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Hypolipidemic Mechanisms of *Mimosa pudica*: Network Pharmacology, Molecular Docking, Molecular Dynamics (MD) Simulation, Density Functional Theory (DFT), and In Vitro Validation

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ABSTRACT

Background: Hyperlipidemia is a primary cardiovascular risk factor, and current synthetic therapies carry significant adverse effects. *Mimosa pudica* has reported lipid-lowering properties; however, the molecular mechanisms underlying this activity remain unclear.

Aim: To investigate the putative hypolipidemic mechanisms of *Mimosa pudica* phytoconstituents using integrated computational approaches, supported by preliminary in vivo cytoprotective evidence from a crude whole-plant extract.

Methods: Network pharmacology, molecular docking, (Schrödinger Glide SP), 100 ns all-atom MD simulation (GROMACS), DFT analysis, and LC-MS characterization were employed. preliminary in vivo activity of crude Soxhlet *Mimosa pudica* extract (yield: 13.52g) was assessed by MTT-based cytoprotection in FFA-induced HepG2 cells. The computational analysis was based on individual predicted phytoconstituents; the in vitro experiment employed a chemically unstandardized crude extract the in silico in vivo connection is therefore indirect.

Result: Ten hub genes including PPARG, IL6, AKT1, and HIF1A were identified, enriched in HIF-1 and PPAR signaling pathways. Cianidanol showed favorable predicted binding to PPAR α (−8.047 kcal/mol), surpassing fenofibrate (−7.798 kcal/mol); quercetin showed predicted binding to HMGCR (−7.912 kcal/mol). MD simulation indicated stable complex dynamics (RMSD~0.20 nm), and DFT identified C3-OH and C4=O as principal reactive sites of quercetin. LC-MS tentatively identified cianidanol (m/z 611.33) and kaempferol/luteolin (m/z 285.04). The crude extract showed concentration-dependent cytoprotection, significant at 200 μ g/mL ($p < 0.0001$ vs. FFA control); lower doses were not significant, while fenofibrate was significant at all concentrations.

Conclusion: This hypothesis-generating study predicts PPAR α and HMGCR as plausible molecular targets for the hypolipidemic activity of *Mimosa pudica* phytoconstituents. While the MTT assay offers preliminary cellular support, it does not directly assess lipid accumulation, HMGCR activity, or PPAR α activation. Chemical standardization, mechanistic in vitro endpoints, and in vivo validation are needed to substantiate these predictions.

Keywords: *Mimosa pudica*; Hyperlipidemia; Network pharmacology; Molecular docking; Molecular dynamics simulation; HepG2

1. Introduction

Hyperlipidemia is a prevalent metabolic disorder characterized by pathologically elevated serum concentration of total cholesterol, triglyceride, low density lipoprotein (LDL), and very low-density lipoprotein (VLDL), accompanied by reduced levels of high-density lipoprotein (HDL). It is widely recognized as one of the primary risk factor for cardiovascular diseases, obesity, high blood pressure, atherosclerosis, and metabolic syndrome(Baroi et al. 2024). The global burden of dyslipidemia continues to escalate, with cardiovascular disease accounting for approximately 19.2 million deaths annually, a trajectory projected to worsen in the absence of improved therapeutic strategies(Roth et al. 2020). Although many synthetic drugs like statins and fibrates are being used to combat hyperlipidemia, these drugs can lead to various complications and toxicity, which includes hepatotoxicity, myopathy, gastrointestinal problems, and drug resistance (Libby 2021). In view of such issues, we have begun focusing on plant-based natural products for the treatment of hyperlipidemia(Adurosakin et al. 2023).

Pharmacologically important plants have always been recognized as valuable reservoirs of medicinal compounds due to their rich chemical profiles and multiple pharmacological actions. One such interesting plant, which has drawn substantial interest over time due to its varied biological effects, is the plant *Mimosa pudica*, generally named as sensitive plant or touch-me-not. It is a member of the Fabaceae family and can be found in tropical and subtropical habitats. In traditional medicine, this plant is employed as an anti-inflammatory agent, remedy for diabetes, wound healing, antidiarrheal drug, treatment for liver diseases, and antibacterial and antimicrobial drug. Numerous phytochemical studies have established that it contains flavonoids, alkaloids, tannins, terpenoids, glycosides, phenols, and saponins(Francis et al. 2002). Extensive phytochemical investigations have established that *Mimosa pudica* harbors a structurally diverse array of secondary metabolites, including flavonoids, saponins, alkaloids, tannins, terpenoids, glycosides, and phenolic acids phytochemical classes with well documented lipid-modulatory properties in independent pharmacological studies. Consistent with this phytochemical profile, prior investigations have reported hypolipidemic activity of crude extract of *Mimosa pudica* in experimental animal modes. demonstrated significant reductions in serum cholesterol, triglycerides, and LD-C in hyperlipidemic rats treated with chloroform leaf extract (Purkayastha et al. 2012) reported lipid normalizing effects of ethanolic extracts of following tetrachloride induced hepatic injury; and documented hypolipidemic activity in diabetics rats. However, these investigations share critical common limitation: all employed crude, unfractionated solvent extracts evaluated empirically in intact animal models, without systematic identification of the molecular targets, protein-protein interaction networks, or signaling pathways through which the plants phytoconstituents exerts their lipid lowering In particular, four mechanistic dimensions are still completely unidentified in the literature: (i) the molecular targets and hub regulatory genes predicted to mediate the hypolipidemic activity of *Mimosa pudica* phytoconstituents at the systems level; (ii) the atomic-resolution binding interactions between individual flavonoid and saponin constituents and their lipid-regulatory protein

targets; (iii) the quantum chemical electronic properties of lead *Mimosa pudica* flavonoids that control their binding complementarity and chemical reactivity; and (iv) the cytoprotective potential of *Mimosa pudica* fractions under in vitro conditions of hepatic lipid overload. In order to move the pharmacological understanding of *Mimosa pudica* from empirical activity reporting to evidence-based mechanistic characterization, it is imperative that these gaps be filled.

Computational pharmacology methods have gained considerable significance over the last decade due to their ability to analyze the interplay between phytoconstituents, target molecules, and pathological process (Atanasov et al. 2021). The field of network pharmacology combines systems biology, bioinformatics, and pharmacological principles to shed light on the multicomponent and multitarget mode of action of herbal drugs. Contrary to the “one drug–one target” framework, the holistic nature of network pharmacology allows us to pinpoint the interconnected signaling networks that play a crucial role in disease regulation (Hopkins 2008). Network pharmacology proves especially valuable when analyzing herbal medicine with numerous interacting constituents targeting metabolic and cardiovascular disorders (Rajendran and Krishnakumar 2010).

In addition to network pharmacology, molecular docking adds to its value by analyzing the molecular interactions between the phytochemicals and target proteins along with predicting their binding affinity (Pinzi and Rastelli 2019). These docking studies assist in recognizing the potential lead molecules that may regulate the proteins associated with the synthesis of cholesterol, lipids, adipose tissue formation, and oxidative stress. In addition, the Density Functional Theory (DFT) technique has recently found applications in the field of phytochemicals, providing information regarding electronic characteristics, molecular stability, chemical reactivity, and electron density of bioactive components (Calais 1993). These include highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO), energy gap, electronegativity, and molecular electrostatic potential of the compounds (Parasuraman et al. 2019).

In the present study, an integrated computational approach combining network pharmacology, molecular docking, and DFT analysis was employed to systematically investigate, for the first time, the putative molecular mechanisms underlying the hypolipidemic activity of *Mimosa pudica*. To provide preliminary cellular evidence supporting the computational predictions, an MTT-based assay was then used to assess the in vitro cytoprotective effect of a *Mimosa pudica* whole-plant extract in free fatty acid-induced HepG2 hepatocellular cells, a clinically relevant in vitro model of hepatic lipid overload. In particular, this study makes the following contributions: (i) the first systems-level identification of hub molecular targets and enriched signaling pathways predicted to mediate *Mimosa pudica* hypolipidemic activity; (ii) the first atomic-resolution characterization of flavonoid–HMGCR and PPAR α binding interactions for *Mimosa pudica* phytoconstituents; (iii) the first DFT-based quantum chemical profiling of lead *Mimosa pudica* flavonoids in the context of lipid-regulatory enzyme interactions; and (iv) preliminary in vitro cellular evidence for a *Mimosa pudica* extract under lipotoxic conditions, offering a mechanistic hypothesis for future in vivo validation.

2. Materials and Methods

2.1. Plant Material Collection and Extraction

Whole plant material of *Mimosa pudica* was collected from Laxmi Garden Jath, district-Sangli, Maharashtra, India during December 2025 and authenticated by Krishna Mandla (Assistant professor department of botany BLDEA's SB Arts and KCP Science college Vijayapura, Karnataka, India) Plant authenticated No. PASSMCP-102. The collected plant material was thoroughly washed under running water to remove adhering soil and debris, shade-dried at ambient temperature for 28 Days and subsequently powdered plant material was passed through a 40-mesh sieve to obtain a uniform particle size and stored in airtight containers at room temperature until further use.

Extraction was performed by Soxhlet extraction using methanol as the extracting solvent. Briefly, 13.52g of dried powdered plant material was loaded into the Soxhlet thimble and extracted continuously with 300ml of methanol at 46 °C for 18hours until the siphon appeared colorless, indicating exhaustive extraction. The resulting extract was filtered through Whatman No.1 filter paper and concentrated under reduced pressure using a rotary evaporator (Buchi, Switzerland) at 40°C to obtain the crude *Mimosa pudica* extract. The dried extract was weighed and the percentage yield was calculated using the formula:

$$\% \text{Yield} = (\text{Weight of dried extract} / \text{Weight of dried plant material}) \times 100$$

2.2. LC-MS Characterization of *Mimosa pudica* whole plant extract

The phytochemical profile of the *Mimosa pudica* was characterized by liquid chromatography-mass spectrometry (LC-MS) using a TSQ Series (Thermo Fisher Scientific) system equipped with an electrospray ionization (ESI) source operating in both positive (ES+) and negative (ES-) ion modes. On a Aquity BEH column kept at 150°C, chromatographic separation was accomplished. The mobile phase consisted of A:0.1% Formic acid in water and B:0.1 Formic acid in Acetonitrile, which were supplied using a gradient elution program at a flow rate of 0.5mL/min. A diode array detector (DAD) at 350 nm, a wavelength typical of flavonoid chromophores, was used for UV detection. The overall run time was 6 minutes, and the injection volume was 2 µL. A scan range of m/z 100–1000 was used to get mass spectra. Mass Lynx was used to acquire and process the data. By comparing the measured [M+H⁺] and [M–H[–]] ions with the published LC-MS data for recognized *Mimosa pudica* phytoconstituents and by searching the PubChem and HMDB databases, compounds were tentatively identified.

2.3. Database used for research process and research flow

An integrated computational and experimental workflow was designed to systematically elucidate the various targets and their pharmacological mechanisms of *Mimosa pudica* phytoconstituents against hyperlipidemia. The workflow comprised six sequential stages i) phytoconstituents collection; ii) pharmacokinetic filtering using their toxicity profile; iii) target identification; iv) construction and topological

analysis of protein-protein interaction (PPI); v) Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis; vi) molecular docking, MD simulation, and DFT analysis. The databases used for this research are summarized in Table 1.

2.4. Prediction of phytochemicals from *Mimosa pudica* and potential targets

Various phytoconstituents of *Mimosa pudica* were systematically retrieved from the IMPPAT 2.0 database (<https://cb.imsc.res.in/imppat/>) and cross verified against peer-reviewed phytochemical literature to ensure completeness and accuracy of the compound inventory. For each identified phytoconstituent, canonical SMILES notations and three-dimensional SDF structure files were obtained from the PubChem repository (Kim et al. 2023). Pharmacokinetic suitability was subsequently evaluated through the Swiss ADME platform and only those compounds satisfying Lipinski's Rule of 5 were retained for downstream computational analysis, thereby eliminating constituents with poor predicted oral bioavailability. The pharmacokinetically screened phytoconstituents were then subjected to target prediction using SwissTargetPrediction (<https://www.swisstargetprediction.ch/>).

2.5. Prediction of Hypolipidemic related genes

Genes associated with hyperlipidemia were obtained from the GeneCards database by using the specific keyword search hyperlipidemia. This search strategy ensured that targets identified through the network pharmacology would retain direct translational relevance, should the phytoconstituents of *Mimosa pudica* demonstrate promising activity in preliminary cell-line studies and proceed to evaluate in experimental animals. Duplicate entries and genes lacking adequate functional annotation were excluded from the retrieved list, while gene nomenclature was standardized through UniProt mapping to maintain consistency across downstream network analyses (Stelzer et al. 2016; UniProt Consortium 2023).

2.6. Predict common targets of *Mimosa pudica* and Hyperlipidemia

The intersection between the predicted common compound targets of *Mimosa pudica* and curated set of high fat diet-induced hyperlipidemia-associated genes was identified using Venny 2.1.0. The common targets were considered as putative therapeutic mediators of the hypolipidemic effects of the phytoconstituents and used for further PPI network construction and hub gene identification.

2.7. PPI network construction and identification of central targets

A PPI network was constructed using the STRING database with a minimum interaction confidence of 0.7 to retain only high-confidence associations, and the interaction landscape of the overlapping hyperlipidemia-associated targets was mapped. The obtained interaction data set was exported and visualized in Cytoscape 3.7.2. The topological characterization was performed by using CytoHubba plugin to rank the nodes and identify the key hub genes based on the degree centrality algorithm (Szkłarczyk et al. 2019). Network topology was further explored using the Network Analyzer plugin in Cytoscape to calculate degree

centrality, betweenness centrality and closeness centrality for each node, to identify genes occupying the positions of highest regulatory influence in the network architecture.

2.8. Functional enrichment analysis via Gene Ontology (GO) and KEGG pathway mapping

The functional characterization of the overlapping hyperlipidemia-associated targets was performed by the STRING database, including the Gene Ontology (GO) annotations in the three major categories, including Biological Process (BP), Molecular Function (MF), and Cellular Component (CC), as well as the KEGG pathway enrichment analysis to identify the major signaling pathways involved in the lipid-modulating activity of *Mimosa pudica*. Statistically significant terms and pathways were visualized by bar plots, interaction network diagrams, and hierarchical tree figures to facilitate interpretation of the underlying biological processes.(Ge et al. 2020).

2.9. Network construction, Linking bioactive phytoconstituents to disease targets

Using Cytoscape, the present study constructed a multilayered network linking the whole plant extract of *Mimosa pudica* to their bioactive constituents, and the corresponding predicted targets, and the hyperlipidemia disease node. The network analyzer plugin was then applied to evaluate node connectivity across this network and node size according to its edge count revealing several bioactive compounds with markedly high centrality values an indication of their potential as principal contributors to the hypolipidemic activity of *Mimosa pudica* under investigation.(Otasek et al. 2019).

2.10. Molecular docking analysis of Network-prioritized ligands and MD simulation

All-atom molecular dynamics (MD) simulation was carried out using GROMACS 2022.3(Abraham et al. 2015) to investigate the conformational stability and dynamics binding behaviour of PPAR α (PDB ID: 2P54) in its apo form and in complex with the top-ranked docking candidate cianidanol and the reference drug fenofibrate over a 100 ns simulation timescale. The CHARMM36 force field parameters were employed for protein parameterization. Ligand topology files and force field parameters were generated using ACPYPE(Sousa da Silva and Vranken 2012). Explicit solvation was performed using the TIP3P water model(Jorgensen et al. 1983) within a cubic periodic boundary box, maintaining a maximum solute-to-box-wall distance of 1.0 nm. Each system was neutralized by additional of Na⁺ and Cl⁻ counterions to an ionic strength of 0.15 M to replicate physiological conditions.

Energy minimization was performed using the steepest descent algorithm until the maximal force was below 1000 kJ mol⁻¹ nm⁻¹. Equilibration was performed in two consequent phases: (i) 100 ps NVT ensemble at 300 K with V-rescale velocity-rescaling thermostat(Bussi et al. 2007) and (ii) 100 ps NPT ensemble at 300 K and 1 bar with Parrinello-Rahman barostat (coupling constant $\tau = 2.0$ ps)(Parrinello and Rahman 1981). Production simulations were performed during 100 ns with a time step of 2 fs. Coordinates were saved every 10 ps. Long-range electrostatics were treated with the Particle Mesh Ewald (PME) method with a real space cutoff of 1.2

nm. All covalent bonds involving hydrogen atoms were restrained using the LINCS algorithm. Trajectory analysis and graphical outputs were obtained using built-in GROMACS analysis tools and Xmgrace.

Four MD parameters were monitored as functions of simulation time:

(i) Root mean square deviation (RMSD) of backbone Ca atoms relative to the initial structure, to assess global conformational stability; (ii) Root mean square fluctuation (RMSF) per residue to map localized flexibility across the PPAR α chain (residues 200–480); (iii) Radius of gyration (Rg) to monitor overall protein compactness; (iv) Solvent accessible surface area (SASA) to evaluate changes in protein surface exposure.

2.11. Density Functional Theory (DFT)-Based quantum chemical analysis

To further characterize the electronic structural and chemical reactivity of the lead phytoconstituents identified through molecular docking, density functional theory (DFT) calculations,(Hohenberg and Kohn 1964; Kohn and Sham 1965) were performed for selected phytoconstituents using Jaguar within Schrödinger Maestro at the B3LYP-D3 and basis set 6-31G** in fine grid density and ultrafine accuracy level of theory(Becke 1993; Lee et al. 1988). Molecular geometries were fully optimized prior to single-point property calculations. Frontier molecular orbitals the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO), along with their respective sub-orbitals (HOMO-1, LUMO+1) were computed and visualized as isosurface plots to evaluate electron density and electron-accepting regions of each molecule .Molecular electrostatic potential (MESP) surfaces were generated to map regions of nucleophilic and electrophilic susceptibility(Murray and Politzer 2011), while average local ionization energy (ALIE) surfaces were computed to identify sites most susceptible to electrophilic attack(Politzer et al. 2010a). Electron density isosurfaces were additionally plotted to visualize overall charge distribution. Global reactivity descriptors ionization potential (IP), electron affinity (EA), energy gap (ΔE), electronegativity (χ), chemical potential (μ), global hardness (η), global softness (S), and electrophilicity index (ω) were derived from the computed HOMO and LUMO energies using Koopmans' theorem-based approximations,(Pal and Chattaraj 2023).

2.12. In Vitro Antihyperlipidemic activity in free fatty acid (FFA)-Induced HepG2 Cells

The cytoprotective and lipid-lowering potential of the sample was evaluated in human hepatocellular carcinoma (HepG2) cells subjected to free fatty acid (FFA) induced lipotoxicity, a widely employed in vitro model recapitulating the hepatocellular lipid overloaded characteristics of hyperlipidemia and non-alcoholic fatty liver disease. HepG2 cells were seeded into sterile 96-well tissue culture plates at a density of 1×10^4 cells/well in 100 μ L of complete growth medium (DMEM supplemented with 10% fetal bovine serum and $1 \times$ antibiotic–antimycotic solution) and allowed to adhere for 24 h at 37°C in a humidified atmosphere containing 5% CO $_2$. Lipotoxicity was subsequently induced by exposing the cells to a free fatty acid mixture comprising oleic acid and palmitic acid in a 2:1 molar ratio at a final concentration of 0.5–1 mM for 24 h, a protocol established to closely mimic the dyslipidemic intracellular lipid burden observed under hyperlipidemic

conditions(Eynaudi et al. 2021). Following FFA incubation, the medium was replaced with fresh medium containing the test samples or the reference standard (Fenofibrate) across a concentration range of 10-200 µg/mL, and cells were incubated for a further 24-48 h.

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric test was used to measure cell viability and cytoprotection.(Mosmann 1983). In order to allow formazan crystal formation by metabolically viable cells, 20 µL of MTT solution (5 mg/mL) was added to each well, and the mixture was then incubated for 3–4 hours at 37°C. After carefully aspirating the medium, 100 µL of DMSO was added to each well to dissolve the formazan product. A microplate reader was used to measure absorbance at 570 nm. Percentage cell viability and percentage protection against FFA-induced lipotoxicity were calculated using following equations:

$$\% \text{ Cell Viability} = (\text{OD}_{\text{c sample}} / \text{OD}_{\text{c normal}}) \times 100;$$

$$\% \text{ Protection} = [(\text{OD}_{\text{c sample}} - \text{OD}_{\text{c FFA}}) / (\text{OD}_{\text{c normal}} - \text{OD}_{\text{c FFA}})] \times 100.$$

where OD denotes the mean optical density of the respective treatment group.

All data are exposed as mean ± SEM (n = 3). Statistical significance was assessed using one-way ANOVA followed by Dunnett 's multiple comparison test (GraphPad Prism), with the FFA control group as the reference for comparison. A p-value < 0.05 was considered statistically significant.

2.13. Tools used for visualization

Schrödinger Maestro ligand interaction was used for all of the 2D and 3D interaction visuals. Cytoscape was used to build and analyze network models, and STRING database and KEGG Mapper were used to create enrichment visualizations. GROMACS was used to carry out MD simulations.

3. Results

3.1. Soxhlet Extraction Yield

Soxhlet extraction of dried *Mimosa pudica* whole plant extract material with Methanol yielded 13.52g of dark green crude extract, corresponding to an extraction yield of 12.16% w/w relative to the starting dry plant material. The extraction exhibited a green herbal odor and was completely soluble in methanol, indicating rich content of polyphenolic phytoconstituents consistent with the known phytochemical profile of *Mimosa pudica*.

3.2. LC-MS Characterization of *Mimosa pudica* whole plant extract

The *Mimosa pudica* extract was subjected to LC-MS analysis using UV detection at 350 nm in both positive (ES+) and negative (ES-) ionization modes. Nine different peaks were identified by the total ion chromatogram (TIC) and UV chromatogram within a retention time range of 1.57–2.73 min. All of these peaks eluted in the early-to-mid chromatographic window, which is compatible with polar polyphenolic contents (Fig. 1). Table 2 provides a summary of the chromatographic peak data.

The two major peaks at RT 1.95 min (area 32.60 %) and RT 1.65 min (area 22.73 %) together contributed to 55.33 % of the total chromatographic area at 350 nm indicating that the *Mimosa pudica* extract is rich in a limited number of major phytoconstituents consistent with flavonoid and polyphenolic compounds absorbing at this wavelength.

Mass spectrometric analysis in the ES+ mode showed three major base ion series. A base peak at m/z 249.12 (intensity 4.69×10^2) and ions at m/z 227.17, 209.06 and 475.32 were found in the scan RT 1.650 min. An additional ion at m/z 617.16 suggested a glycosylated chromone backbone. In ES- mode at the same retention time, a significant ion at m/z 593.01 was found, probably consistent with a di-C-glycosyl flavone $[M-H]^-$ ($M = 594$) previously described in *Mimosa pudica* whole plant extract. The scan at RT 1.841 min produced the highest intensity base peak in ES+ at m/z 453.31 (intensity 3.41×10^7), accompanied by m/z 475.29, 491.12, and a diagnostic ion at m/z 611.33, the latter consistent with rutin $[M+H]^+$ (theoretical: 611.16), a quercetin-3-O-rutinoside previously identified in *Mimosa pudica*. With a base peak at m/z 340.38 (5.23×10^2), along with m/z 276.82, 701.45, and 679.47, the scan at RT 1.962 min produced the highest overall ion intensity. This pattern suggests that a higher-molecular-weight phytoconstituent is undergoing in-source fragmentation.

In ES- mode, the scan at RT 2.371 min revealed a base peak at m/z 332.13 alongside a diagnostic fragment at m/z 285.04, the latter in precise agreement with the $[M-H]^-$ ion of kaempferol or luteolin (theoretical $[M-H]^- = 285.04$ for both aglycones, $MW = 286.24$), consistent with the elution of free flavonoid aglycones at later retention times following hydrolysis or in-source fragmentation of glycosylated precursors. A new ion at m/z 487.26 was found in this scan, tentatively consistent with a flavonoid-O-hexoside $[M-H]^-$.

Collectively, the LC-MS profile of *Mimosa pudica* extract containing putative glycosylated flavones, flavanol glycosides including rutin, free flavonoid aglycones (kaempferol/luteolin), and higher-molecular-weight saponin-related constituents a phytochemical composition corroborating the IMPPAT database-derived compound inventory employed in the network pharmacology component of this study.

3.3. Identification and screening of bioactive compounds

A total of 28 compounds were found through phytochemical mining of whole plant of *Mimosa pudica* using the IMPPAT and Dr. Dukes database and validation against known phytochemical literature. Using Lipinski's rule of five and Drug-likeness score (DLS) as the inclusion criterion, pharmacokinetic filtering of this compound library using SwissADME reduced the dataset to 21 drug-likeness score and follows Lipinski

rule of 5 those phytoconstituents that were kept for downstream network pharmacology and docking analysis Table 3 (Fig 2, step 2).

3.4. Identification and prediction of genes associated with hyperlipidemia

A total of 1769 distinct protein targets linked to the 21 pharmacokinetically filtered phytoconstituents of *Mimosa pudica* were found using reverse target prediction using SwissTargetPrediction. The term “Hyperlipidemia” was used to get 2612 disease associated genes in parallel from the GeneCards database. These genes were then standardized using UniProt mapping to guarantee nomenclature uniformity. The compound target set and the hyperlipidemia gene targets were identified by Venn analysis using Venny 2.1.0 (Fig .2, Step 3; Fig.3). These shared genes were identified as a potential molecular mediator through which *Mimosa pudica* exert their hypolipidemic effects.

3.5. PPI Network construction and identification of key regulatory genes

Using STRING database (confidence score > 0.7), the 224 overlapping hyperlipidemia associated targets were put together into a protein-protein interaction (PPI) network, which was then shown in Cytoscape for topological analysis. Top 10 hub genes PPARG, IL6, AKT1, EGFR, PTGS2, STAT3, ESR1, BCL2, HIF1A, and CASP3 occupying the most significant regulatory locations within the network were found via CytoHubba based ranking using the degree centrality algorithm (Fig.4-5) Table 4. A visual gradient of relative topological importance among the ten hubs was produced by the CytoHubba subnetwork’s node color intensity, which changed from deep red (highest centrality) to yellow (comparative lower centrality).

With a degree score of 13 and centrality 0.152, PPARG was the highest ranked hub gene, which is in line with its well-established function as a master transcriptional regulator of insulin sensitivity, adipogenesis, and lipid metabolism. IL6, which came in second place with degree 12 and betweenness 0.148, illustrates the significant role that systemic low-grade inflammation plays in the pathophysiology of dyslipidemia, especially in hyperlipidemic conditions brought on by high-fat diets. Through the PI3K–AKT pathway, AKT1, the third-ranked hub (degree 12; betweenness 0.147), encodes a key serine/threonine kinase that mediates insulin signaling and hepatic lipid balance. Instead of single-target pharmacology, the coordinated targeting of these 10 hub genes by *Mimosa pudica* phytoconstituents suggests a mechanistically varied, multi-node hypolipidemic effect. The remaining hub genes EGFR, PTGS2, STAT3, ESR1, BCL2, HIF1A, and CASP3 represent interconnected regulators of inflammatory signaling, cell survival, and metabolic stress responses that collectively underpin the multi-system pathophysiology of hyperlipidemia.

3.6. Functional enrichment analysis (GO and KEGG)

Functional annotation of the overlapping targets yielded enriched terms across all three gene ontology categories. Key (Biological Process) BP terms included cellular response to chemical stress, response to oxygen-containing compound, and response to lipid, reflecting the oxidative and lipid-sensing process central

to high-fat diet induced hyperlipidemia (Fig. 6A). Cellular Component (CC) analysis highlighted membrane raft and receptor complex as principal subcellular compartments (Fig. 6B), while Molecular Function (MF) enrichment was dominated by nuclear receptor activity, monooxygenase activity, and monocarboxylic acid binding (Fig. 6C) consistent with PPARG and PPARG as a hub transcription factor. KEGG analysis identified HIF-1 signaling and insulin resistance as the most significantly enriched pathways, followed by the AGE-RAGE signaling pathway in various diabetic complications and prolactin signaling (Fig. 6D), collectively implicating hypoxia-responsive transcriptional regulation and insulin sensitization as key mechanistic axes modulated by *Mimosa pudica* phytoconstituents in hyperlipidemia.

KEGG pathway analysis showed enrichment of HIF-1 signaling, insulin resistance, AGE-RAGE signaling, prolactin signaling, cancer related, and lipid metabolism pathways. Predicted targets of bioactive phytoconstituents of *Mimosa pudica* included several receptors involved in PPAR signaling pathway like PPAR α , PPAR δ , PPAR γ , FABP1, FABP3, CPT-1, LPL, SCD-1, HMGCS2, and CYP7A1 receptors and these were distributed in various axes of lipid transport, fatty acid oxidation, cholesterol metabolism, and lipogenesis (Fig. 7).

3.7. Analysis of Bioactive-Target Pathway-Disease network

Cytoscape was used to construct and visualize a multilayered network integrating the bioactive phytoconstituents of *Mimosa pudica*, their predicted target proteins, associated signaling pathways, and the disease node hyperlipidemia (Fig. 8). Among the compound nodes, Quercetin, luteolin, and chrysoeriol, exhibited the highest degree centrality, identifying them as the principal multi-target bioactive of the flavonoids. Network topology confirmed the multi-target pharmacological capability of *Mimosa pudica* phytoconstituents through dense bioactive-target-pathway-disease connectivity, with the PPAR and HMGCR signaling pathway nodes emerging as the most highly connected pathway hubs, receiving the greatest number of edges from both bioactive compounds and target genes confirming their mechanistic centrality in the hypolipidemic activity of *Mimosa pudica*. Among target gene nodes, PPARG, PPARG, HMGCR, and FABP4 exhibited the largest node sizes, corroborating their designation as hub genes in the preceding CytoHubba analysis.

3.8. Molecular Docking analysis

The lead bioactive compounds of *Mimosa pudica* were docked to two network pharmacology prioritized hub target proteins, HMGCR (PDB ID:6SLG) and PPAR α (PDB ID: 2P54), using Schrödinger Glide SP docking, with Tizaterkib and Fenofibrate serving as reference standards. Glide score was used to evaluate binding affinity, with higher negative values indicating stronger ligand protein binding. (Table 5; Fig. 9A and 9B) summarizes all docking scores, and 2D/3D interaction diagrams for the top-ranked complexes.

Tizaterkib, the reference standard against HMGCR (6SLG), has a Glide score of -9.422 kcal/mol. It established hydrogen bond interactions with LYS54, MET108, and GLU109, as well as significant

hydrophobic contacts. Quercetin had the highest binding affinity (Glide score: -7.912 kcal/mol), making four hydrogen bonds with MET108, GLN105, ASP111, and GLU71, along with hydrophobic interactions with ILE31 and THR110. Riboflavin came second (-7.844 kcal/mol), with hydrogen connections to ILE31 (2.62 Å), ASP111 (1.91 Å), and MET108 (1.83 Å), while Cianidanol (-7.525 kcal/mol) displayed five hydrogen bond interactions with GLN105, GLU71, ASP111, MET108, and SER153. Fenofibrate (standard) inhibited PPAR α (2P54) with a Glide score of -7.798 kcal/mol and a solitary hydrogen bond at SER280 (2.23 Å). Cianidanol outperformed the reference standard with a Glide score of -8.047 kcal/mol. It formed three hydrogen bonds with TYR464 (1.99 Å), HIE440 (2.14 Å), and SER280 (2.50 Å). Riboflavin (-7.601 kcal/mol) and Crocetin (-7.614 kcal/mol) had competitive binding affinities similar to Fenofibrate, with hydrogen bonding at THR279, ALA333, and TYR464, respectively. The constant engagement of residues TYR464, HIE440, and SER280 across numerous test substances indicates common binding within the PPAR α ligand-binding domain (LBD), which governs transcriptional activation of lipid metabolism genes.

3.9. Molecular dynamics simulation

To validate docking predictions, 100 ns GROMACS MD simulations were performed on apo PPAR α (2P54), PPAR α -cianidanol, and PPAR α -fenofibrate systems.

RMSD: Every system reached a plateau and equilibrated. Fenofibrate complex lowest at ~ 0.15 – 0.18 nm (equilibrated fastest, ~ 30 ns); cianidanol complex at ~ 0.20 nm (equilibrated ~ 40 ns); and apo protein stabilized at ~ 0.25 nm (peaks to 0.30 nm, 55 – 65 ns). Fenofibrate exhibited the highest conformational locking, and both complexes demonstrated reduced RMSD than apo, indicating ligand-induced stability (Fig. 10a)

RMSF: In all systems, the core binding domain (residues 280–400) stayed stiff (RMSF < 0.12 nm). Whereas cianidanol displayed a clear peak at residues ~ 255 – 262 (~ 0.34 nm), fenofibrate displayed increased flexibility at residues ~ 225 – 232 (~ 0.40 nm, Ω -loop region). For both ligands, the AF-2 activation helix (~ 415 – 425) displayed similar flexibility (~ 0.20 nm), indicating that cianidanol adopts an agonist-like binding geometry (Fig. 10b).

Radius of Gyration: Unfolding was ruled out because all systems remained stable and compact (1.90 – 1.95 nm). Compared to apo/fenofibrate (~ 1.92 – 1.93 nm), cianidanol displayed slightly tighter packing (~ 1.91 nm) (Fig. 10c).

SASA: Apo protein showed the lowest SASA (~ 133 – 137 nm ^{2}), which decreased after 40 ns. Cianidanol (~ 143 – 147 nm ^{2}) and fenofibrate (~ 139 – 143 nm ^{2}) both exhibited higher SASA, suggesting that ligand binding keeps the pocket more solvent-accessible, with Cianidanol's smaller size permitting greater peripheral exposure (Fig. 10d).

3.10. DFT-Based quantum chemical analysis

As the two most pharmacologically significant flavonoid constituents of *Mimosa pudica* for additional quantum chemical characterization, quercetin and luteolin were chosen for DFT analysis based on their superior docking scores against HMGCR (−7.912 and −7.424 kcal/mol, respectively) and their highest degree centrality among all phytoconstituents nodes in the Bioactive–Target–Pathway–Disease network. Comparable frontier orbital profiles with slight but chemically significant changes were obtained from DFT analysis of both substances Table 6. While luteolin showed a slightly higher HOMO (−4.925 eV) and LUMO (−1.415 eV) with a little narrower energy gap (3.510 eV), quercetin recorded a HOMO energy of −5.034 eV and LUMO energy of −1.469 eV ($\Delta E = 3.565$ eV). In line with its higher global softness ($S = 0.285$ eV^{−1} vs. 0.281 eV^{−1}), luteolin has a somewhat higher chemical reactivity than quercetin, as indicated by its smaller ΔE . Both compounds showed similar global hardness values ($\eta = 1.782$ and 1.755 eV, respectively), indicating similar overall chemical stability, while quercetin showed a higher electrophilicity index ($\omega = 2.966$ eV) compared to luteolin ($\omega = 2.863$ eV), suggesting slightly greater electrophilic character.

The LUMO isosurface was delocalized over the flavone ring system, whereas HOMO isosurfaces of both molecules had localization in the region of catechol B-ring and chromone C-ring carbonyl, which means that these fragments served as the most prominent electron donors (Fig. 11A-B, 12A-B). In the case of quercetin, the ALIE plot showed regions with minimal ionization energy values (181.737 kcal/mol) corresponding to the C3-OH and C4=O positions and serving as the most vulnerable points for electrophilic attack (Fig. 11D, 12D); on the other side, the ESP plot for luteolin showed the predominance of the electron-rich fragment (ESP min = −194.164 kcal/mol; mean = −69.503 kcal/mol), having only one electrophilic zone at the C4=O carbonyl position (ESP max = 39.733 kcal/mol) (Fig. 11C-E, 12C-E)

3.11. FFA-Induced lipotoxicity protection in HepG2 cells

Using the MTT assay and Fenofibrate as the reference standard, the antihyperlipidemic potential of the test sample *Mimosa pudica* whole plant extract was assessed in HepG2 cells induced by free fatty acid (FFA). Table 7 and (Fig. 13) show complete cell viability and % protection data for the concentration range of 10–200 µg/mL.

Exposure of HepG2 cells to the oleic acid-palmitic acid FFA mixture markedly reduced cell viability to 49.36% relative to the normal control (OD = 1.256), confirming successful induction of lipotoxic stress and validating the experimental model. Treatment with *Mimosa pudica* whole plant extract following FFA challenge produced a clear concentration-dependent recovery in both cell viability and cytoprotection. Cell viability increased progressively from 53.66 % at 10 µg/mL to 78.03% at 200 µg/mL, with corresponding percentage protection values rising from 8.49 % to 56.60 % across the same concentration range. The reference standard Fenofibrate demonstrated a markedly stronger and similarly concentration dependent response, with cell viability increasing from 57.72 % to 97.45 % and percentage protection from 16.51 % to 94.97 % over the identical concentration range. While *Mimosa pudica* extract exhibited statistically meaningful

cytoprotection at all tested concentrations, its maximum protective effect at 200 µg/mL (56.60%) remained considerably lower than that of fenofibrate (94.97%) at the same concentration, indicating moderate concentration-dependent anti-hyperlipidemic activity. These in vitro findings are consistent with the HMGCR-inhibitory and PPAR α -modulatory binding interactions predicted through molecular docking for the phytoconstituents of *Mimosa pudica*, and corroborate the computational predictions of this investigation.

A significant difference between research groups was found using one-way ANOVA Mean $\pm$ SEM and SD Table 8 (Fig.14) [F (11,24) = 94.24, $p < 0.0001$]. FFA stimulation dramatically decreased cell viability as compared to the normal control, according to Dunnett's post hoc test ($p < 0.0001$). At all tested concentrations, fenofibrate significantly restored viability in comparison to the FFA control (10 µg/mL: $p = 0.0327$; 50–200 µg/mL: $p < 0.0001$). In comparison to the FFA control, *Mimosa pudica* extract had a concentration-dependent impact, showing no significant protection at 10 µg/mL ($p = 0.556$) or 50 µg/mL ($p = 0.379$), but considerable protection at 100 µg/mL ($p = 0.0019$), 150 µg/mL ($p < 0.0001$), and 200 µg/mL ($p < 0.0001$).

4. Discussion

Hyperlipidemia is a mechanistically complex, multifactorial metabolic disorder whose pathogenesis implicates dysregulated cholesterol biosynthesis, impaired lipoprotein clearance, hepatic lipid overload, chronic low-grade inflammation, and disrupted insulin signaling a breath that renders single target pharmacological strategies inherently insufficient for comprehensive lipid management. In the present investigation, an integrated computational approach encompassing network pharmacology, molecular docking, 100 ns all atom GROMACS molecular dynamics simulation, density functional theory analysis, LC-MS characterization, and preliminary in vivo validation in FFA-induced HepG2 cells was employed to systematically explore the putative hyperlipidemic mechanisms of *Mimosa pudica* phytoconstituents. It must be emphasized at the outset that the computational analyses are based on individually predicted phytoconstituents derived from the IMPPAT database, whereas the in vitro experiment employed a crude, chemically unstandardized whole plant Soxhlet extract. Without chemical standardization or bioactivity guided fractionation linking specific individual compounds to the observed cellular response, the mechanistic connections proposed in the computational component remain predictive and hypothesis generative rather than experimentally validated. The findings should therefore be interpreted as a systems level mechanistic framework warranting further targeted experiment investigation rather than as demonstrated pharmacological mechanisms.

This computationally predicted interconnected regulatory network, comprising of the ten hub genes (PPARG, IL6, AKT1, EGFR, PTGS2, STAT3, ESR1, BCL2, HIF1A and CASP3) derived from CytoHubba analysis, regulates lipid homeostasis, systemic inflammation, insulin sensitization and hepatocyte survival and thus, provides a theoretical multi-target framework for the putative hypolipidemic activity of *Mimosa pudica* in high-fat diet-induced hyperlipidemia(Tontonoz and Spiegelman 2008). However, it is important to note that

the identification of these hub genes is based solely on computational intersection analyses and PPI network topology, and no direct experimental validation of the modulation of these targets by *Mimosa pudica* extracts or individual phytoconstituents was conducted in this study. Predicted interactions would need to be confirmed by experimental validation using target-specific assays (e.g., PPARG reporter gene assays, HMGCR enzyme activity assays, or cytokine quantification).

As the highest degree hub genes, PPARG is a well characterized master transcriptional regulator of adipogenesis, fatty acid storage, and lipid metabolism whose activation modulated downstream targets including PPARG, PPARG, and fatty acid transport (Istvan and Deisenhofer 2001). Molecular docking predicted a favorable binding pose of cianidanol in the PPAR α ligand-binding domain (Glide score: -8.047 kcal/mol; PDB: 2P54) compared to fenofibrate (-7.798 kcal/mol) indicating that cianidanol may potentially bind to PPAR α in a binding geometry similar to fibrate-class agonists. Docking scores, however, are approximations of binding affinity under static, gas-phase conditions and do not account for conformational selection, entropic penalties upon binding, or solvation effects. Moreover, a docking score that is greater than that of fenofibrate does not necessarily correlate with superior biological PPAR α agonism in a cellular or physiological context. To support this predicted interaction, a direct experimental assessment of PPAR α transcriptional activation would be required, e.g. by GAL4-PPAR α reporter gene assays or by expression analysis of downstream target genes.

HMGCR, the rate limiting enzyme of the mevalonate cholesterol biosynthesis pathway and the principal pharmacological target of statins, was identified as one of the most densely connected hub nodes in the PPI network. Molecular docking predicted that quercetin may form hydrogen bond interactions with MET108, GLN105, ASP111, and GLU71 within the HMGCR active site (Glide score: -7.912 kcal/mol; PDB: 6SLG), a binding pose superficially resembling that of competitive statin inhibitors at this locus. This predicted bonding mode may suggest a mechanistically analogous cholesterol suppressive potential; however, the docking score for quercetin (-7.912 kcal/mol) remains substantially below that of the reference compound tizaterkib (-9.422 kcal/mol), raising the possibility that quercetin may achieve only partial engagement of the active site under physiologically relevant concentrations. DFT analysis further indicated that the ALIE minimum of 181.737 kcal/mol, localized at the C3-OH and C4=O positions of quercetin, is consistent with these positions being the most electronically reactive sites of the molecule, providing quantum-chemical plausibility though not experimental confirmation for the predicted HMGCR interaction sites. Direct HMGCR enzyme activity assays using purified recombinant enzyme or microsomal preparations would be required to determine whether quercetin exerts any measurable inhibitory effect on this target.

IL6, the second ranked hub gene, encodes a pleiotropic pro-inflammatory cytokine. The mechanistic role of IL6 in dyslipidemia pathogenesis is well documented in the literature including upregulation of hepatic VLDL secretion, suppression of lipoprotein lipase activity and promotion of systemic insulin resistance via JAK-STAT3 signaling. Its computational identification as a hub target and STAT3 (ranked sixth) tentatively

indicates the putative ability of *Mimosa pudica* phytoconstituents to reduce lipid dysregulation via secondary modulation of the hepatic inflammatory signaling axis, although this hypothesis has not been experimentally tested in the current study. The enrichment of the dominant molecular function term of nuclear receptor activity in GO analysis provides another computational support for the potential involvement of PPARG, PPARA, PPARD and ESR1 in the predicted hypolipidemic mechanism.

AKT, ranked third by degree centrality, encodes the serine/threonine kinase central to the PI3K-AKT insulin signaling cascade, regulating hepatic glucose utilization, lipogenesis via SREBP activation, and fatty acid oxidation through AMPK cross-talk. Given that HIF-1 α is transcriptionally upregulated in hepatic and adipose tissues under lipid overload conditions, potentially driving lipogenesis, glycolytic reprogramming, and inflammatory cytokine production, the KEGG enrichment results identified insulin resistance and HIF-1 signaling as the two most significantly enriched pathways (“HIF-1 α -PDK1 Axis-Induced Active Glycolysis Plays an Essential Role in Macrophage Migratory Capacity | Nature Communications,” n.d.). This offers a theoretical mechanistic connection between the high-fat diet-induced hyperlipidemic phenotype and the HIF1A hub gene; however, this connection is solely based on pathway enrichment analysis of computationally predicted targets and has not been verified through any direct experimental evaluation of HIF-1 α expression or activity in the current study.

Under hyperlipidemic conditions, dysregulated activation of EGFR and PTGS2 (COX-2) has been documented to promote hepatic lipid accumulation and oxidative damage, while ESR1 governs hormonal regulation of cholesterol and triglyceride metabolism. BCL2, CASP3 and HIF1A collectively regulate hepatocellular apoptosis and survival under lipotoxic stress. The crude *Mimosa pudica* extract (MS) produced concentration-dependent protection against FFA-induced cell death, according to preliminary in vitro data from the FFA-induced HepG2 cytoprotection assay; however, the MTT assay used only measures metabolic activity and cell viability and does not provide information on lipid accumulation, apoptotic marker expression, or the specific molecular targets modulated (Feldstein et al. 2003). It is impossible to identify from the MTT data alone whether the observed cytoprotection is due to the engagement of the computationally anticipated targets or to alternative mechanisms such non-specific antioxidant activity, membrane stability, or indirect metabolic effects. Since the phytochemical makeup of the extract is complicated and poorly defined, and the active constituent or constituents responsible for the cytoprotective response have not been separated or identified, the extract's crude nature further complicates mechanistic interpretation.

Molecular docking against the two highest-priority hub targets predicted mechanistically interesting findings. Cianidanol showed a predicted Glide score of -8.047 kcal/mol against PPAR α (2P54), marginally exceeding fenofibrate (-7.798 kcal/mol) making it the only test compound to surpass its respective reference standard in computed binding affinity in this study. A partial or complete agonist binding geometry hypothesis is supported by the three projected hydrogen bond interactions with TYR464, HIE440, and SER280, residues involved in PPAR α ligand-binding domain recognition (“Frontiers | Natural Products in Atherosclerosis

Therapy by Targeting PPARs: A Review Focusing on Lipid Metabolism and Inflammation,” n.d.). In a similar vein, quercetin formed four anticipated hydrogen bonds within the catalytic cleft and ranked as the highest-affinity flavonoid against HMGCR (-7.912 kcal/mol), although it was still lower than the reference compound (-9.422 kcal/mol). When combined, these docking results point to a possible dual-axis mechanism: quercetin may address the enzymatic arm of lipid regulation through HMGCR, while cianidanol may address the transcriptional arm through PPAR α . However, this theory needs to be verified experimentally using biochemical and cell-based target assays (“Synergistic Hypolipidemic Effects and Mechanisms of Phytochemicals: A Review,” n.d.).

The 100 ns GROMACS all-atom MD simulations provided additional computational evidence for the predicted binding mode stability. The RMSD profiles indicated that both complexes reached conformational equilibration by ~ 40 – 50 ns, with the cianidanol PPAR α complex maintaining a stable plateau of ~ 0.20 nm and the fenofibrate complex achieving the lowest RMSD of ~ 0.15 – 0.18 nm. Fenofibrate's recognized status as a complete PPAR α agonist is compatible with its somewhat lower RMSD compared to cianidanol, which may indicate that cianidanol engages the binding site with slightly less conformational constriction. Comparable AF-2 helix flexibility (~ 0.20 nm at residues ~ 415 – 425) between cianidanol and fenofibrate complexes was found by the RMSF analysis, which may be consistent with an agonist-mode interaction for cianidanol. Nevertheless, AF-2 flexibility is not enough to differentiate between full and partial agonism; functional receptor activation assays would be necessary for this determination. It's also crucial to remember that, even though the 100 ns GROMACS simulations significantly outperform static docking in terms of dynamic characterization, they are still only an in-silico approximation of binding behavior and do not provide experimental confirmation of complex stability in a biological setting.

Molecular-level electronic characterization pertinent to the anticipated pharmacological interactions of quercetin and luteolin was obtained by DFT-based quantum chemical analysis. The similar global hardness values (1.782 and 1.755 eV, respectively) and HOMO–LUMO energy gaps (quercetin: 3.565 eV; luteolin: 3.510 eV) show that both flavonoids have balanced chemical reactivity with selective electrophilic character, which are theoretically favorable to particular enzyme–ligand interactions. The anticipated HMGCR binding contacts found in docking studies have quantum-chemical validity since the ALIE minimum of 181.737 kcal/mol at quercetin's C3–OH and C4=O positions are consistent with these locations being the molecule's most electronically reactive regions. Although these interpretations are based on electronic structure calculations for isolated gas-phase molecules and do not take into account solvent effects, protein environment, or conformational dynamics upon binding, luteolin's slightly narrower energy gap (3.510 eV) and slightly higher global softness (0.285 eV $^{-1}$) suggest greater conformational adaptability compared to quercetin, which may be consistent with its predicted accommodation within the more flexible PPAR α ligand-binding domain.

Crude *Mimosa pudica* extract (MS) showed statistically significant, concentration-dependent cytoprotection against oleic acid–palmitic acid-induced lipotoxicity, according to in vitro validation in FFA-induced HepG2 cells. The extract showed significant protection at 100–200 µg/mL (**** $p < 0.0001$ vs. FFA control), according to statistical analysis using one-way ANOVA with Dunnett's post-hoc test. Lower concentrations (10 and 50 µg/mL) did not reach statistical significance ($p > 0.05$), indicating a concentration threshold for meaningful cytoprotective activity. At 200 µg/mL, fenofibrate produced 94.97% protection and 97.45% cell viability, while MS produced 56.60% protection and 78.03% cell viability. Although these findings offer first cellular support for the crude extract's antihyperlipidemic potential, it is vital to recognize a number of significant limitations of this experimental component. First, the MTT assay does not directly measure lipid accumulation, intracellular triglyceride or cholesterol content, HMGCR enzymatic activity, PPAR α transcriptional activation, or any other lipid metabolism-specific endpoint; instead, it only measures mitochondrial metabolic activity as a proxy for cell viability. Second, the tested sample is a crude, chemically unstandardized whole-plant Soxhlet extract whose phytochemical composition has not been quantitatively standardized for individual active ingredients, although being partially described by LC-MS. Therefore, the relationship between the in vitro cytoprotective response of the crude extract and the computationally predicted activities of individual phytoconstituents is still indirect, and further fractionation and mechanistic research are required to attribute the observed cellular protection to particular predicted molecular targets. Third, the HepG2 model is a transformed cancer cell line with different metabolic regulation compared to primary hepatocytes, which may restrict the findings' physiological significance even though it is frequently used as a surrogate for hepatic lipid metabolism(Tie et al. 2021).

With preliminary in vitro cytoprotective evidence from a crude plant extract, the current study offers a systems-level computational framework that predicts that *Mimosa pudica* phytoconstituents may interact with several regulatory targets, such as PPARG, HMGCR, AKT1, and HIF1A, implicated in PPAR-mediated transcriptional regulation, cholesterol biosynthesis, insulin signaling, and hepatic inflammatory pathways. However, these results should be interpreted as mechanistic hypotheses requiring additional experimental substantiation rather than as proven mechanisms of action in the absence of direct target engagement assays, lipid-specific in vitro endpoints, chemical standardization of the extract, and in vivo pharmacological validation(Sowmya and Ananthi 2011). Future research should focus on the following areas: (i) bioactivity-guided fractionation and isolation of individual active phytoconstituents from *Mimosa pudica*; (ii) target-specific enzymatic and reporter gene assays for HMGCR inhibition and PPAR α agonism; (iii) lipid accumulation assays (Oil Red O, BODIPY) and lipid panel quantification in FFA-treated HepG2 cells; (iv) in vivo testing in animal models of high-fat diet-induced hyperlipidemic; and (v) pharmacokinetic profiling of lead compounds to determine oral bioavailability. The translational data base for *Mimosa pudica* as a potential source of plant-derived antihyperlipidemic medicines would be significantly strengthened by addressing these directions.

5. Conclusion

This integrated computational-experimental study explored the putative hypolipidemic potential of *Mimosa pudica* phytoconstituents using network pharmacology, molecular docking, 100 ns MD simulations, DFT analysis, LC-MS, and in vitro MTT assays. Network analysis identified 10 hub genes (including PPARG, IL6, and AKT1) linked to hyperlipidaemia-related pathways. Docking studies predicted favorable binding of cianidanol to PPAR α (comparable to fenofibrate) and quercetin to HMGCR, with MD simulations supporting stable binding poses and DFT analysis identifying quercetin's key reactive sites. LC-MS tentatively confirmed rutin and kaempferol/luteolin as major extract constituents, while in vitro testing showed the crude extract significantly protected HepG2 cells from FFA-induced damage (56.60% protection at 200 μ g/mL vs. 94.97% for fenofibrate). Preliminary in vitro evidence from the MTT-based cytoprotection assay demonstrated statistically significant, concentration-dependent cellular protection by the crude *Mimosa pudica* extract at 100–200 μ g/mL (**** $p < 0.0001$ vs. FFA control; one-way ANOVA, Dunnett's post-hoc test), with 56.60% protection at 200 μ g/mL compared to fenofibrate (94.97%)

Importantly, these findings are hypothesis-generating rather than confirmatory. No direct assays validated HMGCR or PPAR α modulation by the compounds, and the MTT assay measures only cell viability, not lipid metabolism specifically. The tested extract was also crude and chemically unstandardized, making the link between computational predictions and cellular results indirect.

Overall, the study suggests a plausible dual-target mechanism involving PPAR α and HMGCR that could contribute to hypolipidemic activity, but this requires further validation through compound isolation, direct target-engagement assays, extract standardization, in vivo studies, and pharmacokinetic profiling. If confirmed, *Mimosa pudica*-derived phytoconstituents may hold promise as candidate antihyperlipidemic agents.

Ethical approval and consent to the participate

Not Applicable

Availability of data and material

The authors confirm that the data supporting the findings of this study are available within the article.

Consent for publication

I hereby provide consent for the publication of the manuscript detailed above

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CRedit authorship contribution statement

Adarsh R. Patil: Writing – original draft, Writing – review and editing, Methodology, Data curation. **Dr. Banappa S. Unger:** Guide, Supervision, Methodology, Data curation, Conceptualization. **Dr. Shridharkumar S. Biradar:** Guide, Supervision, Methodology, **Muralidhar Dhongadi:** Review and editing, **Apoorva H. Aralikatti:** Review and editing, **Harshith Kariappa C.K:** Resource, review and editing,

Declaration of competing interest

The authors declare there are no conflicts of interest.

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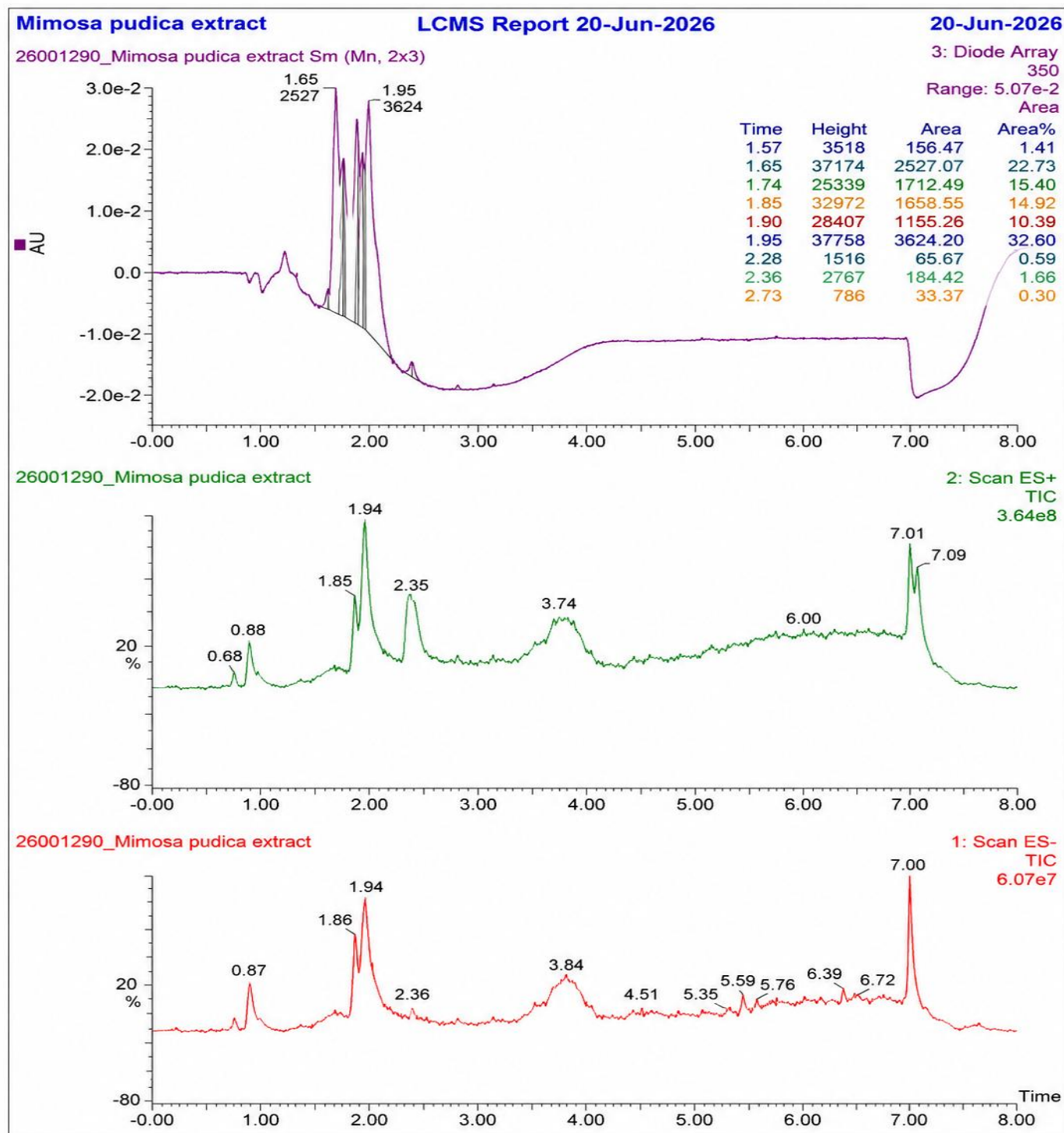


Fig.1. LC-MS chromatographic profile of *Mimosa pudica* extract showing diode-array detection and total ion chromatograms (TIC) recorded in positive (ES+) and negative (ES-) electrospray ionization modes, with major chromatographic peaks at their respective retention times.

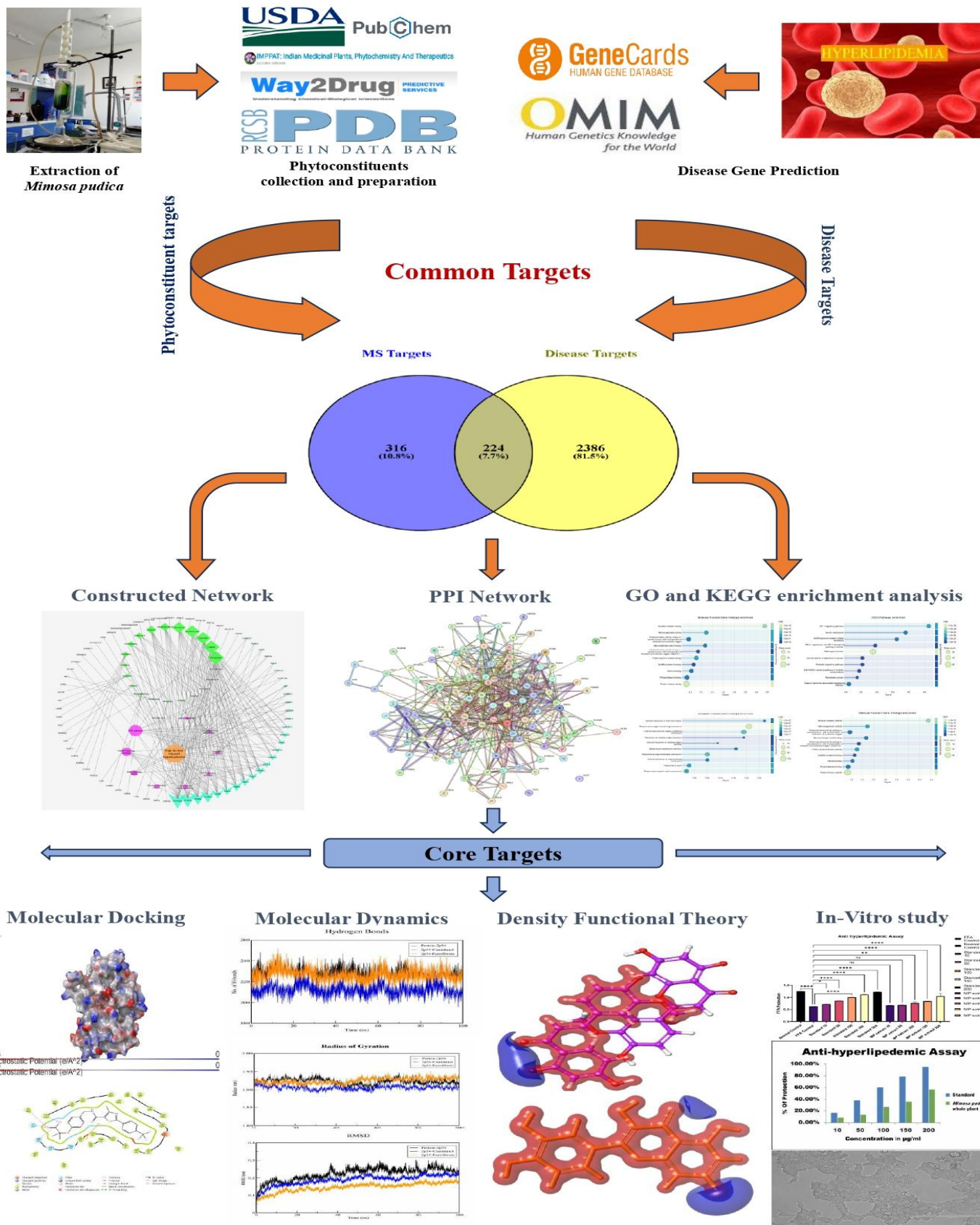


Fig. 2. Workflow of phytoconstituents collection, disease prediction, target prediction, PPI network, enrichment (GO), molecular docking, MD simulation, and in vitro analysis for Anti-hyperlipidemic potential of compounds from *Mimosa pudica*.

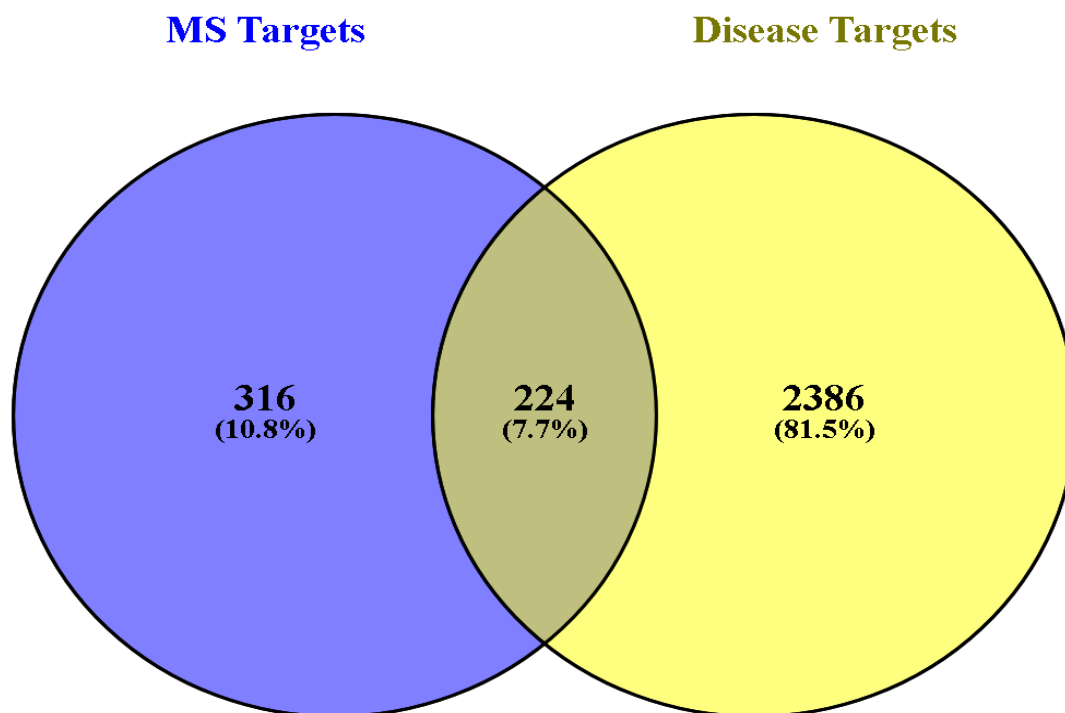


Fig. 3. Venn analysis demonstrated an overlap between the bioactive compounds MS (*Mimosa pudica*) and hyperlipidemic associated genes targeting 224 common targets that are now assumed to play a pivotal role in downstream PPI and enrichment analysis.

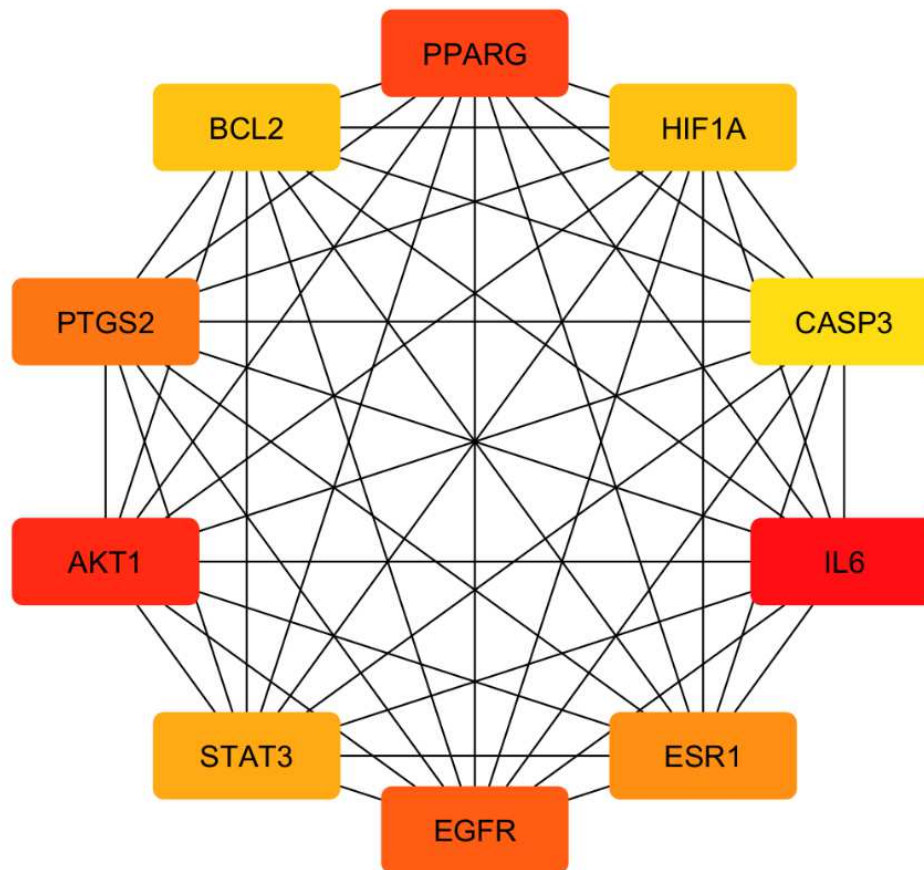


Fig. 5. Subnetwork visualization of the top 10 hub genes obtained from CytoHubba, showing their node colour intensity reflecting degree centrality (Red coloured = highest).

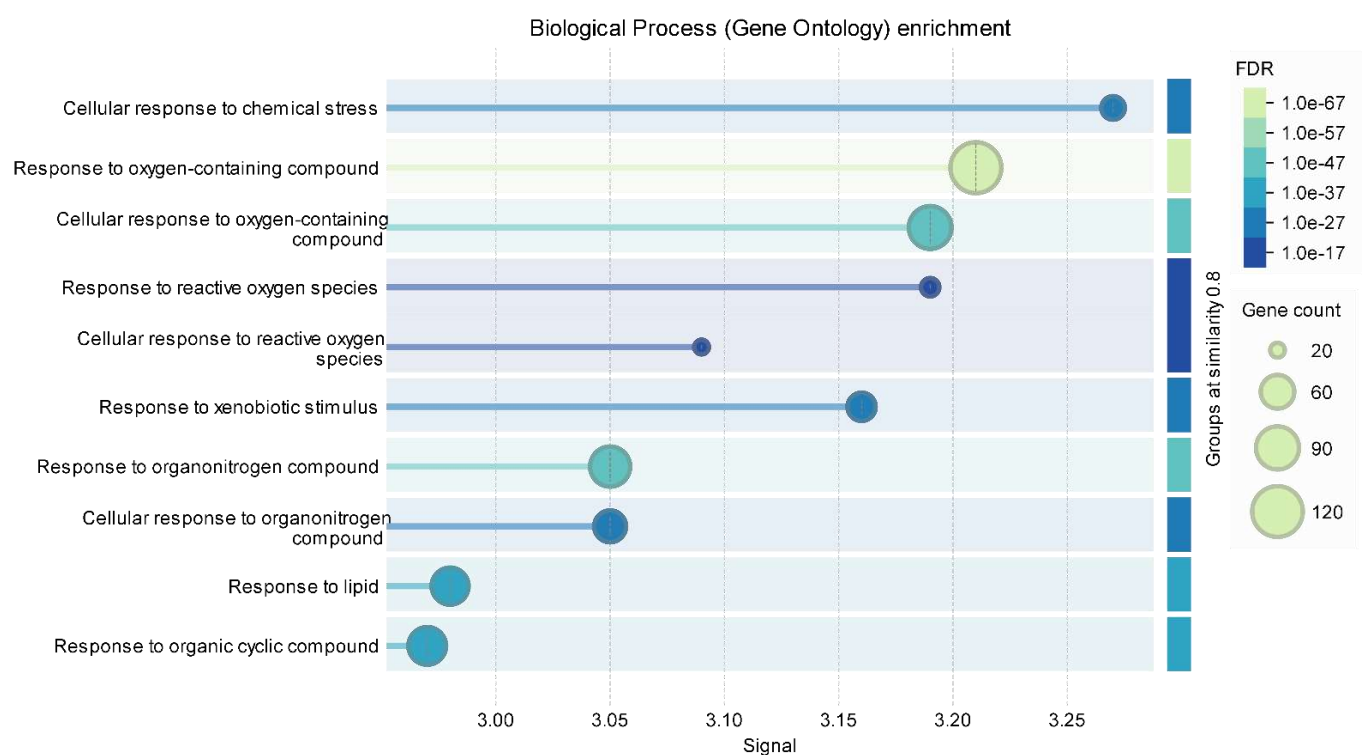


Fig. 6A. Biological Process (BP) GO enrichment showing cellular response to chemical stress as the highest-signal enriched term.

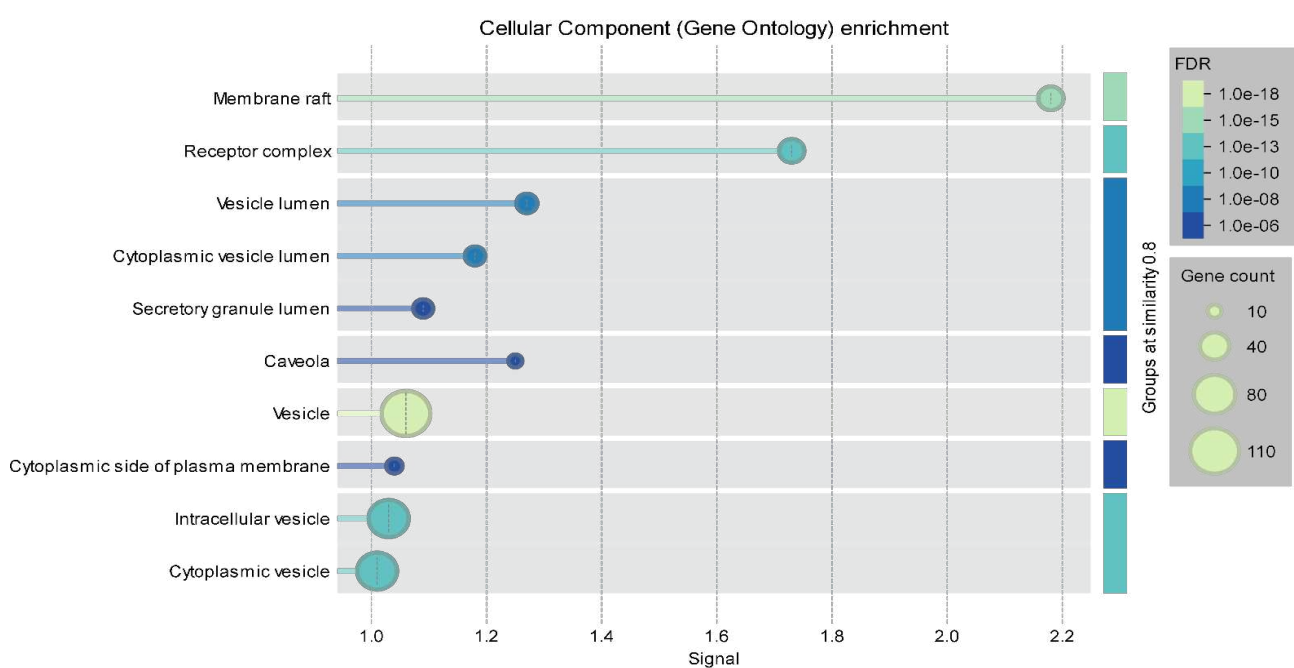


Fig. 6B. Cellular Component (CC) GO enrichment highlighting membrane raft and lipid-related processes as dominant CC terms.

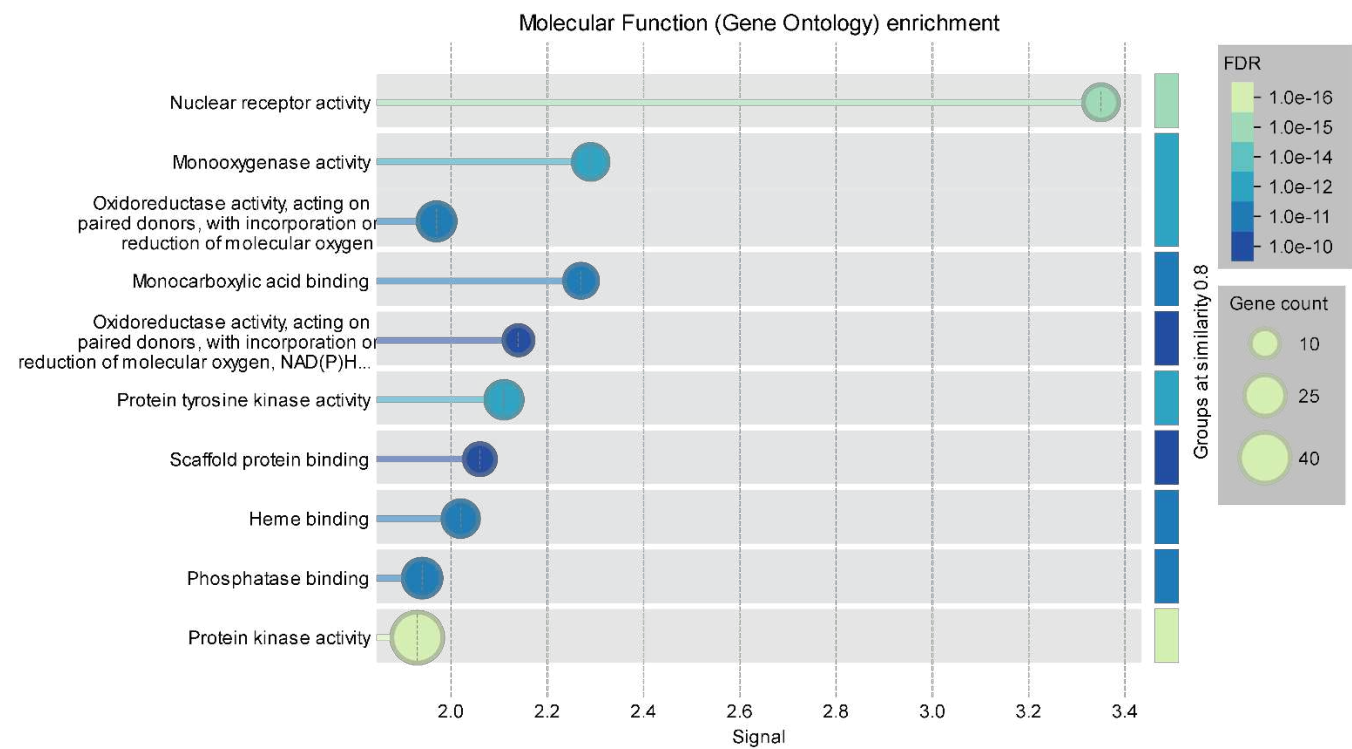


Fig. 6C. Molecular Function (MF) GO enrichment with nuclear receptor activity and receptor complex as the most significantly enriched MF terms.

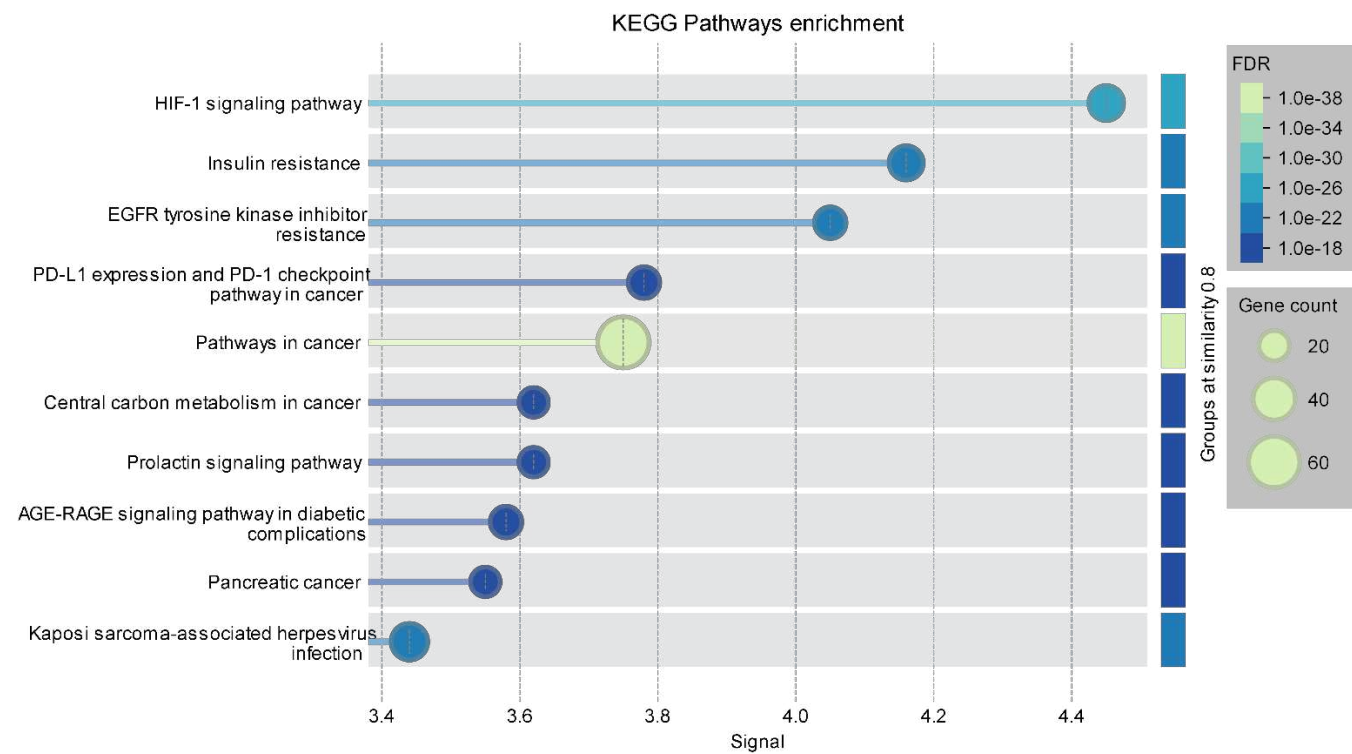


Fig. 6D. KEGG pathway enrichment analysis identifying HIF-1 signaling and insulin resistance as the top-ranked pathways ($FDR \leq 10^{-34}$).

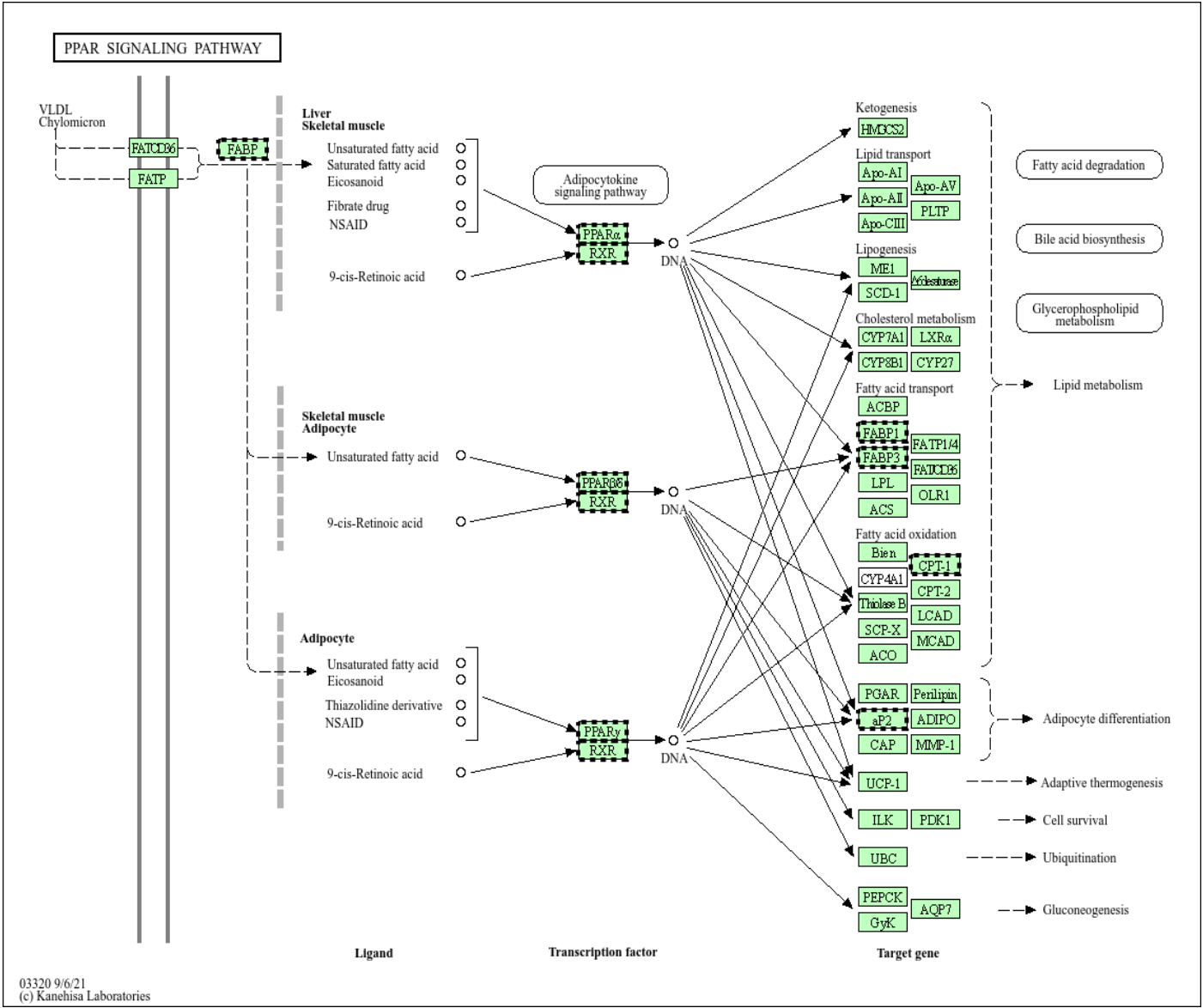


Fig. 7. Mapping of predicted target genes of *Mimosa pudica* onto the KEGG PPAR signaling pathway. Highlighted genes (green boxes) represent potential intervention points through the fractionated phytoconstituents may regulate lipid transport, fatty acid oxidation, cholesterol metabolism, and lipogenesis in high-fat diet induced hyperlipidemia.

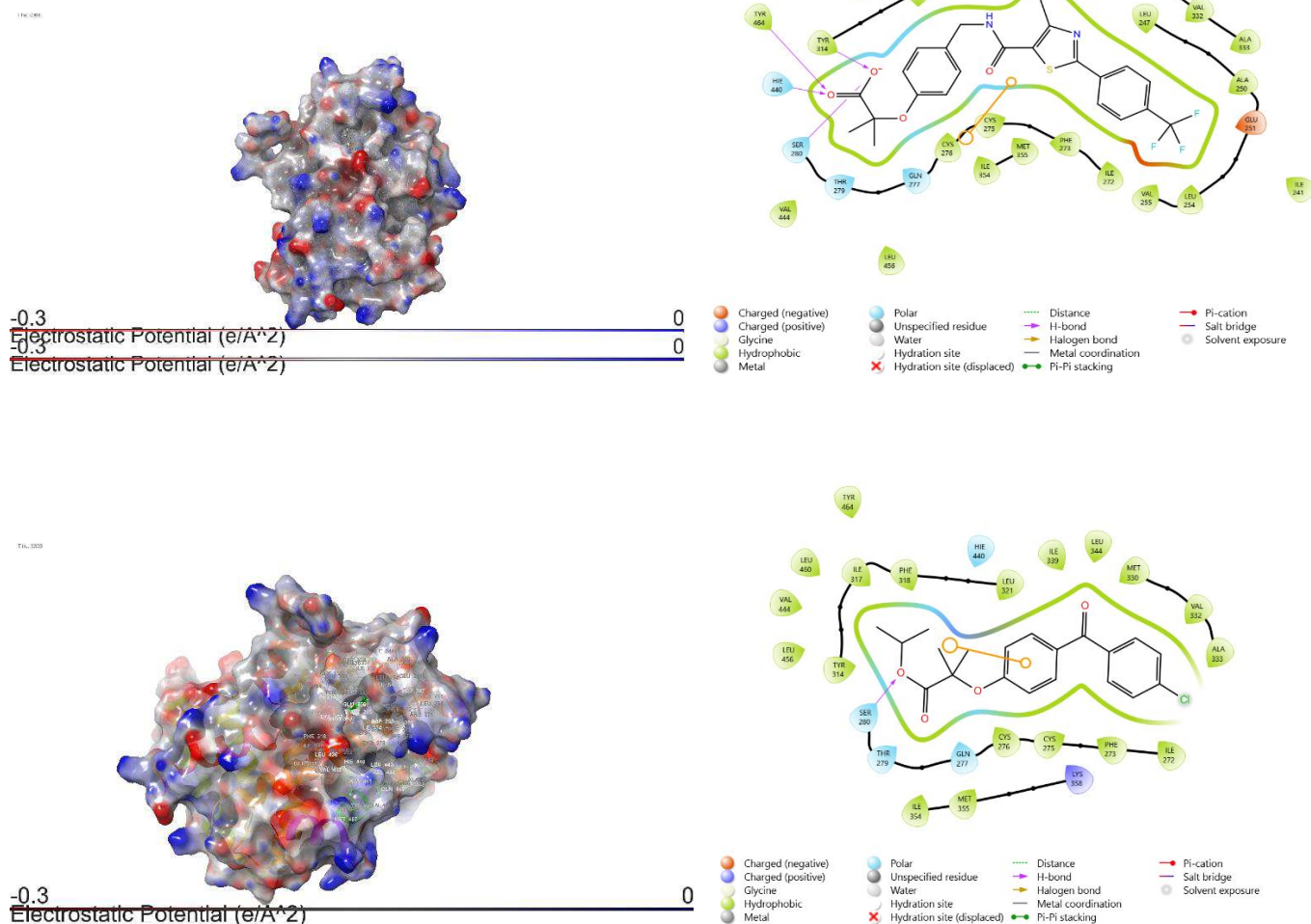


Fig. 9B. 2D and 3D molecular docking interaction diagrams of Cianidanol and Fenofibrate (standard) with PPAR α (PDB ID: 2P54)

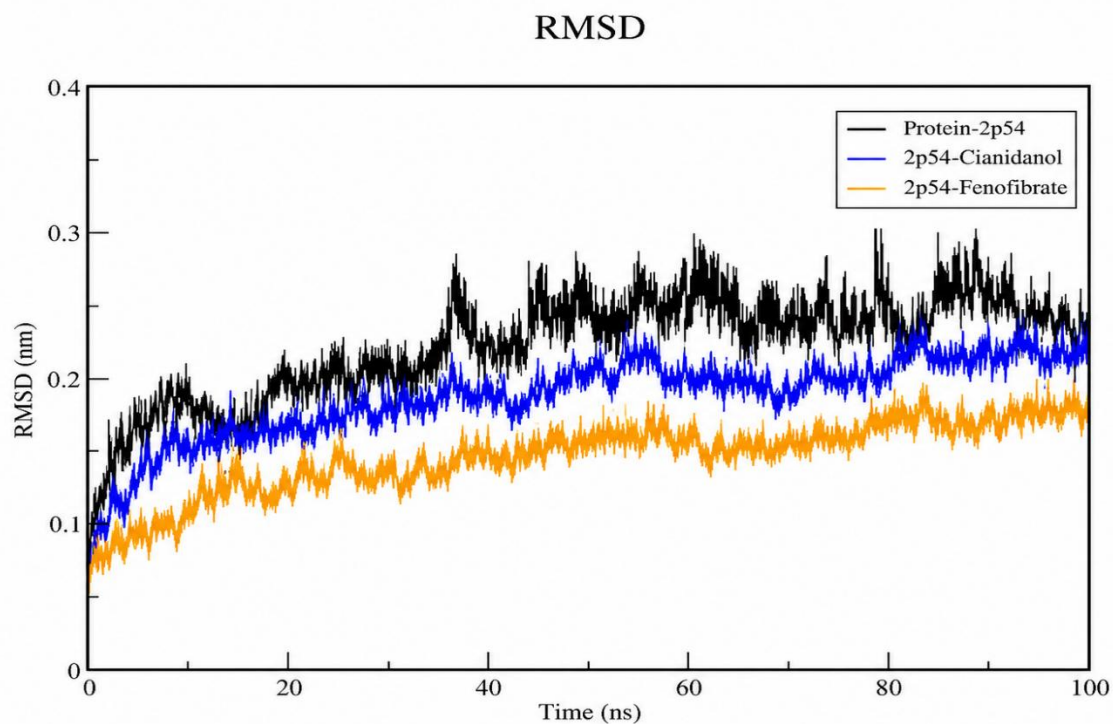


Fig. 10a. RMSD Backbone C α RMSD over 100 ns for apo PPAR α (black), PPAR α -cianidanol (blue), and PPAR α -fenofibrate (orange). All systems equilibrated by ~ 40 – 50 ns. Both ligand complexes showed lower RMSD (cianidanol: ~ 0.20 nm; fenofibrate: ~ 0.15 – 0.18 nm) than apo protein (~ 0.25 nm), confirming ligand-induced stabilization.

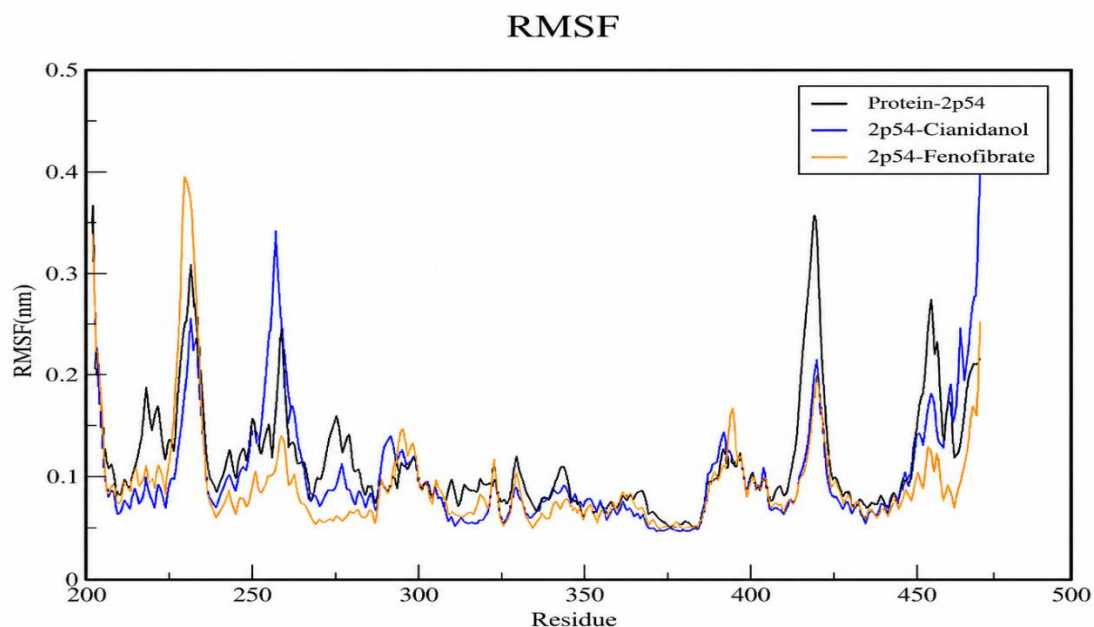


Fig. 10b. RMSF Pre-residue RMSF (residues 200–480) for apo (black), cianidanol (blue), and fenofibrate (orange) complexes. Flexibility peaks at residues ~225–232 and ~255–262 correspond to loop regions flanking the binding domain. Comparable AF-2 helix flexibility (~415–425) between cianidanol and fenofibrate suggests an agonist-like binding mode for cianidanol.

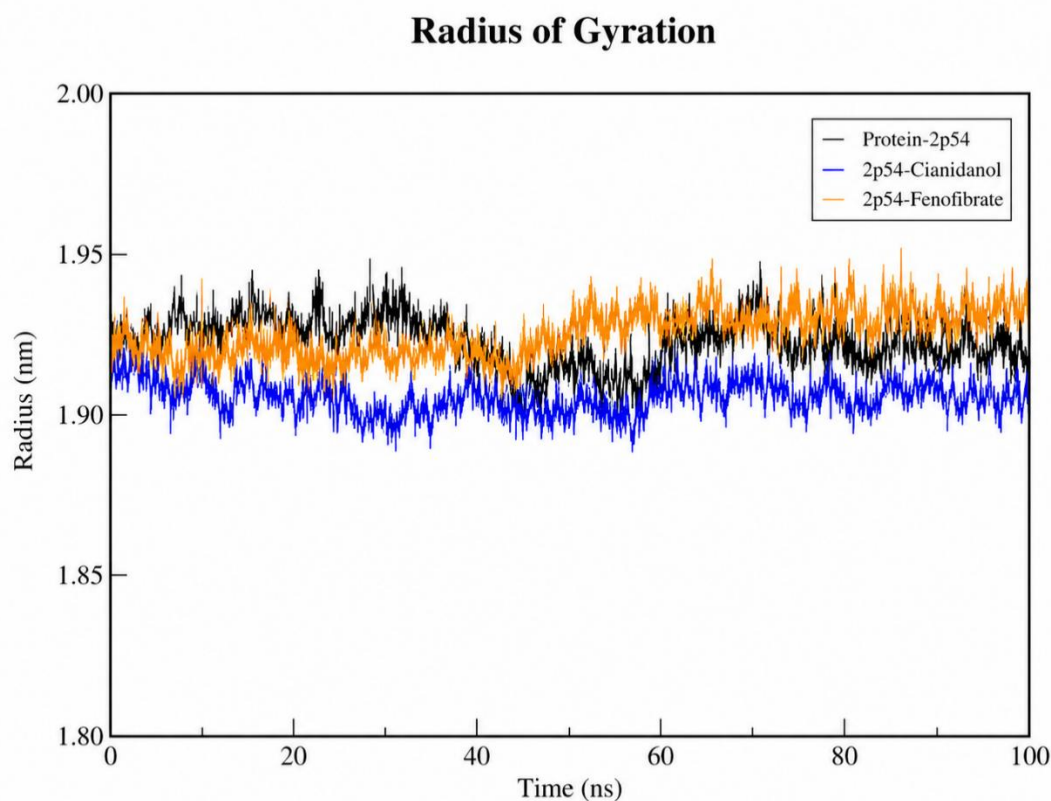


Fig. 10c. Radius of Gyration Rg over 100 ns for apo (black), cianidanol (blue), and fenofibrate (orange) complexes. All systems remained stable within 1.90–1.95 nm, confirming structural integrity with no unfolding or major conformational changes.

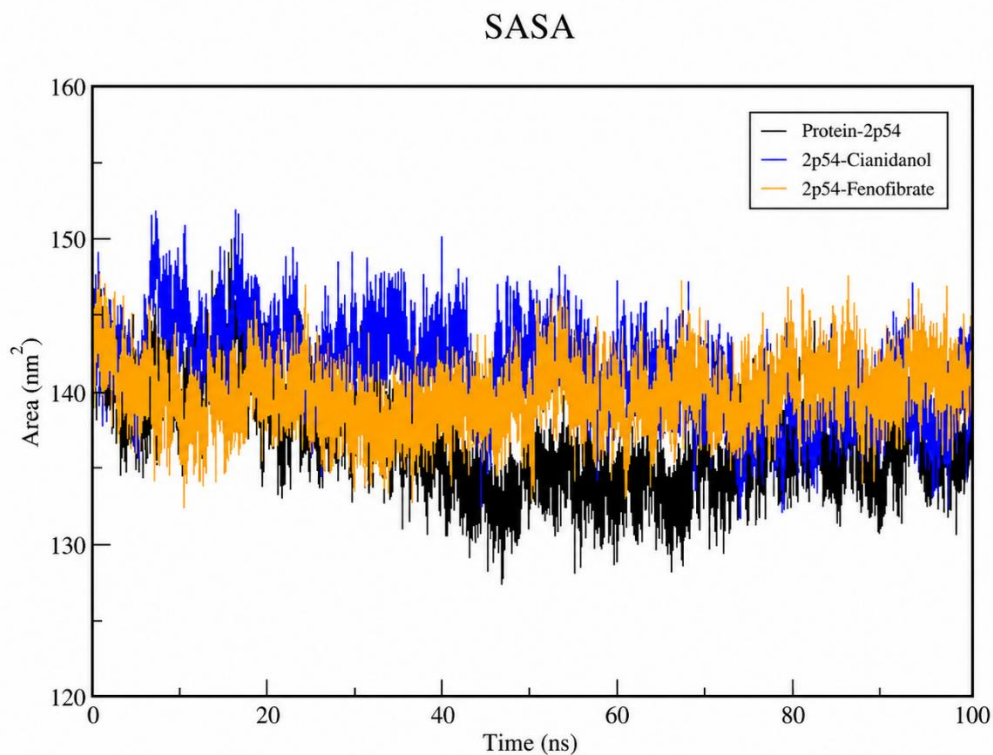


Fig. 10d. SASA over 100 ns for apo (black), cianidanol (blue), and fenofibrate (orange) complexes. Ligand-bound systems showed higher SASA (cianidanol: ~143–147 nm²; fenofibrate: ~139–143 nm²) than apo protein (~133–137 nm²), indicating a more open, solvent-accessible binding pocket upon ligand binding.

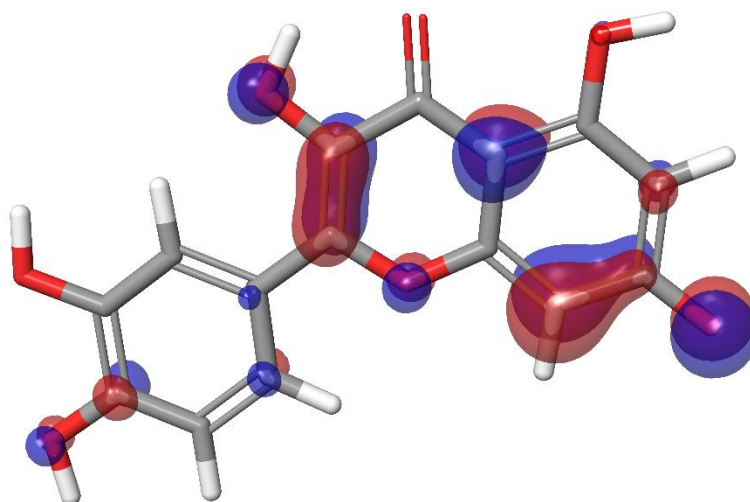


Fig. 11A. HOMO of quercetin electron density localized on catechol B-ring and C-ring carbonyl (principal electron-donating region).

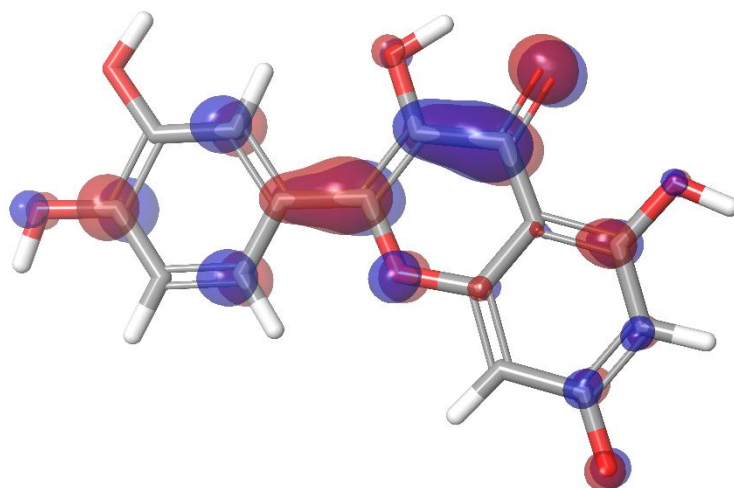


Fig. 11B. LUMO of quercetin delocalized across the flavone scaffold reflecting broad electron accepting capacity

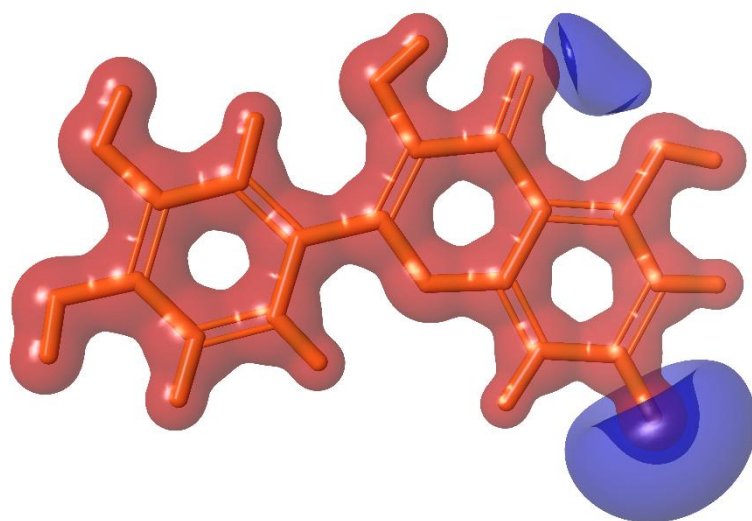


Fig. 11C. ESP Electrostatic potential predominantly electron-rich surface (red/orange) with two electrophilic lobes (blue) at C4=O and C3-OH

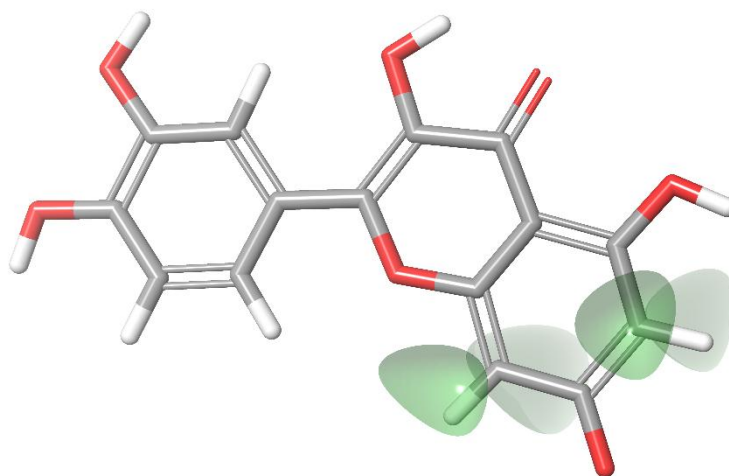


Fig. 11D. ALIE (Average local ionization energy) map of quercetin, green lobes at C3-OH and C4=O (ALIE min = 181.737 kcal/mol) identifying highest electrophilic attack susceptibility.

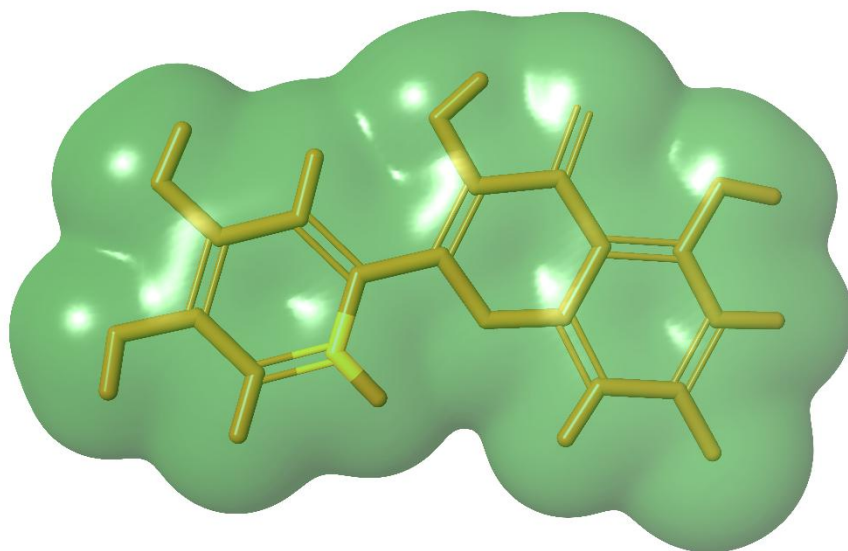


Fig. 11E. Electron density isosurface of quercetin confirming uniform charge delocalization across the aromatic ring system.

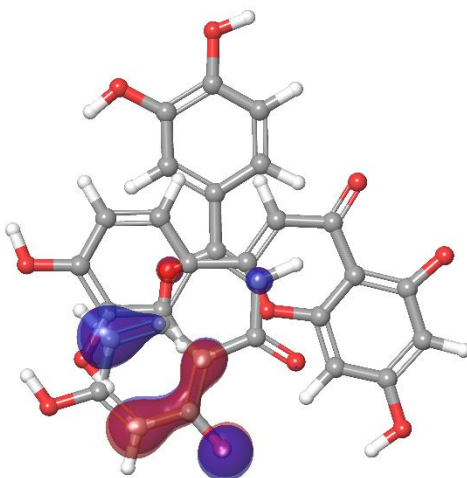


Fig. 12A. HOMO of luteolin, orbital localized over the A-ring/C-ring junction and catechol B-ring, consistent with electron-donation hydroxyl substitution pattern.

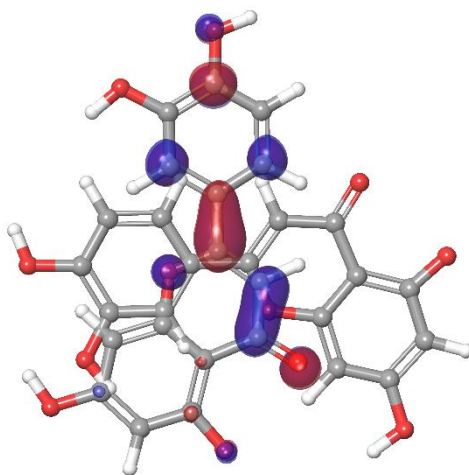


Fig. 12B. LUMO of luteolin, concentrated on the C-ring chromone C2=C3 double bond and C4=O carbonyl region.

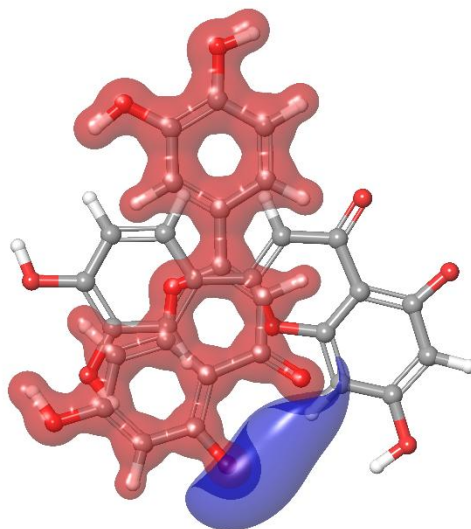


Fig. 12C. ESP (Electrostatic potential) map of luteolin, predominantly nucleophilic surface (red/pink; ESP min = -194.164 kcal/mol) with a single electrophilic lobe (blue) at the C4=O position (ESP max=39.733 kcal/mol).

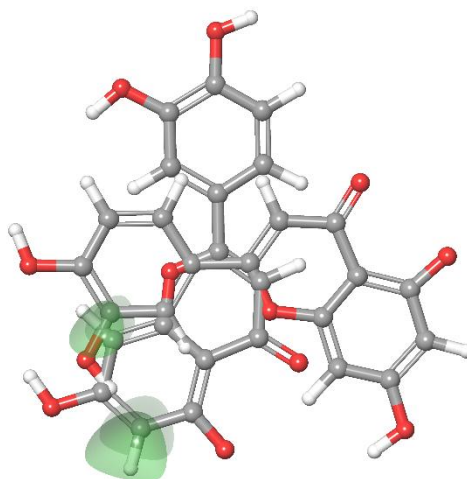


Fig. 12D. ALIE map of luteolin, green lobes at the A-ring catechol-adjacent hydroxyl position indicating sites of greatest electrophilic susceptibility.

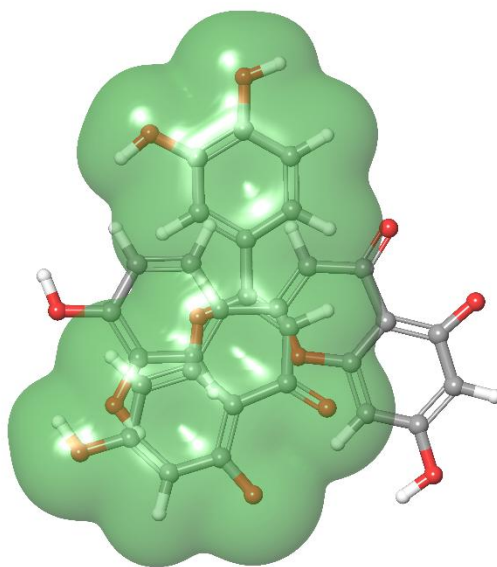


Fig. 12E. Electron density isosurface of luteolin showing uniform charge distribution across the three-ring flavone scaffold.

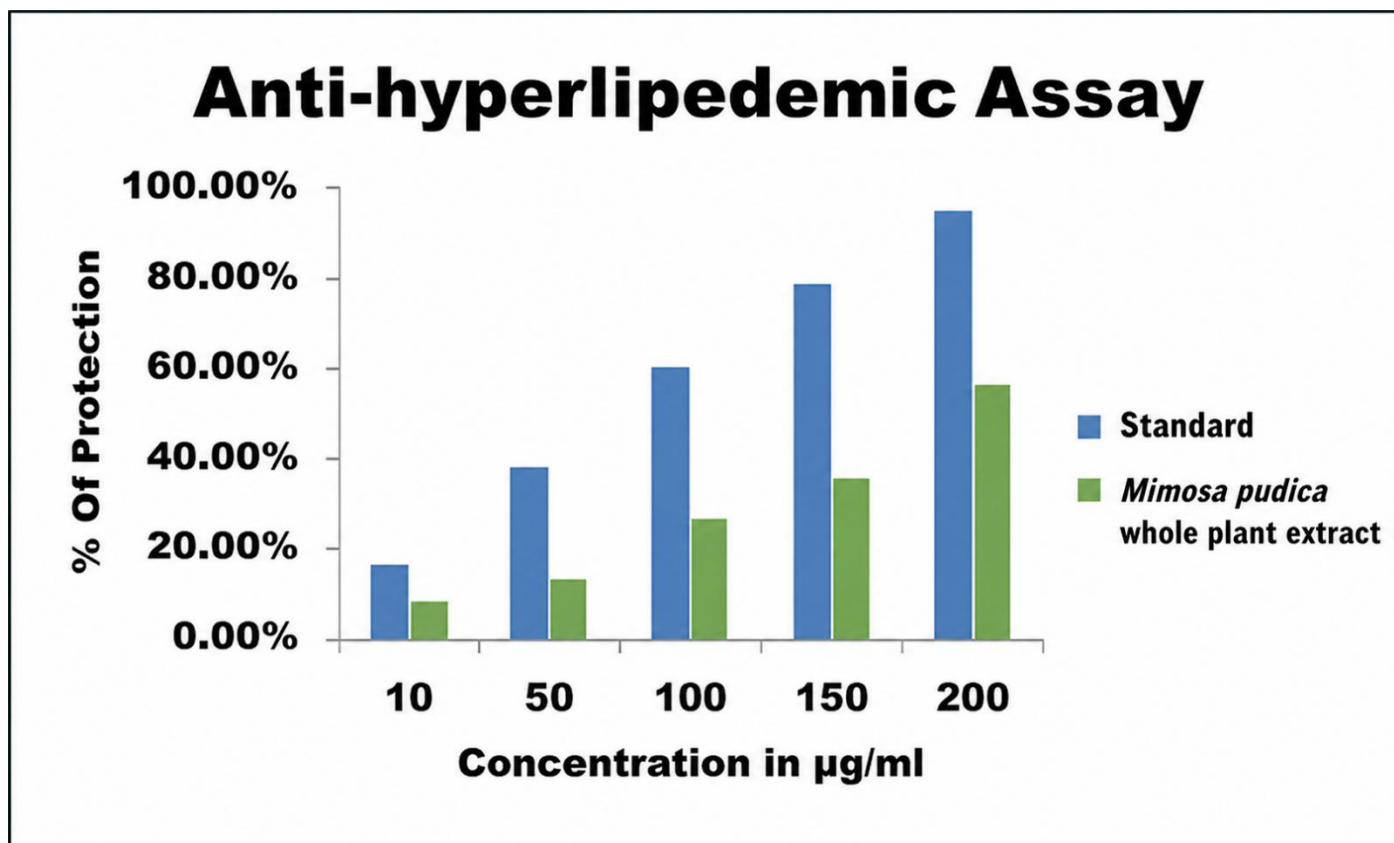


Fig. 13. Concentration-dependent percentage protection of MS and Fenofibrate (standard) against FFA-induced lipotoxicity in HepG2 cells, assessed by MTT assay (10–200 $\mu\text{g/mL}$). MS demonstrated moderate, dose-dependent cytoprotective activity, achieving 56.60% protection at 200 $\mu\text{g/mL}$ compared to 94.97% for Fenofibrate at the same concentration.

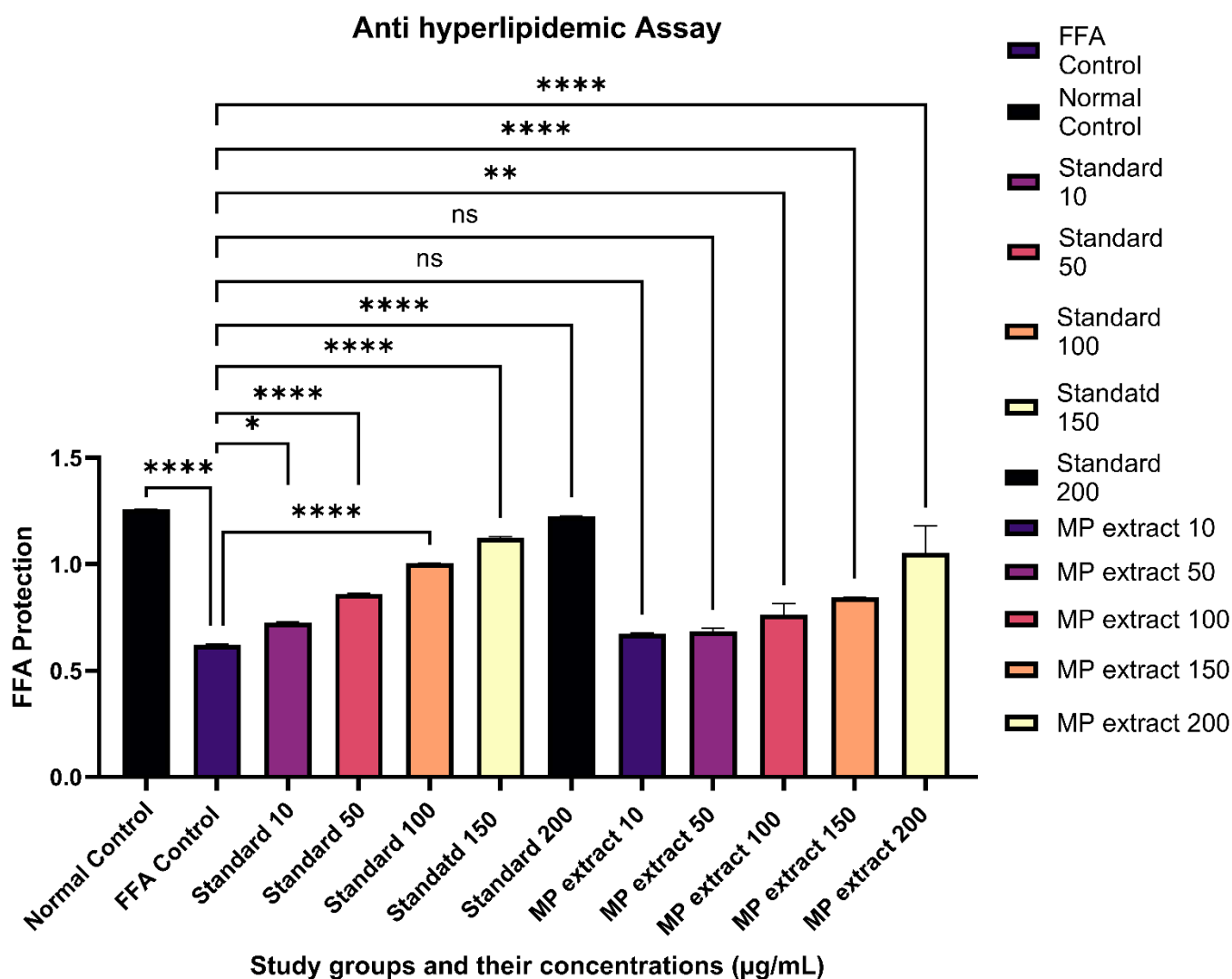


Fig. 14. Antihyperlipidemic cytoprotective activity of MP (*Mimosa pudica* whole plant extract) and fenofibrate (standard) against FFA-induced lipotoxicity in HepG2 cells assessed by MTT assay. Y-axis represents mean absorbance ($OD_{570} \pm SEM$; $n = 3$) as a measure of cell viability/FFA protection. Statistical comparisons were made by one-way ANOVA with Dunnett's post-hoc test (**** $p < 0.0001$; ** $p < 0.01$; * $p < 0.05$; ns = not significant vs. FFA control). Both fenofibrate and MP demonstrated significant concentration-dependent cytoprotection at 10–200 µg/mL.

Table 1

Databases and tool used for this research study

Name	URL
Dr. Duke's Phytochemical and Ethnobotanical Databases	https://phytochem.nal.usda.gov/
IMPPAT	https://cb.imsc.res.in/imppat/basicsearch/phytochemical
ADVERPred	https://way2drug.com/adverpred/
SwissTargetPrediction	https://www.swisstargetprediction.ch/
UniProt	https://www.uniprot.org/
GeneCards	https://www.genecards.org/
VENNY 2.1	https://bioinfogp.cnb.csic.es/tools/venny/
STRING	https://string-db.org/
OMIM	https://www.ncbi.nlm.nih.gov/omim
PDB	https://www.rcsb.org/structure/4CFE
PubChem	https://pubchem.ncbi.nlm.nih.gov/
SRPLOT	https://www.bioinformatics.com.cn/en

Table 2LC-MS chromatographic peak data for *Mimosa pudica*

Peak	RT (min)	Area (UV)	Area (%)	Height	Base m/z (ES+)	Base m/z (ES-)	Tentative LC-MS compound assignment	Phytochemical class
1	1.57	156.47	1.41	3,518	—	—	Minor unassigned component	Quercetin
2	1.65	2527.07	22.73	37,174	249.12	593.01	Putative di-C-glycosyl flavone (e.g. schaftoside)	Flavone C-glycoside
3	1.74	1712.49	15.40	25,339	—	—	Unassigned polyphenol	Unknown polyphenol
4	1.85	1658.55	14.92	32,972	453.31	—	Flavonoid-O-glycoside (M = 452 Da)	Flavonoid glycoside
5	1.90	1155.26	10.39	28,407	—	—	Unassigned polar component	Cianidanol
6	1.95	3624.20	32.60	37,758	340.38	—	Putative saponin aglycone / catechin oligomer	Saponin / Flavan-3-ol
7	2.28	65.67	0.59	1,516	—	—	Trace component	Riboflavin
8	2.36	184.42	1.66	2,767	—	332.13	Putative phenolic; kaempferol/luteolin aglycone	Flavanol / Flavone aglycone
9	2.73	33.37	0.30	786	—	—	Trace component	Thiamine

Table 3.Drug-likeness prediction of bioactive phytoconstituents of *Mimosa pudica*

	Lipinski rule of 5				DLS (>0)
Phytoconstituents	MW (≤500)	HBA (≤10)	HBD (≤5)	Log P (≤5)	
Beta carotene	436.44	0	0	3.93	0.64
Crocin	328.17	4	2	4.96	1.2
D-Glucuronic acid	194.04	7	5	-2.97	0.3
Linoleic acid	280.24	2	1	4.60	1.06
Linolenic acid	278.22	2	1	0.88	0.09
Norepinephrine	169.07	4	5	-1.38	0.98
Oleic acid	282.26	2	1	1.11	1.29
Palmitic acid	256.24	2	1	1.64	0.54
Riboflavin	378.15	7	4	-1.21	0.48
Stearic acid	284.27	2	1	2.65	0.54
Thiamine	283.07	5	4	-0.00	0.82
Quercetin	302.04	7	5	1.19	0.52
Kaempferol	286.05	6	4	1.61	0.5
Luteolin	286.05	6	4	2.78	0.38
Apigenin	270.05	5	3	3.22	0.39
Catechin	290.08	6	5	0.53	0.64
Gallic acid	170.02	5	4	0.78	0.22
Ferulic acid	194.06	4	2	1.61	0.61
Caffeic acid	180.04	4	3	1.27	0.35
Cyanidanol	290.08	6	5	0.53	0.9
Chrysoeriol	300.26	6	3	2.93	0.12

Table 4

Top 10 Hub Genes identifies from the PPI network and their functional relevance in metabolic disorders

Rank	Gene Symbol	Degree Score	Full Gene Name	Major Associated Pathway	Role in Metabolic Disorder
1	PPARG	13	Peroxisome Proliferator-Activated Receptor Gamma	PPAR signalling pathway	Regulates lipid metabolism, adipogenesis, and insulin sensitivity
2	AKT1	12	AKT Serine/Threonine Kinase 1	PI3K-Akt signalling pathway	Controls glucose uptake, insulin signalling, and cell survival
3	IL6	12	Interleukin-6	JAK-STAT signalling pathway	Mediates inflammation and contributes to insulin resistance
4	PTGS2	11	Prostaglandin-Endoperoxide Synthase 2 (COX-2)	Arachidonic acid metabolism	Regulates inflammatory responses and metabolic dysfunction
5	EGFR	11	Epidermal Growth Factor Receptor	EGFR signalling pathway	Influences cell proliferation and metabolic regulation
6	STAT3	10	Signal Transducer and Activator of Transcription 3	JAK-STAT signalling pathway	Regulates inflammation, obesity, and energy homeostasis
7	ESR1	10	Estrogen Receptor 1	Estrogen signalling pathway	Modulates lipid metabolism and insulin sensitivity

8	HIF1A	9	Hypoxia-Inducible Factor 1 Alpha	HIF-1 signalling pathway	Regulates cellular adaptation to hypoxia and glucose metabolism
9	CASP3	9	Caspase-3	Apoptosis pathway	Controls apoptosis associated with metabolic tissue damage
10	BCL2	8	B-Cell Lymphoma 2	Apoptosis pathway	Prevents apoptosis and maintains cellular survival

Table 5

Glide SP molecular docking scores and binding interactions of *Mimosa pudica* phytoconstituents against HMGCR (6SLG) and PPAR α (2P54).

Protein with PDB ID	Compound	Docking Score (kcal/mol)	Glide Score (kcal/mol)	H-Bond Residues with (bond length)	Hydrophobic/Non-H-Bond Residues
HMGCR (PDB ID: 6SLG)	Tizaterkib (Standard)	-9.422	-9.422	LYS54 (2.08 Å), MET108 (2.04 Å), GLU109 (1.12 Å)	TYR36 (1.16 Å), MET38 (4.38 Å), VAL39 (2.12 Å), ILE56 (4.09 Å)
	Quercetin	-7.873	-7.912	MET108 (1.05 Å), GLN105 (2.05 Å), ASP111 (2.045 Å), GLU71 (2.69 Å), ILE84 (1.23 Å)	ILE31 (2.16 Å), THR110 (2.88 Å)
	Riboflavin	-7.844	-7.844	ILE31 (2.62 Å), ASP111 (1.91 Å), MET108 (1.83 Å)	LYS114 (4.05 Å)
	Cianidanol	-7.525	-7.525	GLN105 (2.73 Å), GLU71 (1.92 Å), ASP111 (1.91 Å), MET108 (1.84 Å), SER153 (1.87 Å)	VAL39 (4.17 Å), LYS54 (3.44 Å)
	Luteolin	-7.376	-7.424	ASP111 (1.83 Å), MET108 (1.80 Å), GLU71 (1.79 Å)	LYS114 (2.16 Å)
	Chrysoeriol	-7.064	-7.112	ASP111 (1.88 Å), LYS114 (2.59 Å), MET108 (1.62 Å), LYS54 (2.03 Å)	-

	Apigenin	-7.061	-7.109	ASP111 (1.84 Å), GLU71 (2.07 Å), MET108 (1.81 Å)	-
	Ferulic acid	-6.920	-6.920	MET108 (1.92 Å), LYS54 (1.67 Å), ASP167 (2.19 Å)	LYS54 (4.00 Å)
	Norepinephrine	-6.581	-6.603	SER153 (1.71 Å), MET108 (1.82 Å)	ASP111 (2.12 Å)
	Gallic acid	-6.153	-6.162	LYS114 (2.58 Å), ASP111 (1.83 Å), MET108 (2.17 Å), GLU109 (2.79 Å)	-
	Thiamine	-6.141	-6.165	GLN105 (2.06 Å), LYS114 (2.06 Å)	ASP111 (2.84 Å)
PPAR α (PDB ID: 2P54)	Cianidanol	-8.047	-8.047	TYR464 (1.99 Å), HIE440 (2.14 Å), SER280 (2.50 Å)	-
	Fenofibrate (standard)	-7.789	-7.798	SER280 (2.23 Å)	-
	Riboflavin	-7.601	-7.601	THR279 (1.81 Å), ALA333 (2.88 Å)	-
	Croctin	-7.582	-7.614	TYR464 (1.87 Å)	-
	Kaempferol	-7.548	-7.587	SER280 (2.52 Å)	-
	Gallic acid	-6.690	-6.699	TYR464 (1.81 Å), SER280 (2.17 Å)	HIE440 (4.76 Å)
	Thiamine	-6.415	-6.439	SER280 (2.00 Å)	HIE440 (5.24 Å), PHE273 (5.24 Å)
	Ferulic acid	-5.558	-5.558	TYR314 (1.94 Å), TYR464 (1.85 Å), HIE440 (1.90 Å)	-
	Caffeic acid	-5.558	-5.558	TYR464 (1.89 Å), HIE440 (1.95 Å), TYR314 (1.79 Å)	-
	Mimosine	-5.026	-5.226	TYR464 (2.36 Å)	HIE440 (4.54 Å)
	Norepinephrine	-4.277	-4.245	ALA333 (2.57 Å)	-

Table 6

DFT-derived frontier orbital energies and global reactivity descriptors of quercetin and luteolin calculated at the B3LYP-D3/6-31G** level of theory.

Parameter	Quercetin	Luteolin
E_HOMO (eV)	-5.034	-4.925
E_LUMO (eV)	-1.469	-1.415
Energy gap, ΔE (eV)	3.565	3.510
Ionization potential, IP (eV)	5.034	4.925
Electron affinity, EA (eV)	1.469	1.415
Electronegativity, χ (eV)	3.252	3.170
Chemical potential, μ (eV)	-3.252	-3.170
Global hardness, η (eV)	1.782	1.755
Global softness, S (eV ⁻¹)	0.281	0.285
Electrophilicity index, ω (eV)	2.966	2.863
ESP max (Kcal/mol)	-	39.733
ESP mean (Kcal/mol)	-	-69.503
ESP min (Kcal/mol)	-	-194.164
ALIE max (Kcal/mol)	372.451	-
ALIE mean (Kcal/mol)	258.694	-
ALIE min (Kcal/mol)	181.737	-

Table 7

Effect of MS and Fenofibrate on cell viability and percentage protection against FFA-induced lipotoxicity in HepG2 cells assessed by MTT assay.

SR NO	SAMPLE CODE	Conc. (µg/ml)	OD			Mean	% Of Viability	% Of Protection
1	Normal Control	-	1.256	1.257	1.260	1.257	100.0%	100.0%
2.	FFA Control	-	0.620	0.622	0.625	0.622	49.36%	0.00%
3.	Standard (Fenofibrate)	10	0.720	0.725	0.730	0.725	57.72%	16.51%
		50	0.862	0.859	0.860	0.860	68.47%	37.74%
		100	1.002	1.006	1.004	1.004	79.94%	60.38%
		150	1.126	1.129	1.121	1.125	89.57%	79.40%
		200	1.222	1.225	1.226	1.224	97.45%	94.97%
4.	MS 5L	10	0.675	0.672	0.677	0.674	53.66%	8.49%
		50	0.702	0.705	0.708	0.705	56.13%	13.36%
		100	0.796	0.793	0.790	0.793	63.14%	27.20%
		150	0.842	0.846	0.845	0.844	67.20%	35.22%
		200	0.978	0.983	0.980	1.200	78.03%	56.60%

Table 8

Descriptive statistics (Mean $\pm$ SEM) of optical density values for the anti-hyperlipidemic assay in FFA-induced HepG2 cells

Study groups	Concentration ($\mu\text{g/mL}$)	Standard Deviation	Mean $\pm$ SEM
Normal Control	-	0.002082	1.258 $\pm$ 0.001202
FFA Control	-	0.002517	0.6223 $\pm$ 0.001453
Standard	10	0.005000	0.7250 $\pm$ 0.002887
	50	0.001528	0.8603 $\pm$ 0.0008819
	100	0.002000	1.004 $\pm$ 0.001155
	150	0.004041	1.125 $\pm$ 0.002333
	200	0.002082	1.224 $\pm$ 0.001202
<i>Mimosa pudica</i> whole plant methanolic extract	10	0.002517	0.6747 $\pm$ 0.001453
	50	0.01607	0.6837 $\pm$ 0.009280
	100	0.05090	0.7637 $\pm$ 0.02938
	150	0.002082	0.8443 $\pm$ 0.001202
	200	0.1268	1.054 $\pm$ 0.07318

Supplementary Files

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

- [NPCytoscapeData.xlsx](#)
- [DiseasegeneTargets.xlsx](#)
- [Extractionimage.jpeg](#)
- [NetworkPharmacologyData.xlsx](#)