

Identifying Molecular Mechanisms of *Cinnamomum cassia* in the Treatment of Osteoporosis Based on Network Pharmacology and Validations

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Abstract

Background: Osteoporosis is a common condition among the elderly, characterized by reduced bone density and an increased susceptibility to fractures. The efficacy of *Cinnamomum cassia* in treating osteoporosis is recognized, though its precise molecular mechanisms remain unclear.

Objectives: To investigate the molecular mechanisms underlying the therapeutic effects of *C. cassia* using network pharmacology, molecular docking technology (MDT), and molecular dynamics simulation (MDS).

Materials and Methods: Bioinformatics databases identified active compounds and disease targets. Protein-protein interaction (PPI) networks were constructed, followed by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. MDT and MDS validated the binding affinity between *C. cassia*'s active compounds and key targets.

Results: Ten active compounds of *C. cassia* were identified, modulating the PPAR, HIF-1, AMPK, and cAMP signaling pathways. Key genes include *PPARG*, *PTGS2*, *PPARA*, *BDNF*, and *RXRA*. Molecular docking and simulations confirmed high binding affinity between active compounds and targets, supporting their role in regulating bone metabolism.

Conclusion: This study highlights the multi-target mechanisms of *C. cassia* in treating osteoporosis, emphasizing its therapeutic potential through pathway-based modulation of key genes. These findings provide a basis for further research in modernizing traditional Chinese medicine for osteoporosis management.

Keywords

Cinnamomum cassia, osteoporosis, network pharmacology, molecular docking, molecular dynamics simulation, traditional Chinese medicine, bioinformatics

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Introduction

Network pharmacology (Hopkins, 2008) emphasizes multi-target drug discovery (Nogales et al., 2022), integrating systems biology (Jiashuo et al., 2022; Zhao et al., 2023) to enhance drug design and disease management (Tsuchikama et al., 2024). It has provided novel methodologies and fresh perspectives to contemporary medicine, particularly in navigating the intricate terrain of complex drug investigations (Munnangi et al., 2023). Studies indicate that *Cinnamomum cassia* regulates lipid metabolism and bone remodeling, enhancing osteoblast function and inhibiting osteoclast activity (Dong et al., 2021). These networks represent a pioneering avenue for the design of multi-target drugs and stand as a pivotal facet in the modernization of traditional Chinese medicine (Nogales et al., 2022; Zhang et al., 2023).

This article, rooted in the therapeutic properties of *C. cassia* and firmly grounded in the principles of network pharmacology (Kim et al., 2023; Lim et al., 2022; Zhang et al., 2019), embarks on an incipient exploration into the mechanisms underpinning the efficacy of this traditional Chinese medicine in the treatment

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of osteoporosis (Armas & Recker, 2012; Miller, 2016). It painstakingly constructs a comprehensive network of interactions encompassing the active constituents of *C. cassia* (Lee et al., 2002; Liu et al., 2018), its pharmacological targets, and the genes associated with osteoporosis (Li et al., 2024; Liu et al., 2023; Zhou et al., 2023). This innovative approach challenges the conventional paradigm entrenched in pharmacological thought, one that has traditionally adhered to the concept of “single component, single target, single disease” (Barabási et al., 2011; Li, Liu et al., 2023). The research flowchart is depicted in Figure 1.

Materials and Methods

Collection of Chemical Constituents and Targets

Chemical constituents of *C. cassia* were identified using *TCMSP* (<http://lsp.nwu.edu.cn/tcmsp.php>) and *PubChem* (<https://pubchem.ncbi.nlm.nih.gov/>) databases, with criteria including a drug-likeness (DL) value of ≥ 0.18 and oral bioavailability (OB) of $\geq 30\%$.

Prediction of Disease Targets

Intersection targets between *C. cassia* compounds and osteoporosis were identified using *Venny software* (<https://bioinfogp.cnb.csic.es/tools/venny/>) (Bardou et al., 2014), with genes retrieved from *GeneCards* (<https://www.genecards.org/>) (Safran et al., 2010) and OMIM (<https://www.omim.org/>) (Amberger & Hamosh, 2017) databases. The intersection targets are regarded as potential pivotal targets for the therapeutic use of Chinese herbal compounds in disease treatment (Luo et al., 2019).

“Drug–Target” Network Analysis

Network topology analysis was conducted using Cytoscape 3.9.1 (Shannon et al., 2003) to visualize drug–target interactions (Shang et al., 2023).

Protein–Protein Interaction (PPI) Network Construction and Analysis

PPI networks (Palukuri & Marcotte, 2021) were constructed using *STRING* (<https://cn.string-db.org/>) (Szklarczyk et al., 2023), with topological analyses (Tang et al., 2015) conducted via Cytoscape (Bandettini et al., 2012; Chin et al., 2014).

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Enrichment Analysis

GO and KEGG (Kanehisa & Goto, 2000) pathway enrichment analyses were conducted using DAVID (Ding et al., 2024), with results filtered at a *p* value of < 0.05 .

Molecular Docking Technology (MDT)

Molecular docking involved ligands such as diisobutyl phthalate (DIBP, PubChem CID: 6782), ethyl isocyanate (EIC, PubChem CID: 8022), oleic acid (OA, PubChem CID: 445639), and sativene (PubChem CID: 11830550). The receptor proteins included PPARA (Uniprot ID: Q07869), PPARG (Uniprot ID: P37231), and PTGS2 (Uniprot ID: P35354). Molecular docking analyses were conducted utilizing AutoDock Vina version 1.1.2 (MDT: Molecular Docking Technology). Three-dimensional protein structures were retrieved from the *UniProt database* (<https://www.uniprot.org/>). Protein preprocessing, including the removal of water molecules and extraneous ligands as well as the addition of hydrogen atoms, was performed using PyMOL version 2.4. The generation of PDBQT files for the docking studies was accomplished using AutoDock Tools version 1.5.6.

The docking box for the PPARA protein was configured with dimensions of $100 \text{ \AA} \times 74 \text{ \AA} \times 126 \text{ \AA}$, with a grid spacing of $0.786 \text{ \AA}$, and the coordinates were -4.448 , -4.251 , and -7.491 . For PPARG, the docking box measured $74 \text{ \AA} \times 64 \text{ \AA} \times 116 \text{ \AA}$ with a grid spacing of $0.983 \text{ \AA}$, and the coordinates were -5.943 , -5.943 , -5.943 , and -5.983 . The PTGS2 protein's docking box dimensions were $72 \text{ \AA} \times 82 \text{ \AA} \times 68 \text{ \AA}$, with a lattice spacing of $0.967 \text{ \AA}$, and coordinates at -3.391 , 1.053 , and -3.585 . The conformation with the lowest binding energy was determined to represent the most plausible binding mode between the ligand and the protein. Visualization of the ligand–protein interactions was carried out using PyMOL 2.4.

Molecular Dynamics Simulation (MDS)

MDSs were performed utilizing GROMACS version 2019.6 (Berendsen et al., 1995; Wu et al., 2023) to study protein–ligand interactions. The simulations used amber99sb-ildn and GAFF force fields for proteins and ligands, respectively, for the generation of parameters and topologies. The simulation box was configured so that each protein atom maintained a minimum distance of 1.0 nm from the box edges. The box was filled with SPC216 water molecules as an explicit solvent, Na^+ and Cl^- counterions were introduced to replace water molecules, ensuring electrical neutrality within the system. To minimize unreasonable atomic contacts or overlaps, the optimization of the system was performed using the steepest descent method. Subsequently, pre-equilibration was conducted with 100 ps of the NVT ensemble at 300 K , followed by 100 ps of the NPT ensemble at 1 bar . Under periodic boundary conditions, the main MDS was run for 100 ns , with the V-rescale and Parrinello–Rahman methods used to regulate temperature and pressure, respectively (Kawata & Nagashima, 2001). To solve the Newtonian equations of motion, the leapfrog integration method was employed with a time step of 2 fs . The Particle Mesh-Ewald (PME) method, with a Fourier spacing of 0.16 nm , was utilized to handle long-range electrostatic interactions, and the LINCS method

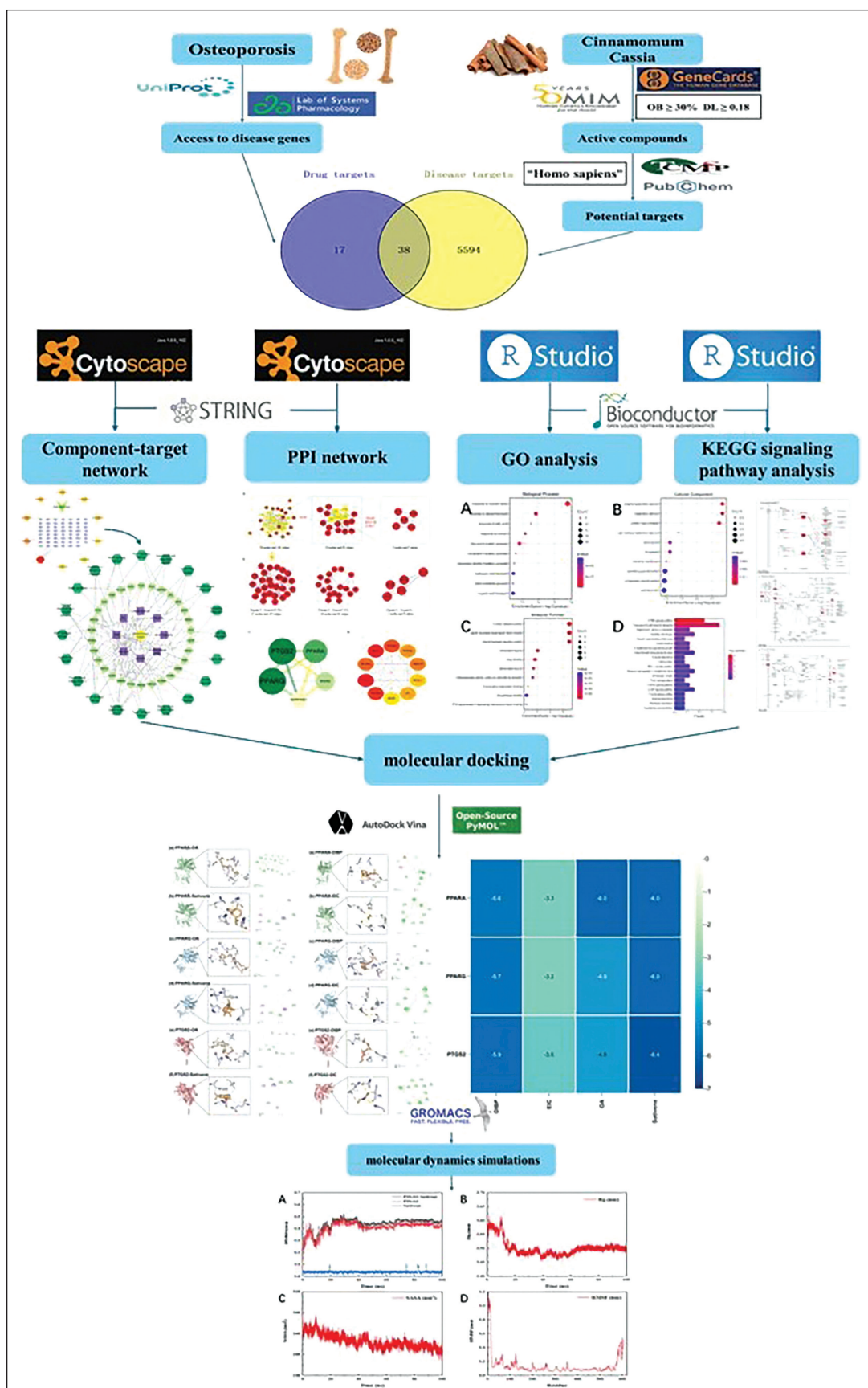


Figure 1. The Research Flowchart.

was applied to constrain all bond lengths. Trajectories were visualized, analyzed, and animated using Visual Molecular Dynamics (VMD) software version 1.9.3 and PyMOL version 2.4.1 (Carretero-González et al., 2005). Binding free energy calculations for the compound were performed using the *gmx_mmpbsa* tool (<http://jerkwin.github.io/gmxtools/>).

Results

Drug Active Component Screening and Target Prediction

Drug components were retrieved from the Traditional Chinese Medicine Systems Pharmacology Database (TCMSP). Components with OB $\geq 30\%$ and DL ≥ 0.18 were considered effective, whereas those without target interactions were excluded. Through this screening process, active drug components were identified, leading to the recognition of 102 targets following gene name conversion. This selection of active components lays the foundation for subsequent disease target identification and their potential therapeutic applications, as explored in the following sections (Table 1) (Ru et al., 2014).

Disease-related Targets

A total of 5,632 targets were retrieved from two disease-related databases: GeneCards (<https://www.genecards.org/>) and OMIM (<https://www.omim.org/>), both of which are widely recognized for their comprehensive coverage of gene-disease associations. By merging the data from both databases and eliminating duplicate genes using Excel, an intersection with drug targets was established, resulting in 38 intersecting target genes (Figure 2). With the identification of these intersection targets, we proceed to establish compound-target interactions, further elucidating the potential molecular mechanisms of *C. cassia*'s action in osteoporosis.

“Drug–Target” Prediction Results

The intersection of drug-related and disease-related targets was identified, resulting in 38 overlapping drug–target genes. A “drug–target” network diagram was created using Cytoscape version 3.9.1, as shown in Figure 3. This network comprises 66 nodes and 122 edges. Key compounds were determined through the Degree algorithm available in the CytoHubba plugin. The principal compounds identified include oleic acid, DIBP, EIC, sativene, and (–)-caryophyllene oxide. Each compound is clearly linked to its respective osteoporosis-related target genes within the drug–target network. The network topology, represented by nodes and edges in Figure 3, visually demonstrates the specific binding interactions and the centrality of these compounds within the overall network. This depiction emphasizes the multi-target therapeutic role of these compounds in mediating the

therapeutic effects of *C. cassia* on osteoporosis through a complex interaction mechanism. The recognition of these key compounds and their corresponding targets forms the foundation for constructing the PPI network, as described in the following section.

PPI Network of Common Targets

To construct the PPI network, 38 shared targets were submitted to the STRING database, resulting in a network structure comprising 38 nodes and 161 edges. Five core targets were identified based on their degree values, betweenness centrality (BC), and closeness centrality (CC), as demonstrated in Figure 4A and C. Cluster analysis, illustrated in Figure 4B, was performed using the MCODE algorithm, which yielded a highly interconnected subnetwork. The identified targets were subsequently classified into three distinct clusters. Additionally, the CytoHubba plugin was utilized to determine the top 10 hub genes by applying the Maximal Clique Centrality (MCC) algorithm, as shown in Figure 4D. The detailed topological parameters of the network are summarized in Table 2.

Biological Function Enrichment Analysis

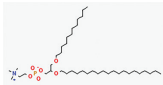
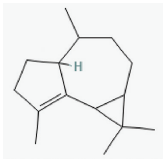
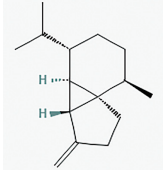
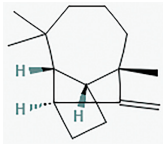
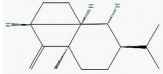
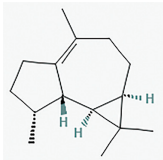
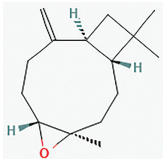
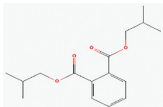
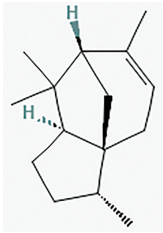
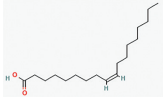
GO Enrichment Analysis

Using R software, a GO gene function enrichment analysis was conducted on the overlapping genes between drug- and disease-related targets. This analysis identified 1,795 biological process (BP) terms, 140 cellular component (CC) terms, and 231 molecular function (MF) terms. The intersecting genes derived from the active components of *C. cassia* and osteoporosis-associated targets were analyzed, specifically focusing on 38 shared genes between the drug and disease. The top 30 significantly enriched terms ($p < 0.05$) related to drug action are presented in Figure 5. In the BP category, key terms include nutrient response, steroid hormone response, fatty acid metabolism, cholesterol metabolism, secondary alcohol metabolism, carboxylic acid transport, sterol metabolism, and organic acid transport (Figure 5A). For CC, the enriched terms primarily involve plasma lipoprotein particles, protein–lipid complexes, high-density lipoprotein particles, peroxisomes, microbodies, and dendritic membranes (Figure 5B). MF terms highlight nuclear receptor activity, ligand-activated transcription factor activity, steroid hormone receptor activity, antioxidant activity, drug binding, and peroxidase activity (Figure 5C). This integrative analysis of BPs and MFs provides a foundation for elucidating the therapeutic mechanisms of *C. cassia*, which are further explored through the enriched signaling pathways discussed in the subsequent section.

KEGG Pathway Enrichment Analysis

The KEGG pathway enrichment analysis was performed using R software, which identified 131 pathways related to

Table 1. The Effective Components of Cinnamomum Cortex (Top 10).

Mol ID	Molecular Name	Molecular Formula	2D Structure	OB (%)	DL
MOL000131	EIC	C ₄₀ H ₈₄ NO ₆ P		41.9	0.14
MOL000208	Aromadendrene	C ₁₅ H ₂₄		55.74	0.1
MOL000266	beta-Cubebene	C ₁₅ H ₂₄		32.81	0.11
MOL002697	Junipene	C ₁₅ H ₂₄		44.07	0.11
MOL003522	Sativene	C ₁₅ H ₂₄		37.41	0.1
MOL003538	Ledene	C ₁₅ H ₂₄		51.84	0.1
MOL002003	Caryophyllene oxide	C ₁₅ H ₂₄ O		32.67	0.13
MOL000057	DIBP	C ₁₆ H ₂₂ O ₄		49.63	0.13
MOL000612	alpha-Cedrene	C ₁₅ H ₂₄		55.56	0.1
MOL000675	Oleic acid	C ₁₈ H ₃₄ O ₂		33.13	0.14

Note: DL, drug similarity; OB, oral bioavailability. Candidates were excluded if they did not meet the target interaction criteria, such as lacking binding affinity with osteoporosis-related targets or demonstrating DL below 0.18 or OB below 30%.

Source: Data retrieved from the Traditional Chinese Medicine Systems Pharmacology Database (TCMSP, <https://old.tcmsp-e.com>), accessed on June 2024.

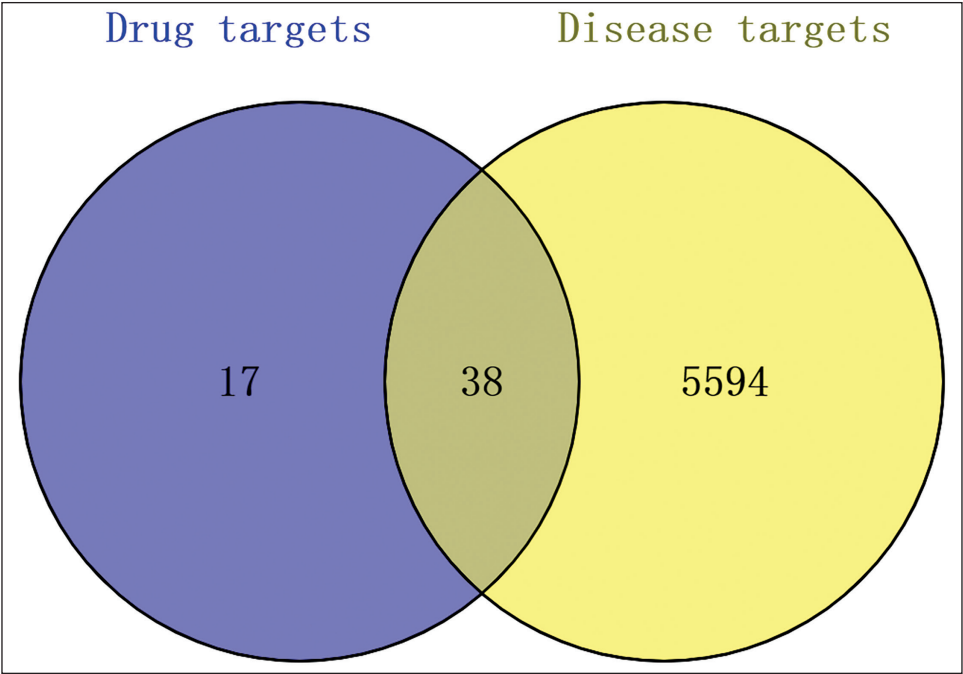


Figure 2. Venn Diagram of Osteoporosis-related Targets and Targets of Cinnamomum Cortex.

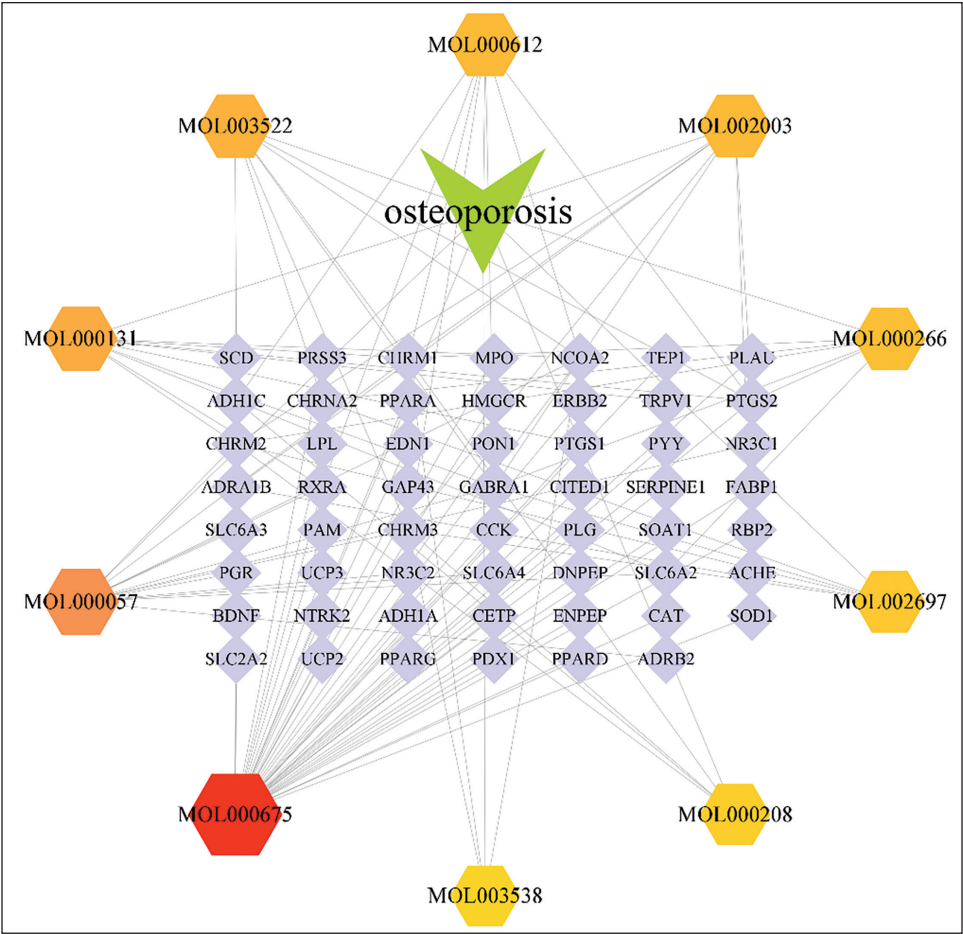


Figure 3. Drug–Target Network.

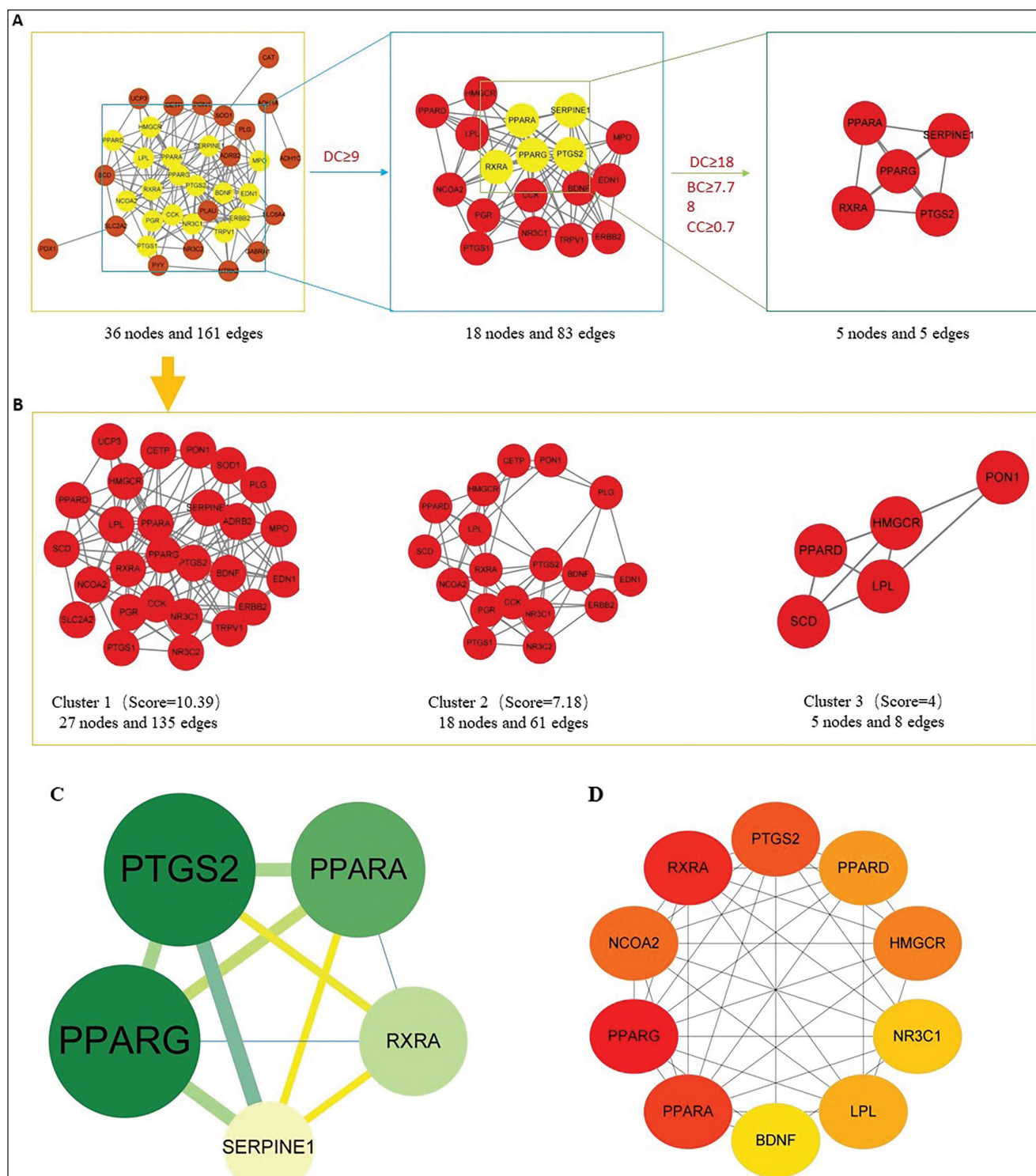


Figure 4. Elucidation of Potential Targets Through Analysis of Protein–Protein Interactions (PPIs). (A) Topological Filtering Process Within the PPI Network. (B) Analysis of the PPI Network Utilizing Cluster Analysis Through the MCODE Plugin. (C) Identification of Five Key Hub Targets from 38 Shared Targets via Degree Centrality (DC), Betweenness Centrality (BC), and Closeness Centrality (CC); the Node Size Reflects the Degree of the Target Within the Network. (D) Selection of Hub Genes from the PPI Network Employing the CytoHubba Plugin, Where Node Color Shifts from Light Yellow to Red Indicating an Increasing Degree.

Table 2. Topology Parameters of Cinnamomum Cortex in the Treatment of Osteoporosis (Top 10 Targets).

Uniprot ID	Target	Degree	Protein Name	Betweenness Centrality	Closeness Centrality	Species
P37231	PPARG	24	Peroxisome Proliferator-activated Receptor Gamma	0.191531205	0.785714286	<i>Homo sapiens</i>
P35354	PTGS2	22	Prostaglandin-Endoperoxide Synthase 2	0.12028249	0.733333333	<i>Homo sapiens</i>
Q07869	PPARA	20	Peroxisome Proliferator-activated Receptor Alpha	0.119691815	0.717391304	<i>Homo sapiens</i>
P23560	BDNF	17	Brain-derived Neurotrophic Factor	0.089400455	0.66	<i>Homo sapiens</i>
PI9793	RXRA	14	Retinoid X Receptor Alpha	0.026391366	0.611111111	<i>Homo sapiens</i>
P04150	NR3C1	13	Nuclear Receptor Subfamily 3 Group C Member 1	0.039704601	0.6	<i>Homo sapiens</i>
P05121	SERPINE1	12	Serpin Family E Member 1	0.016729328	0.589285714	<i>Homo sapiens</i>
P06858	LPL	12	Lipoprotein Lipase	0.032485731	0.568965517	<i>Homo sapiens</i>
P04035	HMGCR	11	3-Hydroxy-3-methylglutaryl-CoA Reductase	0.01257061	0.568965517	<i>Homo sapiens</i>
P05305	EDN1	11	Endothelin 1	0.011662089	0.568965517	<i>Homo sapiens</i>

drug treatment of diseases. The Regulation of Lipolysis in Adipocytes pathway, which includes key genes such as *PTGS2*, is implicated in the metabolic processes related to energy homeostasis. Osteoporosis is associated with changes in fat metabolism, and the regulation of lipolysis in adipocytes may influence bone remodeling by altering the availability of fatty acids and energy substrates required for osteoblast and osteoclast activity. Statistical significance was noted where $p < 0.05$. This analysis covered pathways such as the PPAR signaling pathway, HIF-1 signaling pathway, AMPK signaling pathway, and cAMP signaling pathway, as illustrated in Figure 6 and detailed in Table 3.

The visualization of these pathways, including the PPAR, HIF-1, AMPK, and cAMP signaling pathways, was performed to elucidate their potential therapeutic implications and was achieved using the “pathview” package in R (Figure 7A–D).

The Drug–Target–Pathway (D–T–P) Network

The top 20 pathways were selected and integrated into a Drug–Target–Pathway (D–T–P) network using Cytoscape version 3.9.1. This network, comprising 59 nodes and 119 edges (Figure 8), plays a crucial role in elucidating the

mechanisms underlying of *C. cassia* in the treatment of osteoporosis.

Molecular Docking

The molecular docking results provided detailed information on binding energy, interaction forces, and bond lengths, as shown in Table 4 and Figure 9. Heat maps (Figure 10) illustrated the binding energies of active compounds to the core targets, highlighting that DIBP, OA, and sativene exhibited stronger binding affinities to the three core targets. Hydrophobic interactions were identified as the primary mechanism by which the active compounds interacted with the core targets. The active pocket of PPARA consisted of amino acid residues Ile, Glu, Cys, Ser, Phe, Tyr, Met, Thr, Leu, His, Pro, and Val. The active pocket of PPARG consisted of amino acid residues Asp, Val, Leu, Gln, Lys, Tyr, Gly, Ser, Ile, Phe, Glu, and Pro. The active pocket of *PTGS2* consisted of the amino acid residues Asn, Tyr, Pro, Ile, Gly, Ala, Leu, Phe, Asp, Val, and Gln. Most of the binding pockets of the targets are hydrophobic amino acid residues. Notably, the aromatic amino acid residues Phe, Trp, and Tyr in the target site were able to bind to each other with the active ingredient. This suggests that the active ingredient has some degree

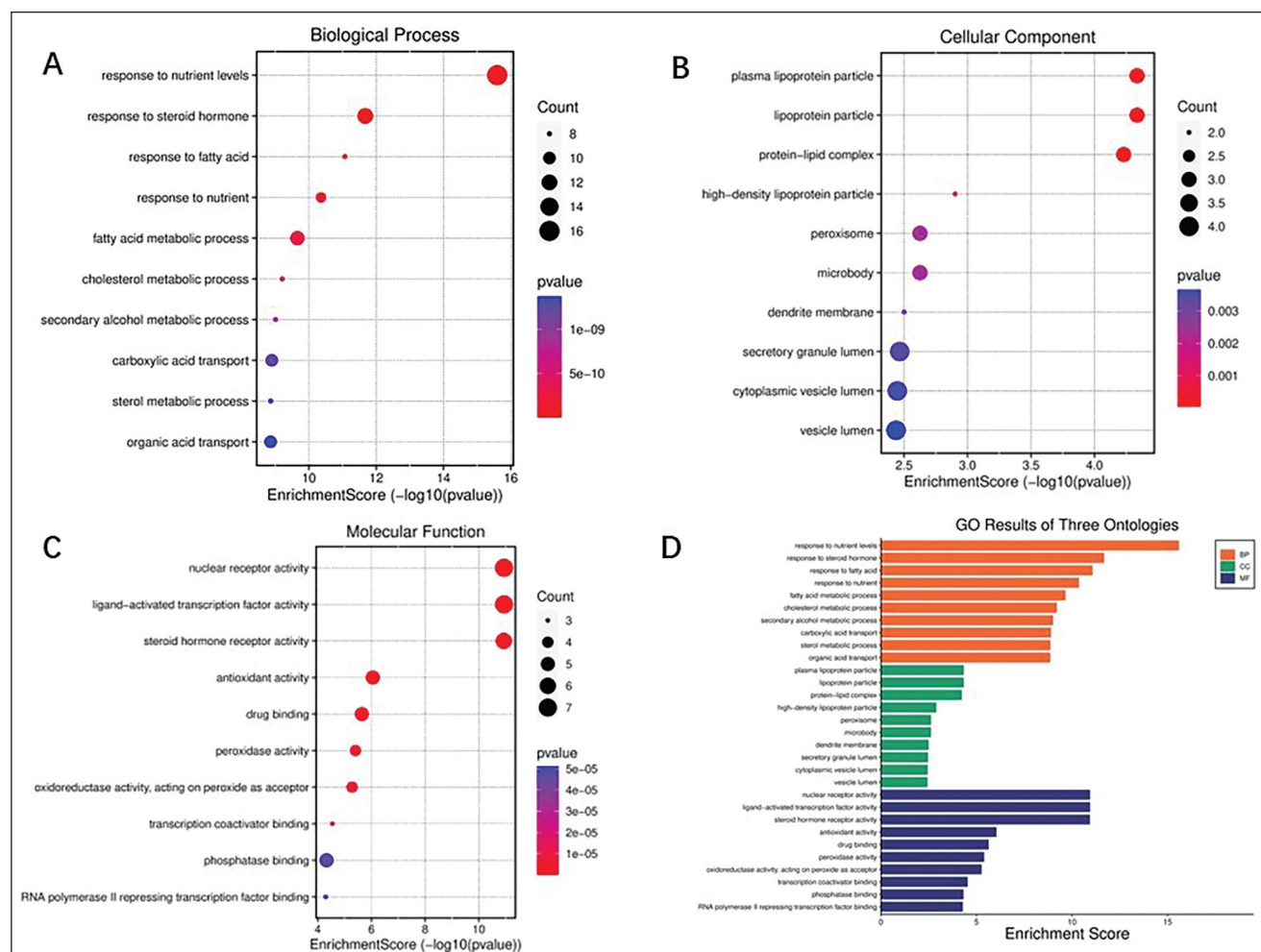


Figure 5. Outcomes of Gene Ontology (GO) Enrichment Analysis. (A–C) Displayed as a Bubble Chart, the Top 10 Terms for Biological Process (BP), Cellular Component (CC), and Molecular Function (MF) in the GO Enrichment Study. (D) The Top 10 Terms for Each Category BP, CC, and MF are Depicted in a Column Chart with Colors Orange, Green, and Purple Representing Each Category, Respectively.

of influence on the fluorescence bursting effect of the target proteins (Lacativa & Farias, 2010).

Molecular Dynamics Simulation

The root mean square deviation (RMSD) measures the average displacement of atomic coordinates relative to a reference model and is commonly employed to evaluate the stabilization of simulation systems. A consistent RMSD indicates equilibrium within the system, while fluctuations in RMSD reflect instability. As shown in Figure 11A, the RMSD values for PTGS2–sativene, PTGS2, and sativene were 0.440 ± 0.049 nm, 0.409 ± 0.048 nm, and 0.039 ± 0.009 nm, respectively, with minimal variation, confirming stable protein–ligand interactions. The radius of gyration (Rg), which represents the compactness of a protein during simulations and is defined as the distance between the center of mass and the outermost atoms, showed a downward

trend throughout the MDS of the complex (Figure 11B). This decline suggests enhanced structural compactness. Simultaneously, the solvent accessible surface area (SASA) exhibited a similar downward trend, reinforcing the observation of increasing protein structural compactness (Figure 11C). Additionally, the root mean square fluctuation (RMSF) quantifies the extent of atomic deviations from their average positions, reflecting the time-averaged structural variability and providing insight into protein regions' flexibility. According to Figure 11D, the average RMSF for the simulated protein complex stood at 0.139 nm, with notably stable fluctuations at the active site, indicating robust PTGS2 protein interaction with sativene.

To analyze the dynamics of protein–ligand interactions, the gmx-mmpbsa script (<https://jerkwin.github.io/gmx-tools/>) was employed to calculate the binding energy of protein–ligand complexes at equilibrium. The MMPBSA method decomposes the total binding energy into four

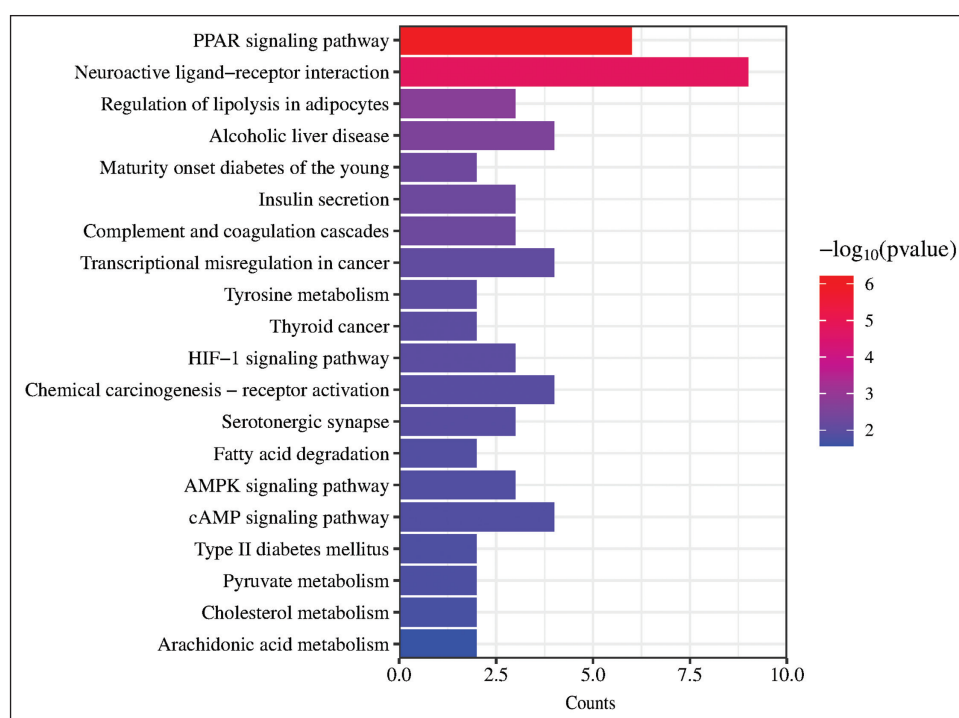


Figure 6. Bar Graph Depicting the Top 20 Pathways as Determined by Kyoto Encyclopedia of Genes and Genomes (KEGG) Enrichment Analysis.

Table 3. Kyoto Encyclopedia of Genes and Genomes (KEGG) Enrichment Results of Key Genes.

ID	Pathway Name	Count	Gene Ratio	Genes	p Value
hsa03320	PPAR signaling pathway	6	6/35	SCD/RXRA/PPARG/PPARD/PPARA/LPL	6.19E-07
hsa04080	Neuroactive ligand–receptor interaction	9	9/35	GABRA1/PYY/PRSS3/PLG/EDN1/CCK/TRPV1/NR3C1/ADRB2	1.60E-05
hsa04923	Regulation of lipolysis in adipocytes	3	3/35	PTGS2/PTGS1/ADRB2	1.86E-03
hsa04936	Alcoholic liver disease	4	4/35	SCD/PPARA/ADH1C/ADH1A	2.95E-03
hsa04950	Maturity onset diabetes of the young	2	2/35	SLC2A2/PDX1	5.37E-03
hsa04610	Complement and coagulation cascades	3	3/35	SERPINE1/PLG/PLAU	5.68E-03
hsa04911	Insulin secretion	3	3/35	SLC2A2/PDX1/CCK	5.68E-03
hsa05202	Transcriptional misregulation in cancer	4	4/35	RXRA/PPARG/PLAU/MPO	8.72E-03
hsa00350	Tyrosine metabolism	2	2/35	ADH1C/ADH1A	1.01E-02
hsa05216	Thyroid cancer	2	2/35	RXRA/PPARG	1.07E-02
hsa04066	HIF-1 signaling pathway	3	3/35	SERPINE1/ERBB2/EDN1	1.09E-02
hsa05207	Chemical carcinogenesis-receptor activation	4	4/35	RXRA/PPARA/PGR/ADRB2	1.20E-02
hsa04726	Serotonergic synapse	3	3/35	PTGS2/PTGS1/SLC6A4	1.26E-02
hsa00071	Fatty acid degradation	2	2/35	ADH1C/ADH1A	1.42E-02
hsa04152	AMPK signaling pathway	3	3/35	SCD/PPARG/HMGCR	1.44E-02
hsa04024	cAMP signaling pathway	4	4/35	PPARA/EDN1/BDNF/ADRB2	1.47E-02
hsa04930	Type II diabetes mellitus	2	2/35	SLC2A2/PDX1	1.62E-02
hsa00620	Pyruvate metabolism	2	2/35	ADH1C/ADH1A	1.69E-02
hsa04979	Cholesterol metabolism	2	2/35	LPL/CETP	1.97E-02
hsa00590	Arachidonic acid metabolism	2	2/35	PTGS2/PTGS1	2.75E-02

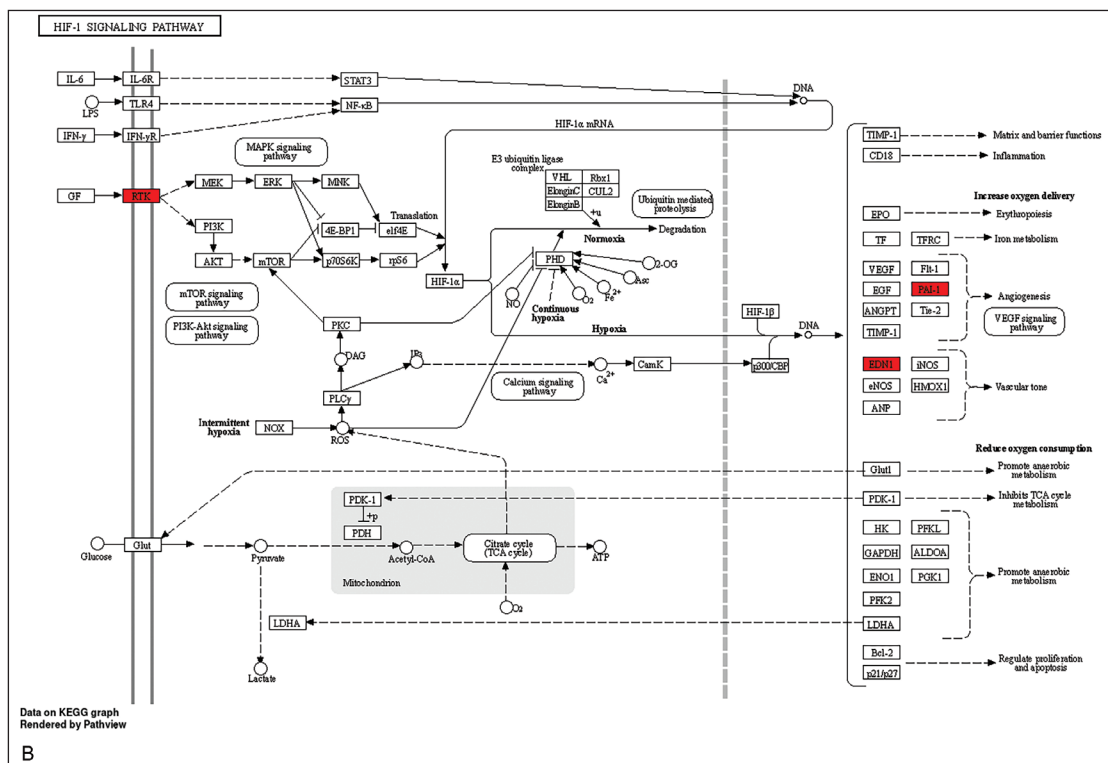


Figure 7. (continued)

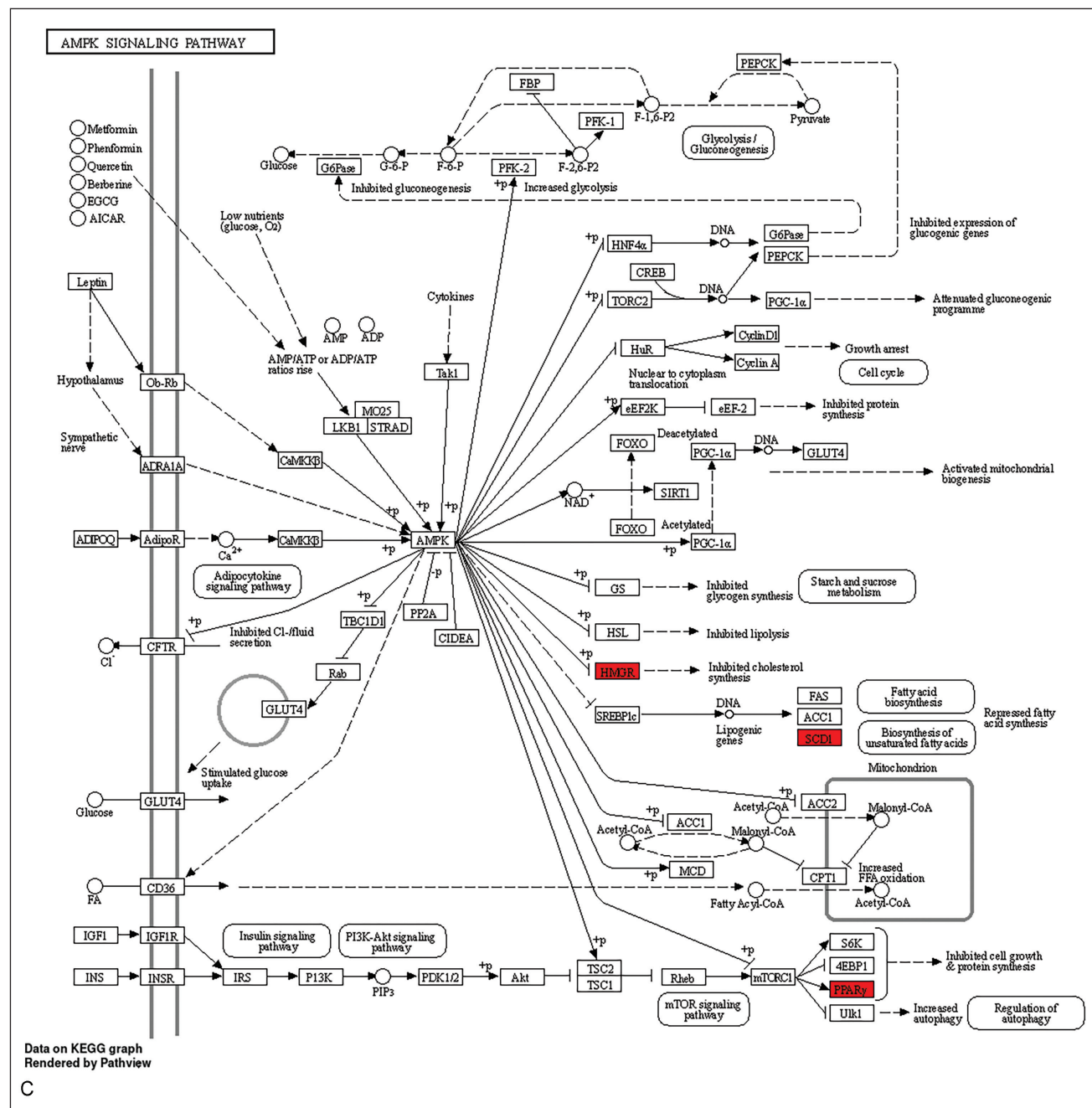


Figure 7. (continued)

distinct components: electrostatic interactions, van der Waals forces, polar solvation, and non-polar solvation (commonly referred to as SASA). The specific binding energy values for each component are detailed in Table 5. In the analysis of the protein–ligand complex, a binding free energy of -78.749 kJ/mol was observed, indicating a favorable interaction between the protein and ligand, signifying a stable binding interaction that aligns with molecular docking results, predominantly characterized by van der Waals forces. In conclusion, the MDSs confirmed that the PTGS2

and sativene complex was securely bound, corroborating the molecular docking findings.

Discussion

Osteoporosis is a systemic bone disorder characterized by reduced bone density and diminished bone quality, resulting from a range of contributing factors, that increases the susceptibility to fractures (Lane et al., 2000; Warriner et al., 2011). This network pharmacology research identified oleic

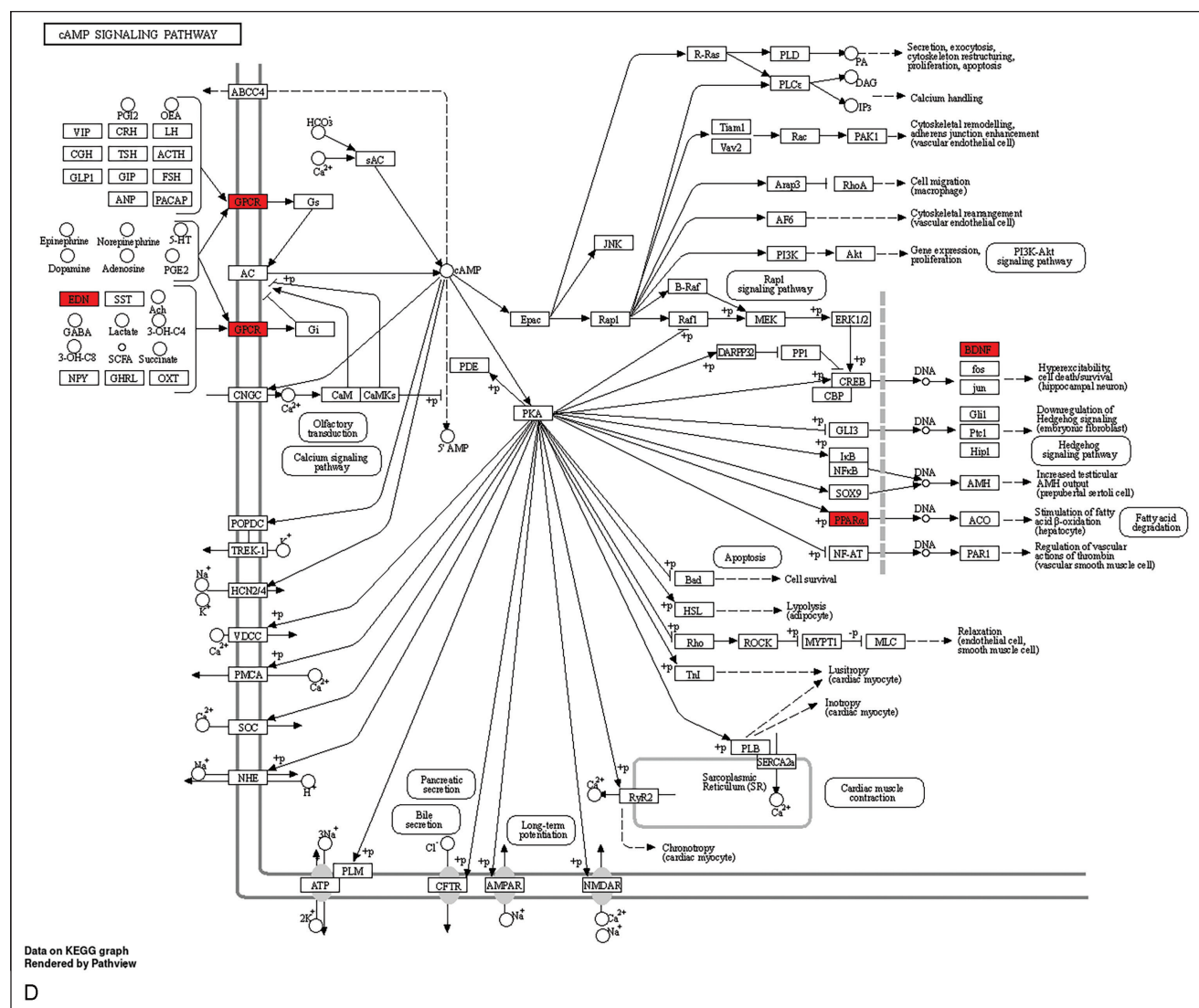


Figure 7. Allocation of Principal Targets Across the Most Pertinent Pathways. (A) Allocation of Principal Targets Within the PPAR Signaling Pathway. (B) Allocation of Principal Targets Within the HIF-1 Signaling Pathway. (C) Allocation of Principal Targets Within the AMPK Signaling Pathway. (D) Allocation of Principal Targets Within the cAMP Signaling Pathway. Key Targets are Represented by a Red Rectangle. The Conjectured Targets and the Genes Involved in Each Pathway are Marked in Red.

acid (Sales-Campos et al., 2013), DIBP, EIC, sativene, and (–)-caryophyllene oxide as key components (Kasonga et al., 2019; Schaepe et al., 2017). Studies have shown that dietary intake of oleic acid improves bone metabolism, a crucial bone marker (Fonolla-Joya et al., 2016). This may be related to oleic acid stimulating nuclear factor κ B ligand and increasing the activity of RANKL in bone cells (Fischer & Haffner-Luntzer, 2022; Song et al., 2019; Tsukasaki, 2021). Another study in rats found that oleic acid affects the formation of melatonin and triglycerides, hindering melatonin's inhibitory effect on triglyceride accumulation, suggesting its role in osteoporosis intervention via melatonin receptor-mediated mechanisms (Sanchez-Hidalgo et al., 2007). Oleic acid influences mesenchymal stem cell lineage

differentiation by modulating the content of bone marrow adipose tissue, a process potentially mediated through interactions between gut microbiota and their metabolic byproducts (Almalki & Agrawal, 2016; Park et al., 2020). Oleic acid intake markedly decreases the number of bone marrow adipocytes, promotes the formation of osteoblasts, and modulates osteoprotegerin expression at both mRNA and protein levels (Zhang et al., 2022). DIBP, a compound strongly associated with osteoporosis progression, suppresses the proliferation of GC-1 spg cells through autophagy activation and the downregulation of the PI3K-AKT and ERK signaling pathways, indicating its potential in osteoporosis regulation through multiple pathways (Kim et al., 2024). DIBP also regulates hormone levels, influencing

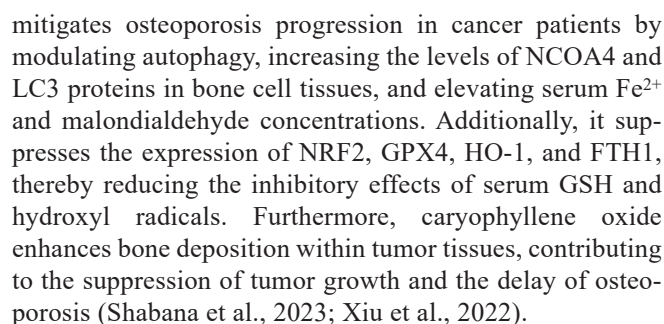


Table 4. Detailed Information of Molecular Docking Analysis.

Core Target	Active Ingredients	Binding Energy (kcal/mol)	Hydrogen Bonds	Hydrophobic Interaction	Salt Bridges
PPARA	DIBP	-5.6	(N/A)	Ile 34A (3.74 Å), Ile 37A (3.93 Å), Ile 41A (3.64 Å), Glu 282A (3.95 Å), Glu 286A (3.82 Å)	(N/A)
	EIC	-3.3	Cys 276A (3.36 Å), Ser 280A (2.97 Å), Tyr 314A (2.99 Å), Tyr 464 (2.84 Å, 2.84 Å)	Phe 273A (3.77 Å)	(N/A)
	OA	-6.0	Ser 280A (3.72 Å), Tyr 314A (3.18 Å), Tyr 464 (2.83 Å)	Met 220A (3.84 Å), Thr 279A (3.54 Å), Thr 283A (3.75 Å), Ile 317A (3.44 Å), Met 320A (3.79 Å), Leu 321A (3.55 Å, 3.92 Å), Val 324A (3.75 Å, 3.67 Å)	His 440A (5.01 Å)
	Sativene	-6.0	(N/A)	Pro 238A (3.77 Å), Leu 229A (3.60 Å), Val 240A (3.73 Å, 3.65 Å), Phe 338A (3.70 Å, 3.64 Å, 3.86 Å, 3.50 Å)	(N/A)
PPARG	DIBP	-5.7	Asp 503A (3.67 Å)	Val 478A (3.47 Å), Leu 481A (3.67 Å), Gln 482A (3.57 Å), Lys 458A (3.67 Å), Leu 493A (3.74 Å), Gln 498A (3.67 Å), Tyr 501A (3.57 Å), Lys 502A (3.81 Å)	Lys 485A (3.65 Å)
	EIC	-3.2	Gly 312A (3.40 Å), Ser 370A (3.09 Å, 2.96 Å)	Ile 295A (3.94 Å), Phe 292A (3.75 Å)	(N/A)
	OA	-4.9	(N/A)	Val 478A (3.60 Å), Leu 481A (3.50 Å), Lys 485A (3.73 Å), Leu 493A (3.78 Å), Gln 498A (3.66 Å, 3.66 Å), Tyr 501A (3.59 Å), Glu 499A (3.45 Å)	(N/A)
	Sativene	-6.0	(N/A)	Pro 274A (3.75 Å), Leu 265A (3.55 Å), Val 276A (3.61 Å, 3.76 Å), Phe 375A (3.71 Å, 3.47 Å, 3.92 Å, 3.72 Å)	(N/A)
PTGS2	DIBP	-5.9	Asn 19A (3.11 Å)	Tyr 122A (3.77 Å, 3.89 Å), Pro 142A (3.77 Å, 3.78 Å)	(N/A)
	EIC	-3.6	Ile 219A (3.15 Å), Gly 274A (4.00 Å), Ala 288A (3.34 Å)	Leu 292A (3.83 Å)	(N/A)
	OA	-4.8	Phe 173A (2.88 Å)	Pro 378A (3.52 Å), Asp 379A (3.76 Å), Val 577A (3.67 Å), Gln 415A (3.78 Å), Phe 173A (3.77 Å, 3.63 Å, 3.79 Å, 3.69 Å)	(N/A)
	Sativene	-6.4	(N/A)	Pro 378A (3.67 Å), Val 577A (3.79 Å), Gln 415A (3.67 Å), Phe 173A (3.79 Å, 3.51 Å, 3.82 Å)	(N/A)

An integrated evaluation of the component-target network and the PPI network in the study suggests that PPARG, PTGS2, PPARA, BDNF, RXRA, NR3C1, SERPINE1, HMGCR, and EDN1 are potential core targets in the treatment of osteoporosis with cinnamon (Ghallab & Seddek, 2020; Jiang et al., 2020; Li, Wang et al., 2023). Evidence indicates that PPARA protein may hinder osteoporosis drug therapy, potentially due to non-expression at binding sites, while RXRA protein specifically binds to ADRB2, facilitating drug effectiveness against osteoporosis (Kim et al., 2019). Increasing evidence shows that proteins like HMGCR and EDN1, which play significant roles in regulating cell apoptosis, differentiation, and tumor suppression, are upregulated in osteoporosis patients (Ren & Zhou, 2024; Zheng et al., 2020; Zhou et al., 2021). Modulating these proteins can reduce cell apoptosis and inflammation, thus treating osteoporosis (Li,

Pang et al., 2023; Muñoz et al., 2020). Osteoporosis is often associated with fluctuations in calcium ion levels at synapses. Activating PTGS2 can reduce calcium levels, enhancing tissue cell plasticity and thereby treating osteoporosis (Kelly et al., 2013).

The findings from the GO enrichment analysis indicated the roles of *C. cassia* in managing osteoporosis primarily involve interactions with various BPs related to nutrient levels, steroid hormones, fatty acids, and nutrition, such as fatty acid metabolic processes, cholesterol metabolic processes, secondary alcohol metabolic processes, and the transport of carboxylic and organic acids. In terms of CCs, significant associations were observed with plasma lipoprotein particles, protein-lipid complexes, high-density lipoprotein particles, peroxisomes, microbodies, and cristae membranes. MFs primarily involve nuclear receptor activity, ligand-activated

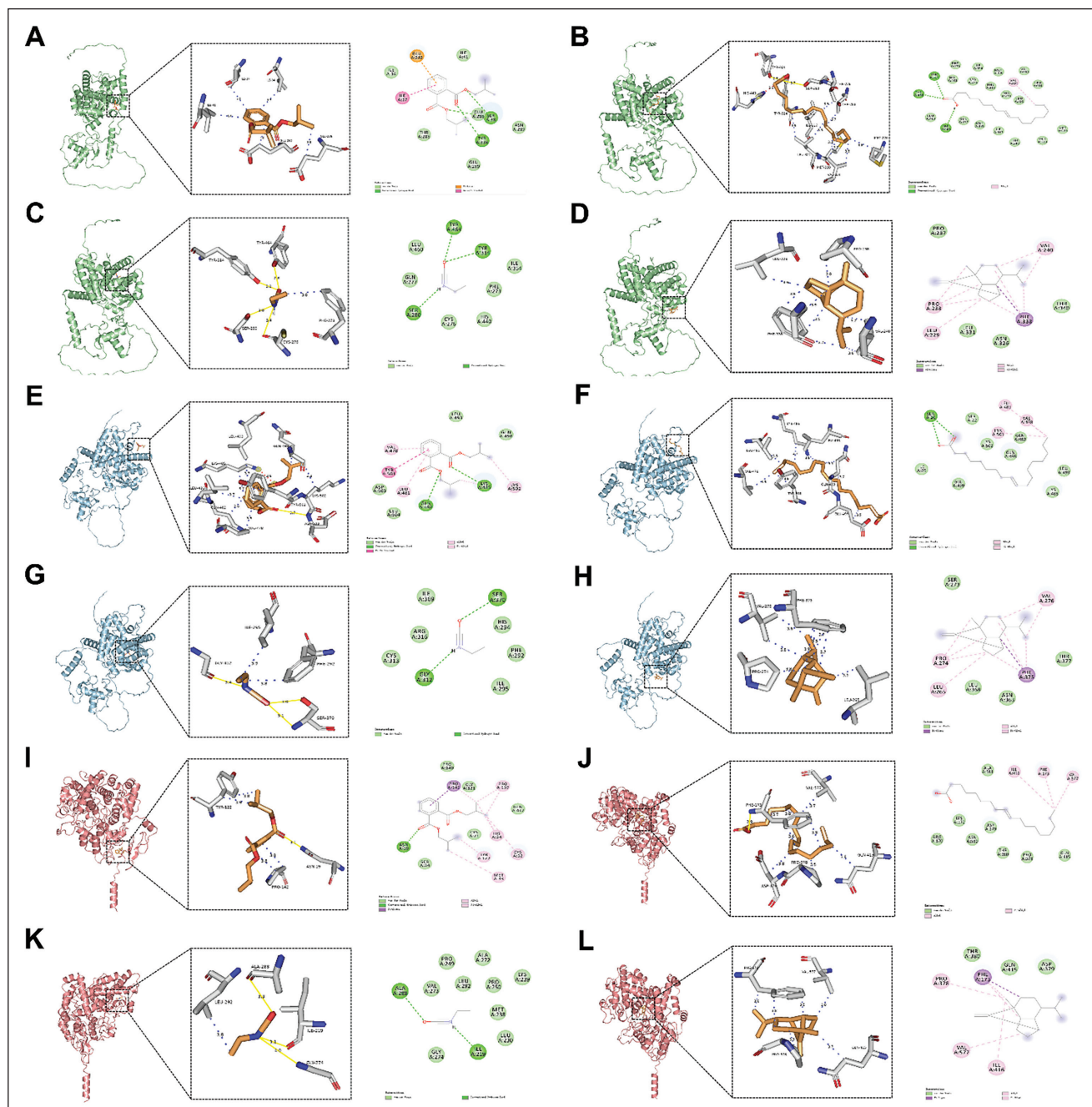


Figure 9. Analysis Of Molecular Docking. (A) Interactions Analysis of the PPARA–DIBP; (B) Interactions Analysis of the PPARA–OA; (C) Interactions Analysis of the PPARA–EIC; (D) Interactions Analysis of the PPARA–Sativene; (E) Interactions Analysis of the PPARG–DIBP; (F) Interactions Analysis of the PPARG–OA; (G) Interactions Analysis of the PPARG–EIC; (H) Interactions Analysis of the PPARG–Sativene; (I) Interactions Analysis of the PTGS2–DIBP; (J) Interactions Analysis of the PTGS2–OA; (K) Interactions Analysis of the PTGS2–EIC; (L) Interactions Analysis of the PTGS2–Sativene. PPARA, PPARG, and PTGS2 were Selected as Receptors and DIBP, OA, EIC, and Sativene were Selected as Ligands. PPARA, PPARG, and PTGS2 are Indicated in Green, Blue and Red, Respectively. Hydrogen Bonds, Hydrophobic Interactions, π –Cation Interactions, and Salt Bridges are Indicated in Yellow, Blue, Green, and Red, Respectively.

Note: DIBP, diisobutyl phthalate; OA, oleic acid; EIC, ethyl isocyanate.

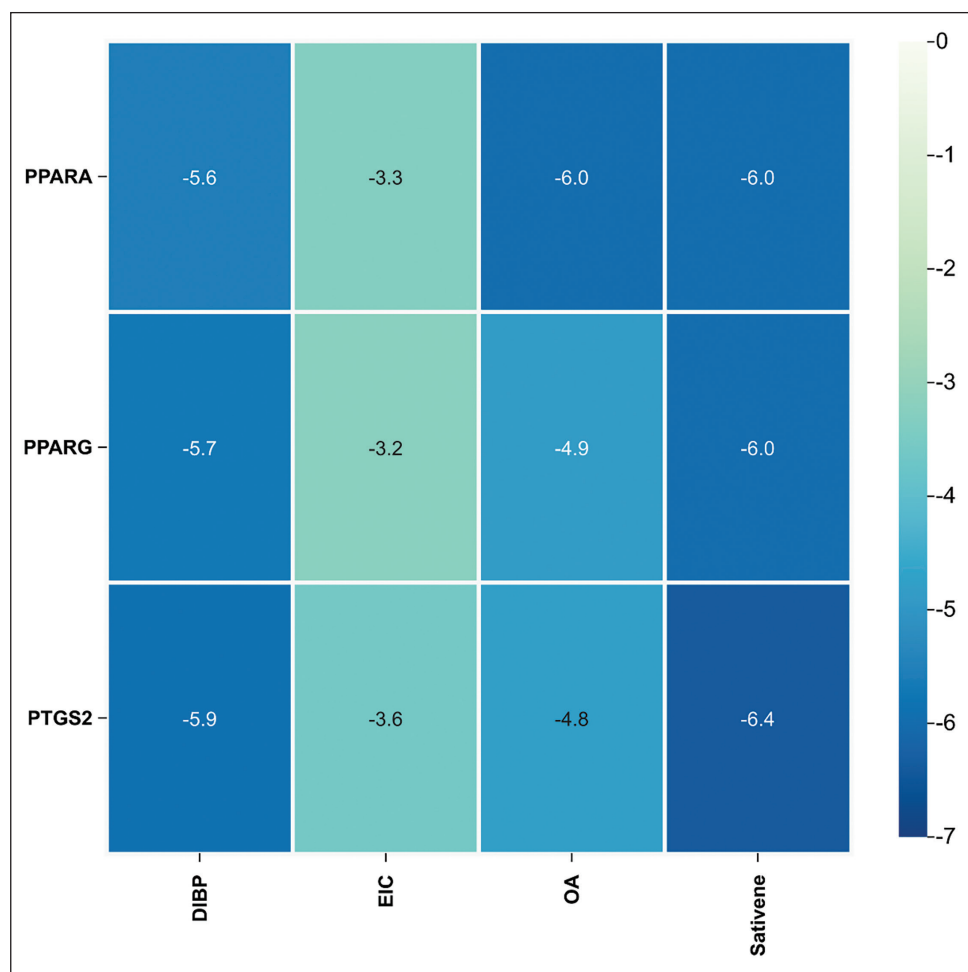


Figure 10. Binding Energy Thermograms of Core Active Ingredients and Core Targets.

transcription factor activity, steroid hormone receptor activity, antioxidant activity, drug binding, and peroxidase activity (Gennari et al., 2007; Lippi et al., 1998; Wang et al., 2023). Furthermore, KEGG enrichment analysis elucidates that *C. cassia*'s intervention in osteoporosis predominantly activates the PPAR, HIF-1, AMPK, and cAMP signaling pathways (Chen et al., 2017).

Research has established a strong correlation between immunity, inflammation, redox processes, ion transport, apoptosis, and the progression of osteoporosis (Iantomasi et al., 2023). Signaling pathways involved in inflammation, including the PPAR, HIF-1, and AMPK pathways, are closely linked to inflammatory responses within the autoimmune system (Bai et al., 2023; Hu et al., 2023; Tao et al., 2021). These signaling pathways immediately participate in autoimmune inflammatory responses upon activation, possibly related to the activation of relevant effective factors. Regulating these signaling pathways can modulate the “neuroimmune inflammation” process of osteoporosis (Lacativa & Farias, 2010). Also, recent research has demonstrated that *C. cassia* mitigates osteoporosis by inhibiting osteoclast

differentiation and activity via suppression of the NF- κ B and MAPK signaling pathways. Notably, it reduces the expression of pro-inflammatory cytokines, including TNF- α and IL-1 β , which play critical roles in driving osteoclastogenesis. Additionally, *C. cassia* influences the RANKL/OPG ratio, enhancing osteoblast function and inhibiting osteoclast activity, which collectively contributes to maintaining bone homeostasis (Liu et al., 2021; Zhang et al., 2019).

Molecular docking analysis confirmed favorable binding interactions between the principal targets and active molecules. Stability assessments of PTGS2–sativene complexes, as evidenced by MDSs, indicated significant stability. This study highlights the potential role of sativene in contributing to the therapeutic effects of *C. cassia* in the treatment of osteoporosis.

This study advances the understanding of *C. cassia* in osteoporosis treatment by integrating network pharmacology, molecular docking, and dynamics simulations. Unlike prior research focusing on single components, our multi-target approach provides a comprehensive view of its therapeutic effects through anti-inflammatory and bone metabolism

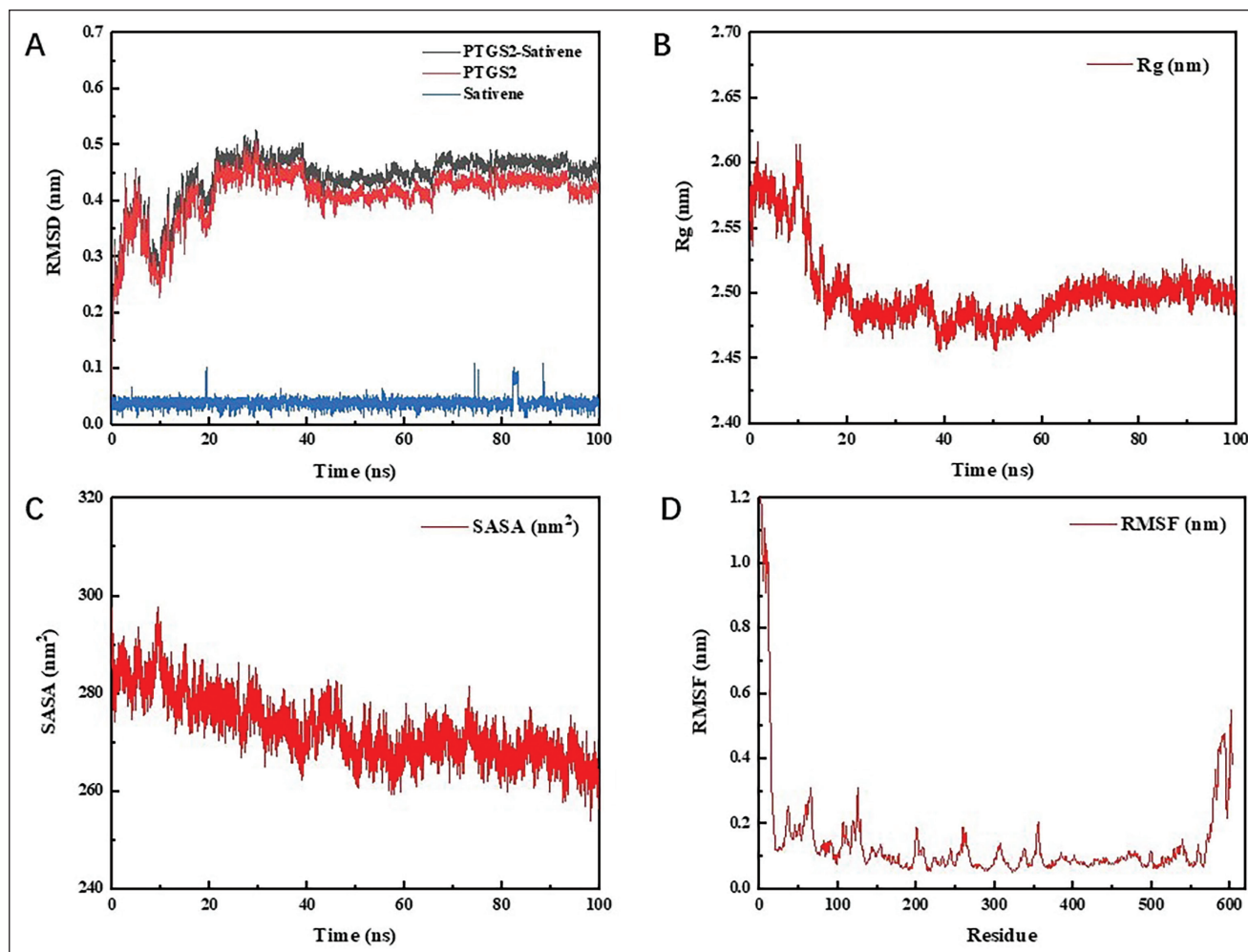


Figure 11. RMSD, Rg, SASA, and RMSF of MDS. (A) The RMSD of PTGS2–Sativene. (B) The Rg of PTGS2–Sativene. (C) The SASA of PTGS2–Sativene. (D) The RMSF of PTGS2–Sativene.

Table 5. MM-PBSA Calculations of the Average Binding Energy between PTGS2 and Sativene.

Energy	PTGS2–Sativene
Van der Waals energy (kJ/mol)	–115.321
Electrostatic energy (kJ/mol)	–0.223
Polar solvation energy (kJ/mol)	41.083
Non-polar solvation energy (kJ/mol)	–16.324
Total binding energy (kJ/mol)	–90.785
–TΔS (kJ/mol)	12.036
Total binding free energy (kJ/mol)	–78.749

pathways. By combining traditional medicine insights with advanced computational tools, this study underscores the value of network pharmacology as a powerful tool in advancing traditional Chinese medicine research.

Despite uncovering some key preliminary insights, this study has certain limitations that should be acknowledged.

The exploration of *C. cassia*'s role in osteoporosis therapy was solely conducted using sophisticated bioinformatics and computational methodologies. Consequently, the predictions' reliability and accuracy require confirmation through additional *in vivo* animal testing (Wang et al., 2024).

Conclusion

This study integrates systematic pharmacology with molecular docking and MDSs to explore the detailed therapeutic mechanisms of *C. cassia* in the treatment and management of osteoporosis. Findings from the experiment proved that some bioactive compounds found in *C. cassia* are oleic acid, DIBP, EIC, sativene, (–)-caryophyllene oxide, which targets only PPARG, PTGS2, and PPARA. It thus activates a few key signaling pathways, that is, PPAR, HIF-1, AMPK, and cAMP, outlining the molecular basis of *C. cassia* effectiveness in the treatment of osteoporosis. This study emphasizes the multi-targeted and pathway-driven therapeutic strategies employed

in traditional Chinese medicine for the treatment of chronic diseases. The study further demonstrates the holistic concept of traditional Chinese medicine and gives theoretical underpinning for future clinical research and application.

The present study findings add more light to the detailed characteristics of the pharmacodynamic properties of *C. cassia* and its mechanisms in the therapy of osteoporosis and provide the base for following *in vivo* animal research.

Abbreviations

DL: Drug-likeness; OB: Oral bioavailability; DC: Degree centrality; BC: Betweenness centrality; BP: Biological process; CC: Cellular component; MF: Molecular functions; MCC: Maximal Clique Centrality; PPI: Protein–protein interaction; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; MDT: Molecular docking technology; MDS: Molecular dynamics simulation.

Consent for Publication

Not applicable.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval and Informed Consent

Not applicable.

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References

- Almalki, S. G., & Agrawal, D. K. (2016). Key transcription factors in the differentiation of mesenchymal stem cells. *Differentiation*, 92(1–2), 41–51. <https://doi.org/10.1016/j.diff.2016.02.005>
- Amberger, J. S., & Hamosh, A. (2017). Searching online mendelian inheritance in Man (OMIM): A knowledgebase of human genes and genetic phenotypes. *Current Protocols in Bioinformatics*, 58, 1.2.1–1.2.12. <https://doi.org/10.1002/cpbi.27>
- Armas, L. A., & Recker, R. R. (2012). Pathophysiology of osteoporosis: New mechanistic insights. *Endocrinology and Metabolism Clinics*, 41(3), 475–486. <https://doi.org/10.1016/j.ecl.2012.04.006>
- Bai, L., Liu, Y., Zhang, X., Chen, P., Hang, R., Xiao, Y., Wang, J., & Liu, C. (2023). Osteoporosis remission via an anti-inflammatory effect by icariin activated autophagy. *Biomaterials*, 297, 122125. <https://doi.org/10.1016/j.biomaterials.2023.122125>
- Bandettini, W. P., Kellman, P., Mancini, C., Booker, O. J., Vasu, S., Leung, S. W., Wilson, J. R., Shanbhag, S. M., Chen, M. Y., & Arai, A. E. (2012). Multicontrast delayed enhancement (MCODE) improves detection of subendocardial myocardial infarction by late gadolinium enhancement cardiovascular magnetic resonance: A clinical validation study. *Journal of Cardiovascular Magnetic Resonance*, 14(1), 83. <https://doi.org/10.1186/1532-429x-14-83>
- Barabási, A. L., Gulbahce, N., & Loscalzo, J. (2011). Network medicine: A network-based approach to human disease. *Nature Reviews Genetics*, 12(1), 56–68. <https://doi.org/10.1038/nrg2918>
- Bardou, P., Mariette, J., Escudié, F., Djemiel, C., & Klopp, C. (2014). Jvenn: An interactive Venn diagram viewer. *BMC Bioinformatics*, 15(1), 293. <https://doi.org/10.1186/1471-2105-15-293>
- Berendsen, H. J., van der Spoel, D., & van Drunen, R. (1995). GROMACS: A message-passing parallel molecular dynamics implementation. *Computer Physics Communications*, 91(1–3), 43–56. [https://doi.org/10.1016/0010-4655\(95\)00042-E](https://doi.org/10.1016/0010-4655(95)00042-E)
- Carretero-González, R., Kevrekidis, P., Kevrekidis, I., Maroudas, D., & Frantzeskakis, D. (2005). A Parrinello–Rahman approach to vortex lattices. *Physics Letters A*, 341(1–4), 128–134. <https://doi.org/10.1016/j.physleta.2005.04.046>
- Chen, L., Zhang, Y. H., Wang, S., Zhang, Y., Huang, T., & Cai, Y. D. (2017). Prediction and analysis of essential genes using the enrichments of gene ontology and KEGG pathways. *PLoS One*, 12(9), e0184129. <https://doi.org/10.1371/journal.pone.0184129>
- Chin, C. H., Chen, S. H., Wu, H. H., Ho, C. W., Ko, M. T., & Lin, C. Y. (2014). cytoHubba: Identifying hub objects and sub-networks from complex interactome. *BMC Systems Biology*, 8(Suppl 4), S11. <https://doi.org/10.1186/1752-0509-8-s4-s11>
- Ding, W., Huang, C., Chen, J., Zhang, W., Wang, M., Ji, X., Nie, S., & Sun, Z. (2024). Exploring the molecular mechanism by which kaempferol attenuates sepsis-related acute respiratory distress syndrome based on network pharmacology and experimental verification. *Current Computer-aided Drug Design*. <https://doi.org/10.2174/0115734099295805240126043059>
- Dong, Y., Tao, B., Xue, X., Feng, C., Ren, Y., Ma, H., Zhang, J., Si, Y., Zhang, S., Liu, S., Li, H., Zhou, J., Li, G., Wang, Z., Xie, J., & Zhu, Z. (2021). Molecular mechanism of Epicedium treatment for depression based on network pharmacology and molecular docking technology. *BMC Complementary Medicine and Therapies*, 21(1), 222. <https://doi.org/10.1186/s12906-021-03389-w>
- Drake, M. T. (2013). Osteoporosis and cancer. *Current Osteoporosis Reports*, 11(3), 163–170. <https://doi.org/10.1007/s11914-013-0154-3>
- Fischer, V., & Haffner-Luntzer, M. (2022). Interaction between bone and immune cells: Implications for postmenopausal osteoporosis. *Seminars in Cell & Developmental Biology*, 123, 14–21. <https://doi.org/10.1016/j.semcdb.2021.05.014>
- Fonolla-Joya, J., Reyes-García, R., García-Martín, A., López-Huertas, E., & Muñoz-Torres, M. (2016). Daily intake of milk enriched with n-3 fatty acids, oleic acid, and calcium improves metabolic and bone biomarkers in postmenopausal women. *Journal of the American College of Nutrition*, 35(6), 529–536. <https://doi.org/10.1080/07315724.2014.1003114>
- Gennari, L., De Paola, V., Merlotti, D., Martini, G., & Nuti, R. (2007). Steroid hormone receptor gene polymorphisms and osteoporosis: A pharmacogenomic review. *Expert Opinion on Pharmacotherapy*, 8(5), 537–553. <https://doi.org/10.1517/14656566.8.5.537>

- Ghallab, A., & Seddek, A. (2020). PPAR γ as therapeutic target for antifibrotic therapy. *EXCLI Journal*, 19, 227–229. <https://doi.org/10.17179/excli2020-1136>
- Hopkins, A. L. (2008). Network pharmacology: The next paradigm in drug discovery. *Nature Chemical Biology*, 4(11), 682–690. <https://doi.org/10.1038/nchembio.118>
- Hu, K., Shang, Z., Yang, X., Zhang, Y., & Cao, L. (2023). Macrophage polarization and the regulation of bone immunity in bone homeostasis. *Journal of Inflammation Research*, 16, 3563–3580. <https://doi.org/10.2147/jir.S423819>
- Iantomasi, T., Romagnoli, C., Palmmini, G., Donati, S., Falsetti, I., Miglietta, F., Aurilia, C., Marini, F., Giusti, F., & Brandi, M. L. (2023). Oxidative stress and inflammation in osteoporosis: Molecular mechanisms involved and the relationship with microRNAs. *International Journal of Molecular Sciences*, 24(4), 3772. <https://doi.org/10.3390/ijms24043772>
- Jiang, Y., Lu, Y., Jiang, X., Hu, J., Li, R., Liu, Y., Zhu, G., & Rong, X. (2020). Glucocorticoids induce osteoporosis mediated by glucocorticoid receptor-dependent and -independent pathways. *Biomedicine & Pharmacotherapy*, 125, 109979. <https://doi.org/10.1016/j.biopha.2020.109979>
- Jiashuo, W. U., Fangqing, Z., Zhuangzhuang, L. I., Weiyi, J., & Yue, S. (2022). Integration strategy of network pharmacology in traditional Chinese medicine: A narrative review. *Journal of Traditional Chinese Medicine*, 42(3), 479–486. <https://doi.org/10.19852/j.cnki.jtcm.20220408.003>
- Kanehisa, M., & Goto, S. (2000). KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Research*, 28(1), 27–30. <https://doi.org/10.1093/nar/28.1.27>
- Kasonga, A. E., Kruger, M. C., & Coetzee, M. (2019). Free fatty acid receptor 4- β -arrestin 2 pathway mediates the effects of different classes of unsaturated fatty acids in osteoclasts and osteoblasts. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, 1864(3), 281–289. <https://doi.org/10.1016/j.bbalip.2018.12.009>
- Kawata, M., & Nagashima, U. (2001). Particle mesh Ewald method for three-dimensional systems with two-dimensional periodicity. *Chemical Physics Letters*, 340(1–2), 165–172. [https://doi.org/10.1016/S0009-2614\(01\)00393-1](https://doi.org/10.1016/S0009-2614(01)00393-1)
- Kelly, O. J., Gilman, J. C., Kim, Y., & Ilich, J. Z. (2013). Long-chain polyunsaturated fatty acids may mutually benefit both obesity and osteoporosis. *Nutrition Research*, 33(7), 521–533. <https://doi.org/10.1016/j.nutres.2013.04.012>
- Kim, Y. H., Jang, W. G., Oh, S. H., Kim, J. W., Lee, M. N., Song, J. H., Yang, J. W., Zang, Y., & Koh, J. T. (2019). Fenofibrate induces PPAR α and BMP2 expression to stimulate osteoblast differentiation. *Biochemical and Biophysical Research Communications*, 520(2), 459–465. <https://doi.org/10.1016/j.bbrc.2019.10.048>
- Kim, S. M., Kim, Y. H., Han, G. U., Kim, S. G., Bhang, D. H., Kim, B. G., Moon, S. H., Shin, S. H., & Ryu, B. Y. (2024). Diisobutyl phthalate (DiBP)-induced male germ cell toxicity and its alleviation approach. *Food and Chemical Toxicology*, 184, 114387. <https://doi.org/10.1016/j.fct.2023.114387>
- Kim, M., Tran, P., Yin, J., Song, J., & Kim, H. (2023). *Cinnamomum cassia* and *Rosa laevigata* mixture improves benign prostatic hyperplasia in rats by regulating androgen receptor signaling and apoptosis. *Nutrients*, 15(4), 818. <https://doi.org/10.3390/nu15040818>
- Lacativa, P. G., & Farias, M. L. (2010). Osteoporosis and inflammation. *Archives of Endocrinology and Metabolism*, 54(2), 123–132. <https://doi.org/10.1590/s0004-27302010000200007>
- Lane, J. M., Russell, L., & Khan, S. N. (2000). Osteoporosis. *Clinical Orthopaedics and Related Research*, (372), 139–150. <https://doi.org/10.1097/00003086-200003000-00016>
- Lee, H. S., Kim, B. S., & Kim, M. K. (2002). Suppression effect of *Cinnamomum cassia* bark-derived component on nitric oxide synthase. *Journal of Agricultural and Food Chemistry*, 50(26), 7700–7703. <https://doi.org/10.1021/jf020751f>
- Li, C., Lin, X., Lin, Q., Lin, Y., & Lin, H. (2024). Jiangu granules ameliorate postmenopausal osteoporosis via rectifying bone homeostasis imbalance: A network pharmacology analysis based on multi-omics validation. *Phytomedicine*, 122, 155137. <https://doi.org/10.1016/j.phymed.2023.155137>
- Li, X., Liu, Z., Liao, J., Chen, Q., Lu, X., & Fan, X. (2023). Network pharmacology approaches for research of traditional Chinese medicines. *Chinese Journal of Natural Medicines*, 21(5), 323–332. [https://doi.org/10.1016/s1875-5364\(23\)60429-7](https://doi.org/10.1016/s1875-5364(23)60429-7)
- Li, X. H., Pang, W. W., Zhang, Y., Liu, D. Y., Yi, Q. R., Wang, N., Zhang, F. R., Deng, Y., Chen, X. D., Greenbaum, J., Xiao, H. M., Deng, H. W., & Tan, L. J. (2023). A Mendelian randomization study for drug repurposing reveals bezafibrate and fenofibric acid as potential osteoporosis treatments. *Frontiers in Pharmacology*, 14, 1211302. <https://doi.org/10.3389/fphar.2023.1211302>
- Li, Y. Y., Tan, X. M., Yang, J., Guo, L. P., & Ding, G. (2020). Naturally occurring seco-sativene sesquiterpenoid: Chemistry and biology. *Journal of Agricultural and Food Chemistry*, 68(37), 9827–9838. <https://doi.org/10.1021/acs.jafc.0c04560>
- Li, H., Wang, C., Jin, Y., Cai, Y., Yao, J., Meng, Q., Wu, J., Wang, H., Sun, H., & Liu, M. (2023). Anti-Postmenopausal osteoporosis effects of isopsoralen: A bioinformatics-integrated experimental study. *Phytotherapy Research*, 37(1), 231–251. <https://doi.org/10.1002/ptr.7609>
- Lim, J. O., Kim, Y. H., Lee, I. S., Kim, W. I., Lee, S. J., Pak, S. W., Shin, I. S., & Kim, T. (2022). *Cinnamomum cassia* (L.) J.Presl alleviates allergic responses in asthmatic mice via suppression of MAPKs and MMP-9. *Frontiers in Pharmacology*, 13, 906916. <https://doi.org/10.3389/fphar.2022.906916>
- Lippi, G., Braga, V., Adami, S., & Guidi, G. (1998). Modification of serum apolipoprotein A-I, apolipoprotein B and lipoprotein(a) levels after bisphosphonates-induced acute phase response. *Clinica Chimica Acta*, 271(1), 79–87. [https://doi.org/10.1016/s0009-8981\(97\)00212-x](https://doi.org/10.1016/s0009-8981(97)00212-x)
- Liu, X., Fu, J., Yao, X. J., Yang, J., Liu, L., Xie, T. G., Jiang, P. C., Jiang, Z. H., & Zhu, G. Y. (2018). Phenolic constituents isolated from the twigs of *Cinnamomum cassia* and their potential neuroprotective effects. *Journal of Natural Products*, 81(6), 1333–1342. <https://doi.org/10.1021/acs.jnatprod.7b00924>
- Liu, D., Hu, Z., Tang, Z., Li, P., Yuan, W., Li, F., & Chen, Q. (2023). Identification of biomarkers associated with oxidative stress-related genes in postmenopausal osteoporosis. *Cellular and Molecular Biology*, 69(6), 186–192. <https://doi.org/10.14715/cmb/2023.69.6.28>
- Liu, S., Yang, L., Zheng, S., Hou, A., Man, W., Zhang, J., Wang, S., Wang, X., Yu, H., & Jiang, H. (2021). A review: the botany, ethnopharmacology, phytochemistry, pharmacology of *Cinnamomi* cortex. *RSC Advances*, 11(44), 27461–27497. <https://doi.org/10.1039/d1ra04965h>

- Luo, H., Vong, C. T., Chen, H., Gao, Y., Lyu, P., Qiu, L., Zhao, M., Liu, Q., Cheng, Z., Zou, J., Yao, P., Gao, C., Wei, J., Ung, C. O. L., Wang, S., Zhong, Z., & Wang, Y. (2019). Naturally occurring anti-cancer compounds: shining from Chinese herbal medicine. *Chinese Medicine*, 14, 48. <https://doi.org/10.1186/s13020-019-0270-9>
- Miller, P. D. (2016). Management of severe osteoporosis. *Expert Opinion on Pharmacotherapy*, 17(4), 473–488. <https://doi.org/10.1517/14656566.2016.1124856>
- Munnangi, S. R., Youssef, A. A. A., Narala, N., Lakkala, P., Narala, S., Vemula, S. K., & Repka, M. (2023). Drug complexes: Perspective from Academic Research and Pharmaceutical Market. *Pharmaceutical Research*, 40(6), 1519–1540. <https://doi.org/10.1007/s11095-023-03517-w>
- Muñoz, M., Robinson, K., & Shibli-Rahhal, A. (2020). Bone health and osteoporosis prevention and treatment. *Clinical Obstetrics and Gynecology*, 63(4), 770–787. <https://doi.org/10.1097/grf.0000000000000572>
- Nogales, C., Mamdouh, Z. M., List, M., Kiel, C., Casas, A. I., & Schmidt, H. (2022). Network pharmacology: Curing causal mechanisms instead of treating symptoms. *Trends in Pharmacological Sciences*, 43(2), 136–150. <https://doi.org/10.1016/j.tips.2021.11.004>
- Ru, J., Li, P., Wang, J., Zhou, W., Li, B., Huang, C., Li, P., Guo, Z., Tao, W., Yang, Y., Xu, X., Li, Y., Wang, Y., & Yang, L. (2014). TCMSP: A database of systems pharmacology for drug discovery from herbal medicines. *Journal of Cheminformatics*, 6(1), 13. <https://doi.org/10.1186/1758-2946-6-13>
- Palukuri, M. V., & Marcotte, E. M. (2021). SuperComplex: A supervised machine learning pipeline for molecular complex detection in protein-interaction networks. *PLoS One*, 16(12), e0262056. <https://doi.org/10.1101/2021.06.22.449395>
- Park, J. W., Fu, S., Huang, B., & Xu, R. H. (2020). Alternative splicing in mesenchymal stem cell differentiation. *Stem Cells*, 38(10), 1229–1240. <https://doi.org/10.1002/stem.3248>
- Ren, Z., & Zhou, L. (2024). Association of statin use with osteoporosis risk: A drug-targeted Mendelian randomization study. *Inflammopharmacology*, 32(2), 1253–1261. <https://doi.org/10.1007/s10787-024-01441-y>
- Safran, M., Dalah, I., Alexander, J., Rosen, N., Iny Stein, T., Shmoish, M., Nativ, N., Bahir, I., Doniger, T., Krug, H., Sirotamadi, A., Olender, T., Golan, Y., Stelzer, G., Harel, A., & Lancet, D. (2010). GeneCards Version 3: The human gene integrator. *Database (Oxford)*, 2010, baq020. <https://doi.org/10.1093/database/baq020>
- Sales-Campos, H., Souza, P. R., Peghini, B. C., da Silva, J. S., & Cardoso, C. R. (2013). An overview of the modulatory effects of oleic acid in health and disease. *Mini Reviews in Medicinal Chemistry*, 13(2), 201–210.
- Sanchez-Hidalgo, M., Lu, Z., Tan, D. X., Maldonado, M. D., Reiter, R. J., & Gregerman, R. I. (2007). Melatonin inhibits fatty acid-induced triglyceride accumulation in ROS17/2.8 cells: Implications for osteoblast differentiation and osteoporosis. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 292(6), R2208–R2215. <https://doi.org/10.1152/ajpregu.00013.2007>
- Schaepe, K., Werner, J., Glenske, K., Bartges, T., Henss, A., Rohnke, M., Wenisch, S., & Janek, J. (2017). ToF-SIMS study of differentiation of human bone-derived stromal cells: New insights into osteoporosis. *Analytical and Bioanalytical Chemistry*, 409(18), 4425–4435. <https://doi.org/10.1007/s00216-017-0386-7>
- Sedha, S., Gautam, A. K., Verma, Y., Ahmad, R., & Kumar, S. (2015). Determination of in vivo estrogenic potential of di-isobutyl phthalate (DIBP) and di-isononyl phthalate (DINP) in rats. *Environmental Science and Pollution Research*, 22(22), 18197–18202. <https://doi.org/10.1007/s11356-015-5021-6>
- Shabana, S. M., Gad, N. S., Othman, A. I., Mohamed, A. F., & El-Missiry, M. A. (2023). β -Caryophyllene oxide induces apoptosis and inhibits proliferation of A549 lung cancer cells. *Medical Oncology*, 40(7), 189. <https://doi.org/10.1007/s12032-023-02022-9>
- Shang, L., Wang, Y., Li, J., Zhou, F., Xiao, K., Liu, Y., Zhang, M., Wang, S., & Yang, S. (2023). Mechanism of Sijunzi Decoction in the treatment of colorectal cancer based on network pharmacology and experimental validation. *Journal of Ethnopharmacology*, 302(Pt A), 115876. <https://doi.org/10.1016/j.jep.2022.115876>
- Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., Amin, N., Schwikowski, B., & Ideker, T. (2003). Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Research*, 13(11), 2498–2504. <https://doi.org/10.1101/gr.1239303>
- Song, C., Yang, X., Lei, Y., Zhang, Z., Smith, W., Yan, J., & Kong, L. (2019). Evaluation of efficacy on RANKL induced osteoclast from RAW264.7 cells. *Journal of Cellular Physiology*, 234(7), 11969–11975. <https://doi.org/10.1002/jcp.27852>
- Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., Gable, A. L., Fang, T., Doncheva, N. T., Pyysalo, S., Bork, P., Jensen, L. J., & von Mering, C. (2023). The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Research*, 51(D1), D638–D646. <https://doi.org/10.1093/nar/gkac1000>
- Tang, Y., Li, M., Wang, J., Pan, Y., & Wu, F. X. (2015). CytoNCA: A cytoscape plugin for centrality analysis and evaluation of protein interaction networks. *Biosystems*, 127, 67–72. <https://doi.org/10.1016/j.biosystems.2014.11.005>
- Tao, H., Li, W., Zhang, W., Yang, C., Zhang, C., Liang, X., Li, H., Xu, Y., Hao, Y., Liu, Y., & Geng, D. (2021). Urolithin A suppresses RANKL-induced osteoclastogenesis and postmenopausal osteoporosis by, suppresses inflammation and downstream NF- κ B activated pyroptosis pathways. *Pharmacological Research*, 174, 105967. <https://doi.org/10.1016/j.phrs.2021.105967>
- Tsuchikama, K., Anami, Y., Ha, S. Y. Y., & Yamazaki, C. M. (2024). Exploring the next generation of antibody-drug conjugates. *Nature Reviews Clinical Oncology*, 21(3), 203–223. <https://doi.org/10.1038/s41571-023-00850-2>
- Tsukasaki, M. (2021). RANKL and osteoimmunology in periodontitis. *Journal of Bone and Mineral Metabolism*, 39(1), 82–90. <https://doi.org/10.1007/s00774-020-01165-3>
- Wang, W., Chen, Z. Y., Lv, F. Y., Tu, M., & Guo, X. L. (2023). Apolipoprotein A1 is associated with osteocalcin and bone mineral density rather than high-density lipoprotein cholesterol in Chinese postmenopausal women with type 2 diabetes mellitus. *Frontiers in Medicine*, 10, 1182866. <https://doi.org/10.3389/fmed.2023.1182866>
- Wang, Y., Xu, T., Chen, X., Ye, Y., Liu, L., Wang, Y., & Zhang, P. (2024). Network Pharmacology and Molecular Docking approach to investigate the mechanism of a Chinese herbal formulation Yougui pills against steroid-related osteonecrosis of

- the femoral head. *Arabian Journal of Chemistry*, 17(3), 105609. <https://doi.org/10.1016/j.arabjc.2024.105609>
- Warriner, A. H., Patkar, N. M., Curtis, J. R., Delzell, E., Gary, L., Kilgore, M., & Saag, K. (2011). Which fractures are most attributable to osteoporosis? *Journal of Clinical Epidemiology*, 64(1), 46–53. <https://doi.org/10.1016/j.jclinepi.2010.07.007>
- Wu, J., Ge, F., Zhu, L., & Liu, N. (2023). Potential toxic mechanisms of neonicotinoid insecticides in rice: Inhibiting auxin-mediated signal transduction. *Environmental Science & Technology*, 57(12), 4852–4862. <https://doi.org/10.1021/acs.est.2c09352>
- Xiu, Z., Zhu, Y., Han, J., Li, Y., Yang, X., Yang, G., Song, G, Li, S., Li, Y., Cheng, C., Li, Y., Fang, J., Li, X., & Jin, N. (2022). Caryophyllene oxide induces ferritinophagy by regulating the NCOA4/FTH1/LC3 pathway in hepatocellular carcinoma. *Frontiers in Pharmacology*, 13, 930958. <https://doi.org/10.3389/fphar.2022.930958>
- Zhang, C., Fan, L., Fan, S., Wang, J., Luo, T., Tang, Y., Chen, Z., & Yu, L. (2019). *Cinnamomum cassia* Presl: A review of its traditional uses, phytochemistry, pharmacology and toxicology. *Molecules*, 24(19), 3473. <https://doi.org/10.3390/molecules24193473>
- Zhang, J., Zhang, Q., Liu, H., Liu, X., Yu, Y., Han, D., He, X., Zeng, P., & Wang, J. (2022). Soy-whey dual-protein alleviates osteoporosis of ovariectomized rats via regulating bone fat metabolism through gut-liver-bone axis. *Nutrition*, 103–104, 111723. <https://doi.org/10.1016/j.nut.2022.111723>
- Zhang, P., Zhang, D., Zhou, W., Wang, L., Wang, B., Zhang, T., & Li, S. (2023). Network pharmacology: Towards the artificial intelligence-based precision traditional Chinese medicine. *Briefings in Bioinformatics*, 25(1), bbad518. <https://doi.org/10.1093/bib/bbad518>
- Zhao, L., Zhang, H., Li, N., Chen, J., Xu, H., Wang, Y., & Liang, Q. (2023). Network pharmacology, a promising approach to reveal the pharmacology mechanism of Chinese medicine formula. *Journal of Ethnopharmacology*, 309, 116306. <https://doi.org/10.1016/j.jep.2023.116306>
- Zheng, J., Brion, M. J., Kemp, J. P., Warrington, N. M., Borges, M. C., Hemani, G., Richardson, T. G., Rasheed, H., Qiao, Z., Haycock, P., Ala-Korpela, M., Davey Smith, G., Tobias, J. H., & Evans, D. M. (2020). The effect of plasma lipids and lipid-lowering interventions on bone mineral density: A Mendelian randomization study. *Journal of Bone and Mineral Research*, 35(7), 1224–1235. <https://doi.org/10.1002/jbmr.3989>
- Zhou, M., Lu, W., Li, B., Yuan, X., Liu, M., Han, J., Liu, X., & Li, A. (2021). Roquin2 suppresses breast cancer progression by inhibiting tumor angiogenesis via selectively destabilizing pro-angiogenic factors mRNA. *International Journal of Biological Sciences*, 17(11), 2884–2898. <https://doi.org/10.7150/ijbs.59891>
- Zhou, S., Sosina, O. A., Bovijn, J., Laurent, L., Sharma, V., Akbari, P., ... Lotta, L. A. (2023). Converging evidence from exome sequencing and common variants implicates target genes for osteoporosis. *Nature Genetics*, 55(8), 1277–1287. <https://doi.org/10.1038/s41588-023-01444-5>