

Reporting the Anti-neuroinflammatory Potential of Selected Spondias mombin Flavonoids through Network Pharmacology and Molecular Dynamics Simulations

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Abstract

Introduction: Neuroinflammation plays a pivotal role in the development and progression of neurodegenerative diseases, with a complex interplay between immune responses and brain activity. Understanding this interaction is crucial for identifying therapeutic targets and developing effective treatments.

Aim: This study aimed to explore the neuroprotective properties of flavonoid compounds from *Spondias mombin* against neuroinflammation using a comprehensive in-silico approach, including network pharmacology, molecular docking, and dynamic simulations.

Materials & Methods: Active flavonoid ingredients from *S. mombin* were identified, and their potential protein targets were predicted through Network Pharmacology. Molecular docking was conducted to determine the binding affinities of these compounds to cyclooxygenase-2 and FYN kinase, prioritizing docking scores ≥ -8.0 kcal/mol. Molecular dynamic simulations (MDS) assessed the stability and interaction profiles of these ligand-protein complexes.

Results: The docking studies highlighted cyclooxygenase-2 and FYN kinase as significant targets, with catechin and epicatechin displaying specificity towards FYN. However, these compounds failed the blood-brain barrier permeability test. MDS confirmed the stability of catechin and a reference ligand at the FYN active site, with notable interactions involving hydrogen bonds, hydrophobic contacts, and water bridges. GLU54 emerged as a key residue in the catechin-FYN complex due to its prolonged hydrogen bond interaction stability.

Conclusion: The findings underscore the potential of *S. mombin* flavonoids as therapeutic agents against neuroinflammation, though optimization and nanotechnology-based delivery methods are suggested to enhance drug efficacy and overcome blood-brain barrier limitations.

INTRODUCTION

The human brain was previously thought to be an organ with special immunity, however, it is now understood to be a dynamic location of immune activity that is home to a complex web of inflammatory events. The word "neuroinflammation," which was developed to characterize the inflammatory reactions that occur within the central nervous system (CNS), has become essential to comprehending the complex relationships that exist between the immune system and the brain. Glial cell activation, the production of inflammatory mediators, and the ensuing immunological reactions inside the brain tissue are all components of this event. Neuroinflammation functions as a defensive mechanism, safeguarding the brain by eliminating or hindering various pathogens (Wyss-Coray *et al.*, 2002). This protective response brings about beneficial effects, fostering tissue repair and the removal of cellular debris. Nevertheless, prolonged inflammatory responses prove detrimental, impeding the regenerative processes (Kempuraj *et al.*, 2016). The persistence of inflammatory stimulation may stem from endogenous factors, such as genetic mutations and protein aggregation, or environmental influences like infection, trauma, and drugs (Glass *et al.*, 2010). These sustained inflammatory responses, orchestrated by microglia and astrocytes, contribute to the development of neurodegenerative diseases (Kempuraj *et al.*, 2016).

Inflammation in tissue pathology indicates the long-term effects of inflammatory stimuli or a malfunction in the regular repair mechanisms, which may then result in the production of neurotoxic substances that worsen disease states (Lull *et al.*, 2010; Sarma 2014). As a result, the particular inflammatory triggers associated with neurodegenerative illnesses tend to be centered around the mechanisms that sense, transduce, and enhance inflammatory processes. Neurotoxic mediators, such as cytokines and interleukins, are produced as a result of these pathways (Glass *et al.*, 2010; Teeling *et al.*, 2009). Neurotoxic mediators are commonly associated with a number of neurodegenerative illnesses, such as amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), multiple sclerosis (MS), and Alzheimer's disease (AD).

Intracellular processes such as apoptosis, mitochondrial dysfunction, axonal transport abnormalities, and protein degradation are frequently linked to these illnesses (Taylor *et al.*, 2002; Chen *et al.*, 2009). Consequently, neuroinflammation has been linked to be one of the main cellular events that cause neurodegeneration, others including oxidative stress, mitochondria dysfunction, protein aggregation etc (Hoglund and Septa 2013).

Since polyphenols can affect and modify these important cellular processes linked to neurodegeneration, they may be useful neuroprotective agents. Particularly interesting are the class of flavonoids, the most abundant subgroup of polyphenols (Solanki 2015; Vaouzour 2008). Since the early 2000s, a large number of reviews have been published in the literature detailing the neuroprotective properties of these substances (Youdim 2008), hence, the reason we explored this specific group of phytochemicals in order to screen for the best candidate with the most promising ameliorative effect against neuroinflammation.

One promising avenue is the use of natural medicinal plants which are rich in compounds such as flavonoids that have been shown to have anti-oxidative and neuroprotective effects (Wyss-Coray and Mucke, 2002; Zhang *et al.*, 2019; Fasooto *et al.*, 2024). There are mounting evidences that project the crucial roles that Inflammation plays in the onset and progression of several neurodegenerative disorders. In fact, several studies have identified and suggested immune-related genetic mutations as the underlying mechanism for neurodegeneration. Thus, provides compelling evidence for developing therapeutic strategies that regulate neuroinflammation to prevent central nervous system pathologies. Flavonoids have been shown to have anti-inflammatory properties (Zhang *et al.*, 2019).

Spondias mombin is a plant member of the Anacardiaceae family commonly found in the tropical Americas, encompassing the West Indies. Moreover, it has been successfully naturalized in various regions of Africa, notably Ghana, as well as certain parts of Asia. Different components of *S. mombin*, including the stem bark, leaves, and roots, have been used in ethnomedicine to cure a variety of ailments. *S. mombin* has been shown to have antiviral (Corthout *et al.*, 1988), antibacterial properties (Amadi *et al.*, 2007), hematinic (Hamenoo 2010), anthelmintic (Ademola *et al.*, 2005), and anti-inflammatory qualities (Nworu *et al.*, 2011). This plant contains several compounds in their hundreds that are either tannins, saponins, alkaloids, flavonoids, or phenols (Awogbindin *et al.*, 2014). However, this study is focused on the flavonoid fraction of the plant as several studies have presented compounds in this fraction to have higher binding affinity for protein targets involved in various neurodegenerative diseases (John *et al.*, 2023)

This present study aims to explore the neuroprotective properties of various compounds in the flavonoids enriched fraction of *S. mombin* against neuroinflammation by employing several in-silico tools including network pharmacology, molecular docking, pharmacokinetics screening and molecular dynamic simulation.

MATERIALS AND METHODS

Network Pharmacology

Screening of Active Ingredients and Related Targets

Data Collection and Compound Retrieval:

We initiated our study by obtaining the PubChem Compound Identifier (CID) for eight flavonoids (Eugenol, Estradiol, Linalool, Catechin, Tangeretin, Epicatechin, 2-Nitroethylbenzene, Quercetin) present in *S. mombin*. Subsequently, the Simplified Molecular Input-Line Entry System (SMILES) and structural data file (MOL.SDF) format representations of these flavonoids from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) were retrieved.

Prediction of Protein Targets:

To predict potential protein targets for the active ingredients in *S. mombin*'s flavonoids, Swiss Target Prediction tool (<http://www.swisstargetprediction.ch/>) was employed. We considered protein targets with a probability score of ≥ 0 . Any duplicate data were meticulously removed, and the names of these protein targets along with their Uniprot IDs were recorded.

Screening of Co-targets for *Spondias mombin* and Neuroinflammation

Identification of Neuroinflammation Targets:

The next step involved the identification of relevant targets associated with neuroinflammation. Comprehensive searches were conducted on GeneCards (<https://www.genecards.org/>) using the keyword 'Neuroinflammation' and on OMIM (<https://www.omim.org/>) using the same keyword to retrieve Homo sapiens-specific targets. Subsequently, we carefully removed any duplicate data and excluded false positive genes.

Matching Gene Targets:

To identify potential gene targets that could be influenced by *S. mombin*'s flavonoid compounds in the context of the neuroinflammation, the gene targets of *S. mombin* were cross-referenced with those associated with neuroinflammation. This co-gene target dataset was prepared for visualization using a bioinformatics web server tool (<https://www.bioinformatics.com.cn/>).

Construction of Active Ingredient-Gene Network

Network Visualization:

Cytoscape Version 3.6.0, an open-source software platform renowned for its ability to integrate, analyze, and visualize molecular interaction networks and biological pathways was utilized (Otasek, et al., 2019). To create the *S. mombin* active ingredient-gene target network, the gene targets obtained from OMIM and GeneCards were incorporated along with the active ingredients of *S. mombin*.

Topology Analysis:

Topological parameters, including degree values, betweenness centrality, and closeness centrality, were computed to identify the ten gene targets with the highest centrality measures. These genes were designated as pivot genes within the network.

Construction of Protein-Protein Interaction (PPI) Networks

We utilized String version 11.0 (<https://string-db.org/>) to explore protein-protein interactions among the identified targets. A minimum combined score threshold of 0.400 was applied to retain high-confidence protein interactions for Homo sapiens. Targets lacking interactions were removed, and the resultant data were saved as SIF files. Subsequently, we generated the PPI network by importing node1, node2, and the combined score into Cytoscape. Cluster analysis was performed to identify core targets using the Cytohubba plugin.

Gene Ontology Function and KEGG Pathway Analysis

We employed the Database for Annotation, Visualization, and Integrated Discovery (DAVID, <https://david.ncifcrf.gov/>, Version 6.8) to perform comprehensive functional annotation analysis for neuroinflammation-related genes. We selected "official gene symbol" as the identifier, specified "gene list" as the list type, and chose "Homo sapiens" as the species background. Gene Ontology (GO) functions, including Biological Process (BP), Cellular Component (CC), and Molecular Function (MF), were analyzed using a bioinformatics web server tool (<https://www.bioinformatics.com.cn/>). KEGG

pathway analysis results were visualized using an advanced bubble chart also generated by the same web server. Significant results were reported based on a threshold of $P < 0.05$.

Retrieval and Preparation of Proteins and Ligands

3D Crystal Structure Retrieval and Preparation:

The ten targets with the highest degree values from the topology analysis were retrieved, and their 3D crystal structures were obtained from the Protein Data Bank (PDB) RCSB in PDB file format. The respective PDB IDs are listed in Table 1. We prepared the proteins using the Protein Preparation Wizard module in Schrödinger Maestro 12.8 as described by Ogunbiyi et al. (Ogunbiyi et al., 2023). This process involved assigning bond orders and hydrogen bonds, adding hydrogens, optimizing, minimizing the proteins, and deleting waters beyond 4Å from the het group.

Ligand Preparation: Eight flavonoid phytochemicals from *S. mombin* having been retrieved were prepared using LigPrep in the Schrödinger suite, employing the OPLS4 force field module for minimization, desalting, retaining specified chiralities, and generating the chemical compound states at $\text{pH } 7.0 \pm 2.0$ were carried out.

Molecular Docking Using Glide

Ligand Docking: Molecular docking computations were performed using the Glide's ligand docking module. Extra-precision docking was employed for stringent screening of ligand binding affinities.

Binding Free Energy Calculation: The relative binding-free energy (ΔG_{bind}) of the compounds with the best binding poses (hit ligands) was calculated using the Prime MM/GBSA method accessible in Maestro. This assessment aimed to evaluate the stability of the complexes formed by the hit ligand(s) and the corresponding protein(s). The formula is given below: $\Delta G_{\text{bind}} = \Delta G_{\text{solv}} + \Delta E_{\text{MM}} + \Delta G_{\text{SA}}$ where,

ΔG_{solv} is the difference in GBSA solvation energy of the proteins-ligands complex and the sum of the solvation energies for the free proteins and hit ligands

ΔE_{MM} is a difference in the minimized energies between proteins-ligands complex and the sum of the energies for free proteins and hit ligands.

ΔG_{SA} is a difference in surface area energies of the complex and the sum of the surface area energies for the free proteins and hit ligands.

Prime MM-GBSA computes the energy of optimized free receptors, free ligands, and ligand-receptor complexes. Similarly, calculating the ligand strain energy by immersing the ligand in a solution generated by the VSGB suite (Borkotoky et al., 2016).

Pharmacokinetics Screening

The drug-likeness, physicochemical properties, and pharmacokinetic properties of the flavonoid compounds from *S. mombin* were predicted using SwissADME (<http://www.swissadme.ch/index.php>). Additionally, the compounds were screened for toxicity using the DataWarrior program version 4.6.1.

Molecular Dynamic Simulation

Molecular dynamics simulations (MDS) were conducted for hit ligands with up to -8.0 kcal/mol binding energies or more to investigate the structural dynamics and stability of the system of interest (Olugbogi et al., 2023). The simulations were performed using the Maestro software suite (Schrödinger, LLC), leveraging the computational power of an NVIDIA GeForce RTX 3080 graphics processing unit (GPU) for enhanced performance. The OPLS3e force field was employed for

all simulations, providing an advanced level of accuracy in modeling molecular interactions and energetics (Harder et al., 2016).

The system was initially prepared and minimized using the standard protocols within the Maestro environment. Following energy minimization, the system was equilibrated in an NPT ensemble at 300 K and 1 atm, using a Berendsen thermostat and barostat to maintain constant temperature and pressure, respectively (Berendsen et al., 1984). The equilibration phase lasted for 100 ps, ensuring adequate system stability before the production run.

The production MD simulation was conducted over a duration of 200 nanosecond (ns), allowing for extensive sampling of the conformational space. A time step of 2 fs was utilized, with periodic boundary conditions applied to mimic an infinite system. Long-range electrostatic interactions were calculated using the Particle Mesh Ewald (PME) method, with a non-bonded cutoff of 10 Å (Darden et al., 1993). The SHAKE algorithm was used to constrain all bond lengths involving hydrogen atoms, enabling the use of a larger integration time step while preserving system stability (Ryckaert et al., 1977)

RESULTS

Docking Computation

Table 1 provides a quantitative evaluation of the interaction between *S. Mombin* flavonoids and two protein targets, COX2 and FYN, by presenting both the XP Glide Scores and MM/GBSA binding energies. Compounds such as Eugenol and the reference ligand show significant binding affinities to both proteins. In contrast, compounds like Catechin and Epicatechin, despite having no affinity to COX2, display a notable preference for binding with FYN, suggesting potential specificity in their therapeutic action.

Figure 1 visually represents these interactions through a heatmap and bar graph, offering a color-coded display and quantifiable data on the binding affinity and stability of these compounds when docked with the target proteins. The cooler colors in the heatmap correlate with stronger binding affinities, which is consistent with the numerical data provided in the bar graph.

This combination of data illustrates a varied landscape of molecular interactions. Compounds with stronger binding scores and negative delta G binding energies are indicative of potentially more stable and potent interactions, which could influence the pharmacological effectiveness of these compounds (Feng et al., 2022).

Table 1: Comparative Binding Profiles of *S. mombin* Flavonoid Phytochemicals to COX2 and FYN Proteins *(MM/GBSA & XP Score are in kcal/mol)

PROTEINS	COX2(5F1A)		FYN(2DQ7)	
PARAMETERS	XP GScore (kcal/mol)	MM/GBSA dG Bind (kcal/mol)	XP GScore (kcal/mol)	MM/GBSA dG Bind (kcal/mol)
2-Nitroethylbenzene	-5.202	-33.81	-3.333	-31.64
Catechin	0	0	-8.504	-47.53
Epicatechin	0	0	-9.446	-38.22
Estradiol	0	0	-6.696	-41.92
Eugenol	-6.454	-14.22	-6.85	-38.34
Linalool	-4.494	0.65	-3.78	-30.47
Quercetin	0	0	-5.358	-37.28
Tangeretin	0	0	-7.999	-48.71
Reference ligand	-6.291	-5.13	-8.501	-76.79

ADME Screening

Part A of table 2 delineates the absorption, distribution, metabolism, and excretion (ADME) properties of various flavonoid compounds from *S. mombin*. It includes data on molecular weight (MW), oral bioavailability (OB), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), blood-brain barrier permeability (BBB), topological polar surface area (TPSA), gastrointestinal absorption (GI), calculated LogP (cLogP), and adherence to Lipinski's Rule of Five (RO5#). These parameters are critical for assessing the drug-like qualities of these compounds (Doak et al., 2014).

Part B of the table presents the toxicity screening results for the same set of compounds. This includes assessments of their potential mutagenic, tumorigenic, reproductive effects, and irritant properties. This information is vital for understanding the safety profile of these compounds (Tharwat et al., 2016).

The results from these tables indicate a diverse range of pharmacokinetic properties and toxicity risks among the compounds. For instance, Catechin and Epicatechin, despite having similar molecular weights and high HBA and HBD counts, differ markedly from compounds like Eugenol and Tangeretin in terms of their toxicity profiles. Such distinctions are crucial for evaluating the potential therapeutic applications and safety considerations of these compounds.

Table 2: ADME Properties(A) and Toxicity Screening(B) of Flavonoid Compounds from *S. mombin*

(A)Molecule Name	PubChem ID	MW	OB	HBA	HBD	BBB	TPSA	GI	cLogP	RO5#
Eugenol	3314	164.20	0.55	2	1	Yes	29.46	High	2.2723	0
Estradiol	5757	272.40	0.55	2	2	Yes	40.46	High	3.8766	0
Linalool	6549	154.25	0.55	1	1	Yes	20.23	High	3.2311	0
Catechin	9064	290.27	0.55	6	5	No	110.38	High	1.5087	0
Tangeretin	68077	372.40	0.55	7	0	Yes	76.36	High	3.0228	0
Epicatechin	72276	290.27	0.55	6	5	No	110.38	High	1.5087	0
2-Nitroethylbenzene	80208	151.16	0.55	2	0	Yes	45.82	High	0.8873	0
Quercetin	5280343	302.24	0.55	7	5	No	131.36	High	1.4902	0

(B)Molecule Name	Mutagenic	Tumorigenic	Reproductive Effective	Irritant
Eugenol	high	high	none	high
Estradiol	none	none	none	none
Linalool	none	none	none	high
Catechin	none	none	none	none
Tangeretin	high	high	none	none
Epicatechin	none	none	none	none
2-Nitroethylbenzene	none	none	none	none
Quercetin	high	high	none	none

This Figure details the investigative approach to identifying and understanding the molecular players in neuroinflammation. By discerning the primary actors and their interrelations, the figure provides insight into the potential pathways that could be modulated in therapeutic interventions.

This visual representation elucidates the intricate relationship between the flavonoids found in *S. mombin* and genes associated with neuroinflammatory pathways. It emphasizes the potential of these phytochemicals to influence gene expression or protein function, providing a basis for the exploration of novel treatments derived from this plant source.

These visualizations collectively reveal the various biological pathways, processes, and functional categories enriched in response to *S. mombin* treatment for neuroinflammation. This detailed analysis helps in understanding how the natural compounds from *S. mombin* may exert their therapeutic effects at the molecular and functional levels, shedding light on their potential mechanisms of action.

Molecular Interaction Profile

FYN Protein Interactions: Catechin, epicatechin, and a reference ligand docking with the FYN protein, resulted in different interaction profiles. These profiles include hydrogen bonds (HB) and hydrophobic bonds (HPB), with amino acids like threonine (THR82), glutamic acid (GLU54), and aspartic acid (ASP148) involved in the binding. The average bond

distances range from 3.48 Å with catechin to 3.93 Å with the reference ligand, indicating the strength and proximity of these interactions.

COX2 Protein Interactions: Eugenol and a reference ligand were examined for their binding to the COX2 protein. Similar to the FYN interactions, hydrogen and hydrophobic bonds were identified with amino acids such as leucine (LEU352) and glycine (GLY526) participating in the interaction. The bond distances are slightly longer here, with eugenol showing an average distance of 4.36 Å.

These interactions are important for understanding how these flavonoids might influence the activity of these proteins. The 3D representations give a spatial understanding of the interactions, while the 2D diagrams provide a clearer schematic of the types of bonds formed and the specific amino acids involved.

Table 3: Summarized interaction data between the lead proteins and the investigated ligands from *S.mombin*, detailing: ^aName of lead target ^bInteraction/bond types(*HB- Hydrogen bond *HPB- Hydrophobic bond) ^cInteracting amino acids residues ^dThe average distances of the bonds, measured in angstroms (Å), signifying the proximity of the ligand to the target site.

PROTEINS ^a	BOND TYPE ^b	INTERACTING AMINO ACIDS ^c	AVERAGE BOND DISTANCE ^d (Å)
FYN	5 _{HB} ; 8 _{HPB} (catechin)	T82,E54,D148,M85,L17,L137,V25,K39,A147,A37,E83,N135	3.48(catechin)
	2 _{HB} ;		3.79(epicatechin)
	6 _{HPB} (epicatechin)		3.93(coligand)
	6 _{HB} ;14 _{HPB} (coligand)		
COX2	4 _{HB} ; 3 _{HPB} (eugenol)	L352,G526,A527,V349,L531,L384,M522,V523,Y385,W387,	4.36(eugenol)
	3 _{HB} ; 3 _{HPB} (coligand)	S530	3.67(coligand)

Molecular Dynamic Simulations

Protein RMSD analysis, as depicted in the given plot, measures the protein's structural stability throughout the simulation by aligning all frames to the reference frame backbone and calculating RMSD based on selected atoms. This technique helps to evaluate the protein's conformational dynamics over time. RMSD values serve as an indicator of simulation equilibration, with fluctuations around a thermal average structure suggesting stability. For small, globular proteins, RMSD changes within the range of 1-3 Å are considered normal, while larger shifts might denote significant conformational alterations. Furthermore, achieving convergence, indicated by RMSD values stabilizing around a consistent figure, is crucial to confirm system equilibration. Inadequate equilibration, marked by continuously increasing or decreasing RMSD at the simulation's end, suggests the need for extended simulation for reliable analysis.

Ligand RMSD, represented on the right Y-axis, assesses ligand stability within the protein's binding pocket. The plot's 'Lig fit Prot' metric calculates the ligand's RMSD post alignment of the protein-ligand complex against the protein backbone of the reference frame, focusing on the ligand's heavy atoms. Discrepancies significantly larger than the protein's RMSD could imply that the ligand has migrated from its initial docking site, affecting the interaction stability and efficacy.

RMSD analyses across all trajectory frames revealed that among the studied complexes, only the epicatechin-FYN complex demonstrated instability over the 200 ns simulation. This suggests significant conformational changes in the epicatechin ligand throughout the simulation period.

In the plot, observed peaks highlight regions within the protein that experience the highest degree of fluctuation throughout the simulation. Commonly, the protein's extremities, specifically the N- and C-terminals, exhibit greater fluctuations compared to other areas. This is in contrast to secondary structural elements such as alpha helices and beta strands, which due to their rigidity, show lesser fluctuations and are more stable than loop regions, which are typically unstructured and thus more prone to fluctuation.

The epicatechin-FYN complex demonstrated remarkable stability throughout the simulation, showing minimal fluctuation with root mean square fluctuation (RMSF) peaks not exceeding 2.5 Å. This stability, characterized by lower RMSF values compared to other complexes studied, identifies the epicatechin-FYN complex as the most stable among the evaluated complexes.

Throughout a simulation, protein-ligand interactions are continuously monitored and can be categorized into hydrogen bonds, hydrophobic interactions, ionic interactions, and water bridges. Each category encompasses subtypes that detail the nature of these contacts, with a 'Simulation Interactions Diagram' providing an in-depth view. Stacked bar charts, normalized over the trajectory, indicate the duration of these interactions, where, for example, a value of 0.7 signifies that an interaction is present for 70% of the simulation time. Values exceeding 1.0 occur when a residue forms multiple contacts of the same type with the ligand.

Hydrogen bonds are critical for ligand binding, influencing drug specificity, metabolism, and absorption. They are subdivided into interactions involving backbone acceptors and donors, and side-chain acceptors and donors, with specific geometric criteria defined for identifying such bonds.

Hydrophobic contacts, essential for the ligand's interaction with hydrophobic amino acids and aromatic or aliphatic groups, include π -Cation, π - π stacking, and other nonspecific interactions. These are determined by proximity and orientation criteria.

Ionic interactions occur between oppositely charged atoms not involved in hydrogen bonding, with specific distance criteria applied, including Protein-Metal-Ligand interactions.

Water Bridges are hydrogen-bonded interactions between the protein and ligand mediated by water molecules, with slightly relaxed geometric criteria from standard hydrogen bonds.

GLU54 in the bar graph maintained hydrogen bond interaction for more than 175% of the simulation time, registering the highest in all the residues observed.

DISCUSSION

Network Pharmacology & Molecular Docking

Neuroinflammation has been extensively studied over the years for its critical roles in the pathobiology and adverse progression of neurodegenerative diseases, neurocognitive disorders, and cancer (Zhang et al., 2023; Mayne et al., 2020; Stephenson et al., 2018; Alghamri et al., 2021). Numerous signaling pathways comprising key biomolecules have been reported to contribute to the progression of neuroinflammation (Shih et al., 2015; Marino et al., 2022). Furthermore, there is ongoing research into therapeutic interventions designed to disrupt the activities of these biomolecules, aiming to mitigate neuroinflammation (Cheng et al., 2022; Bogár et al., 2022). Thus, this study focuses on assessing ethnopharmacological interventions to regulate these molecular hotspots.

In this study, we evaluated *S. mombin*, a plant known for its significant therapeutic benefits (Ogunro et al., 2023; Gomes et al., 2020; Cabral et al., 2016), by screening eight of its known flavonoid phytochemicals. This follows our previous

study, which screened the plant's entire phytochemical library (Olanrewaju et al., 2023). We began by analyzing target genes that are structurally compatible with the flavonoids and implicated in neuroinflammation. SwissTargetPrediction was used to predict potential target proteins for the flavonoids, yielding a total of 279 targets, while GeneCard and OMIM were employed to identify genes associated with neuroinflammation, resulting in 2033 target genes (Fig. 2a, 3a, & 3b).

To pinpoint potential gene-targets for these flavonoids in neuroinflammation, we cross-referenced the flavonoids' gene-targets with those linked to neuroinflammation using a Venn diagram, identifying a total of 108 targets (Fig. 2a). These targets were then imported into STRING and Cytoscape software for Protein-Protein Interaction (PPI) network and centrality analyses, respectively. This led to the identification of ten core targets: MAPK1, APP, FYN, GSK3B, TGF β 1, PTGS2, NF κ B1, EGFR, SRC, and AKT1 (Fig. 2c). KEGG pathway and Gene Ontology function analyses confirmed that these core targets play major roles in the biochemical pathways and processes of neurodegeneration, cancer, kinase activities, inflammatory responses, and apoptotic processes (Demuro et al., 2021; Guglietti et al., 2021; Zou et al., 2023), thus validating their activity in neuroinflammation (Fig. 4).

Extra-precision molecular docking was employed to assess the inhibitory properties of the flavonoids on these targets and FYN Kinase (FYN) showed greater binding affinities with the flavonoids. Although eugenol scored up to -6.45 kcal/mol with Cyclooxygenase Two (COX2), we prioritize binding scores of about -8.0 kcal/mol or better. COX2 is a well-known prostaglandin synthase isoform, and a common target for several drugs, including Non-steroidal Anti-inflammatory Drugs (NSAIDs) and COX2-Selective inhibitors, used to treat a wide range of inflammatory infections and/or diseases (Kirchheiner et al., 2003; Rowlinson et al., 2003). Additionally, its potential as a target for Alzheimer's disease and cancer has been highlighted (Sil and Ghosh., 2016; Pu et al., 2021). FYN, a tyrosine kinase, plays a significant role in neuroinflammatory processes, cell proliferation, differentiation, and adhesion, and its expression has been reported in various cancer types (Marotta et al., 2022; Saminathan et al., 2020; Elias et al., 2015).

The docking results revealed that these flavonoids had better structural compatibility and alignment with FYN, as indicated by their strong docking scores (in -kcal/mol), with Epicatechin showing the highest binding affinity of -9.446kcal/mol, followed by Catechin (-8.504kcal/mol), compared to the reference ligand (staurosporine) which registered -8.501kcal/mol (Fig. 1; Table 1). These promising scores may be attributed to intricate factors like bond types and distances with key amino acid residues, suggesting FYN's molecular novelty (Table 3; Fig. 6). These compounds exhibited a high number of hydrophobic and hydrogen bonds with short average bond distances, potentially explaining their strong docking scores, as studies have shown the importance of bond types and distances in determining the strength of drug-protein complexes (Majewski et al., 2019; Chen et al., 2016).

The flavonoids' structural compatibility with COX2 was less pronounced, with only Eugenol showing a high docking score (-6.454kcal/mol), while the standard ligand recorded a binding affinity of -6.291kcal/mol. This observation is supported by the minimal number of hydrophobic and hydrogen bonds formed with key amino acid residues at the binding site of this synthase (Table 3; Fig. 7).

Additionally, the hit ligand-protein complexes showed promising binding free energies, as depicted by their MMGBSA scores, reflecting the likely stability level of their ligand-protein complex (Table 1). This promising free binding energy score can also be linked to the intrinsic roles of the bond types formed between these hit flavonoids and proteins (Majewski et al., 2019).

Pharmacokinetics (ADMET)

In addition to assessing the pharmacodynamic potentials of these flavonoids, their pharmacokinetic properties were evaluated using SwissADME and DataWarrior software (Daina et al., 2017; Sander et al., 2015). This screening process aids in understanding their probable side effects, toxicity levels, and bioavailability—crucial indicators of a ligand's suitability for drug development (Pires et al., 2018). The results encompassed a range of descriptors, intended to evaluate

the drug-likeness and pharmacokinetic attributes inherent in our compounds. Key parameters such as molecular weight, the number of hydrogen bond acceptors and donors, adherence to Lipinski's Rule of Five, and the potential for blood-brain barrier permeability were paramount in this evaluation. This intricate array of characteristics provided a comprehensive picture of the drug-like attributes of our compounds. Tables 2a and 2b present detailed insights into the diverse physicochemical properties (e.g., HBA, HBD, MW, cLOGP) of these flavonoids, affirming their druggability, as the screening showed that all flavonoids fell within the accepted ranges for these parameters, thus supporting their potential for drug development.

Moreover, oral bioavailability, a critical aspect of drug candidacy, was rigorously assessed using Lipinski's Rule of Five. This well-established set of criteria is used to predict the likelihood of oral administration of compounds (Lipinski et al., 2001). It is posited that if a compound satisfies at least three out of the five rules, it is likely to have favorable oral bioavailability (Lipinski et al., 2001). Notably, all our ligands successfully met Lipinski's criteria, highlighting their potential for oral bioavailability which is a vital consideration in their development (Table 1; Fig. 8). Additionally, the results offered a promising outlook, as most of the flavonoids demonstrated the ability to cross the blood-brain barrier (Fig. 9; Table 2), with the exceptions of epicatechin and catechin.

The comprehensive analyses conducted here firmly establish the drug-like properties and favorable pharmacokinetic profiles of these flavonoids. These findings enhance their prospects as candidates for further development, positioning them as promising therapeutic agents in the fight against neuroinflammation.

Molecular Dynamic Simulation

Data from molecular dynamics simulations (MDS) were meticulously analyzed to gauge the structural stability, conformational alterations, and dynamic attributes of the system. Two pivotal metrics employed were the root mean square deviation (RMSD) and root mean square fluctuation (RMSF) to validate the ligands with -8.0 kcal/mol, which serve to quantify the overall structural steadiness and the local flexibility of individual amino acid residues, respectively. RMSD provides a measure of the average deviation of the system's atomic positions from a reference structure over time, thus reflecting the system's stability (Karplus and Kuriyan, 2005). RMSF, on the other hand, offers insights into the flexibility of specific residues within the protein, with higher values indicating regions of greater dynamic movement (Levitt and Warshel, 1975). Collectively, these analyses afford a comprehensive understanding of the molecular underpinnings governing the behavior of protein-ligand complexes during simulations.

RMSD line plot

Figure 10 represents the ligand RMSD value, depicted on the Y-axis, serves as a measure of the stability of the docked ligand pose within the binding pocket. It provides insights into how well the ligand interacts with the target protein. Additionally, the term "Lig Fit Prot" refers to the RMSD values of the ligand concerning the protein backbone, offering further details on the conformational changes and structural compatibility between the ligand and the protein. The value for "Lig Fit Prot" is expected to be slightly higher than that of the protein RMSD, indicating some degree of flexibility or movement in the ligand with respect to the protein backbone. However, a significantly higher value suggests substantial changes in the ligand pose compared to its initial docked position. This could indicate a less stable interaction between the ligand and the protein or significant conformational adjustments within the binding pocket, potentially impacting the effectiveness of the ligand in binding to its target.

During the 200 ns MDS, the stability and conformational behavior of catechin, epicatechin, and a reference ligand when bound to the FYN protein were assessed through the "Lig Fit Prot" values, which measure the deviation of the ligand from its initial position relative to the protein. For the catechin-FYN complex, the Lig Fit Prot value remained relatively stable, with a minor fluctuation around 2.0 ± 1 Å, indicating a stable interaction throughout the simulation period. In contrast, the

epicatechin-FYN complex exhibited a wider range of Lig Fit Prot values between 2 and 9 Å, suggesting that the epicatechin underwent significant conformational changes and did not maintain a stable interaction with FYN over the course of the 200 ns simulation. Conversely, the reference ligand-FYN complex demonstrated enhanced stability, as evidenced by a more consistent Lig Fit Prot value of 2.0 ± 0.5 Å, indicating a stable and less fluctuating interaction with the FYN protein throughout the simulation.

RMSF line plot

In the MDS, RMSF values were employed to discern the subtle changes across the entire protein structure when bound with the reference ligand and other tested ligands (catechin and epicatechin). RMSF plots are instrumental in identifying regions within the protein that exhibit considerable variability during the simulation, with pronounced peaks indicating areas of constant and frequent fluctuation, thus suggesting less stability (Leach, 2001; Karplus and Kuriyan, 2005). Typically, the peripheral or tail regions of proteins are less stable compared to their core, leading to higher fluctuations observed in these areas, a phenomenon that was noted in the reference ligand complex. The catechin-FYN complex, on the other hand, showed decreased stability particularly at residues around 50 (3.6 Å) and 160 (4.8 Å), yet it demonstrated a tendency towards stabilization as the simulation progressed. In contrast, the epicatechin-FYN complex maintained a relatively stable profile with RMSF peaks not surpassing 2.5 Å, marking it as the most stable complex among those studied, as sharp peaks in RMSF plots typically signify areas of higher dynamicity within the protein-ligand complex (Grant, 2007).

Interaction fraction analyses

To elucidate the molecular interactions within the protein-ligand complexes during simulation, analyses focused on hydrogen bonds, hydrophobic interactions (including Pi-cation and Pi-Pi stacking), water bridges, and ionic interactions, as depicted in Fig. 12 for catechin, epicatechin, and the reference ligand. Ionic interactions were notably present in both the reference ligand and epicatechin complexes. Hydrogen bonding, in particular, plays a pivotal role in defining drug specificity, metabolism, and absorption, underscoring its significance in drug design and pharmacodynamics (Liu and Nussinov, 2013; Bissantz et al., 2010). The quantification of these interactions over the simulation period was expressed as a percentage (%), indicating the persistence of ligand-residue contacts, which is essential for understanding ligand efficacy and stability within the binding site.

In the molecular dynamics simulation depicted in Fig. 12, catechin established hydrogen bonds with six specific amino acids like GLU54, THR82, GLU83, MET85, LYS87, and ASP147, each exhibiting varying durations of interaction. Notably, interactions with GLU83, MET85, and LYS87 were sustained for less than 40% of the simulation duration, whereas THR82 and ASP147 maintained bonds for over 75% of the time, with GLU54 showing the most prolonged engagement, extending beyond 175%. These findings align with initial docking results presented in Table 3. Additionally, MET85 and ASP148 participated in water bridge formations, a type of hydrogen bond facilitated by water molecules, for approximately 80% and 150% of the simulation, respectively, highlighting the dynamic nature of ligand-protein interactions (Kumar and Nussinov, 2002; Skyner et al., 2015). During the simulation, hydrophobic interactions with MET85 were observed for less than 40% of the time.

Epicatechin demonstrated hydrogen bond formation with several residues, notably with MET85 for 90% of the simulation, ASN86 for more than 50% of the duration, and ASP92 for 60%, while other interactions persisted for less than 30% of the time. Hydrophobic contacts across all residues did not surpass 30% of the simulation duration. Notably, GLU83, MET85, ASN86, ASP92, and ASP148 engaged in water bridge interactions for over half the simulation period, with MET85 and ASP92 showing increased interaction frequency towards the end. Ionic bonds, which are critical electrostatic interactions between charged ions, were particularly notable between ASP92 and the ligand for over 60% of the

simulation, emphasizing their role in stabilizing protein-ligand complexes and affecting binding affinities and enzymatic activities (Pan et al., 2019; Zhou et al., 2019).

In the MDS, the reference ligand engaged in five hydrogen bond interactions, with GLU83 and MET85 showing significant stability by maintaining interactions for over 80% of the simulation, and ALA134 for 40%. This observation aligns with the initial docking results, highlighting the crucial roles of GLU83 and MET85 in the reference ligand binding. Additionally, out of five water bridge interactions, only those involving ASP92 and ALA134 were sustained for more than 40% of the simulation duration. A noteworthy hydrophobic interaction with LEU137 persisted for 60% of the simulation, underscoring its importance in the stability of the protein-ligand complex. Meanwhile, an ionic bond with ASP92 was maintained for approximately 30% of the time.

Remarkably, within the catechin-FYN complex, GLU54 emerged as a key residue due to its substantial hydrogen bond interaction stability, exceeding 175%. The comprehensive analysis of the simulation data corroborates the initial docking study's findings, underscoring the consistency and reliability of the identified key interactions (Zhou et al., 2019; Skolnick et al., 2015).

Conclusion

In this study, a comprehensive in silico approach, including network pharmacology and molecular docking simulations, was undertaken to identify key pathways and target proteins involved in the pathogenesis of neurodegenerative disorders. Cyclooxygenase-2 and FYN kinase were identified as potential targets for S. mombin ligands, with particular attention paid to ligands displaying docking scores greater than or equal to -8.0 kcal/mol. Although the pharmacokinetic analysis suggested the potential of these compounds as therapeutic agents, catechin and epicatechin were found unable to cross the blood-brain barrier, limiting their direct utility in treating neurodegenerative diseases. However, molecular dynamics simulations over 200 ns demonstrated the stability of catechin and a reference ligand at FYN's active site, with the epicatechin-FYN complex also showing stability, as indicated by RMSF peaks not exceeding 2.5 Å. Notably, the catechin-FYN complex highlighted GLU54 as a crucial residue for its significant hydrogen bond interaction stability (175%). Given these findings, we advocate for the further optimization of these ligands for drug development purposes. The application of nanotechnology could offer a novel means of delivering these drugs effectively to their intended targets, circumventing the blood-brain barrier challenge and potentially offering new therapeutic avenues for neurodegenerative disorders.

Declarations

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Conceptualization and Validation of results: O.A.J, E.A.O, S.E.O, and A.O.L.

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Data curation: S.E.O, E.A.O, and P.O.A.

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Figures

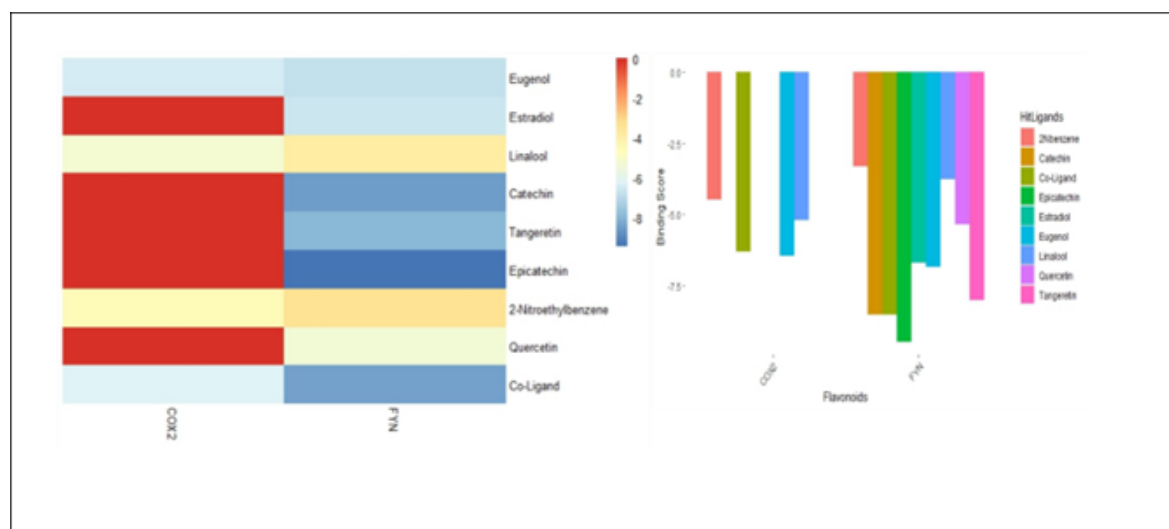


Figure 1

Heatmap and Bar Graph Depicting Docking Scores of Phytochemicals with COX2 and FYN Targets

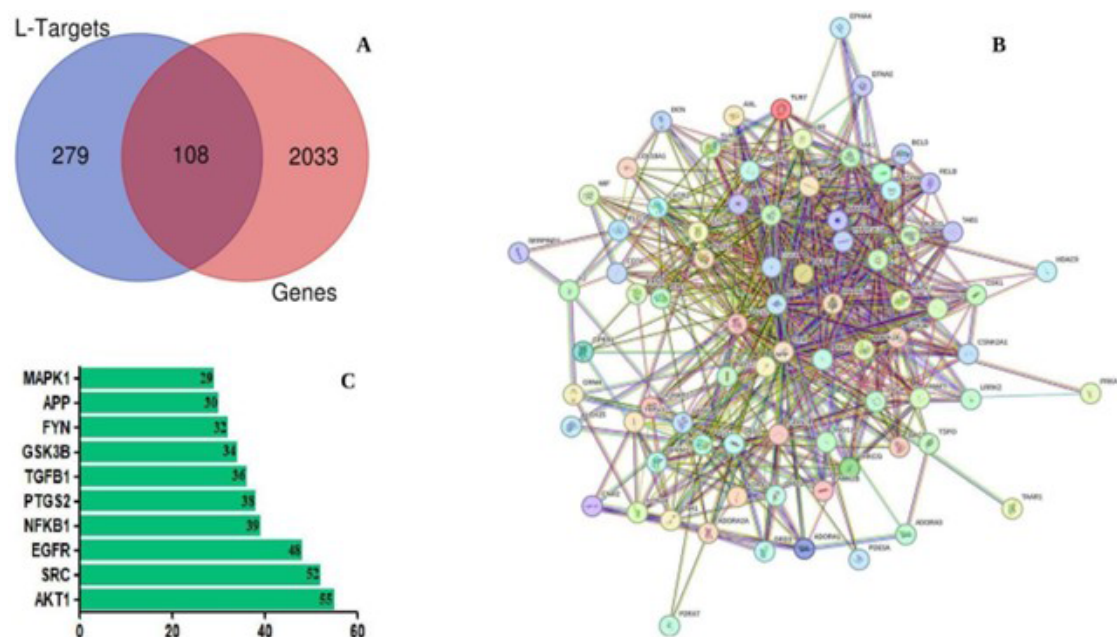


Figure 2

Neuroinflammation-Associated Targets and Interaction Analysis

- **(A)** displays a Venn diagram highlighting the overlap between the flavonoids targets (L-Targets) and a comprehensive set of genes implicated in neuroinflammation, underscoring the potential focus genes.
- **(B)** shows a Protein-Protein Interaction (PPI) network, visualized using data from the STRING database, where nodes represent individual proteins and lines illustrate the known or predicted interactions between them, emphasizing the complexity of neuroinflammatory pathways.
- **(C)** lists the top 10 implicated targets ranked by their connectivity within the PPI network, with the bar lengths representing the degree values, indicating the prominence of each target within the network.

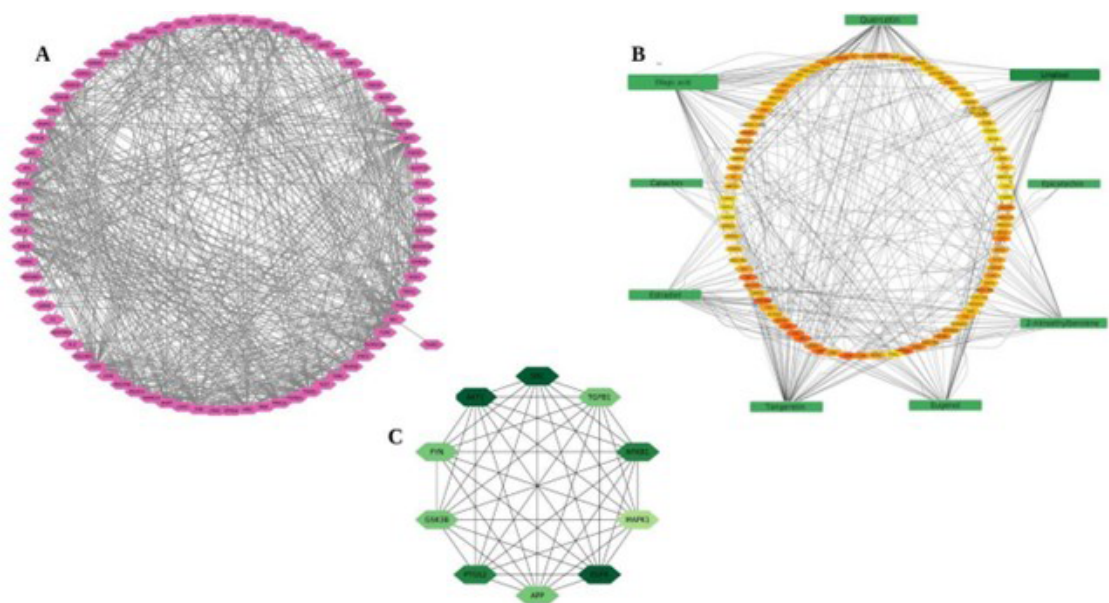


Figure 3

Interaction Network of *S. Mombin* Flavonoid Phytochemicals and Neuroinflammatory Genes

- (A) depicts a network with pink hexagonal nodes representing genes implicated in neuroinflammation, mapping the scope of genetic involvement in the condition.
- (B) features green rectangular nodes that correspond to the flavonoid phytochemicals of *S. mombin*, as predicted by SwissTargetPrediction to intersect with the aforementioned genes, suggesting potential sites for therapeutic action.
- (C) highlights core targets in the treatment of neuroinflammation with green hexagons, with connecting lines denoting the strength and complexity of interactions between the S.Mombin compounds and the neuroinflammatory genes.

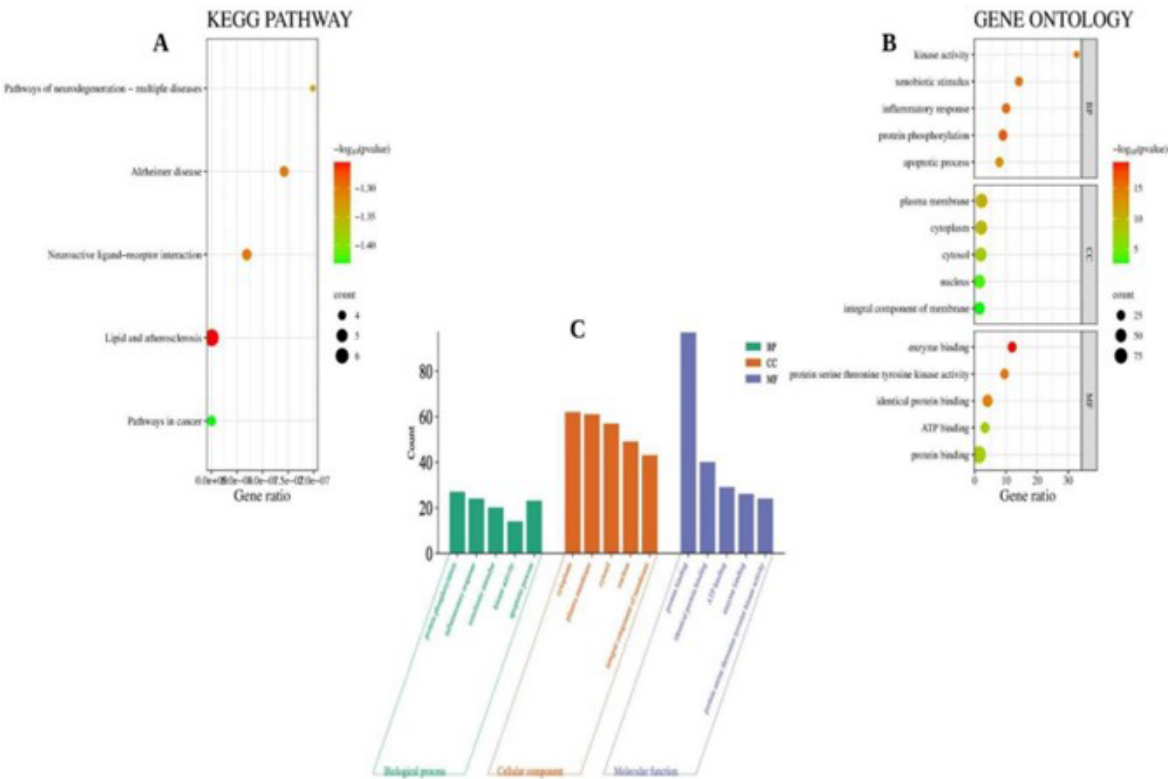


Figure 4

Functional Enrichment Analyses of *S. Mombin* in Neuroinflammation Treatment

- (A) presents a bubble diagram illustrating the enriched KEGG pathways associated with the treatment of neuroinflammation using *S. mombin*, with bubble sizes and colors representing the significance and relevance of each pathway.
- (B) displays a bubble diagram encompassing enrichments in biological processes (BP), cellular components (CC), and molecular functions (MF), offering insights into the diverse functional aspects impacted by *S. mombin*'s treatment.
- (C) provides a comprehensive Gene Ontology (GO) function analysis that includes biological processes (BP), cellular components (CC), and molecular functions (MF), providing a comprehensive overview of the functional attributes and cellular localization influenced by *S. mombin* in the context of neuroinflammation treatment.

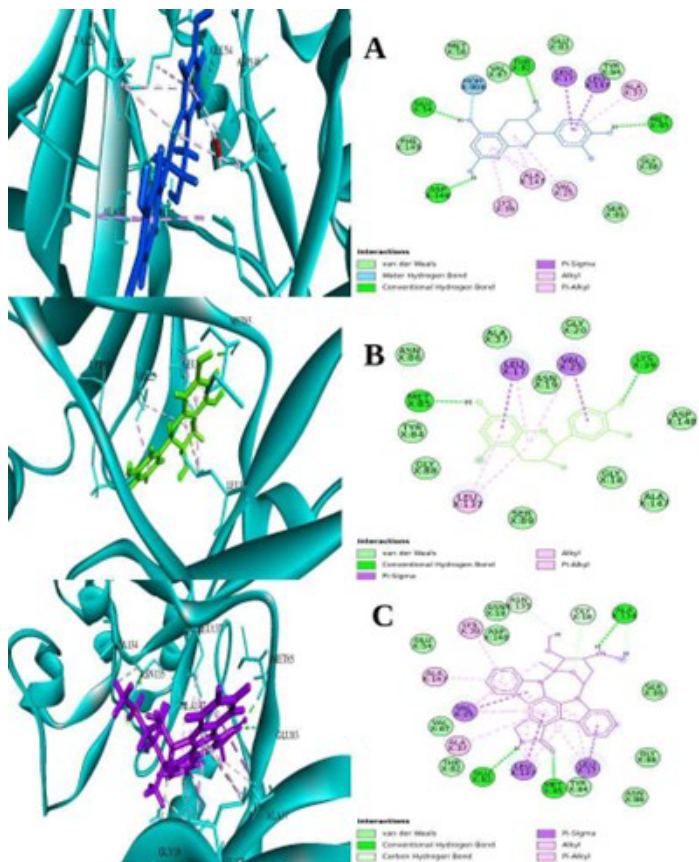


Figure 5

Figure 6: 3D and 2D interaction profile of hit_ligand-protein complexes post-molecular docking, revealing the types of interactions between these ligands and the amino acid residues at the binding pockets of the protein **(A)** FYN-catechin complex **(B)** FYN-epicatechin complex **(C)** FYN-coligand complex

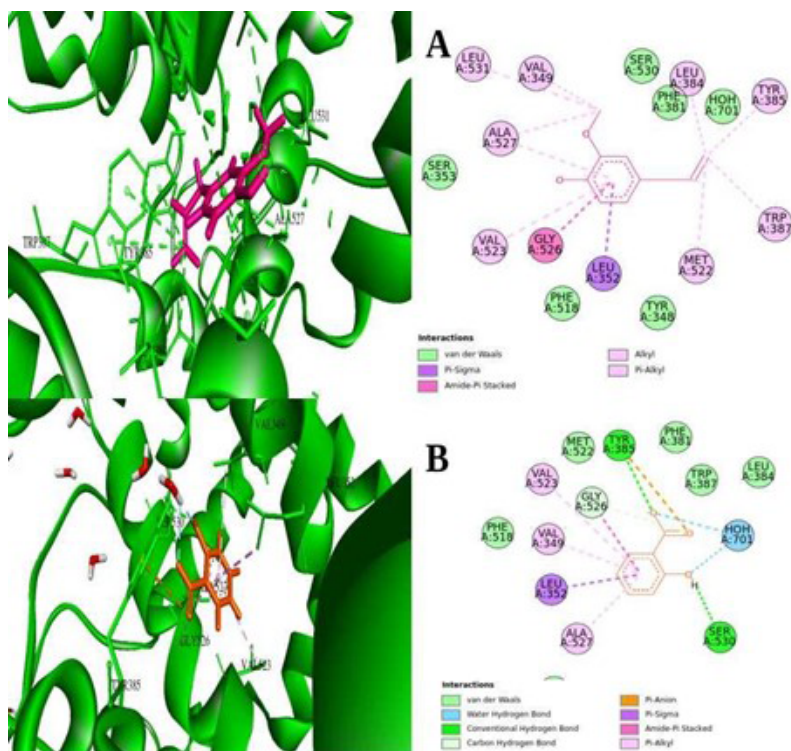


Figure 6

Figure 7: 3D and 2D interaction profile of hit_ligands-protein complexes post-molecular docking, revealing the types of interactions between these ligands and the amino acid residues at the binding pockets of the protein. (A) COX2-eugenol complex (B) COX2-coligand complex

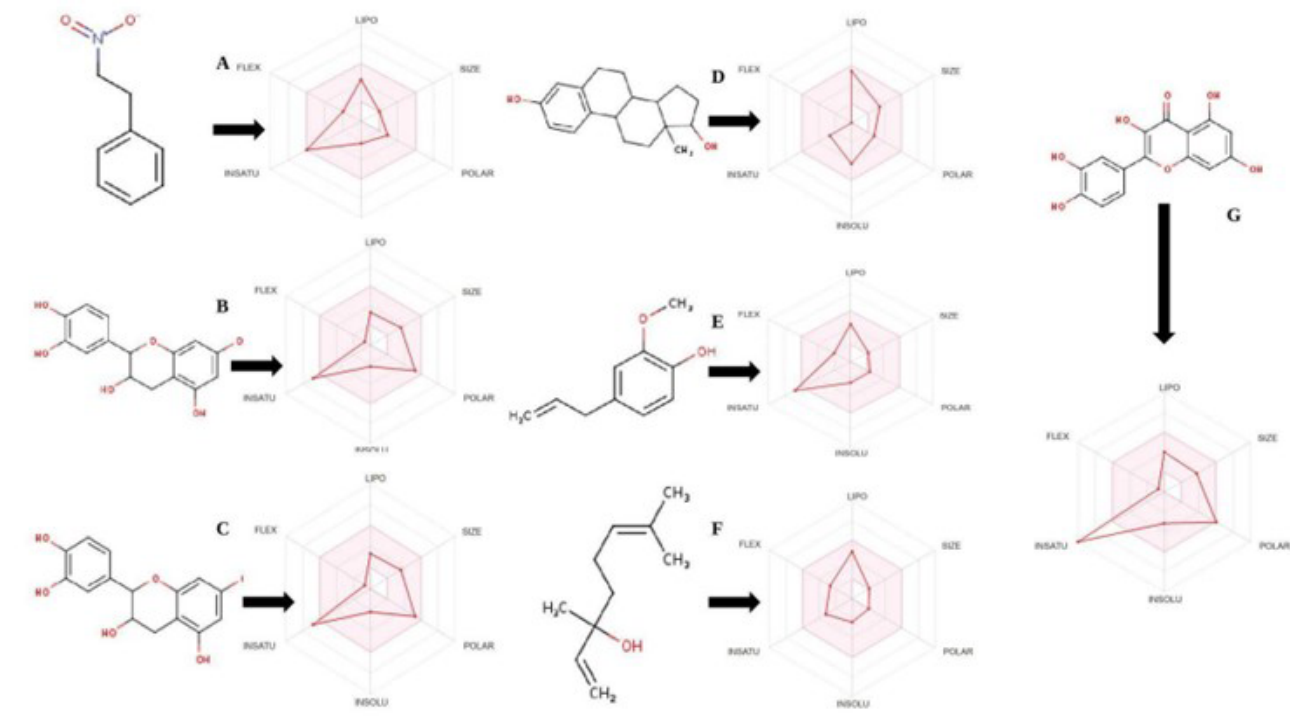


Figure 7

Figure 8: Structural formula and bioavailability radar-chart of S.mombin flavonoid hit phytocompounds (A) 2-nitroethylbenzene (B) Catechin (C)Epicatechin (D) Estradiol (E) Eugenol (F) Linalool (G)Quercetin

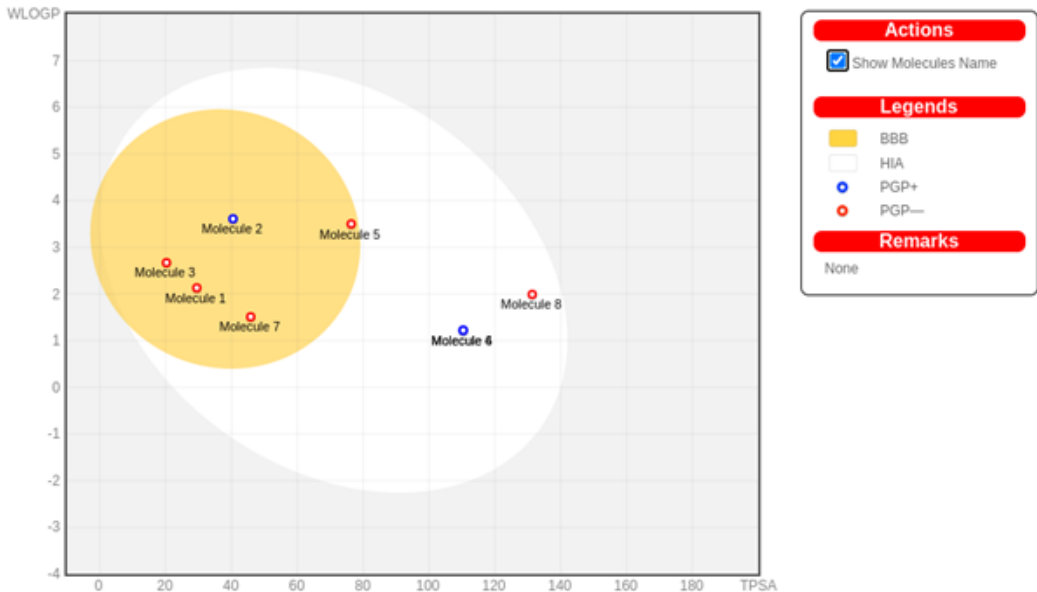


Figure 8

Figure 9: Blood brain barrier(BBB) penetration of *S.mombin* compounds as generated by SwissADME

***molecule 1-** Eugenol **molecule 2-** Estradiol **molecule 3-** Linalool **molecule 4-** Catechin **molecule 5-** Tangeretin **molecule 6-** Epicatechin **molecule 7-** 2-Nitroethyl Benzene **molecule 8-** Quercetin

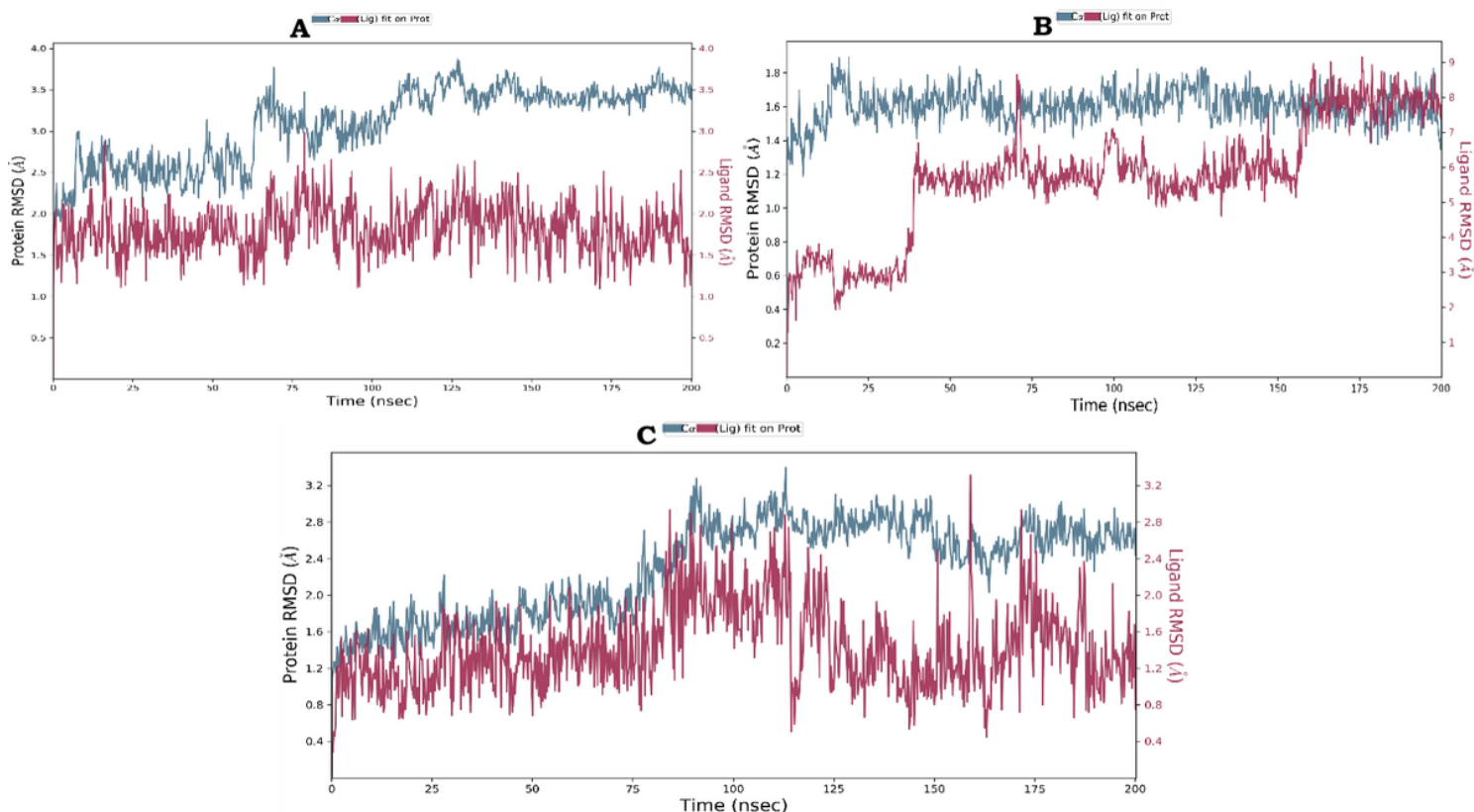


Figure 9

Figure 10: The Root Mean Square Deviation (RMSD) line plot used to measure the average change in displacement of a selection of atoms for a particular frame with respect to a reference frame.

- (A) presents the RMSD values of catechin in complex with FYN (2DQ7)
- (B)presents the RMSD values of epicatechin in complex with FYN (2DQ7)
- (C)presents the RMSD values of reference ligand in complex with FYN (2DQ7)

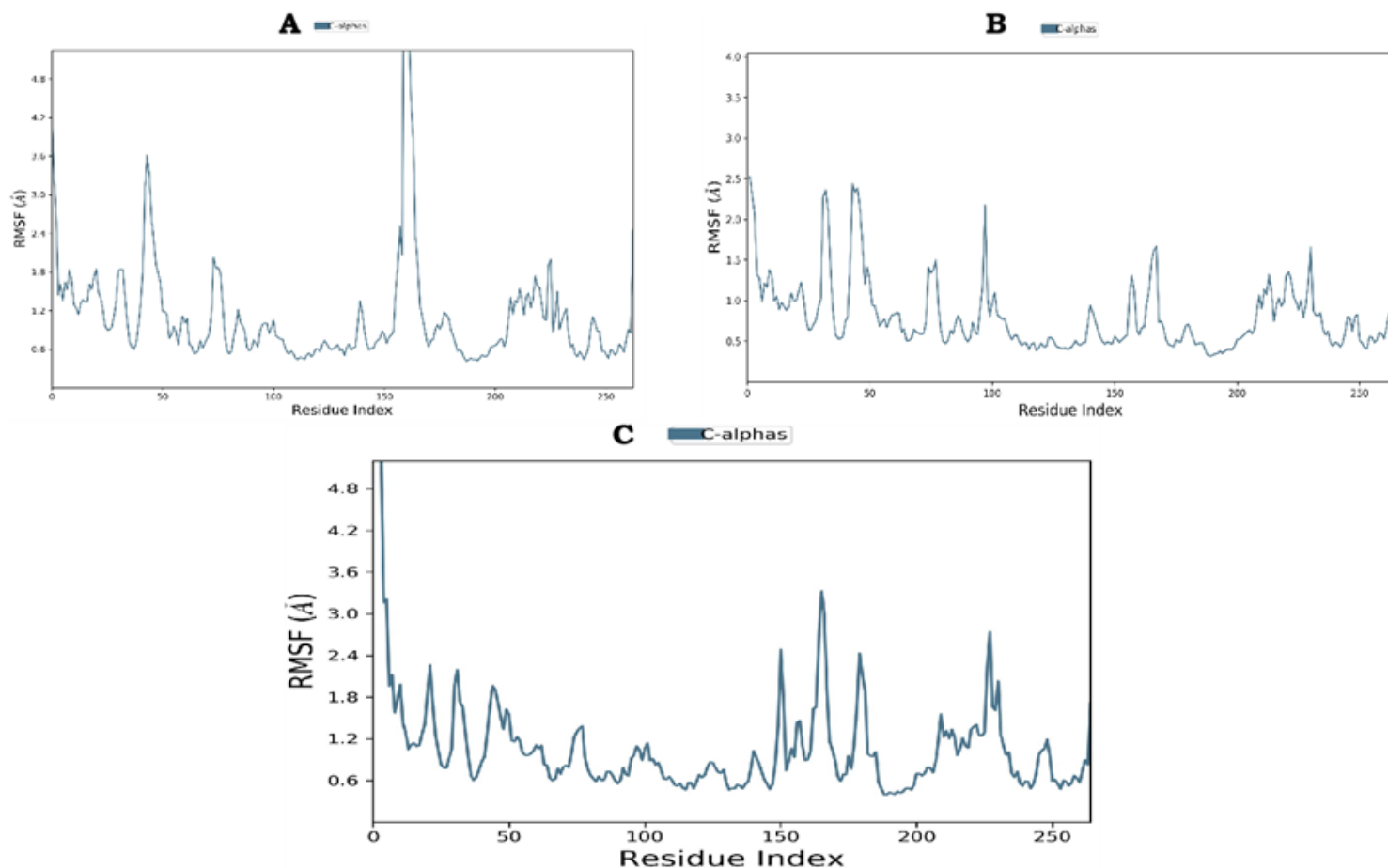


Figure 10

Figure 11: The Root Mean Square Fluctuation (RMSF) line plot used for characterizing local changes along the protein chain.

-(A) presents the RMSD values of catechin in complex with FYN (2DQ7)

-(B)presents the RMSD values of epicatechin in complex with FYN (2DQ7)

-(C)presents the RMSD values of reference ligand in complex with FYN (2DQ7)

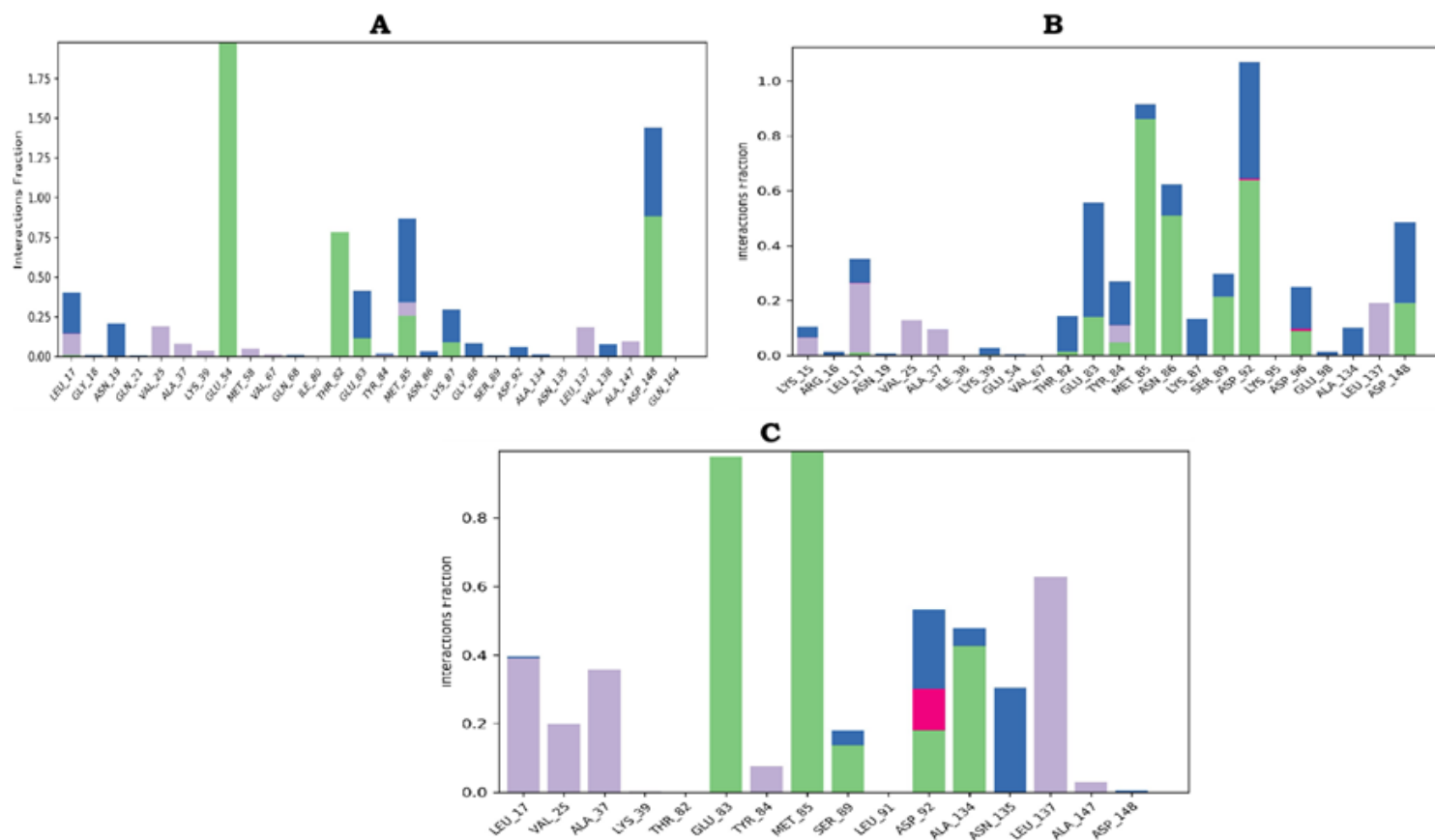


Figure 11

Figure 12: The protein-ligand contact histogram used for comprehensive interaction fraction within the simulation trajectory.

-(A) presents the protein-ligand contact histogram of catechin and FYN (2DQ7)

-(B)presents the protein-ligand contact histogram of epicatechin and FYN (2DQ7)

-(C)presents the protein-ligand contact histogram of the reference ligand and FYN (2DQ7)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [GraphicalAbstract.png](#)