtain energetically favorable conformations [20]. These structures were then formatted appropriately for downstream molecular docking and simulation studies.

2.2 | ADMET Analysis of Organosulfur Compounds From *A. sativum*

The pharmacokinetic properties of the nine curated organosulfur compounds from *A. sativum* were evaluated through in silico ADMET analysis to predict their drug-likeness and systemic behavior [21, 22]. Canonical SMILES notations and 3D structures of all compounds were retrieved from the PubChem

database. The ADMET predictions were carried out using two web-based platforms: SwissADME (<http://www.swissadme.ch/>) and ADMETlab 2.0 (<https://admetmesh.scbdd.com/>) [23, 24]. For each compound, physicochemical parameters such as molecular weight, hydrogen bond donors and acceptors, topological polar surface area (TPSA), number of rotatable bonds, and logP values were computed. Gastrointestinal (GI) absorption was predicted based on the BOILED-Egg model, and blood–brain barrier (BBB) permeability was assessed to determine the potential of the compounds to exert central nervous system (CNS) activity [25]. Skin permeability coefficients ($\text{Log } K_p$) were also calculated to evaluate the potential for transdermal delivery [26]. Drug-likeness was assessed according to Lipinski's Rule of Five, and compounds were screened for any rule violations [27]. Additionally, metabolism-related parameters such as CYP450 enzyme inhibition profiles, and toxicity endpoints, including hepatotoxicity and Ames mutagenicity, were predicted to ensure safety profiles [28, 29]. Compounds exhibiting high GI absorption, favorable BBB permeability (where applicable), zero violations of Lipinski's rules, and acceptable toxicity profiles were considered suitable for further molecular docking and simulation studies.

2.3 | Preparation of α -Synuclein Structure

For molecular docking and simulation studies, the α -synuclein protein structure was prepared using the structure available in the Protein Data Bank (PDB ID: 1XQ8) [30]. The structure was retrieved in PDB format from the RCSB PDB [31]. Initial protein preparation steps were carried out using the Protein Preparation Wizard in Schrödinger's Maestro suite [32]. This process included assigning proper bond orders, adding missing hydrogen atoms, and correcting any structural anomalies. Protonation states of ionizable residues were adjusted to pH 7.4 using the Epik module, ensuring physiological relevance of the protein's charge state [33]. The structure was then energy-minimized using the OPLS3e force field, with restraints applied to the heavy atoms to maintain the overall backbone geometry [34].

2.4 | Molecular Docking of Selected *A. sativum* Compounds With α -Synuclein

To investigate the binding interactions between α -synuclein and selected organosulfur compounds from *A. sativum*, molecular docking was performed using a structure-based approach. The protein and ligands' structures were prepared as described above. The binding site of α -synuclein was defined based on literature-reported aggregation-prone regions, especially the central hydrophobic NAC domain and surrounding helices, which are critical in early oligomer formation. A receptor grid was generated around this region to confine the docking search space. Molecular docking was performed using Glide (Schrödinger) in standard precision (SP) mode to predict the most favorable binding poses based on GlideScore and interaction profiles [35]. Each ligand was docked flexibly into the defined site, allowing for sampling of different conformational orientations. The best-ranked docking poses were selected based on binding affinity scores and visual inspection of molecular interactions, including hydrogen bonds, hydrophobic contacts, π – π stacking, and

salt bridges. Special attention was given to interactions involving residues known to influence α -synuclein misfolding and aggregation. The docking output was further analyzed using Maestro's interaction analysis tools [36] and PyMOL for 3D visualization [37].

2.5 | MD Simulation of α -Synuclein–Ligand Complexes

MD simulations were conducted to evaluate the dynamic stability, conformational flexibility, and interaction persistence of α -synuclein complexes with four selected *A. sativum*-derived compounds: alliin, allicin, E-ajoene, and DADS. The docking-derived complexes were used as initial structures for simulation. All MD simulations were carried out using the Desmond simulation engine implemented within the Schrödinger Maestro suite (Schrödinger Release 2023-1) [36].

Each protein–ligand complex was first placed in an orthorhombic simulation box with a 10 Å buffer distance and solvated using the TIP3P explicit water model [38]. The system was neutralized by adding appropriate counterions (Na^+ or Cl^-) and brought to physiological salt concentration (0.15 M NaCl) to mimic biological conditions. The system was then subjected to energy minimization using the OPLS3e force field to remove steric clashes and optimize initial geometries. After minimization, the system was equilibrated using the default NPT relaxation protocol in Desmond, which includes several short runs under NVT and NPT ensembles [36]. The production MD run was then performed for 100 ns at constant temperature (300 K) and pressure (1 atm), maintained using the Nose–Hoover thermostat and Martyna–Tobias–Klein barostat, respectively [39, 40]. A time step of 2 fs was used, and snapshots were recorded at 100 ps intervals for trajectory analysis.

Post-simulation, root mean square deviation (RMSD) and root mean square fluctuation (RMSF) analyses were conducted to assess the stability of the protein backbone and fluctuations of individual residues, respectively. The radius of gyration (R_g) was calculated to examine the overall compactness of the protein during simulation. Ligand RMSD plots were also analyzed to determine the positional stability of the compounds within the binding site. Additionally, secondary structure elements (SSEs) of α -synuclein during the simulation were analyzed using the simulation interactions diagram (SID) tool in Desmond, which provides time-resolved data on helices, strands, and coil regions [36].

Protein–ligand interaction profiling was carried out across the trajectory to identify persistent interactions such as hydrogen bonds, hydrophobic contacts, salt bridges, and water bridges. Interacting residues were mapped, and the frequency of interactions over simulation time was quantified to determine interaction stability and specificity. Binding free energy calculations were performed using the MM-GBSA (Molecular Mechanics–Generalized Born Surface Area) method implemented in Prime (Schrödinger) on representative frames extracted from the equilibrated portions of each trajectory [41]. These calculations provided insight into the thermodynamic favorability of ligand binding.

2.6 | PCA of MD Trajectories

PCA was employed to investigate the collective conformational motions and dominant dynamic modes of α -synuclein in complex with selected *A. sativum*-derived compounds—alliin, allicin, E-ajoene, and DADS—based on the 100-ns MD simulation trajectories. For each complex, trajectory files generated by Desmond were pre-processed using the Simulation Event Analysis tool in Maestro (Schrödinger suite). The trajectories were first aligned to a reference frame based on the protein's C α atoms to remove translational and rotational motions, ensuring that the analysis focused only on internal conformational changes. The covariance matrix of atomic positional fluctuations (C α atoms) was calculated over the simulation time, and the resulting matrix was diagonalized to yield a set of eigenvectors (principal components) and corresponding eigenvalues, which represent the magnitude of motion along each vector [42]. The first few principal components (typically PC1–PC3), which accounted for the majority of structural variance, were used to generate 2D and 3D projection plots. These plots illustrate the conformational space sampled by the protein during the simulation, with each point corresponding to a specific frame in the trajectory. The transitions from blue to red in the projection plots indicate the temporal progression from the start to the end of the simulation, allowing visualization of structural convergence or divergence over time. The degree of clustering of these points was used to assess the structural stability and conformational flexibility of each protein–ligand complex. In addition, scree plots were generated to visualize the eigenvalue distribution and determine how much variance was captured by the top principal components. Complexes with tightly clustered projections and low variance beyond PC2 were considered more stable, while broader distributions indicated enhanced flexibility or less stable binding conformations. Comparative PCA analysis among the four ligand-bound systems provided insights into the relative ability of each compound to stabilize α -synuclein and suppress large-scale conformational rearrangements associated with its aggregation-prone behavior.

2.7 | Cell-Based Assays for α -Synuclein Aggregation and Cytotoxicity

To evaluate the effect of *A. sativum*-derived compounds alliin and allicin on α -synuclein aggregation and associated cytotoxicity, cell-based assays were conducted using the human neuroblastoma cell line SH-SY5Y [43]. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, and 1% penicillin–streptomycin, maintained at 37°C in a humidified incubator with 5% CO₂. Cells were seeded in 6-well or 96-well plates depending on the downstream assay, and allowed to reach 70%–80% confluency before transfection.

To induce intracellular aggregation of α -synuclein, SH-SY5Y cells were transfected with a plasmid encoding human α -synuclein fused to green fluorescent protein (α -synuclein–GFP) using Lipofectamine 2000. Transfected cells were incubated for 24–48 h to allow for adequate protein expression. Following transfection, cells were treated with alliin or allicin at final

concentrations of 1, 5, and 10 μ M diluted in culture medium. Untreated cells served as controls. After 24 h of compound treatment, α -synuclein aggregation was assessed by fluorescence microscopy. Aggregation was identified by the formation of punctate GFP signals in the cytoplasm, while diffuse fluorescence indicated non-aggregated protein. Images were captured, and the number of aggregate-positive cells was quantified from multiple fields using ImageJ software [44].

To evaluate cytotoxicity, the MTT assay was performed [45]. SH-SY5Y cells were seeded in 96-well plates and treated under the same experimental conditions as described above. After 24 h of compound exposure, 20 μ L of MTT reagent (5 mg/mL in PBS) was added to each well and incubated at 37°C for 4 h. Formazan crystals formed by viable cells were solubilized using 100 μ L of DMSO, and absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated relative to untreated controls. Additional MTT assays were performed on non-transfected SH-SY5Y cells treated with alliin or allicin alone to ensure that the compounds were not inherently cytotoxic.

All assays were performed in triplicate, and data were analyzed statistically using Student's *t* test or ANOVA, with *p* < 0.05 considered significant.

3 | Results

3.1 | ADMET Analysis of Bioactive Compounds From *A. sativum*

Nine bioactive compounds isolated from *A. sativum* were subjected to a detailed in silico ADMET analysis to assess their pharmacokinetic behavior and drug-likeness (Table 1). Each of the nine compounds exhibited high GI absorption, suggesting favorable oral bioavailability. This property was attributed to their relatively low molecular weights, ranging from 88.17 to 234.40 g/mol, which support efficient passive diffusion across the intestinal epithelium. Compounds such as AMS (88.17 g/mol) and DAS (114.21 g/mol), being the smallest in size, are particularly likely to be rapidly and extensively absorbed upon oral administration.

Positive BBB permeability was predicted for six of the nine compounds, including allicin, 2-vinyl-4H-1,3-dithiin, DAS, DADS, DATS, and AMS. This indicates that these compounds may have CNS activity and could potentially be utilized in neurological applications. In contrast, alliin, E-ajoene, and Z-ajoene were not predicted to cross the BBB, suggesting a limited ability to exert effects within the CNS. Additionally, skin permeability, as indicated by Log₁₀*K_p* values, varied significantly among the compounds. Alliin showed the lowest skin permeation (–9.89 cm/s), implying minimal dermal absorption, whereas DAS (–5.46 cm/s) and 2-vinyl-4H-1,3-dithiin (–5.55 cm/s) had relatively higher skin permeability, which may make them suitable candidates for transdermal delivery systems.

All nine compounds were evaluated against Lipinski's Rule of Five. Remarkably, all tested compounds complied fully with these criteria, registering zero violations. This suggests that

TABLE 1 | ADMET analysis of nine compounds isolated from *Allium sativum*.

Name of compound	Molecular weight	GI absorption	BBB permeant	Log K_p (skin permeation) (cm/s)	Lipinski
Alliin	177.22	High	No	−9.89	Yes
Allicin	162.27	High	Yes	−6.36	Yes
E-ajoene	234.40	High	No	−6.52	Yes
Z-ajoene	234.40	High	No	−6.52	Yes
2-Vinyl-4H-1,3-dithiin	144.26	High	Yes	−5.55	Yes
Diallyl sulfide	114.21	High	Yes	−5.46	Yes
Diallyldisulfide	146.27	High	Yes	−5.63	Yes
Diallyltrisulfide	178.34	High	Yes	−5.51	Yes
Allyl methyl sulfide	88.17	High	Yes	−5.76	Yes

each compound possesses physicochemical characteristics conducive to oral administration and systemic bioavailability.

Overall, the ADMET analysis supports the potential of these *A. sativum*-derived compounds as promising drug candidates. Their favorable absorption, acceptable distribution properties, and strong compliance with drug-likeness rules position them well for further computational and experimental pharmacological investigation.

3.2 | Molecular Docking Analysis of *A. sativum*-Derived Compounds With α -Synuclein

Four molecules (alliin, allicin, E-ajoene, and DADS) were subjected to docking simulations on α -synuclein to explore the binding affinity of each compound at the predicted active site of α -synuclein (Figure 1A–D). The results revealed that the binding affinities of these compounds ranged from −5.1 to −10.8 kcal/mol, indicating varying degrees of interaction strength between the ligands and α -synuclein. Among these, alliin and DADS exhibited the highest binding affinities of −10.8 kcal/mol, followed closely by allicin and E-ajoene, each with a binding energy of −10.4 kcal/mol. Due to their strong interaction potential, these four compounds—alliin, allicin, E-ajoene, and DADS—were selected for detailed structural analysis and considered for further validation.

The α -synuclein–alliin complex showed that alliin fits stably into the cavity by interacting with key residues such as K32, G36, L38, V40, and K43 (Figure 1A). The molecule engages in strong hydrogen bonding and electrostatic interactions through its sulfoxide and carboxylic acid functional groups. Residues like A124 and A125 provide additional van der Waals contacts, stabilizing the ligand within the protein's structure.

In the α -synuclein–allicin complex, allicin was found to be stabilized primarily by hydrogen bonding with residues K32, E35, G36, L38, Y39, V40, and K43 (Figure 1B). The sulfoxide moiety of allicin plays a pivotal role in anchoring the molecule through polar interactions. Hydrophobic interactions involving residues

L38, E35, and V40 contribute further to the overall stability of the complex.

The α -synuclein–E-ajoene complex demonstrated that E-ajoene interacts with a shallow groove on the protein surface through hydrogen bonding and π – π stacking with residues Y39, G41, K43, and T44 (Figure 1C). Its disulfide linkage and vinyl group facilitate distinct conformational anchoring, supported by additional electrostatic interactions with E36 and hydrophobic contacts with L38.

Similarly, the α -synuclein–DADS complex showed extensive interactions involving residues E35, L38, Y39, G41, T44, and K45 (Figure 1D). The disulfide bond of DADS forms stable interactions with both polar and nonpolar residues, while the hydrophobic diallyl moieties contribute to favorable van der Waals interactions.

Overall, these findings indicate that sulfur-containing compounds from *A. sativum*, particularly alliin, allicin, E-ajoene, and DADS, exhibit strong and specific interactions with α -synuclein.

3.3 | MD Simulation and Structural Stability Analysis of α -Synuclein–Ligand Complexes

To evaluate the conformational stability and dynamic behavior of α -synuclein upon binding with alliin, diallyldisulfide, allicin, diallyltrisulfide, and E-ajoene, a 100-ns MD simulation was carried out for each protein–ligand complex.

The results of RMSD profiles of the C α atoms of the protein across all five complexes show that each complex undergoes a rapid structural relaxation phase within the initial 10–20 ns, after which the protein backbone reaches equilibrium (Figure 2A). Among the complexes, α -synuclein–alliin and α -synuclein–allicin exhibit the lowest average RMSD values (Figure 2A), suggesting greater conformational stability. In contrast, the α -synuclein–E-ajoene complex shows consistently higher RMSD values (~23–25 Å) (Figure 2A), indicating

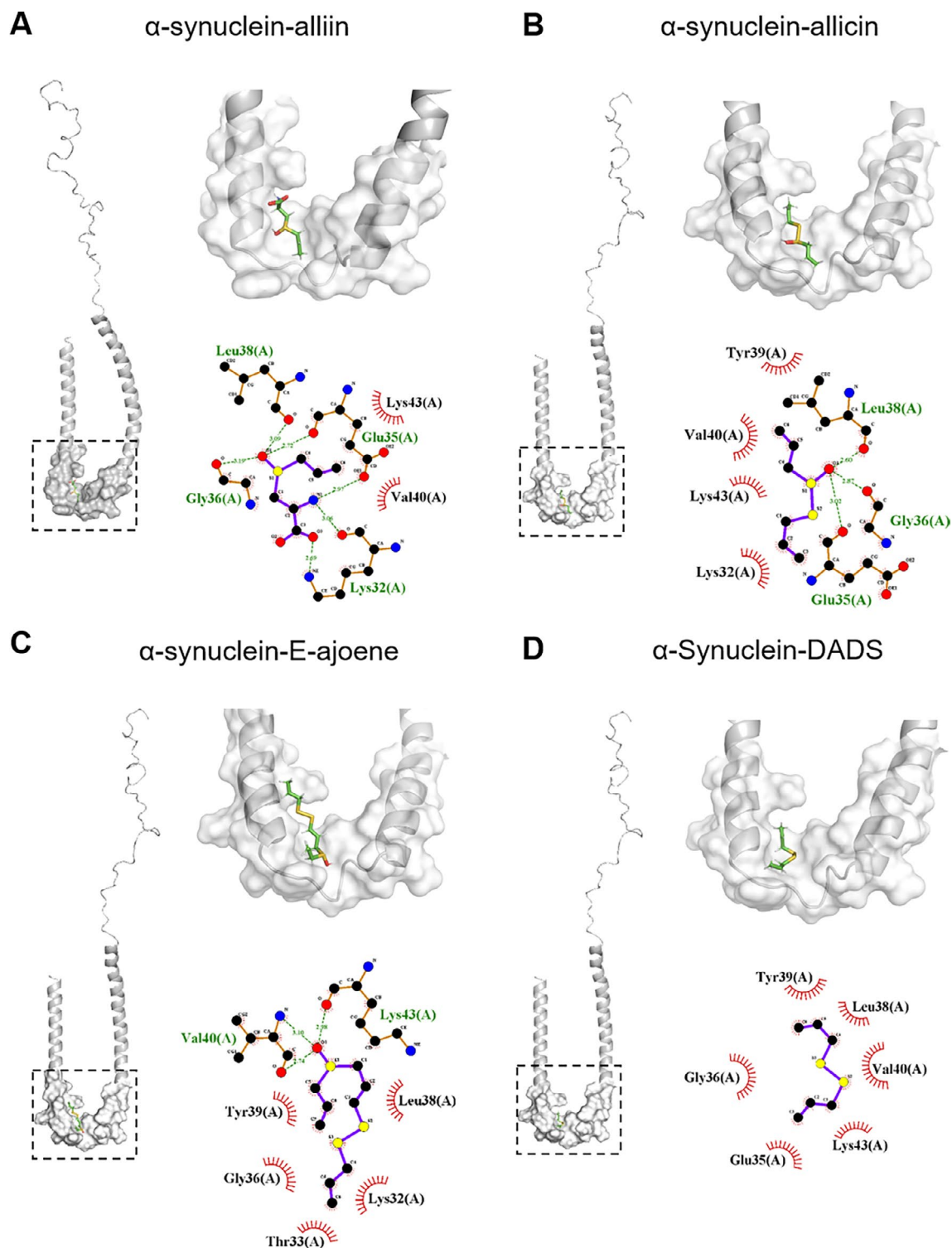


FIGURE 1 | Molecular docking of *Allium sativum*-derived compounds with α -synuclein. (A–D) Predicted binding conformations of alliin (A), allicin (B), E-ajoene (C), and diallyl disulfide (DADS) (D) within the helical groove of α -synuclein (PDB ID: 1XQ8). Enlarged upper panels show the docked ligands (stick models) within the surface representation of the α -synuclein binding pocket. Lower panels provide an enlarged view of the interaction interface, highlighting the key residues involved in salt bridges, hydrogen bonding, and hydrophobic interactions with each ligand. Residues such as Lys32, Glu35, Gly36, Leu38, Val40, and Lys43 are recurrent across complexes, suggesting a conserved interaction hotspot near the aggregation-prone region of α -synuclein. The binding orientation and interaction profiles suggest stabilization of the native helical structure, potentially preventing pathological unfolding and aggregation.

substantial conformational rearrangement or structural flexibility upon ligand binding. The other complexes (of diallyldisulfide and of diallyltrisulfide) maintained moderate stability,

with RMSD values plateauing around 16–18 Å (Figure 2A), suggesting these ligands moderately stabilize α -synuclein structure.

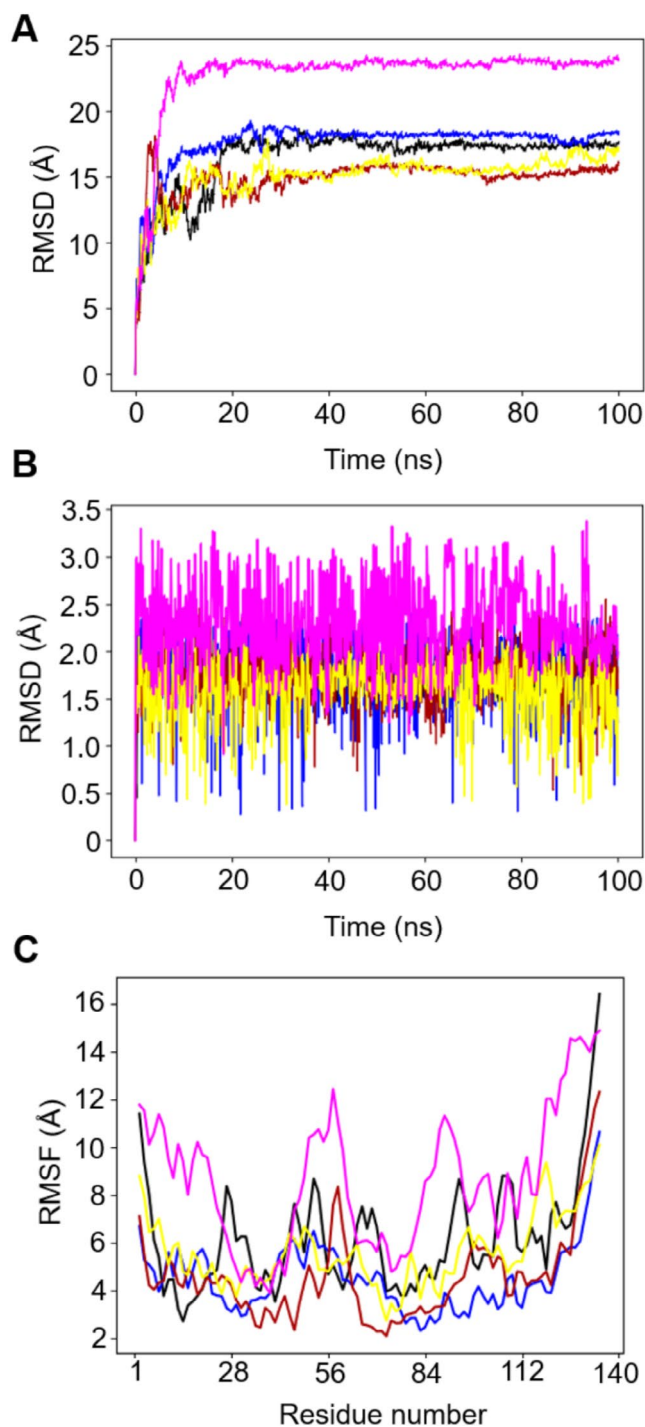


FIGURE 2 | Molecular dynamics simulation analysis of α -synuclein in complex with *Allium sativum*-derived ligands over 100 ns. (A) Root mean square deviation (RMSD) plot showing backbone fluctuations of α -synuclein in the presence of five different ligands. Each trace represents one ligand-bound complex: Alliin (red), allicin (yellow), E-ajoene (black), diallyl disulfide (DADS, blue), and diallyl trisulfide (DATS, pink). The results indicate that α -synuclein–alliin and α -synuclein–allicin complexes exhibit lower RMSD values, reflecting enhanced conformational stability, while α -synuclein–DATS shows the highest RMSD, indicating greater structural flexibility. (B) Ligand RMSD plot demonstrating the positional stability of the ligands within the α -synuclein binding site throughout the simulation. Alliin, allicin, and DADS show relatively stable binding with low RMSD values ($<2\text{Å}$), whereas E-ajoene and DATS display higher fluctuations, particularly DATS, suggesting more dynamic interactions or pose changes during the simulation. (C) Root mean square fluctuation (RMSF) plot of α -synuclein residues, highlighting residue-wise flexibility in each complex. The N-terminal and C-terminal regions show expected flexibility, while central residues involved in ligand binding display lower fluctuations in the alliin and allicin complexes, supporting their role in stabilizing aggregation-prone regions of α -synuclein. Elevated RMSF values in the DATS-bound complex correlate with its higher global RMSD, reflecting reduced stabilization.

ultimately achieved a stabilized configuration, maintaining its association with the protein.

For all the complexes, residues located in the N-terminal and C-terminal regions, as well as in loop segments, showed elevated RMSF values (Figure 2C), indicative of their inherent flexibility. Notably, the α -synuclein–E-ajoene complex exhibited the highest fluctuations across multiple residue segments (Figure 2C), consistent with its elevated RMSD profile. In contrast, α -synuclein–allicin and –alliin complexes displayed the least fluctuation (Figure 2C), particularly in the central structured regions, suggesting that these ligands contribute to conformational rigidity and localized stabilization of α -synuclein. Importantly, residues associated with ligand binding sites demonstrated reduced RMSF values, signifying a stable ligand-protein interaction interface.

The binding free energies (ΔG binding) of all complexes were calculated using the MM-GBSA approach (Figure 3A). Among the studied ligands, alliin and E-ajoene exhibited the most favorable ΔG values, reflecting stronger binding affinities with α -synuclein (Table 2, Figure 3A). While the helical content of the unbound α -synuclein decreased over the simulation time, indicating the helix-to-disorder transition of α -synuclein during simulation (Figure 3B), the binding of the ligands prevented the loss of helical structure of the ligand-bound α -synuclein (Figure 3B).

Total contacts (Figure 4A), including hydrogen bonding analysis revealed differential interaction profiles among the five complexes. For α -synuclein–alliin, key interacting residues included Met1, Lys21, Lys58, Thr59, and Gln62, which formed persistent hydrogen bonds throughout the simulation. The α -synuclein–allicin complex displayed interactions predominantly through residues Glu28, Lys32, Lys34, and Glu35.

The ligand RMSD plots represent the positional deviations of the ligands relative to their initial bound poses throughout the simulation (Figure 2B). Most ligands remained relatively stable after the initial equilibration phase (~ 20 ns), exhibiting fluctuations within an acceptable range ($<2.5\text{Å}$), which indicates their consistent stability within the binding pocket (Figure 2B). However, E-ajoene displayed high fluctuation throughout the trajectory (up to $\sim 3.5\text{Å}$), likely due to conformational rearrangements or a potential flipping of the ligand within the active site during the 50–90 ns timeframe (Figure 2B). Despite these transient fluctuations, the ligand

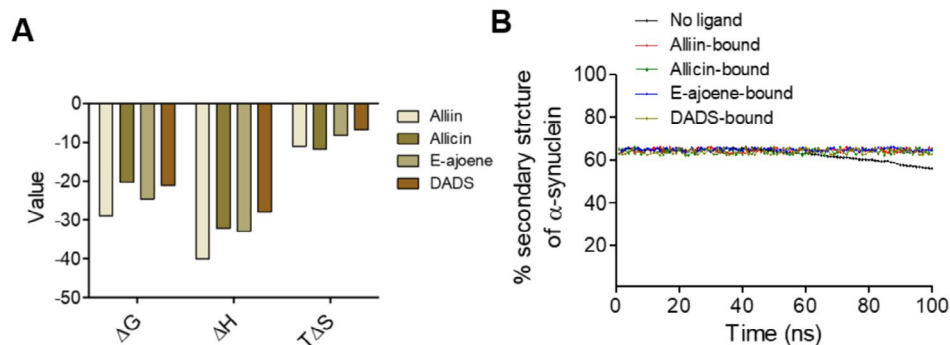


FIGURE 3 | Thermodynamic and structural analysis of α -synuclein–ligand complexes. (A) Bar graph representing the thermodynamic parameters of binding—Gibbs free energy (ΔG), enthalpy (ΔH), and entropy contribution ($T\Delta S$)—for four *Allium sativum*-derived ligands (alliin, allicin, E-ajoene, and DADS) with α -synuclein. Alliin exhibits the most favorable binding energetics with the lowest ΔG and ΔH values, suggesting strong enthalpy-driven binding. (B) Time-dependent evolution of α -synuclein's secondary structure (percentage of helical content) during 100 ns molecular dynamics simulations in the absence and presence of ligands. The presence of alliin, allicin, E-ajoene, and DADS maintains or slightly enhances α -helical stability throughout the simulation period, in contrast to the unbound protein which shows a gradual loss of helical structure.

TABLE 2 | Binding affinities of compounds.

Name of compound	PubChem CID	Binding affinities (kcal/mol)
Alliin	9576089	−10.8
Allicin	65036	−10.4
E-ajoene	5386591	−10.4
Z-ajoene	9881148	−5.1
2-Vinyl-4H-1,3-dithiin	133337	−6.0
Diallyl sulfide (DAS)	11617	−6.8
Diallyldisulfide (DADS)	16590	−10.8
Diallyltrisulfide (DATS)	16315	−7.5
Allyl methyl sulfide (AMS)	66282	−6.2

Diallyldisulfide showed fewer hydrogen bonds, suggesting hydrophobic interactions may be more dominant in maintaining the complex. In the Diallyltrisulfide complex, critical hydrogen bond-forming residues included Lys10, Glu28, Gly84, Gly86, Ser87, and Lys96. For E-ajoene, residues Lys21, Ala89, and thr92 contributed significantly to hydrogen bonding. These observations collectively suggest that although all ligands interacted favorably with α -synuclein, the nature and extent of interactions varied based on the chemical features of each ligand.

In conclusion, the MD simulation analyses confirm that alliin and allicin are potent stabilizers of α -synuclein conformation, whereas E-ajoene, despite a strong binding affinity, introduces notable structural flexibility.

3.4 | Time-Resolved Interaction Dynamics of α -Synuclein With *A. sativum*-Derived Ligands During MD Simulation

During the simulation, allicin is observed to engage in a network of stabilizing interactions with key residues within the central binding groove of α -synuclein, including Lys43, Tyr39, Leu38, Val40, Glu35, and Gly36 (Figure 5). These interactions involve hydrogen bonding and hydrophobic contacts that stabilize the sulfoxide moiety of allicin. Notably, these contacts are largely conserved at the end of the simulation (Figure 5), indicating a persistent binding pose. However, slight reorientation of allicin within the groove is visible, likely due to local relaxation of side chains, though the core interaction network (Lys43, Tyr39, Glu35) remains intact (Figure 5), reflecting stable and robust ligand retention.

For alliin, the binding configuration during simulation reveals strong hydrogen bonding and electrostatic interactions with residues Glu35, Gly36, Lys32, and Leu38 (Figure 5). These contacts are indicative of alliin anchoring itself within the NAC-adjacent helical region, contributing to structural stabilization. At the ending phase of simulation, these interactions are preserved with minor positional adjustment of the alliin molecule, suggesting a well-defined and energetically favorable binding mode (Figure 5). The consistent participation of Lys32 and Glu35 in hydrogen bonding across the simulation period underscores alliin's strong binding affinity and potential to prevent conformational drift of α -synuclein.

The E-ajoene-bound complex shows a relatively dynamic interaction pattern. E-ajoene engages Glu35, Val40, Lys43, and Tyr39 through a combination of polar and hydrophobic interactions (Figure 5). However, by the end of simulation, there is a visible shift in the binding orientation, with new contacts forming with Thr33 and a rearrangement in the positions of interacting residues such as Leu38 and Lys32 (Figure 5). This evolving interaction profile suggests that while E-ajoene remains bound within the groove, its pose is more flexible and adaptive, possibly indicating a less rigid, though still relevant, stabilizing influence on α -synuclein.

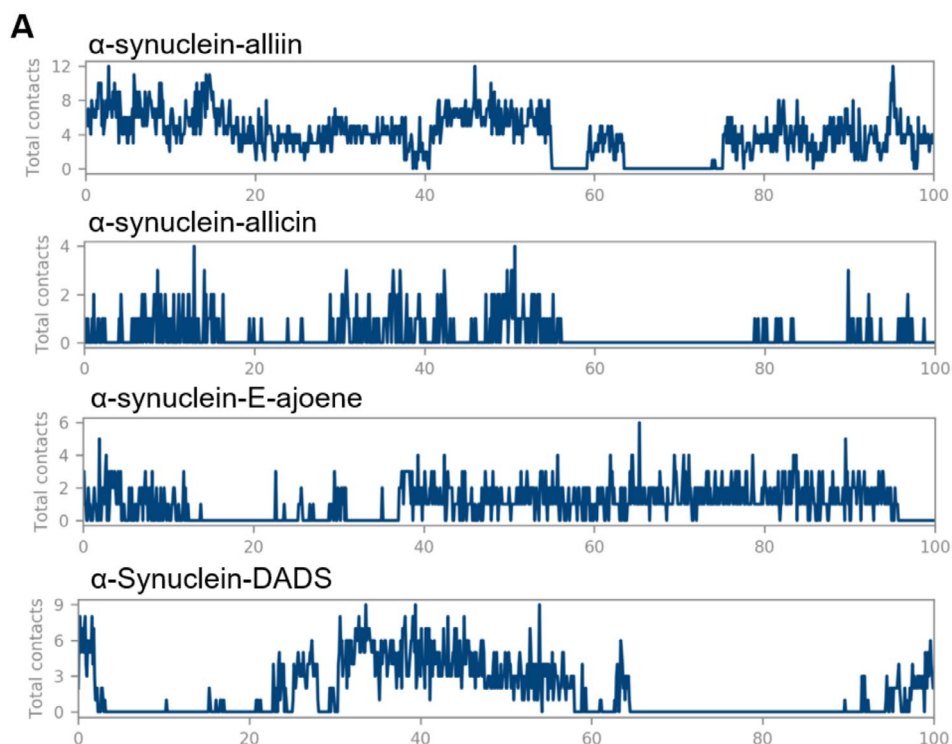


FIGURE 4 | Interaction dynamics and compactness of α -synuclein–ligand complexes during molecular dynamics simulations. (A) Plots showing the total number of atomic contacts between α -synuclein and each ligand—alliin, allicin, E-ajoene, and diallyl disulfide (DADS)—over the course of 100 ns simulation. Alliin and E-ajoene form relatively stable and consistent interactions with α -synuclein, while allicin and DADS exhibit more transient or intermittent contact patterns. The number of persistent contacts reflects the stability of ligand engagement within the binding site.

DADS is stabilized predominantly through hydrophobic interactions involving Leu38, Val40, Tyr39, and Gly36, as well as limited electrostatic interactions with Lys32 and Glu35 (Figure 5). These interactions suggest a preference for nonpolar contacts, consistent with the aliphatic and disulfide-rich nature of DADS. By end of simulation, the DADS molecule retains its orientation and maintains most of its original contacts, highlighting a stable binding configuration despite the absence of extensive hydrogen bonding (Figure 5). The persistence of interactions with Leu38 and Tyr39 at both time points supports DADS' capacity to contribute to conformational stabilization via hydrophobic interactions.

Across all four complexes, certain residues—particularly Tyr39, Leu38, Gly36, and Glu35—emerge as common interaction hotspots, indicating their central role in mediating ligand-induced stabilization (Figure 5). While allicin and alliin form extensive and persistent hydrogen bonds over time, E-ajoene exhibits more dynamic repositioning, suggesting moderate flexibility in its binding. DADS, in contrast, relies predominantly on hydrophobic contacts but maintains a relatively fixed pose throughout the simulation. These observations indicate that the structural and chemical features of each ligand govern their interaction dynamics with α -synuclein. Allicin and alliin, through consistent polar interactions, appear to contribute to more stable complexation, potentially inhibiting the structural rearrangements necessary for α -synuclein aggregation. E-ajoene, although interactive, shows more conformational plasticity, while DADS secures its position via nonpolar forces, with limited conformational perturbation.

3.5 | PCA Reveals Ligand-Dependent Stabilization Dynamics of α -Synuclein

PCA was employed to capture the essential collective motions and dominant conformational changes of α -synuclein upon ligand binding, using the 100 ns MD trajectories of the protein–ligand complexes. For the PCA results for each α -synuclein–ligand complex, the scatter plots represent projections of the MD trajectories onto the first three principal components (PC1–PC3). Each dot corresponds to a conformational snapshot in time, colored from blue (initial frames) to red (final frames), thus illustrating the conformational progression throughout the simulation.

For all complexes, the major conformational transitions are predominantly captured by PC1 and PC2. In the α -synuclein–alliin complex, PC1 accounts for 41.12% of the total variance, followed by PC2 (29.85%) and PC3 (7.09%) (Figure 6). The clustering of red points in a compact zone and their limited dispersion in PC1–PC2 and PC1–PC3 subspaces suggests a stable conformational state attained after an initial dynamic rearrangement. This behavior reflects effective stabilization of the α -synuclein structure by alliin.

Similarly, the α -synuclein–allicin complex shows even higher contributions from PC1 (47.85%) and PC2 (16.15%) (Figure 6), suggesting that allicin also induces substantial motion along the principal axes. However, the convergence of red-colored snapshots in a restricted region further supports the hypothesis of stable complex formation. Notably, the conformational space

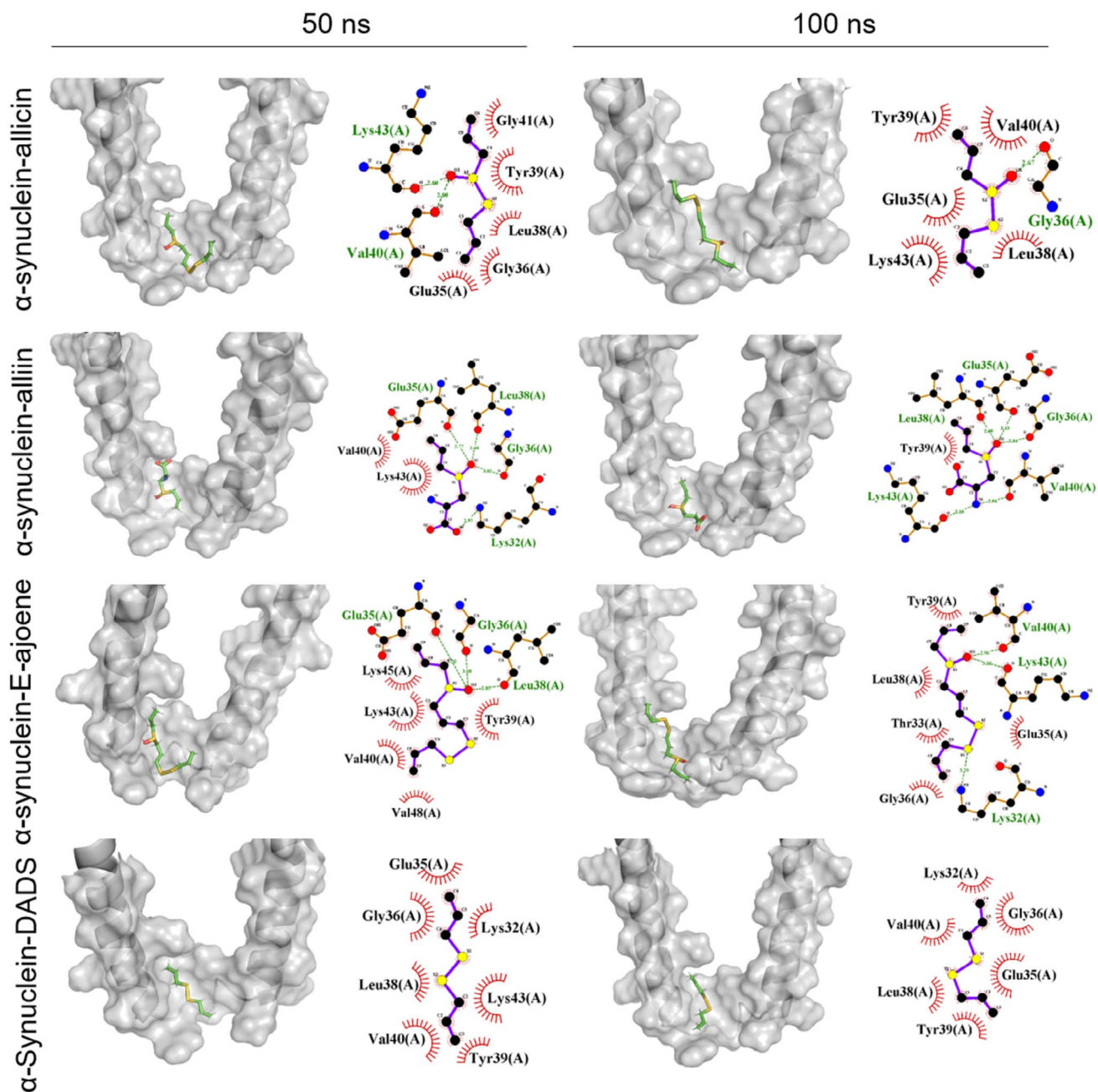


FIGURE 5 | Snapshots of ligand-bound α -synuclein complexes at representative time points—50 and 100 ns—during the 100 ns molecular dynamics simulation. For each complex, the left panels show the 3D surface representation of α -synuclein with the bound ligand within the binding groove, while the right panels show detailed 2D interaction diagrams depicting residues involved in hydrogen bonding, hydrophobic, and electrostatic interactions. Interacting residues such as Lys32, Glu35, Gly36, Leu38, Tyr39, Val40, and Lys43 are consistently observed across different ligands.

sampled by this complex appears to be narrower than that observed for alliin, indicating more restricted motion and potentially stronger binding-induced rigidity.

In contrast, the α -synuclein-E-ajoene complex exhibits relatively higher variability in conformational space, as shown by the scattered red and blue dots in the PC projections. PC1 and PC2 together explain 55.01% of the variance (38.06% and 16.94%, respectively), while PC3 adds another 10.6% (Figure 6). The broader dispersion of points, even in the later stages of simulation, suggests a less stable binding mode and greater structural plasticity upon E-ajoene interaction.

The α -synuclein-DADS complex exhibits moderate dynamic behavior, with PC1 (34.85%) and PC2 (17.23%) capturing a total of ~52% of the variance (Figure 6). Although the transition of colors from blue to red indicates a progression to a somewhat stable state, the wide scattering of red points implies that conformational stabilization is less pronounced compared to alliin and allisin complexes.

The scree plots adjacent to each set of PCA projections display the eigenvalue distribution across the first 10 eigenvectors. The eigenvalue corresponding to PC1 is significantly higher than the subsequent components in each complex, confirming that

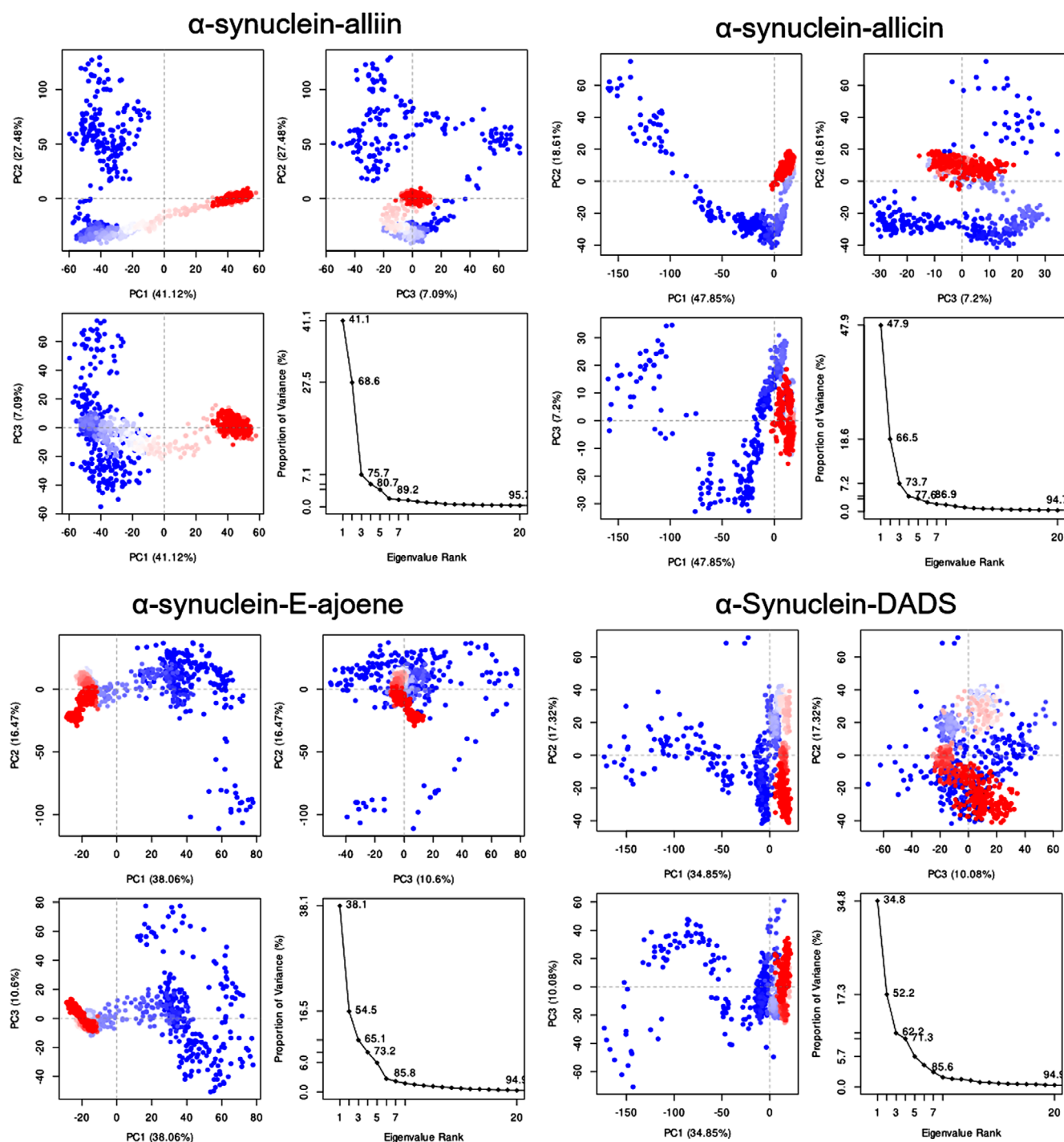


FIGURE 6 | Principal component analysis of α -synuclein–ligand complexes from the molecular dynamics simulations. Scatter plots represent the conformational sampling of α -synuclein in complex with alliin, allicin, E-ajoene, and DADS, projected onto combinations of the top three principal components (PC1, PC2, and PC3). Each dot represents a snapshot from the trajectory, color-coded from blue (early time points) to red (later time points), showing the temporal evolution of conformations. For α -synuclein–alliin and α -synuclein–allicin complexes, tight clustering of red points indicates conformational convergence and structural stabilization during simulation. Broader distribution of conformers in the DADS complex suggests increased structural variability. Accompanying scree plots depict the eigenvalue distribution and cumulative variance explained by each principal component. In all complexes, the first few components (PC1–PC3) capture the majority of the system's dynamic motion, with α -synuclein–allicin showing the highest variance captured by PC1.

PC1 represents the most dominant mode of motion (Figure 6). In all complexes, the cumulative variance reaches a plateau by the seventh or eighth component, indicating that the majority of the system's motion is adequately described by the first few principal components.

Overall, PCA clearly indicates that alliin and allicin promote more defined and stable conformational landscapes of α -synuclein compared to E-ajoene and DADS. The clustering patterns, motion amplitudes, and eigenvalue distributions together suggest that alliin and allicin contribute to stronger

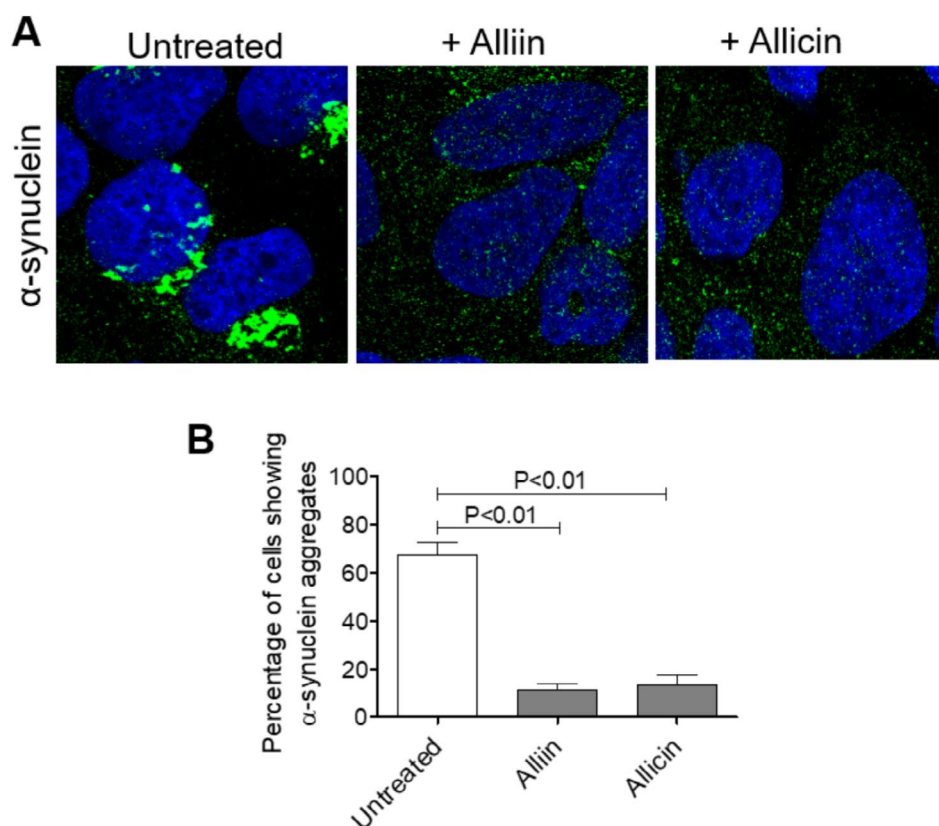


FIGURE 7 | Effect of alliin and allicin on intracellular α -synuclein aggregation in SH-SY5Y cells. (A) Representative fluorescence microscopy images showing α -synuclein-GFP aggregation in untreated cells and cells treated with 10 μ M of alliin or allicin. In untreated cells, distinct punctate GFP-positive aggregates are visible, indicating misfolded α -synuclein accumulation. In contrast, both alliin- and allicin-treated cells exhibit a diffuse cytoplasmic GFP signal with markedly reduced aggregate formation. Scale bar: 5 μ m. (B) Quantification of the percentage of cells exhibiting α -synuclein aggregates under each condition. Treatment with alliin or allicin significantly reduced the proportion of aggregation-positive cells compared to untreated controls ($p < 0.01$, Student's t test). Data are represented as mean $\pm$ SEM from three independent experiments.

and more stable interactions with the intrinsically disordered α -synuclein, potentially suppressing its pathogenic misfolding or aggregation.

3.6 | Alliin and Allicin Reduce α -Synuclein Aggregation in SH-SY5Y Cells

To assess the modulatory effect of alliin and allicin, SH-SY5Y neuroblastoma cells were transfected with a plasmid encoding α -synuclein tagged with green fluorescent protein (α -synuclein-GFP). In untreated control cells, a substantial proportion of the α -synuclein-GFP-expressing cells exhibited distinct punctate aggregates distributed throughout the cytoplasm, indicating pathological misfolding and oligomerization of α -synuclein.

Upon treatment with 10 μ M concentrations of alliin and allicin, a marked reduction in α -synuclein aggregation was observed compared to the untreated group. As shown in the representative fluorescence images, control cells expressing α -synuclein-GFP displayed robust and widespread aggregation patterns, characterized by intense green puncta (Figure 7A). In contrast, cells treated with either alliin or allicin exhibited significantly fewer fluorescent puncta, and the GFP signal appeared more diffusely distributed throughout the cytoplasm (Figure 7A), indicative of a reduction in aggregated species.

Quantitative analysis of the aggregation-positive cells revealed a statistically significant decline in the number of α -synuclein aggregates upon treatment (Figure 7B). The reduction was evident in both treatment conditions, with alliin and allicin both showing similar efficacy in mitigating aggregate formation. These findings suggest that both compounds interfere with the intracellular aggregation pathway of α -synuclein, likely by stabilizing its native conformation or enhancing its clearance via proteostatic mechanisms.

3.7 | Alliin and Allicin Alleviate α -Synuclein-Induced Cytotoxicity in SH-SY5Y Cells

To evaluate the cytotoxic potential of alliin and allicin in neuronal cells, SH-SY5Y cells were treated with varying concentrations (1, 5, and 10 μ M) of each compound in the absence of α -synuclein overexpression (Figure 8A). None of the tested concentrations of alliin or allicin elicited significant cell death. The percentage of viable cells remained comparable to that of untreated controls, indicating that both compounds are nontoxic to SH-SY5Y cells up to a 10 μ M concentration.

To evaluate the potential impact of alliin and allicin on cell cycle progression, SH-SY5Y cells were treated with 10 μ M concentrations of each compound, and flow cytometry-based cell cycle

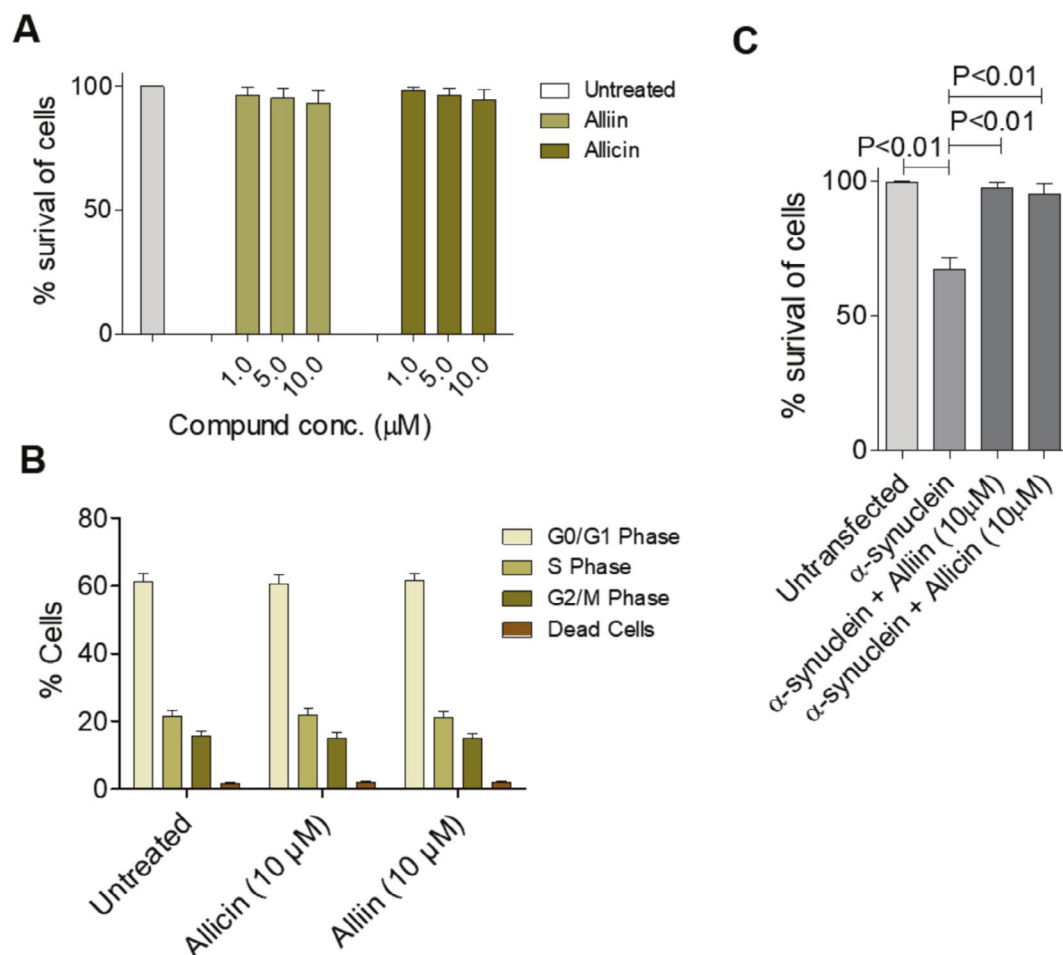


FIGURE 8 | Cytotoxicity assessment of alliin and allicin in SH-SY5Y cells using MTT assay. (A) Cell viability of SH-SY5Y cells treated with increasing concentrations (1, 5, and 10 μM) of alliin and allicin for 24 h. No significant cytotoxicity was observed at any concentration, with survival rates comparable to untreated control cells, indicating that both compounds are nontoxic up to 10 μM. (B) Cell cycle (cell phase) analysis of untreated, 10 μM allicin-treated, and 10 μM alliin-treated cells. (C) Effect of 10 μM alliin and allicin on α-synuclein-GFP-overexpressing SH-SY5Y cells. Overexpression of α-synuclein significantly reduced cell viability compared to untransfected cells ($p < 0.01$). Treatment with alliin or allicin markedly improved cell survival, suggesting that both compounds effectively attenuate α-synuclein-induced cytotoxicity ($p < 0.01$). Data represent mean $\pm$ SEM from three independent experiments.

analysis was performed. The distribution of cells across the G0/G1, S, and G2/M phases was comparable among untreated controls and treated groups. As shown in the Figure 8B, untreated cells exhibited 61.2% in the G0/G1 phase, 21.5% in the S phase, and 15.6% in the G2/M phase. Cells treated with allicin displayed a similar distribution, with 60.8% in G0/G1, 22.0% in S phase, and 15.2% in G2/M phase (Figure 8B). Likewise, alliin-treated cells showed 61.6% in G0/G1, 21.3% in S phase, and 15.1% in G2/M phase (Figure 8C). Importantly, the percentage of dead cells remained consistently low (<2%) across all groups, with no statistically significant differences observed (Figure 8C). These results indicate that treatment with alliin or allicin at 10 μM does not alter the normal cell cycle distribution or induce cytotoxic effects in SH-SY5Y cells, affirming the cellular safety and compatibility of these compounds at the tested concentration.

To assess the effect of these compounds on α-synuclein-mediated cytotoxicity, SH-SY5Y cells were transfected with α-synuclein-GFP and cell survival was quantified. Expression of α-synuclein led to a marked reduction in cell viability compared to untransfected control cells (Figure 8C), corroborating previous reports

that aberrant α-synuclein accumulation is detrimental to neuronal viability. Interestingly, treatment with 10 μM of alliin or allicin significantly rescued α-synuclein-expressing cells from cell death (Figure 8C). Both compounds conferred significant protection, restoring viability to levels much closer to that of untransfected controls.

4 | Discussion

PD is a progressive neurodegenerative disorder primarily characterized by the accumulation of misfolded α-synuclein aggregates, which form intracellular inclusions known as Lewy bodies [3, 46]. Under physiological conditions, α-synuclein exists as a soluble, intrinsically disordered protein with a propensity to adopt α-helical conformations upon membrane binding. However, in pathological states, the protein misfolds into β-sheet-rich fibrils through a nucleation-dependent process [47]. This transition is influenced by multiple intrinsic factors such as mutations, posttranslational modifications, and oxidative stress, leading to the accumulation of toxic oligomers and fibrillar

aggregates that disrupt cellular homeostasis, impair mitochondrial function, and ultimately trigger neuronal death [48]. Targeting α -synuclein aggregation and its associated toxicity is thus a compelling strategy for developing disease-modifying therapies in PD [49].

In recent years, phytochemicals have emerged as promising neuroprotective agents due to their multitarget capabilities, antioxidant properties, and low toxicity [50]. Compounds derived from *A. sativum* are particularly notable for their rich content of sulfur-containing bioactives [13]. Our study focused on four such compounds—alliin, allicin, E-ajoene, and DADS—and investigated their capacity to bind α -synuclein and modulate its aggregation propensity through integrated computational and cellular approaches.

Molecular docking and dynamic simulations revealed that all four compounds bind with high affinity to α -synuclein. Detailed binding analyses demonstrated that these compounds engage key aggregation-prone regions and anchor themselves through extensive hydrogen bonding, electrostatic interactions, and hydrophobic contacts. Alliin was shown to stabilize residues K32, G36, L38, V40, and K43, which lie near the β NAC region crucial for fibril formation. Similarly, allicin also primarily stabilized *N*-terminal residues such as K32, E35, G36, L38, Y39, V40, and K43, potentially preventing early-stage conformational transitions that promote aggregation. E-ajoene formed multiple interactions along loop and helical regions but introduced more flexibility in protein structure, while DADS established hydrophobic contacts that may reinforce the native conformation. Importantly, these interactions were observed to stabilize α -helical domains and restrict β -sheet conversion, thereby maintaining the protein in a less aggregation-prone state.

MD simulations further supported these findings, showing that alliin and allicin maintained low RMSD values of the protein backbone and reduced RMSF of residues in the central structured domains of α -synuclein, indicating enhanced conformational stability. In contrast, E-ajoene exhibited higher RMSD and RMSF values, suggesting greater flexibility and possibly less effective stabilization. The Rg analysis revealed that all complexes maintained compact structures throughout the 100 ns simulation, although minor fluctuations in the E-ajoene complex reflected its relatively looser binding conformation. MM-GBSA binding energy calculations further confirmed the thermodynamic favorability of alliin and E-ajoene, aligning well with docking results.

PCA provided additional insight into the large-scale motions of the α -synuclein complexes. Alliin and allicin displayed compact clustering of trajectory frames along PC1 and PC2 axes, indicating minimal conformational drift and suggesting stable protein-ligand interactions. These ligands reduced the amplitude of dominant motions in α -synuclein, potentially lowering the entropic drive towards misfolded states. Conversely, E-ajoene demonstrated broader dispersion in conformational space, reflecting structural plasticity and potentially transient binding, which may limit its aggregation-inhibitory capacity under certain conditions.

Corroborating the computational findings, cellular assays using SH-SY5Y neuroblastoma cells expressing α -synuclein-GFP

revealed that both alliin and allicin significantly reduced intracellular aggregation. Untreated α -synuclein-expressing cells displayed numerous cytoplasmic puncta representing aggregated species, whereas treatment with 10 μ M of either compound led to diffuse GFP distribution and a marked reduction in aggregate-positive cells. This anti-aggregation effect likely results from the compounds' ability to interfere with oligomerization and promote α -synuclein clearance or refolding [51]. Furthermore, MTT-based cytotoxicity assays demonstrated that while α -synuclein expression alone significantly reduced cell viability, treatment with alliin or allicin effectively restored cell survival to near-control levels. Importantly, these compounds showed no inherent cytotoxicity at doses up to 10 μ M, underscoring their safety for therapeutic applications.

Together, these findings highlight the multifaceted neuroprotective potential of *A. sativum*-derived sulfur compounds. The ability of alliin and allicin to stably bind α -synuclein, suppress its aggregation, and alleviate associated cytotoxicity positions them as promising leads for future drug development in PD. Their dual capacity to modulate protein structure and protect neuronal viability makes them especially attractive candidates for further preclinical validation, including *in vivo* efficacy and bioavailability studies. Additionally, their natural origin and favorable ADMET profiles support their long-term therapeutic applicability with minimal side effects.

One of the critical limitations of this study lies in the structural model used for α -synuclein in our *in silico* experiments. α -Synuclein is intrinsically disordered in its soluble native monomeric state under physiological conditions, lacking a stable secondary or tertiary structure. A significant fraction of its structure attains helical conformation during its insertion in membrane structure. Due to the absence of a complete high-resolution structure of the full-length, disordered α -synuclein, we employed the micelle-bound α -helical conformation as a surrogate model. This structure has been commonly used in previous computational studies as it captures transiently ordered regions, particularly the *N*-terminal helices and the NAC domain, which are critically involved in early aggregation and membrane interactions. While this choice enables reproducible docking and simulation analyses, it also represents the dynamic and flexible nature of some of the native disordered state. Therefore, the interaction patterns and stabilizing effects observed in our study can be interpreted in the context of early structural intermediates of α -synuclein, rather than its fully unstructured form. Future studies incorporating ensemble-based or coarse-grained approaches to model disordered proteins, or experimental validation with aggregation-prone variants, will be essential to refine our understanding of ligand binding under physiologically relevant conditions. Nonetheless, the consistency between our computational predictions and experimental aggregation assays supports the biological relevance of our findings and underscores the potential of the selected compounds in modulating α -synuclein pathology.

In conclusion, this study provides compelling evidence that specific garlic-derived bioactive molecules—particularly alliin and allicin—offer a rational, multitargeted approach to modulating α -synuclein pathology.

Author Contributions

S. Rehan Ahmad: conceptualization, investigation, writing – original draft, methodology, validation, visualization, software, formal analysis, data curation, supervision, resources. **Md. Zeyauallah:** investigation, methodology, validation, visualization. **Abdullah M. AlShahrani:** investigation, methodology, validation, software, formal analysis. **Khursheed Muzammil:** investigation, funding acquisition, methodology, validation, software, data curation. **Adam Dawria:** investigation, writing – original draft, methodology, validation, data curation, formal analysis, software. **Md. Faruque Ahmad:** investigation, methodology, validation. **Ahmed Salih:** investigation, methodology, validation.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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