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In Silico Study of Selected Bambara Groundnut Bioactive Compounds on Anti-Lipogenic Protein Targets in Non-Alcoholic Fatty Liver Disease

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ABSTRACT
Non-alcoholic fatty liver disease (NAFLD) is characterised by excessive hepatic triglyceride accumulation independent of significant alcohol intake and is strongly associated with obesity, insulin resistance and type 2 diabetes. Bambara groundnut (Vigna subterranea) is a nutrient-dense legume rich in phenolic compounds with documented antioxidant and anti-inflammatory properties. This study employed an integrated network pharmacology and molecular docking approach to investigate the potential of selected Bambara groundnut bioactive compounds against anti-lipogenic protein targets in NAFLD. Phytochemicals were subjected to target prediction (SwissTargetPrediction), disease-gene mapping (GeneCards), consensus target identification (Venny 2.1.0), Gene Ontology and KEGG enrichment (ShinyGO), protein–protein interaction network analysis (STRING/Cytoscape) and molecular docking with MM-GBSA binding free energy calculations (Schrödinger Maestro). A total of 329 consensus genes were identified. Hub genes included EGFR, SRC, ERBB2, PIK3CA and PIK3R1. Molecular docking revealed that isoquercitrin exhibited the strongest binding affinity for EGFR (Glide score −13.520 kcal/mol; MM-GBSA ΔGbind −59.60 kcal/mol). Rutin, quercitrin, ellagic acid, myricetin and luteolin also showed favourable interactions across multiple targets. These findings suggest that Bambara groundnut flavonoids, particularly isoquercitrin and rutin, may modulate key signalling pathways involved in hepatic lipid accumulation and inflammation, supporting their potential as nutraceutical candidates for NAFLD management. Experimental validation is recommended.

Keywords: Bambara Groundnut, Molecular Docking, Isoquercitrin, Network Pharmacology, Nutraceuticals, Non-Alcoholic Fatty Liver Disease.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) represents the most common chronic liver disorder globally and is characterised by excessive hepatic fat accumulation in the absence of significant alcohol consumption or other secondary causes of liver injury (Chalasani et al., 2018; Powell et al., 2021). The rising prevalence of NAFLD is strongly linked to increasing rates of obesity, physical inactivity and nutritionally poor dietary patterns, making it a major public health concern worldwide (Younossi et al., 2018).

Epidemiological evidence indicates that NAFLD affects an estimated 882 million individuals globally, although this figure is likely underestimated due to underdiagnosis and limited screening in asymptomatic populations (Ge et al., 2020). In Nigeria, NAFLD is increasingly recognised as an important contributor to chronic liver disease, particularly among individuals with metabolic risk factors. Hospital-based studies have reported significantly higher prevalence among obese individuals (36.4%) compared with non-obese controls (0.4%), with strong associations observed with obesity, type 2 diabetes mellitus, hypertension and dyslipidaemia (Poochanasri et al., 2025). NAFLD affects roughly 30–40% of men and 15–20% of women in the general population. The burden is even greater among people with type 2 diabetes mellitus (T2DM), where up to 70% may have NAFLD (Feyisa, 2026).

Oxidative stress causes damage to DNA, affecting both mitochondrial and nuclear genetic material. These changes can lead to mutations and interfere with the cell's normal repair systems. In addition, reactive oxygen species (ROS) can oxidise proteins, causing structural and functional changes through post-translational modifications. As a result, essential metabolic and structural proteins may lose their proper function, contributing to disease progression (Qin et al., 2026). NAFLD is also closely linked to an increased risk of cardiovascular disease (CVD), which remains the leading cause of death among people with this condition. A more severe form of NAFLD, known as non-alcoholic steatohepatitis (NASH), involves liver inflammation in addition to fat accumulation and represents a major and growing public health issue (Chiriac et al., 2021).

Bambara groundnut (Vigna subterranea) is an underutilised African legume with high nutritional value (carbohydrates 55–70%, proteins 17–25%, dietary fibre 5.2–6.4% and fat 1.4–12%) and reported antioxidant, antimalarial, anti-inflammatory and antimicrobial properties (Hillocks et al., 2012; Okafor et al., 2022). Its phenolic profile includes quercetin, isoquercitrin, rutin, kaempferol, myricetin, luteolin, catechin, epicatechin, caffeic acid, ellagic acid, chlorogenic acid and gallic acid.

The aim of this study was to determine the potential therapeutic effect of selected Bambara groundnut bioactive compounds on anti-lipogenic genes and proteins via an in

silico approach integrating network pharmacology and molecular docking.

MATERIALS AND METHODS

Phytochemical Profiling

The bioactive phenolic compounds of Bambara groundnut were retrieved from the literature (Okafor et al., 2022). The compounds considered and their approximate concentrations (mg/g) in raw and cooked samples are summarised in Table 1.

Table 1: Phenolic Compounds Identified in Bambara Groundnut (Adapted from Okafor Et Al., 2022)

Phenolic Compound	Raw (mg/g)	Cooked (mg/g)
Quercetin	0.007–6.39	3.94
Quercitrin	2.05	1.58
Isoquercitrin	0.42	0.29
Kaempferol	0.052–2.18	3.15
Rutin	0.427–24.46	3.16
Myricetin	0.062–1.800	–
Luteolin	1.09	1.67
Catechin	0.01–2.34	–
Epicatechin	1.15	0.39
Caffeic acid	3.65	3.75
Ellagic acid	0.005–1.09	1.42
Chlorogenic acid	0.03–2.37	0.50
Gallic acid	0.05–1.03	0.41

Target Prediction

The bioactive phytochemicals identified from Bambara groundnut were subjected to target prediction using the SwissTargetPrediction web tool (http://www.swisstargetprediction.ch/) (Daina et al., 2019; Gao et al., 2020). The SMILES structures of each compound were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and inputted into SwissTargetPrediction. The tool estimates the most probable protein targets based on a combination of 2D and 3D similarity with known ligands, specifically focusing on Homo sapiens as the species of interest. The resulting predicted protein targets for all compounds were compiled and used in subsequent network pharmacology and enrichment analyses.

Identification of NAFLD-Associated Targets

Gene targets associated with NAFLD were retrieved from the GeneCards database (https://www.genecards.org/), a comprehensive compendium of human gene-centric information (Luo et al., 2022). The keyword "NAFLD" was used to query the database, and genes were filtered based on their relevance scores, with higher-scoring genes considered more significantly associated with the disease condition. The resulting gene list was compiled as disease-associated targets.

Identification of Consensus Targets

The predicted protein targets of Bambara groundnut-derived phytochemicals were compared with genes linked to NAFLD to identify possible therapeutic targets. The overlap of the two gene sets was assessed using Venny 2.1.0, an online Venn diagram tool (https://bioinfogp.cnb.csic.es/tools/venny/). The overlapping genes, known as consensus targets, signify candidate proteins that are connected with the disease and may be influenced by the bioactive substances found in Bambara groundnut. The consensus genes were further analysed for gene ontology enrichment, pathway assessment and network-based evaluations.

Gene Ontology and KEGG Pathway Enrichment Analysis

Functional enrichment analysis was performed on the consensus target genes to examine their biological roles and participation in disease-associated pathways (Zhang et al., 2024). Gene Ontology (GO) analysis, encompassing Biological Process (BP), Molecular Function (MF) and Cellular Component (CC), in conjunction with Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway analysis, was conducted using ShinyGO v0.82 (http://bioinformatics.sdstate.edu/go/) (Ge et al., 2020).

Protein–Protein Interaction Network Construction and Hub Gene Identification

To explore the functional associations among the consensus target genes, a Protein–Protein Interaction (PPI) network was constructed using the STRING database (https://string-db.org/) (Bu et al., 2021). The consensus gene list was uploaded to the platform with the species set to Homo sapiens, and the interaction network was generated using a highest confidence score threshold of 0.9. Disconnected nodes were excluded to enhance network clarity.

The resulting network was downloaded and imported into Cytoscape software (version 3.10.0) for visualisation and topological analysis. CytoHubba, a Cytoscape plugin, was employed to identify hub genes based on network topology, using metrics such as node degree, betweenness centrality and maximal clique centrality (MCC). The top-ranking hub genes were selected for further molecular docking analysis as key targets potentially modulated by Bambara groundnut-derived phytochemicals in NAFLD.

Molecular Docking and MM-GBSA Binding Free Energy Calculation

Molecular docking studies were conducted to evaluate the interaction and binding affinity between Bambara groundnut-derived bioactive compounds and the identified hub proteins. The 3D structures of target proteins were retrieved from the RCSB Protein Data Bank (PDB) (https://www.rcsb.org/) (Burley et al., 2023), while the chemical structures of the bioactive compounds were obtained from the PubChem database in SDF format (Kim et al., 2019).

All computational procedures were carried out using the Maestro molecular modelling suite (Schrödinger, Release 2021-2). Protein structures were prepared using the Protein

Preparation Wizard, where water molecules beyond 5 Å were removed. The proteins were processed by assigning bond orders, adding hydrogen atoms, optimising hydrogen bonds and minimising the structure using the OPLS4 force field. Ligands were prepared using the LigPrep module. This included generating ionisation states at pH 7.0 ± 2.0, optimising geometries and generating low-energy conformers while preserving original chiral centres. Potential binding sites were identified using SiteMap, and the most druggable site was selected for each target protein. A receptor grid was generated around the predicted binding pocket using the Receptor Grid Generation module. Docking simulations were performed using the Glide module in Extra Precision (XP) mode. Each prepared ligand was docked into the binding site of the respective protein, and the best binding poses were selected based on docking score and protein–ligand interaction profile. The Prime MM-GBSA module was used to calculate the binding free energies (ΔG_bind) of the protein–ligand complexes employing the VSGB solvation model and the OPLS4 force field.

RESULTS AND DISCUSSION

Consensus Targets between Bambara Groundnut Bioactives and NAFLD

Integration of the predicted Bambara groundnut targets with NAFLD-associated genes via a two-way Venn diagram revealed key overlapping and unique gene populations. A small subset of 49 genes (0.5%) was exclusive to the Bambara groundnut predicted targets. Overlaps included 329 genes (3.3%) shared between Bambara groundnut targets and NAFLD genes, representing potential points of interaction between Bambara groundnut bioactivity and NAFLD pathways. The NAFLD gene set contained a large exclusive population of 9,662 genes (96.2% of the total combined gene universe), reflecting the broad genetic landscape of NAFLD not linked to Bambara groundnut targets in this analysis (Figure 1).

The core finding of this analysis was the identification of 329 consensus genes present in both datasets. These 329 genes represent high-priority candidates for follow-up functional validation, as they directly bridge the study's two focal areas.

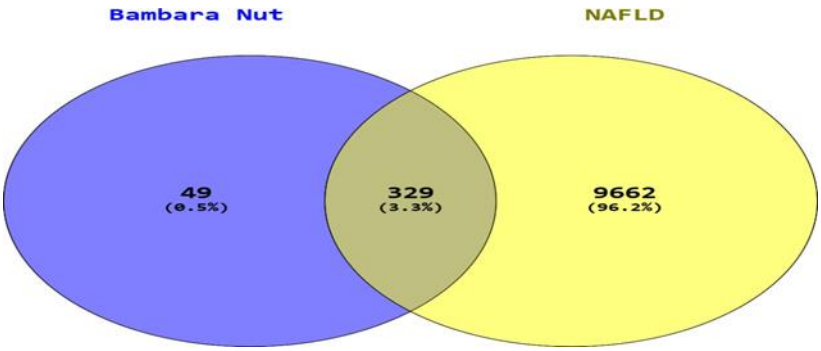

Figure 1: Venn Diagram Showing the Consensus Genes Between Bambara Groundnut Predicted Targets and NAFLD-Associated Genes

Gene Ontology and KEGG Pathway Enrichment

To further characterise the biological roles of the 329 consensus genes, Gene Ontology (GO) enrichment analysis was performed across three domains: Molecular Function (MF), Cellular Component (CC) and Biological Process (BP). In the Molecular Function category (Figure 2), the consensus genes exhibited significant enrichment in enzyme binding. The most significantly enriched terms included transcription coactivator binding and transcription coregulator binding, indicating extensive modulation of gene expression networks. Additionally, RNA polymerase II-specific DNA-binding transcription factor binding was significantly enriched, further supporting active transcriptional control. Notably, protein kinase activity and kinase binding were also enriched, suggesting the involvement of signalling cascades that regulate metabolic and inflammatory responses. Other enriched functions, including phosphotransferase activity and nucleotide binding, reflect alterations in enzymatic and metabolic processes.

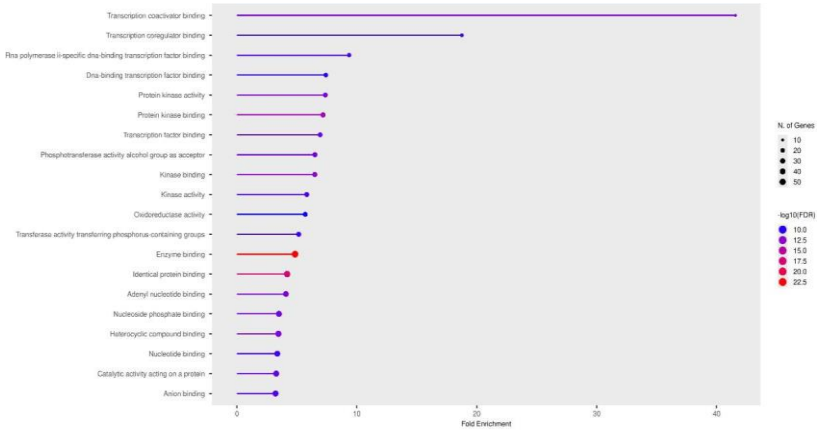

Figure 2: Bar Plot Showing the Gene Ontology Molecular Function Enrichment Analysis of The 329 Consensus Genes

Cellular Component analysis (Figure 3) indicated that these genes are predominantly localised to vesicles. The most enriched terms included ficolin-1-rich granule lumen, vesicle lumen and secretory granule lumen, indicating enhanced involvement of intracellular trafficking and secretory pathways. Significant enrichment was also observed in cytoplasmic vesicle lumen and extracellular vesicle-related compartments, suggesting active intercellular communication and molecular transport. Furthermore, enrichment of receptor complexes and transcription regulator complexes highlights the spatial organisation of signalling and gene regulatory machinery. Importantly, mitochondrion and endoplasmic reticulum were among the enriched components, underscoring the role of organelle dysfunction in NAFLD.

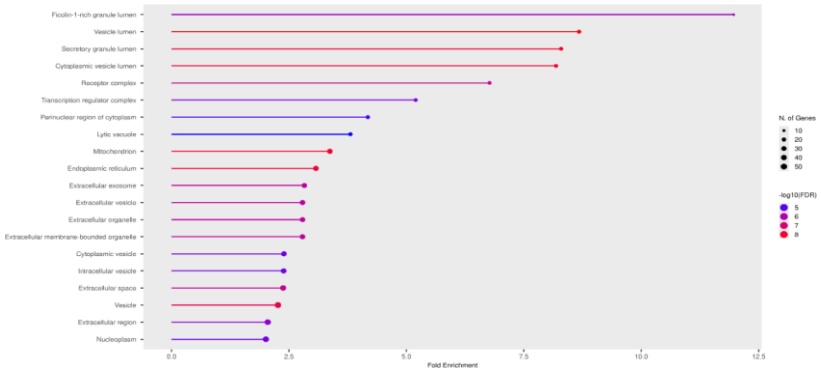

Figure 3: Bar Plot Showing the Gene Ontology Cellular Component Enrichment Analysis of The 329 Consensus Genes

Biological Process analysis (Figure 4) highlighted the involvement of these genes in response to chemical stimuli and inflammatory regulation. The most significantly enriched term was response to lipid, highlighting the central role of lipid metabolism in the dataset. Additional enriched processes included cellular response to oxygen-containing compounds and response to nitrogen compounds, indicating oxidative and metabolic stress. Processes related to apoptosis and programmed cell death were also significantly enriched, suggesting activation of pathways involved in hepatocellular injury. Furthermore, enrichment of response to chemical stimulus, regulation of signalling and cell communication reflects the involvement of inflammatory and stress-response mechanisms.

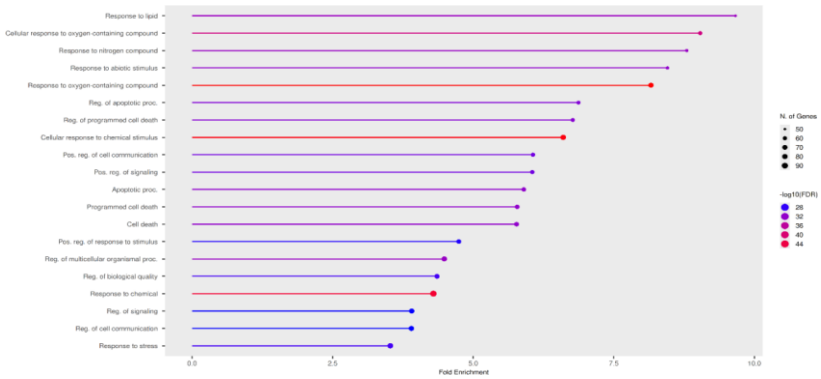

Figure 4: Bar plot showing the Gene Ontology Biological Process enrichment analysis of the 329 consensus genes

Pathway enrichment and KEGG-based mapping (Figure 5) revealed that the identified targets are extensively involved in key molecular processes underlying NAFLD, including insulin resistance, lipid metabolism, inflammation, cellular stress and apoptosis. Dysregulation of the insulin signalling cascade, marked by altered activity of IRS-1/2, PI3K, Akt and GSK-3, indicates impaired insulin sensitivity, while concurrent activation of lipogenic regulators such as SREBP-1c, LXR and RXR suggests enhanced de novo fatty acid synthesis and hepatic lipid accumulation. The PPAR signalling axis further showed metabolic imbalance, with reduced AMPK activity and differential regulation of PPAR isoforms favouring lipid storage over fatty acid oxidation. Inflammatory pathways were prominently activated through the TNF-α/NF-κB signalling axis, accompanied by increased expression of pro-inflammatory cytokines including IL-6 and IL-1, indicating progression toward steatohepatitis. Additionally, adipocytokine signalling was disrupted, with elevated leptin and reduced adiponectin activity contributing to both inflammation and metabolic dysfunction. Evidence of endoplasmic reticulum stress was observed via activation of the PERK–eIF2α–ATF4–CHOP pathway, alongside increased oxidative stress characterised by reactive oxygen species generation and mitochondrial dysfunction. These stress conditions were associated with activation of apoptotic pathways, as indicated by upregulation of caspase-3 and caspase-7, while increased levels of fibrogenic mediators such as TGF-β1 suggest progression toward fibrosis.

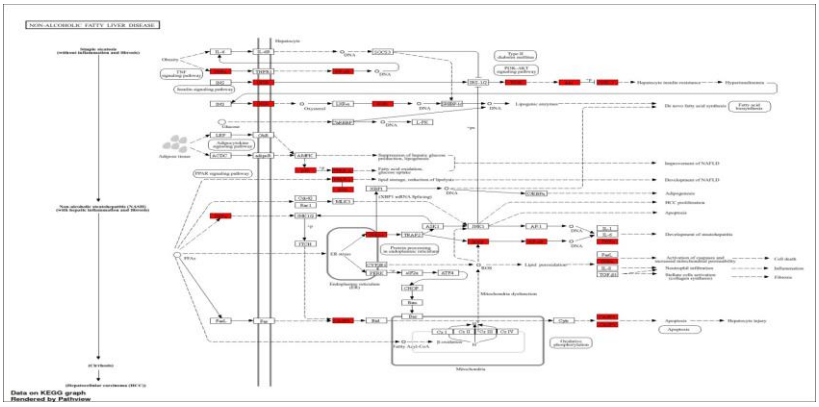

Figure 5: KEGG Pathway Map of Non-Alcoholic Fatty Liver Disease (NAFLD) Highlighting Key Signalling Nodes Modulated by The Consensus Targets

Figure 5 illustrates how factors such as obesity, insulin resistance and inflammation activate key signalling pathways including TNF, insulin (INS/INSR), PI3K-AKT and NF-κB. These pathways promote lipid accumulation (steatosis), increased fatty acid synthesis and reduced lipid breakdown in hepatocytes. As the condition progresses, oxidative stress, endoplasmic reticulum (ER) stress and mitochondrial dysfunction trigger inflammatory responses and cell death (apoptosis). Key mediators such as cytokines (e.g., TNF-α, IL-6), transcription factors and stress-related proteins contribute to liver inflammation, fibrosis and eventually more severe stages such as non-alcoholic steatohepatitis (NASH), cirrhosis and hepatocellular carcinoma (HCC).

Protein–Protein Interaction Network and Hub Gene Identification

The protein–protein interaction (PPI) network for the consensus genes associated with NAFLD was constructed using the STRING database (Figure 6). The resulting network displayed a highly complex and densely interconnected architecture, characterised by a prominent central core of tightly linked nodes with numerous radiating interactions to peripheral proteins. Multiple functional clusters were evident, reflecting the involvement of diverse biological pathways and molecular modules in NAFLD pathogenesis.

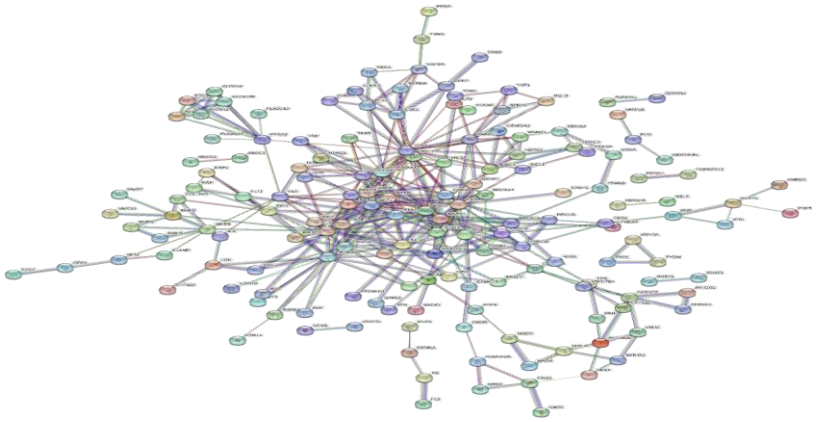

Figure 6: Protein–Protein Interaction Network of the Consensus Genes Between NAFLD and Bambara Groundnut Targets (STRING, Confidence ≥ 0.9)

To pinpoint the most influential regulatory elements within this network, topological analysis was performed using Cytoscape. The top 10 hub genes were identified based on key network parameters including degree of connectivity, betweenness centrality and maximal clique centrality (MCC). These hub genes—TP53, SRC, HSP90AA1, ESR1, PIK3CA, IGF1R, EGFR, ERBB2, PTK2 and PIK3R1—were visualised as a tightly interconnected subnetwork (Figure 7). The hub gene network demonstrated extensive cross-talk among the nodes. A colour gradient was applied to indicate relative importance/ranking, with red nodes (SRC, EGFR and ERBB2) representing the highest-ranking hubs with the greatest connectivity, followed by orange nodes (ESR1, PIK3CA, IGF1R and PIK3R1) and yellow nodes (TP53, HSP90AA1 and PTK2). This analysis highlights the central roles of tyrosine kinase signalling (SRC, EGFR, ERBB2, PTK2), PI3K-AKT pathway components (PIK3CA, PIK3R1, IGF1R), tumour suppression and cellular stress responses (TP53), oestrogen receptor signalling (ESR1) and molecular chaperone activity (HSP90AA1) within the NAFLD interactome.

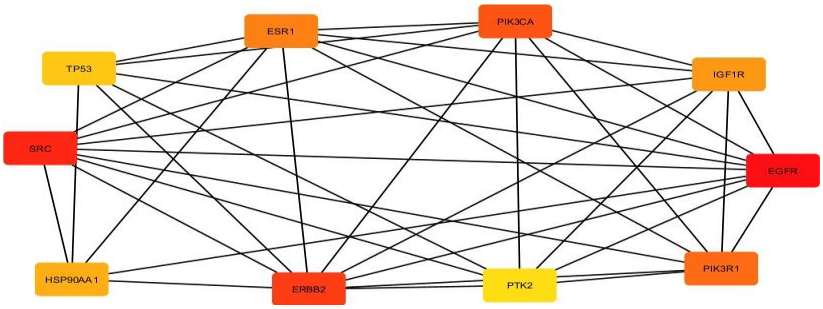

Figure 7: Top 10 Hub Genes of the Consensus NAFLD–Bambara Groundnut Network Identified by Cytohubba

Molecular Docking and MM-GBSA Analysis

Molecular docking and MM-GBSA analyses demonstrated strong binding interactions between bioactive compounds identified from Bambara groundnut and key protein targets implicated in NAFLD, including ERBB2 (PDB: 1MFG), PIK3R1 (PDB: 7RNS), EGFR (PDB: 8A27), PIK3CA (PDB: 9CWY) and SRC (PDB: 9OFX). Overall, several phytoconstituents showed favourable docking scores and binding free energies, indicating potential modulatory activity against pathways involved in hepatic lipid accumulation, inflammation, insulin signalling and progression of NAFLD.

For ERBB2 (1MFG), rutin exhibited the best docking affinity with a Glide score of −7.541 kcal/mol, while ellagic acid showed the most favourable MM-GBSA binding free energy (−44.31 kcal/mol), closely followed by quercitrin (−44.17 kcal/mol) and epicatechin (−40.05 kcal/mol) (Table 2). These strong binding interactions suggest that these compounds may modulate ERBB2-mediated signalling associated with metabolic dysfunction and hepatic injury in NAFLD.

Table 2: Molecular Docking and MM-GBSA Results of Bambara Groundnut Bioactive Compounds Against ERBB2 (PDB: 1MFG)

Ligand	CID	Glide gscore	MMGBSA dG Bind
Rutin	5280805	−7.541	−38.02
Ellagic acid	5281855	−6.791	−44.31
Gallic acid	370	−6.007	−18.75
Isoquercitrin	5484006	−5.974	−36.08
Chlorogenic acid	1794427	−5.912	−26.85
Epicatechin	72276	−5.356	−40.05
Quercitrin	5280459	−5.305	−44.17
Caffeic acid	689043	−5.193	−29.46
Quercetin	5280343	−4.841	−35.89
Myricetin	5281672	−4.796	−32.18
Catechin	9064	−4.702	−37.31
Luteolin	5280445	−4.643	−34.78
Kaempferol	5280863	−4.094	−35.24

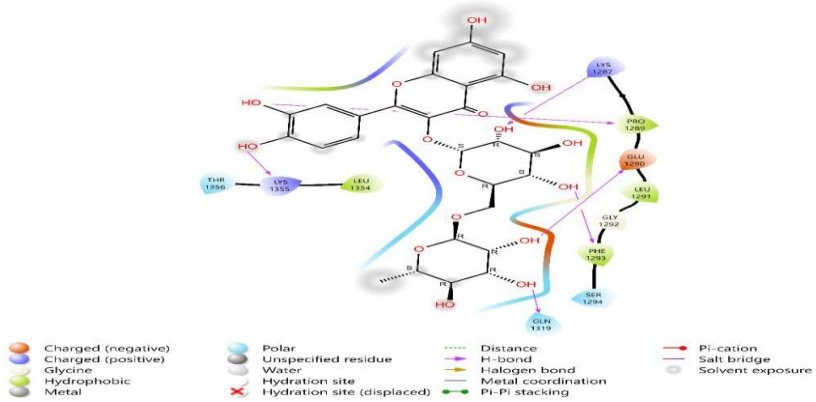

Figure 8: 2D Interaction Diagram of the Best-Scoring Ligand with ERBB2 (1MFG)

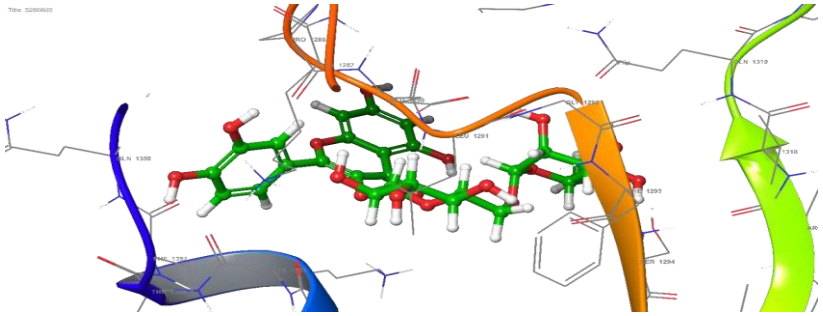

Figure 9: 3D Binding Pose of the Best-Scoring Ligand with ERBB2 (1MFG)

Against PIK3R1 (7RNS), isoquercitrin showed the strongest docking score (−6.518 kcal/mol), whereas myricetin produced the most favourable MM-GBSA energy (−40.42 kcal/mol), followed by rutin (−37.96 kcal/mol) and epicatechin (−35.91 kcal/mol) (Table 3). These findings suggest possible regulation of the PI3K signalling axis, which is critical in insulin resistance and hepatic lipid metabolism.

Table 3: Molecular Docking and MM-GBSA Results of Bambara Groundnut Bioactive Compounds Against PIK3R1 (PDB: 7RNS)

Ligand	CID	Glide gscore	MMGBSA dG Bind
Isoquercitrin	5484006	−6.518	−35.01
Rutin	5280805	−5.425	−37.96
Quercitrin	5280459	−5.296	−21.37
Gallic acid	370	−5.111	−19.93
Chlorogenic acid	1794427	−4.749	−31.31
Quercetin	5280343	−3.997	−32.65
Epicatechin	72276	−3.863	−35.91
Catechin	9064	−3.515	−28.83
Myricetin	5281672	−3.331	−40.42
Luteolin	5280445	−3.199	−16.60
Kaempferol	5280863	−2.967	−20.77
Caffeic acid	689043	−2.822	−23.08
Ellagic acid	5281855	−2.317	−28.94

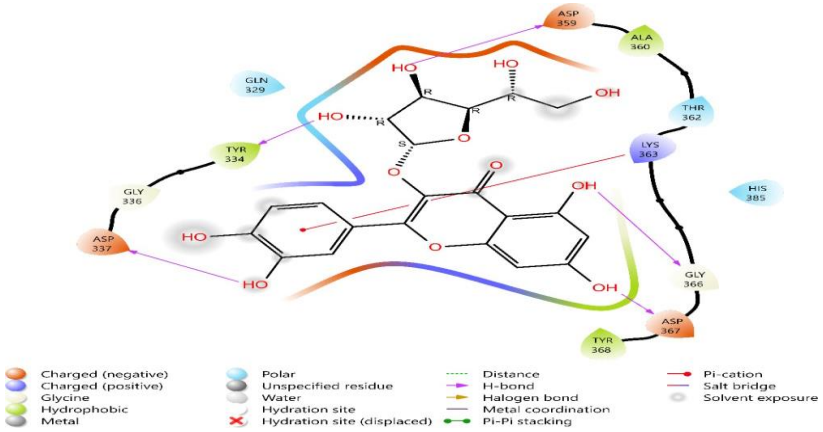

Figure 10: 2D Interaction Diagram of the Best-Scoring Ligand with PIK3R1 (7RNS)

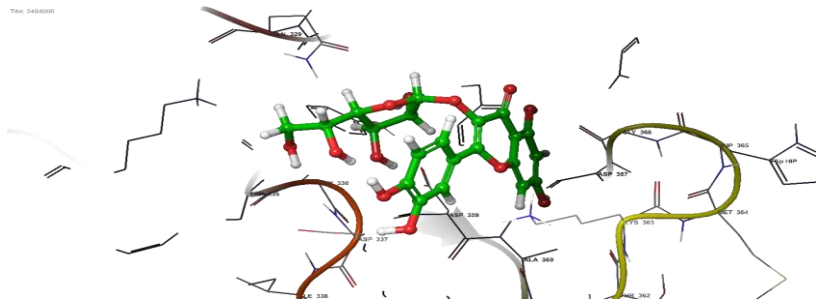

Figure 11: 3D Binding Pose of the Best-Scoring Ligand with PIK3R1 (7RNS)

For EGFR (8A27), the strongest interactions were observed overall. Isoquercitrin recorded the best docking score (−13.520 kcal/mol) and the most favourable binding free energy (−59.60 kcal/mol), outperforming all other ligands.

Quercitrin, myricetin and quercetin also showed strong binding free energies of −53.98, −53.09 and −52.81 kcal/mol, respectively (Table 4). These results indicate highly stable ligand–protein complexes and suggest EGFR as a major target through which Bambara groundnut phytochemicals may attenuate NAFLD progression.

Table 4: Molecular Docking and MM-GBSA Results of Bambara Groundnut Bioactive Compounds Against EGFR (PDB: 8A27)

Ligand	CID	Glide gscore	MMGBSA dG Bind
Isoquercitrin	5484006	−13.520	−59.60
Quercitrin	5280459	−12.464	−53.98
Myricetin	5281672	−10.560	−53.09
Quercetin	5280343	−9.980	−52.81
Rutin	5280805	−9.493	−45.34
Epicatechin	72276	−9.148	−41.55
Luteolin	5280445	−8.763	−49.80
Chlorogenic acid	1794427	−8.584	−44.82
Catechin	9064	−8.197	−46.07
Kaempferol	5280863	−7.246	−47.98
Gallic acid	370	−6.918	−21.21
Caffeic acid	689043	−6.037	−22.20
Ellagic acid	5281855	−6.099	−37.83

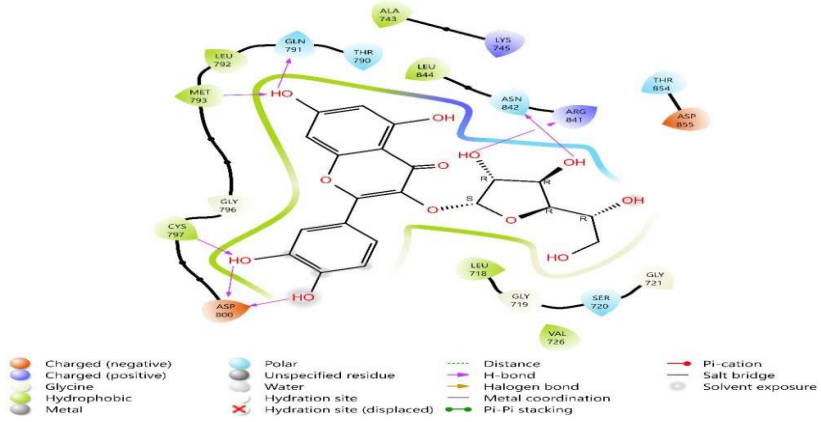

Figure 12: 2D Interaction Diagram of Isoquercitrin (Best Score) With EGFR (8A27)

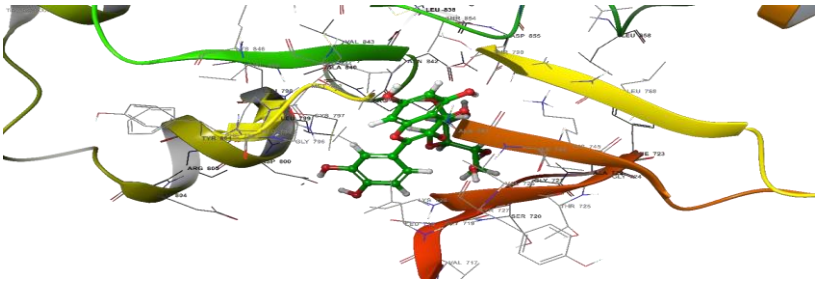

Figure 13: 3D binding pose of isoquercitrin with EGFR (8A27)

For PIK3CA (9CWY), rutin displayed the strongest docking affinity (−10.077 kcal/mol), while luteolin had the most favourable MM-GBSA binding free energy (−40.98 kcal/mol), followed by ellagic acid (−40.70 kcal/mol) and chlorogenic acid (−40.52 kcal/mol) (Table 5). These compounds may contribute to modulation of PI3K-mediated lipid metabolic pathways relevant to steatosis development.

Table 5: Molecular Docking and MM-GBSA Results of Bambara Groundnut Bioactive Compounds Against PIK3CA (PDB: 9CWY)

Ligand	CID	Glide gscore	MMGBSA dG Bind
Rutin	5280805	−10.077	−28.04
Quercitrin	5280459	−7.761	−37.91
Chlorogenic acid	1794427	−7.507	−40.52
Isoquercitrin	5484006	−7.436	−30.34
Quercetin	5280343	−6.548	−38.11
Ellagic acid	5281855	−6.058	−40.70

Myricetin	5281672	−5.920	−38.57
Catechin	9064	−5.733	−31.04
Caffeic acid	689043	−5.698	−15.45
Luteolin	5280445	−5.744	−40.98
Epicatechin	72276	−5.546	−30.45
Gallic acid	370	−5.336	−14.43
Kaempferol	5280863	−5.075	−34.97

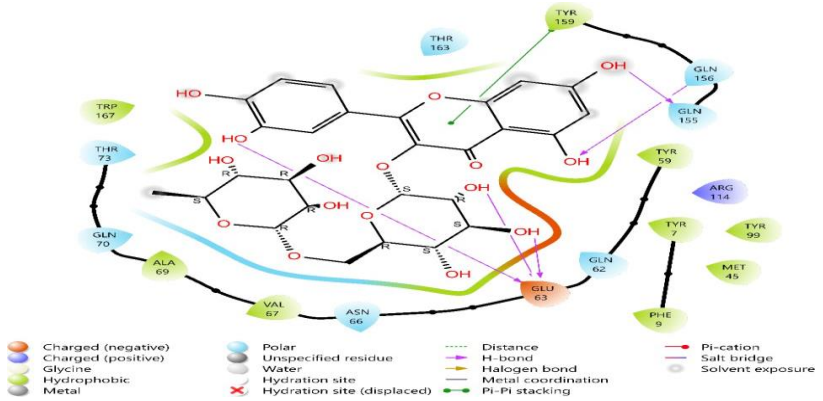

Figure 14: 2D Interaction Diagram of the Best-Scoring Ligand with PIK3CA (9CWY)

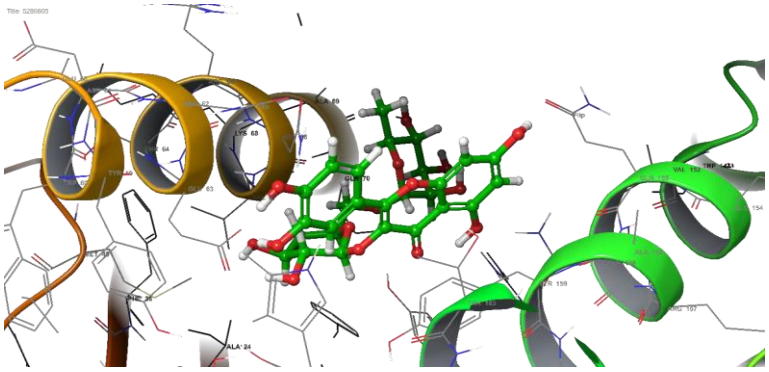
Figure 15: 3D binding pose of the best-scoring ligand with PIK3CA (9CWY)

Similarly, for SRC (9OFX), rutin showed the strongest docking score (−11.945 kcal/mol) and the most favourable MM-GBSA binding energy (−51.29 kcal/mol), followed by isoquercitrin (−43.16 kcal/mol) and ellagic acid (−41.11 kcal/mol) (Table 6). The strong interaction profile suggests potential inhibition of SRC-associated inflammatory and fibrotic signalling implicated in NAFLD progression.

Table 6: Molecular Docking and MM-GBSA Results of Bambara Groundnut Bioactive Compounds Against SRC (PDB: 9OFX)

Ligand	CID	Glide gscore	MMGBSA dG Bind
Rutin	5280805	−11.945	−51.29
Isoquercitrin	5484006	−7.513	−43.16
Quercitrin	5280459	−7.426	−33.70
Epicatechin	72276	−6.108	−33.53
Quercetin	5280343	−6.042	−34.86
Ellagic acid	5281855	−5.995	−41.11
Luteolin	5280445	−5.842	−32.63
Myricetin	5281672	−5.778	−32.85
Catechin	9064	−5.127	−23.55
Gallic acid	370	−4.925	−15.79
Chlorogenic acid	1794427	−4.863	−22.08
Kaempferol	5280863	−4.510	−32.48
Caffeic acid	689043	−3.575	−6.36

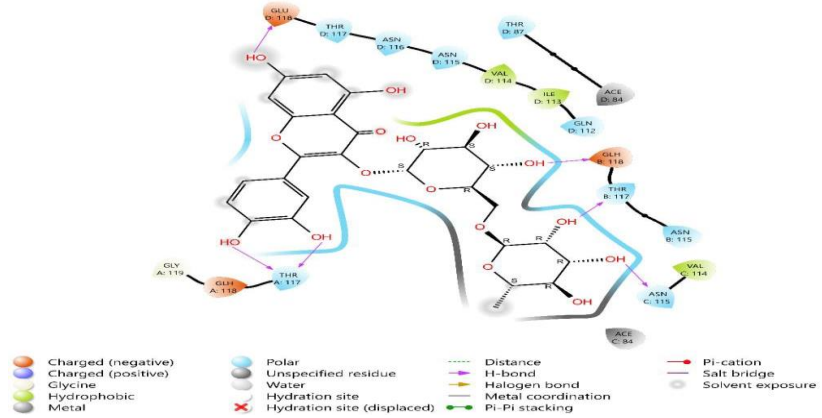

Figure 16: 2D Interaction Diagram of the Best-Scoring Ligand with SRC (9OFX)

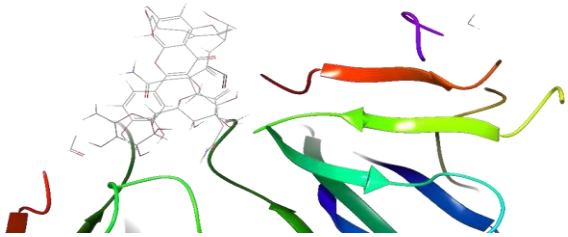

Figure 17: 3D Binding Pose of the Best-Scoring Ligand with SRC (9OFX)

Collectively, rutin, isoquercitrin, quercitrin, ellagic acid, myricetin and luteolin emerged as the most promising bioactive compounds based on both docking performance and binding free energy across multiple targets. Among all proteins studied, EGFR demonstrated the strongest ligand-binding interactions, particularly with isoquercitrin, suggesting that modulation of EGFR-associated pathways may represent a key mechanism underlying the hepatoprotective and anti-NAFLD potential of Bambara groundnut phytochemicals.

Discussion

The present study employed an integrated network pharmacology and molecular docking strategy to explore the potential of Bambara groundnut bioactive compounds against anti-lipogenic targets in NAFLD. The identification of 329 consensus genes that are both predicted targets of Bambara groundnut phenolics and associated with NAFLD provides a focused set of candidates for multi-target intervention. Enrichment analysis indicated that these genes are involved in lipid metabolism, insulin/PI3K–Akt signalling and inflammation, consistent with the well-established "multiple-hit" pathogenesis of NAFLD (Chalasani et al., 2018; Powell et al., 2021).

Key hub genes identified—EGFR, SRC, ERBB2, PIK3CA and PIK3R1—are central nodes in tyrosine kinase and PI3K signalling. Dysregulated PI3K/Akt signalling is recognised as a major driver of hepatic insulin resistance and steatosis (Younossi et al., 2018). The strong enrichment of response-to-lipid and kinase-related GO terms further supports the relevance of these targets to the metabolic dysregulation characteristic of NAFLD.

Molecular docking results highlight isoquercitrin and rutin as particularly promising ligands. Isoquercitrin exhibited the highest affinity for EGFR (Glide score −13.520 kcal/mol; MM-GBSA ΔGbind −59.60 kcal/mol). EGFR signalling has been implicated in hepatic inflammation, regeneration and fibrogenesis; its pharmacological modulation is therefore of therapeutic interest (Chiriac et al., 2021). Rutin displayed the strongest binding to SRC (−11.945 kcal/mol; −51.29 kcal/mol) and also performed well against PIK3CA. These findings align with experimental literature demonstrating that rutin alleviates NAFLD by activating AMPK, suppressing de novo lipogenesis and reducing inflammatory signalling (Gao et al., 2020; Zhang et al., 2024). Similarly, isoquercitrin has been reported to improve hepatic steatosis through modulation of the AMPK/ACC and SREBP-1 pathways, including TLR4/AMPK crosstalk.

The enrichment of AMPK-related and PPAR signalling pathways in the KEGG analysis is consistent with the known ability of many botanical flavonoids to activate AMPK, thereby shifting the metabolic balance away from lipid storage toward fatty-acid oxidation. The concurrent enrichment of TNF-α/NF-κB and apoptotic pathways indicates that the same compounds may also attenuate the inflammatory and cell-death cascades that drive progression from simple steatosis to NASH.

Nevertheless, the present analysis is entirely computational. Compound–target predictions rely on similarity-based algorithms, and docking scores, while informative, do not fully capture binding kinetics or cellular permeability. No ADMET or toxicity profiling was performed. Consequently, the results should be regarded as hypothesis-generating. Future experimental work should include biochemical kinase assays (particularly for the isoquercitrin–EGFR interaction), cellular models of hepatic steatosis, and ultimately animal studies of NAFLD to confirm whether the predicted interactions translate into measurable reductions in lipid accumulation, insulin resistance and inflammation.

CONCLUSION

This in silico investigation demonstrates that selected phenolic compounds from Bambara groundnut, most notably isoquercitrin and rutin, exhibit strong predicted binding

affinities for key anti-lipogenic and inflammatory protein targets (EGFR, SRC, ERBB2, PIK3CA and PIK3R1) implicated in the pathogenesis of non-alcoholic fatty liver disease. Network pharmacology further revealed that these compounds are linked to a core set of 329 consensus genes enriched in insulin signalling, lipid metabolism and inflammatory pathways. The findings support the potential of Bambara groundnut bioactives as multi-target nutraceutical candidates for the management of NAFLD. Experimental validation of the top compound–target interactions, particularly the isoquercitrin–EGFR complex, is strongly recommended before any translational application.

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