Int J Adv Life Sci Res. Volume 8(2) 74-94
https://doi.org/10.31632/ijalsr.2025.v08i02.007

International Journal of Advancement in Life Sciences Research

Online ISSN: 2581-4877

Journal homepage http://ijalsr.org

Original Article

A Network Pharmacology-based Method to Examine Folium Artemisiae Argyi's Mechanism of Action in the Management of Hepatocellular Carcinoma

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Abstract

Hepatocellular carcinoma (HCC) mortality rates have risen significantly in recent years. Folium Artemisiae Argyi (FAA) has demonstrated anticancer properties, yet its specific mechanisms of action in HCC treatment remain unclear. This study used network pharmacology and molecular docking to investigate these mechanisms. HCC-related targets were sourced from GeneCards, NCBI, and GEPIA2 databases, while active FAA compounds and targets were identified through Swiss Target Prediction. Protein-protein interaction (PPI) networks were constructed using the STRING database and visualized with Cytoscape software. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted using ShinyGO. Autodock was employed for molecular docking, and gene expression profiling was performed to assess the prognosis and survival of HCC patients. The study identified 19,467 predicted HCC targets and 292 FAA compound targets, with 207 overlapping targets. GO/KEGG enrichment analyses indicated that FAA influences HCC by regulating biological processes related to cell proliferation and survival, particularly through pathways such as cancer pathways, proteoglycans in cancer, and the PI3K-AKT pathway. Key targets identified included AKT1, SRC, EGFR, PPARG, ESR1, BCL2, PTGS2, HSP90AA1, HIF1A, and MAPK3, with most showing upregulation linked to poor prognosis, reduced disease-free survival, and lower overall survival in HCC patients. Molecular docking analysis confirmed strong interactions between the top five core targets and FAA compounds. Among them, Quercetin, Mandenol, and Ethyl Oleate demonstrated high binding affinities with EGFR, scoring -7.98 kcal/mol, -7.17 kcal/mol, and -6.97 kcal/mol, respectively. In contrast, (R)-Naringenin showed the strongest interaction with AKT1, exhibiting a binding affinity of -8.51 kcal/mol.These findings suggest that FAA exerts a therapeutic effect on HCC via multipathway pharmacological mechanisms, offering the potential to improve patient outcomes. The study provides a foundation for clinical validation and the development of novel anti-cancer drugs.

Keywords: *Folium Artemisiae Argyi; Hepatocellular Carcinoma; Network Pharmacology; Pharmacological Mechanism*

Introduction

One of the primary malignant tumours, liver cancer is distinguished by its great aggressiveness, dismal prognosis, and high death rate (Tang *et al.,* 2022). With 830,200 fatalities and 905,700 new cases reported in 2020, it ranks third globally in terms of cancer-related mortality, after colorectal and lung cancer (Rumgay *et al.,* 2022). A poor prognosis is associated with liver cancer, with a mortality/incidence ratio of 0.92. With 75–85% of cases, hepatocellular carcinoma (HCC) is the most prevalent form of primary liver cancer (Rumgay *et al.,* 2022). It frequently coexists with underlying liver diseases such chronic hepatitis or cirrhosis. Alcohol use, non-alcoholic steatohepatitis (NASH),

Received on :1st December 2024; Revised version received on :8th January 2025; Accepted: 30thMarch 2025

hepatitis B virus (HBV), and hepatitis C virus (HCV) are the primary risk factors (Mittal & El-Serag, 2013). Although surgery is now the best treatment choice for HCC, the age-standardized relative 5-year survival rate is only 18.1%. After removal, tumour recurrence is frequent (Hassanipour *et al.,* 2020). A positive prognosis for patients with HCC is not always guaranteed by a number of treatment modalities, especially when applied in their early phases. These include biological therapy, transcatheter arterial chemoembolization, resection, tumour ablation, liver transplantation, interventional radiology, and chemotherapy (Balogh *et al.,* 2016; El-Serag 2011; Hilmi *et al.,* 2019). Furthermore, treatment with medications like Sorafenib and Lenvatinib barely prolong the life of patients identified in an advanced stage by no more than three months (Casadei-Gardini *et al.,* 2021; Terashima *et al.,* 2020). Therefore, there is a pressing need for novel, more effective, and less toxic treatment approaches that avoid the adverse effects associated with conventional therapies, such as radiation (Majeed & Gupta 2025). Moreover, using a medication repurposing technique can efficiently identify and develop therapeutics for HCC therapy (Pfab *et al.,* 2021). This strategy decreases the chance of safety and toxicity failures while also being a time-efficient and cost-effective alternative to traditional drug design and development, hence speeding the process of bringing novel medications to market (Nosengo 2016; Pushpakom *et al.,* 2019).

Natural compounds derived from traditional medicinal plants have increasingly attracted interest due to their wide range of pharmacological properties along with their relatively low toxicity and minimal side effects. Folium Artemisiae Argyi (FAA) is among these medicinal plants. With its antipyretic, analgesic, warming, and haemostatic functions, it is used internally to warm the channels, stop bleeding, dispel cold, and relieve pain, and externally to eliminate moisture and reduce itching (Gu *et al.,* 2022; Song *et al.,* 2019). FAA contains a variety of bioactive components, including flavonoids, glycosides, triterpenoids, tannins, sterols, and essential oils (Lv *et al.,* 2013; Zhang *et al.,* 2013). FAA can exhibit antiasthmatic, antitussive and expectorant, liver-protective, anticancer, antioxidant (Xia *et al.,* 2019; Xiao *et al.,* 2019), antibacterial, and antiviral properties (Guan *et al.,* 2019). A recent study found that Folium Artemisiae Argyi can decrease malignant hepatoma cell proliferation by causing apoptosis (Liu *et al.,* 2018). However, the mechanism of action of this plant in HCC therapy has not been well investigated.

In order to evaluate biological system networks and choose important signal nodes for the creation of multi-target drugs, network pharmacology is a unique paradigm that combines network science, pharmacology, and systems biology. Studies on drug development and discovery can benefit from this approach. It employs machine learning to create disease-gene-target-drug interaction networks to investigate multi-component, multi-target, and multi-molecule processes (Tang *et al.,* 2022; Zhang, Shi & Wang, 2023). To predict ligand-target interactions at the molecular level, molecular docking simulation studies are also often employed in drug development (Pinzi & Rastelli, 2019). Thus, the fundamental purpose of the current study is to apply network pharmacology and molecular docking to find in silico Folium Artemisiae Argyi targets and predict the mechanism of action of its bioactive components in HCC treatment.

Methodology

Screening for active ingredients and target genes in Folium Artemisiae argyi

The TCMSP database (http://lsp.nwu.edu.cn/tcmsp.php) was used to collect thorough information on the active components of the medicinal plant FAA. Oral bioavailability (OB) is the quantity of a medicine that enters the circulation by oral absorption and works on tissues and organs to achieve the desired pharmacological effects. Drug-likeness (DL) is the degree to which a compound's chemical structure resembles that of recognised medications. These two qualities are necessary for determining the potential therapeutic usefulness of substances (Dai *et al.,* 2022). The active ingredients were selected based on their drug-likeness (DL) >= 0.18 and oral bioavailability (OB) > 30% (Ru *et al.,* 2014). The Canonical Smile structure was then obtained from the Swiss Target Prediction database (http://www.swisstargetprediction.ch), and the names of each active chemical were entered into the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Microsoft Excel was used to screen and

aggregate all active chemicals, and the anticipated target genetic data was acquired in CSV format. To generate a compound-target network and rank the top compounds according to the number of targets, the anticancer targets of FAA's essential components were imported into Cytoscape 3.10.2 (https://cytoscape.org/).

Screening for Hepatocellular Carcinoma (HCC) and Drug-Disease Intersection Targets

Three databases were used to identify HCC-related targets: GeneCards (https://www.genecards.org/), NCBI (https://www.ncbi.nlm.nih.gov), and GEPIA2 (http://gepia2.cancer-pku.cn). By merging the findings, an HCC-related gene set was found. The Folium Artemisiae Argyi target list and the HCC-related gene set were loaded into a Venn Diagram (https://bioinformatics.psb.ugent.be/webtools/Venn/) for further investigation. The primary possible treatment targets were discovered using overlapping gene targets.

Protein-Protein Interaction Analysis and Core Target Screening

A protein-protein interaction (PPI) network was constructed to find interacting proteins. A PPI network for Folium Artemisiae Argyi's anticancer impact was constructed using the STRING database version 12.0 (Szklarczyk *et al.,* 2021) (http://string-db.org), with the species limited to "Homo sapiens" and an interaction score > 0.4. The "Send Network to Cytoscape" option in the String App was then used to instantly send the PPI network to Cytoscape 3.10.2. Topological metrics were analyzed and important therapeutic targets were found using the Cytoscape plug-ins Cytohubba and MCODE.

Pathway enrichment analyses with Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG)

The common targets of Folium Artemisiae Argyi and HCC were functionally analysed using ShinyGO V0.80 (http://bioinformatics.sdstate.edu/go/). This program makes use of numerous R/Bioconductor tools and a large collection of annotations and routes from diverse sources. The GO enrichment study has three major modules: biological process (BP), molecular function (MF), and cellular component (CC). To further understand the interactions and activities of these targets, a KEGG pathway analysis was undertaken (Ge, Jung & Yao, 2020).

Active compounds-Target Molecular docking

Preparation of target protein structures

The major five target proteins' crystal structures were collected from the Protein Data Bank (https://www.rcsb.org/). Before docking with other compounds, the co-crystallized ligands were removed using the Discovery Studio Client v24.1 (Pawar & Rohane, 2021). The starting directory was set to a specific docking folder and each target was imported into the software Autodock 4.0 (Morris *et al.,* 2009) workspace. The missing atoms have been checked first of all and repaired. Then the polar hydrogen was added, and the Gasteiger and Kollman charges were determined. Each protein was then used as a target after being saved in the pdbqt format.

Preparation of active compounds structures

For molecular docking, the structures of the nine active ingredients were obtained from the PubChem database. After downloading these structures as sdf, they were imported into Chem3D software version 14.0 for energy reduction and optimization. Open Babel (https://sourceforge.net/projects/openbabel/files/openbabel/2.4.0/) was then used to convert them to pdb format. After that, the optimized structures were imported into the AutoDock 4.0 workspace, where the root, torsions, and number of torsions were defined and translated to pdbqt format.

Docking processing and analysis

The grid was carefully created to encircle the active site of each target after loading the pdbqt files of each compound with the corresponding target into the workspace of Autodock 4.0 for docking simulation. The Genetic algorithms were configured with a population size of 300, 27,000 generations, 1,000,000 evaluations, and 100 Genetic Algorithm (GA) runs to optimize docking results. The five

targets were then docked against the four active compounds, and a post-docking analysis was used to choose the optimal binding poses. The generated protein-ligand complexes were examined with Discovery Studio Client v21.1 (Pawar & Rohane, 2021).

Core target Gene expression and Survival analysis

Gene Expression Profiling Interactive Analysis was used to examine the top ten anti-HCC core target genes in liver hepatocellular carcinoma (LIHC) from the GEPIA database (http://gepia.cancer-pku.cn/). Using GEPIA, the expression levels of 10 significant target genes were examined for their impact on disease-free survival (DFS) and overall survival (OS), with the hypothesis being tested using the Log-rank test. A 95% confidence interval (CI) hazard ratio (HR) from the Cox proportional hazards model was employed in the analysis. Using a 50% median expression criterion, the samples were separated into cohorts with high and low expression. P-values less than 0.05 were regarded as statistically significant.

Results

Active Ingredients and Target Genes for Folium Artemisiae Argyi

Nine acceptable bioactive chemicals were selected from the TCMSP database based on the compounds' screening criteria (Table 1). The compilation of the Swiss Target Prediction findings for each of the nine chemicals in the Excel datasheet, together with the exclusion of repetitive drug targets, resulted in the identification of 500 FAA prospective targets. Figure 1 shows the active ingredient/drug-target network graph constructed in Cytoscape 3.10.2, which reflects the correspondence of the compound's targets.

Table 1: The top key active ingredients ranked by degree method using Cytoscape software

Mol ID	Names	MW	PubChem ID	OB (%)	DL	Target Score
MOL000098	Quercetin	302.25	5280343	46.43	0.28	100
MOL001494	Mandenol	308.56	5282184	42	0.19	100
MOL001040	(R)-naringenin	272.27	667495	42.36	0.21	97
MOL002883	Ethyl oleate	310.58	5363269	32.4	0.19	58
MOL000358	Beta-sitosterol	414.79	222284	36.91	0.75	44
MOL000449	Stigmasterol	412.77	5280794	43.83	0.76	41
MOL005735	Dammaradienyl acetate	454.81	14137679	44.83	0.83	30
MOL005741	Cycloartenol acetate	468.84	17750996	41.11	0.8	23
MOL005720	24-methylenecyloartanone	438.81	634880	41.11	0.79	7

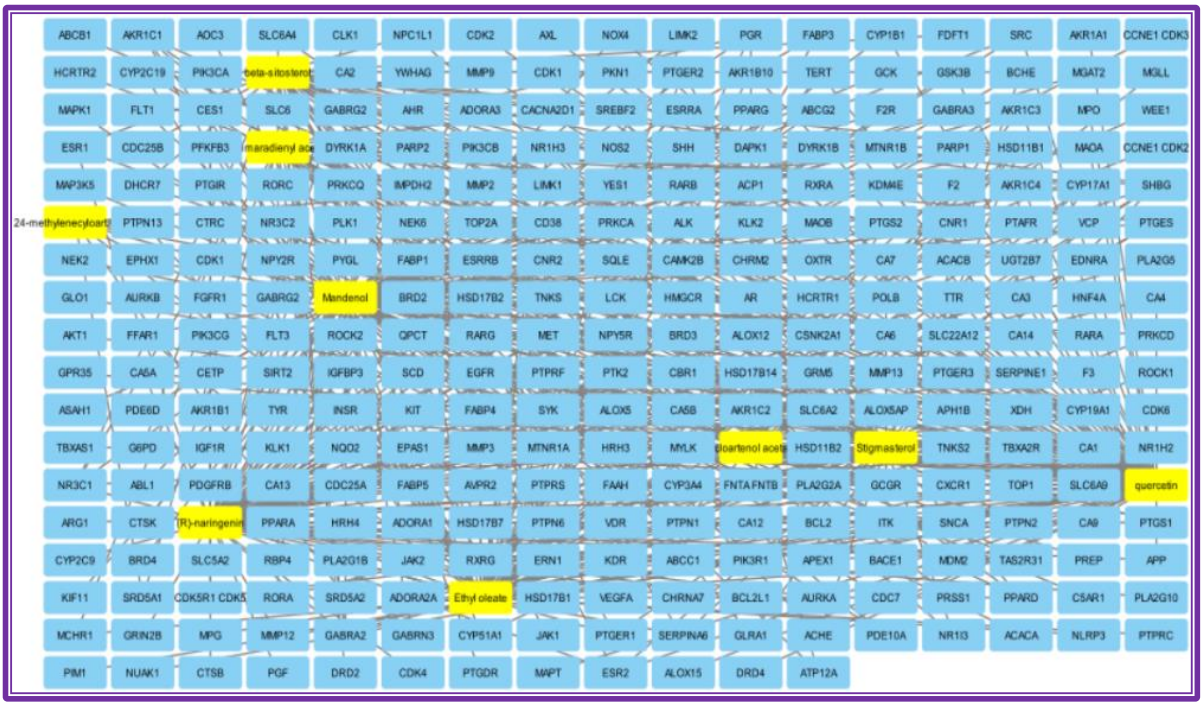

Figure 1: *The Drug-target network graph. The yellow nodes represent the different compounds of FAA, the blue nodes represent the different interacted targets, and the gray edges represent the interactions between the drugs and targets.*

Hepatocellular Carcinoma (HCC) Targets and Drug-Disease Intersection Targets

The GeneCards database yielded 18753 predicted HCC targets, whereas the NCBI database yielded 11950 targets and the GEPIA2 database yielded 262. After deleting the duplicates, we had 19467 possible HCC targets. Using the Venn Diagram to combine the active chemical and HCC target lists yielded 277 overlapping targets that were deemed prospective treatment gene targets (Figure 2).

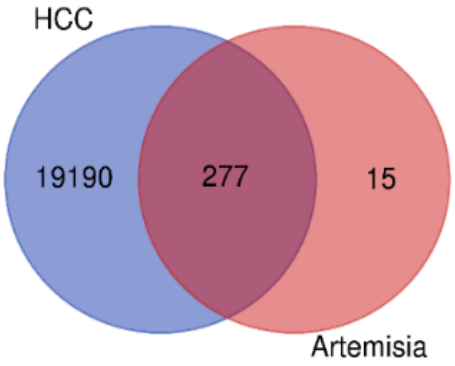

Figure 2: *Venn diagram of HCC (blue) and Folium Artemisiae Argyi (red) gene targets.*

Examining interactions between proteins and looking for key targets

By entering the official gene symbols of 277 potential targets into the STRING 12.0 database (http://string-db.org), a PPI network was constructed. The network's complexity was evident from its 277 nodes and 2970 edges (Figure 3A). After that, we created a network diagram of the main target interactions by importing the PPI network into Cytoscape 3.10.2. The colors red and yellow represent low and high degree values, respectively. Based on these values, we identified the top 100 and ten major nodes, including AKT1, SRC, EGFR, PPARG, ESR1, BCL2, PTGS2, HSP90AA1, HIF1A, and MAPK3 (Table 2, Figures 3B and 3C), indicating the ten main therapeutic targets for HCC therapy using Artemisia's bioactive chemicals.

Table 2: Top 10 key gene Targets of Artemisia's bioactive ingredients

Rank	Gene Target	Uniprot ID	Degree
1	AKT1	P31749	172
2	SRC	P12931	157
3	EGFR	P00533	137
4	PPARG	P37231	129
5	ESR1	P03372	126
6	BCL2	P10415	122
7	PTGS2	P35354	119
8	HSP90AA1	P07900	118
9	HIF1A	Q16665	116
10	MAPK3	P27361	112

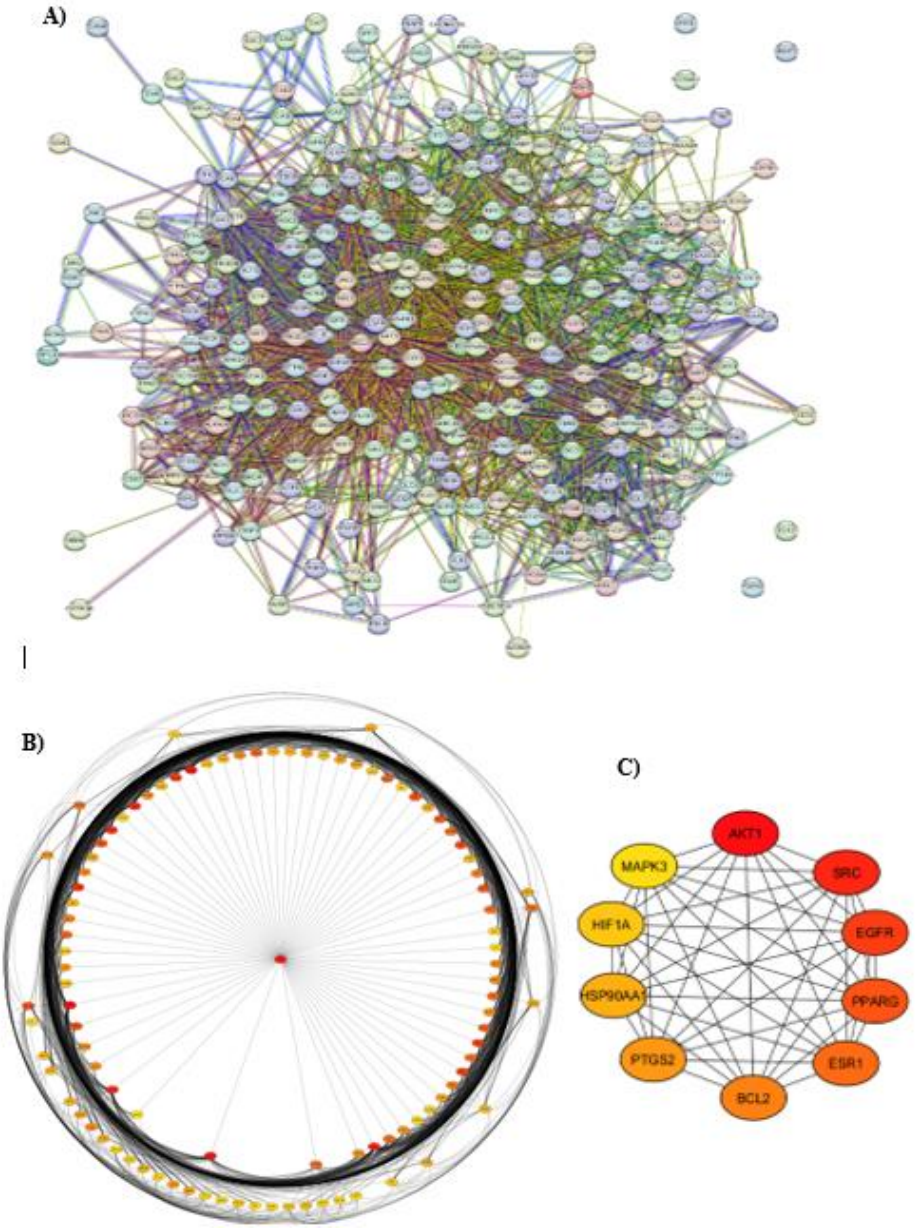

Figure 3: *Protein-protein interaction (PPI) analysis. (A) The PPI network was built using a String database. (B-C) Cytoscape ranks the top 100 and ten core targets in the PPI network based on maximal clique centrality. Proteins are shown as nodes (colours ranging from red to yellow indicate the degree of interaction amongst anti-HCC targets). Edges reflect protein–protein interactions*

GO and KEGG Pathway Enrichment results analysis

The anti-HCC benefits of FAA's active components and related molecular processes were further examined by GO and KEGG pathway enrichment analysis of the top 100 intersecting targets, using an adjusted filter with a P-value < 0.05. The top ten most enriched GO words (BP, MF, and CC) were discovered. Figure 4 (A-C) shows the results. The major active substances' anti-HCC targets are engaged in a variety of biological processes (BP), including chemical response, programmed cell death control, cellular response to chemical stimuli, apoptotic process regulation, and so on. Furthermore, the targets engaged in the therapy of HCC with FAA drugs are linked to a variety of cellular components (CC), such as vesicles, receptor complexes, Phosphatidylinositol 3-kinase complexes IA and I, the endoplasmic reticulum, and others. Furthermore, the targets through which FAA compounds exert their effects on HCC are involved in a variety of molecular functions (MF), including protein kinase activity, protein serine/threonine/tyrosine kinase activity, phosphotransferase activity with an alcohol group as acceptor, small molecule binding, and others.

Figure 4D also includes a dot map of the top ten KEGG pathways. The molecular mechanisms linked to the anti-HCC activities of these bioactive chemicals may include cancer pathways such as the PI3K-Akt signaling pathway, microRNAs in cancer, chemical carcinogenesis-receptor activation, and EGFR tyrosine kinase inhibitor resistance, among others. Figure 5 depicts the relevant targets in the FAA and PI3K-Akt signaling pathways, respectively.

Molecular docking analysis

Using molecular docking, it was shown that the five main target proteins AKT1 (PDB ID: 6HHG), SRC (PDB ID: 1O48), EGFR (PDB ID: 7U98), PPARG (PDB ID: 1KNU), and ESR1 (PDB ID: 2BJ4) interact with four related significant compounds. The docking data are shown in Table 3, and the protein-ligand interactions of the docked complexes are shown in Figures 6, 7, 8, and 9. With the highest score of -7.98 kcal/mol, the results showed that quercetin had a substantial affinity for each of the five targets. However, ethyl oleate and mandenol showed poor binding affinities of -2.67 kcal/mol and -2.49 kcal/mol, respectively, to SRC, whereas the other two showed substantial binding affinities to all the targets. Their greatest binding scores were -7.17 kcal/mol for mandenol and -6.97 kcal/mol for ethyl oleate. Furthermore, (R)-naringenin showed higher binding energy with the five targets than the other compounds, with the maximum score of -8.51 kcal/mol.

Overall, hydrogen bonding dominated the protein-ligand interactions in quercetin-protein complexes. We found six hydrogen bonds with PPARG, four with AKT1, five with SRC, four with ESR1, and eight with EGFR. In the complexes produced with the bioactive chemical (R)-naringenin, we found two hydrogen bonds with PPARG, three with AKT1, five with SRC, five with ESR1, and six with EGFR. Furthermore, alkyl/pi-alkyl interactions were the most common between mandenol and proteins. We discovered two hydrogen bonds with PPARG but none with AKT1, two with SRC, one with ESR1, and two with EGFR. Moreover, with ethyl oleate, we found just one hydrogen bond with SRC, ESR1, and EGFR receptors, but none with PPARG or AKT1 receptors.

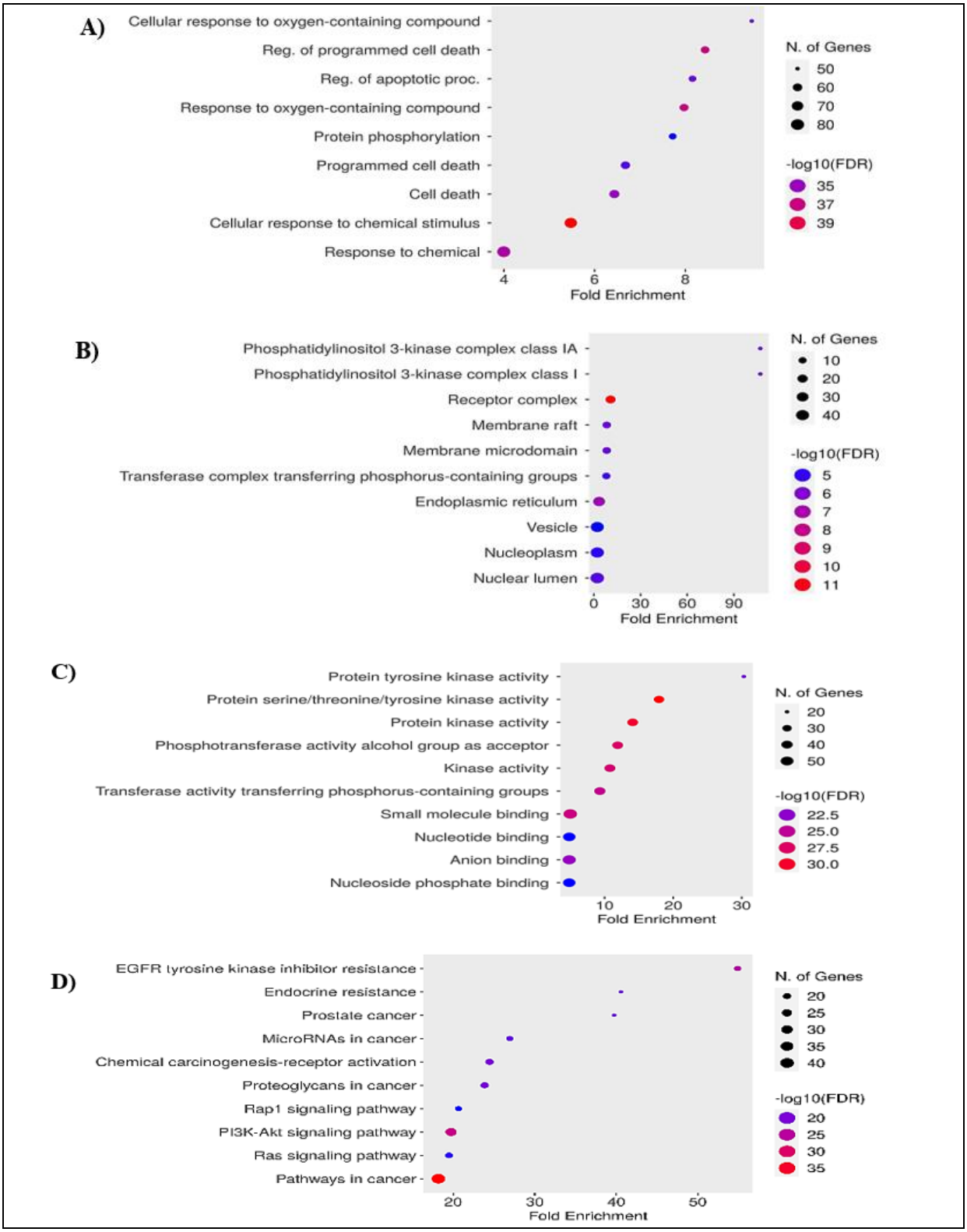

Figure 4: *Results of GO and KEGG pathway enrichment analyses of target proteins that interact with active substances. The y-axis displays biological processes, cellular components, and molecular function terms, while the x-axis displays the degree of enrichment. The size of the dots represents the number of genes; a larger point suggests that more genes are engaged in the relevant process. (D) The y-axis lists the pathway names, while the x-axis shows the number of enriched genes in each pathway*

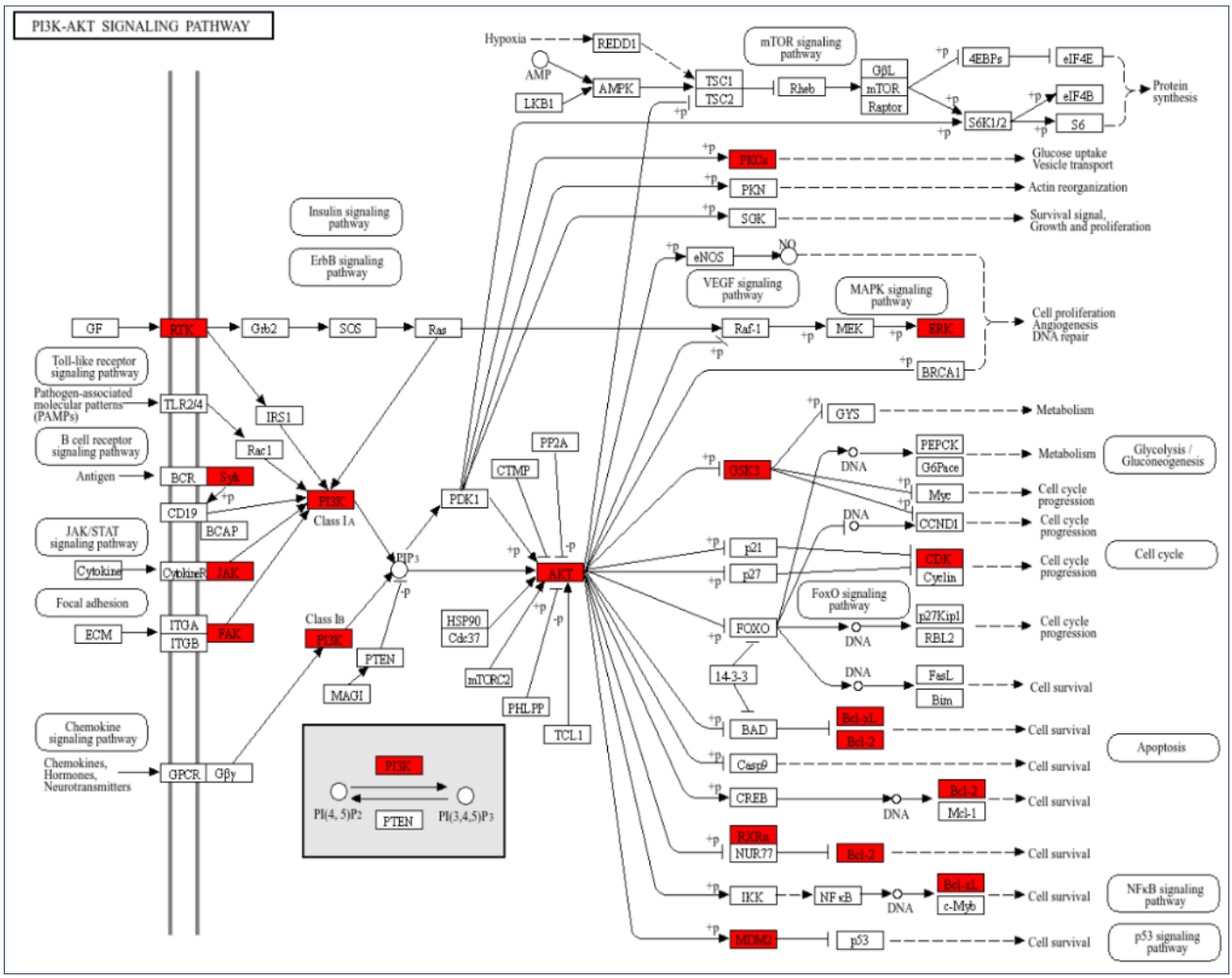

Figure 5: *Relevant targets in the FAA ingredient signalling pathway as well as the PI3K-Akt signalling pathway. The red rectangles represent our discovered target proteins*

The presence of hydrogen bonds is generally important in molecular recognition and contributes significantly to the stability of protein-ligand complexes.

Table 3: Molecular docking results of the four main active compounds of Artemisiae with the top five anti-HCC core targets.

Compounds	Names	Molecular docking results (kcal/mol)				
		PPARG	AKT1	SRC	ESR1	EGFR
MOL000098	Quercetin	-6.86	-7.80	-6.16	-6.45	-7.98
MOL001494	Mandenol	-7.05	-5.86	-2.49	-6.05	-7.17
MOL002883	Ethyl oleate	-6.95	-5.90	-2.67	-5.65	-6.97
MOL001040	(R)-naringenin	-7.33	-8.51	-5.91	-7.47	-8.40

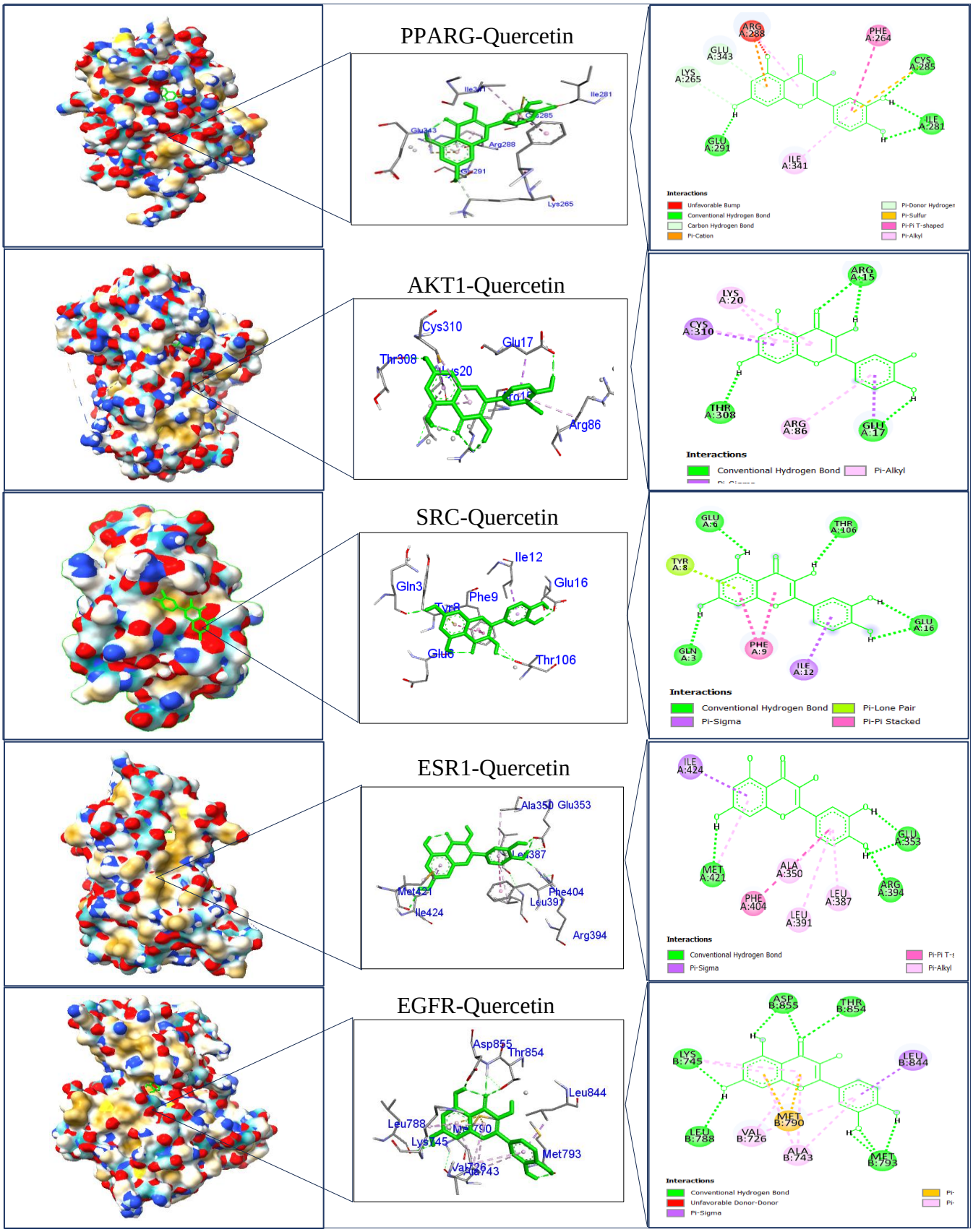

Figure 6: *Quercetin's protein-ligand interactions with key targets include PPARG, AKT1, SRC, ESR1, and EGFR. From left to right, we see a global view of the complex, 3D receptor-ligand, and 2D receptor-ligand interaction*

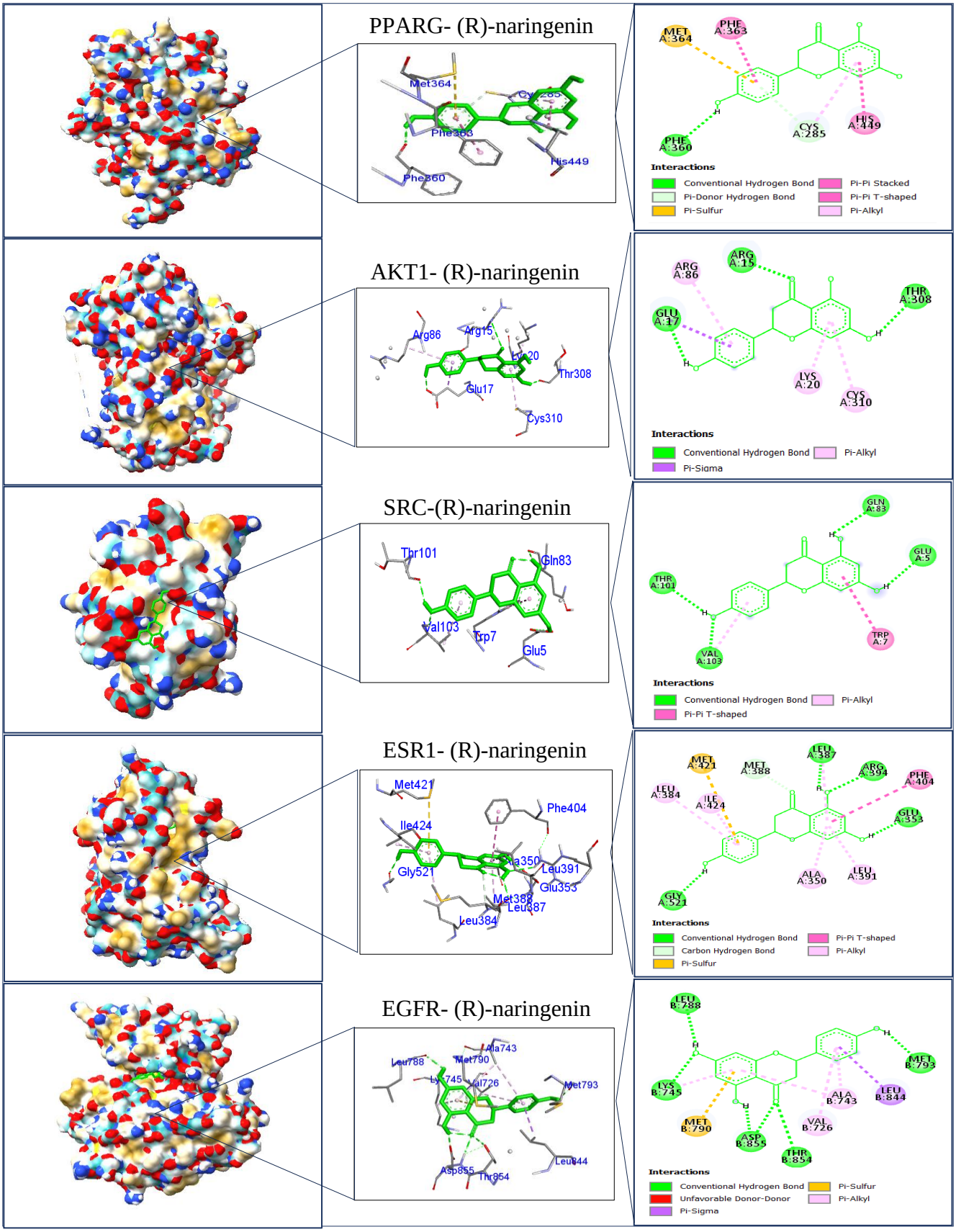

Figure 7: *(R)-naringenin's protein-ligand interactions with main targets PPARG, AKT1, SRC, ESR1, and EGFR. The global view of the complex, 3D receptor-ligand, and 2D receptor-ligand interactions are shown from left to right*

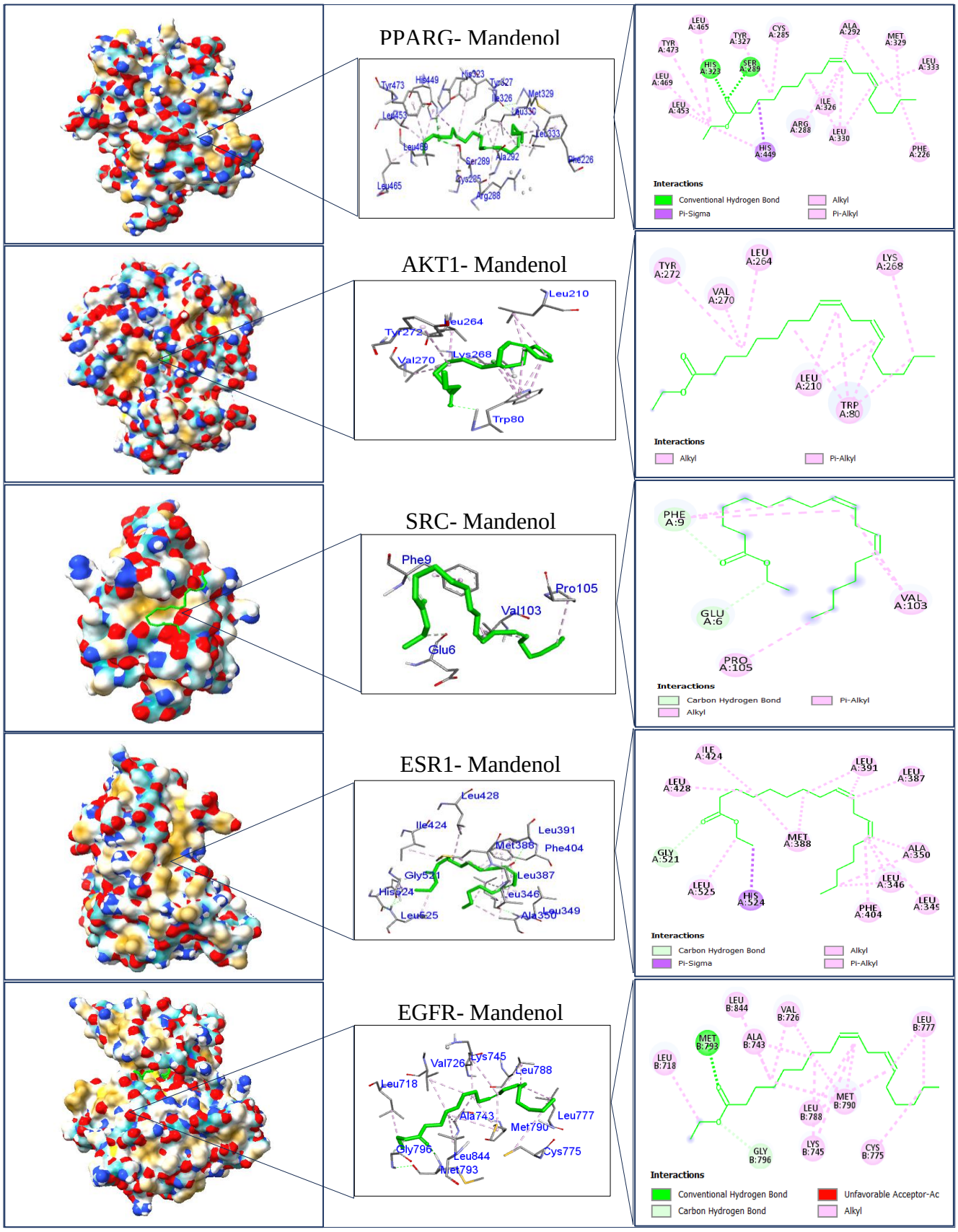

Figure 8: *Mandenol protein-ligand interactions with key targets include PPARG, AKT1, SRC, ESR1, and EGFR. From left to right, we see the complex's 3D receptor-ligand and 2D receptor-ligand interactions.*

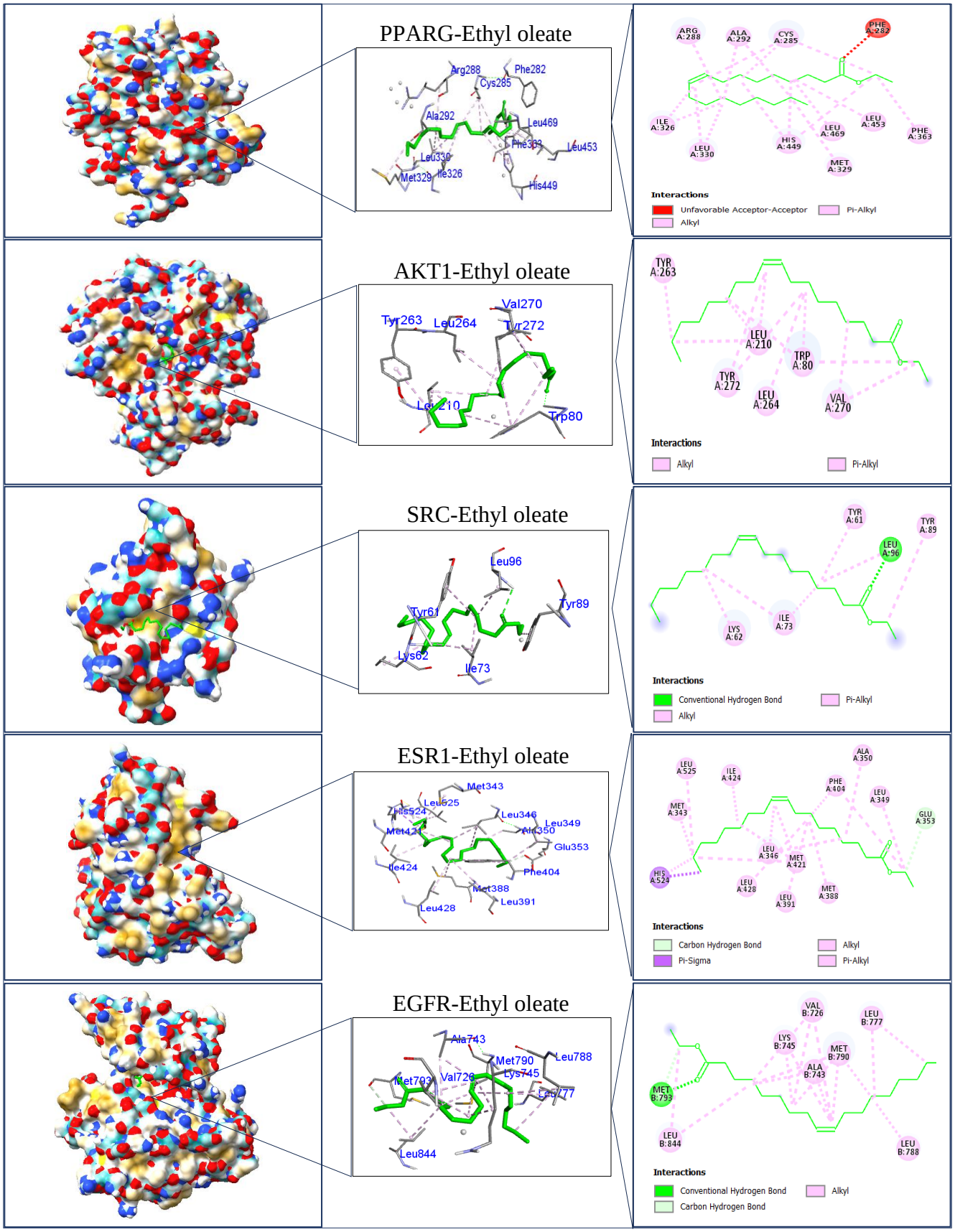

Figure 9: Ethyl oleate's protein-ligand interactions with main targets PPARG, AKT1, SRC, ESR1, and EGFR. The complicated, 3D receptor-ligand, and 2D receptor-ligand interactions are shown in a global perspective from left to right.

Expression of anti-HCC core targets

The GEPIA2 database was utilized to analyze the expression levels of the top ten anti-HCC core targets AKT1, SRC, EGFR, PPARG, ESR1, BCL2, PTGS2, HSP90AA1, HIF1A, and MAPK3 in both LIHC and normal tissue samples (Figure 10A). The observed differences in the expression of these core targets between LIHC and normal samples suggest the involvement of multiple regulatory mechanisms in LIHC.

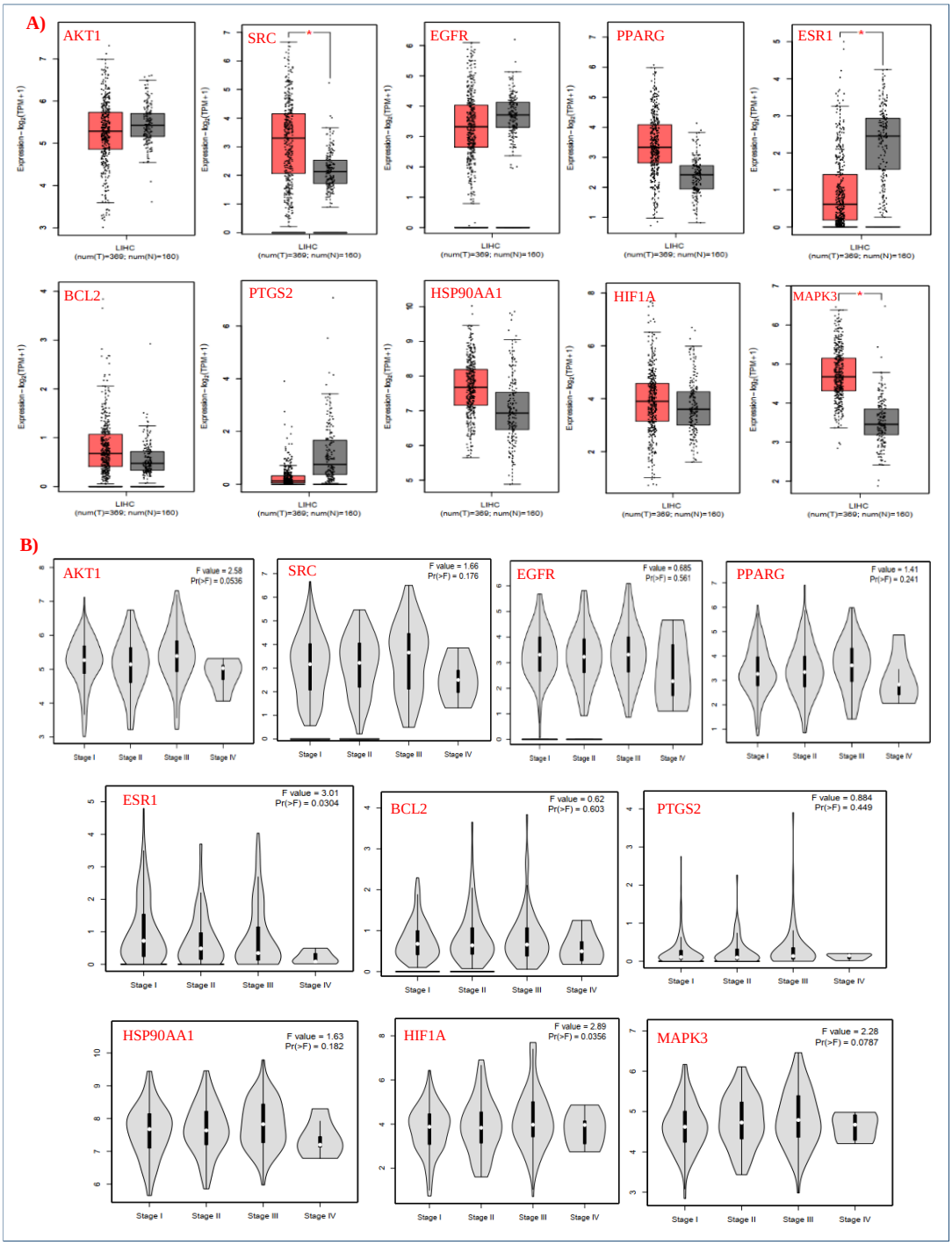

Figure 10: Expression of the top ten anti-HCC core targets in LIHC (the red and grey boxes indicate tumour and normal cells, respectively) and pathological phases. (A) Expression of the 10 key target genes in LIHC. (B) Correlation between core target gene expression levels and LIHC pathological stages using TCGA data.

A core target cancer stage plot analysis further revealed significant correlations between the expression levels of these targets and the pathological stages of LIHC, with *p*-values of 0.0536 (AKT1), 0.176 (SRC), 0.561 (EGFR), 0.241 (PPARG), 0.0304 (ESR1), 0.603 (BCL2), 0.449 (PTGS2), 0.182 (HSP90AA1), 0.0356 (HIF1A), and 0.0787 (MAPK3) (Figure 10B).

Additionally, the prognostic significance of these targets was also assessed. Among the ten core targets, the overexpression of ESR1 and HIF1A was strongly associated with poor prognosis and reduced overall survival (OS) in LIHC patients. Similarly, elevated levels of ESR1 and HIF1A were significantly linked to worse disease-free survival (DFS) outcomes in LIHC patients (Figure 11B).

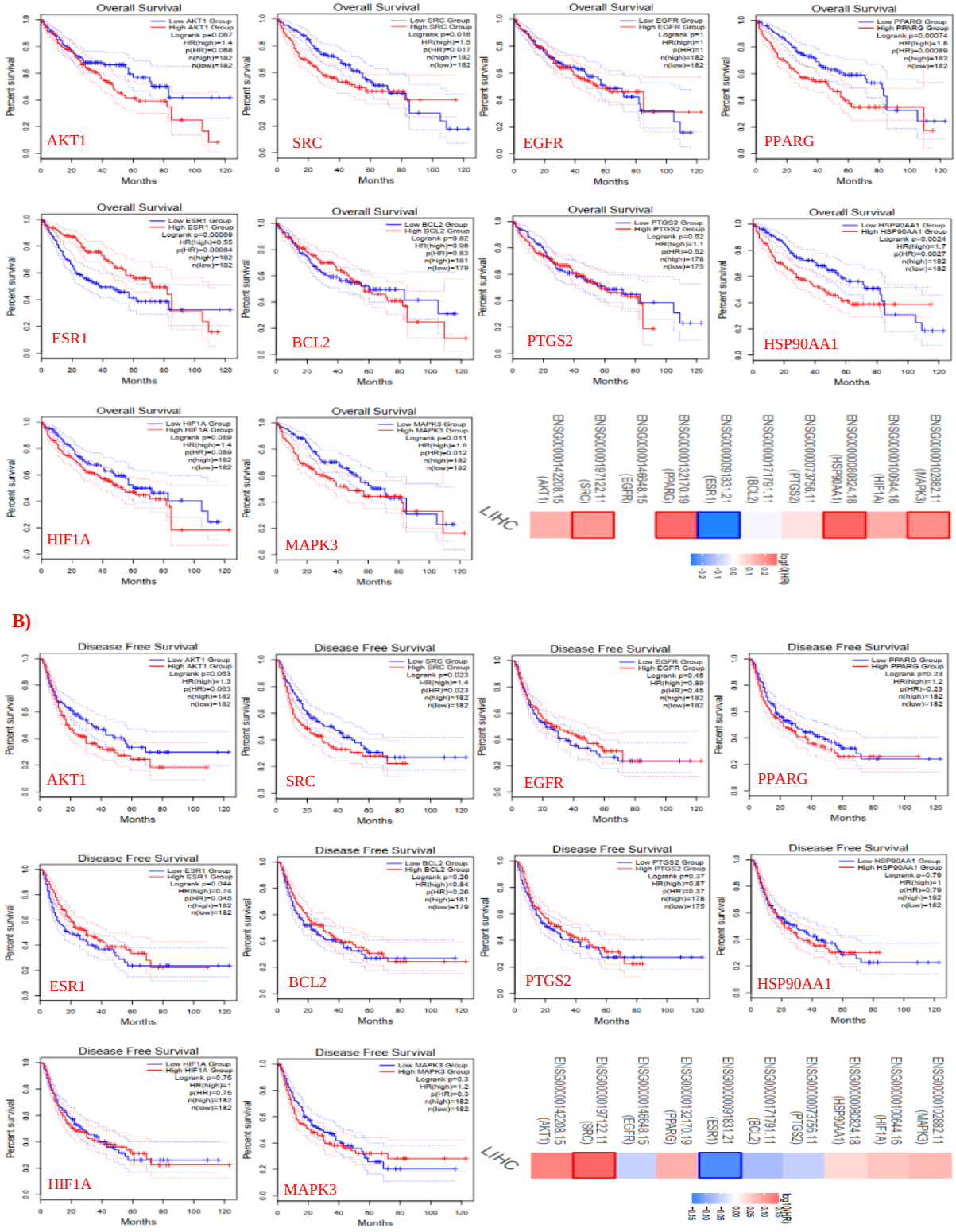

Figure 11: *The relationship between core target gene expression and prognosis in patients with LIHC tumours. There was a strong link between increased gene expression and overall survival (A) and disease-free survival (B) of patients with tumours, as shown by the Survival map*

Discussion

HCC is difficult to diagnose, with limited therapy possibilities. While early surgical intervention can be favorable, the hidden nature of HCC makes early detection challenging. Because of this, it is usually found late in the course of the disease, which makes therapy challenging. In clinical practice, this is a significant challenge that has to be resolved right now (Mroweh *et al.,* 2021; Tang *et al.,* 2022). The introduction of new bioinformatics research methodologies, such as network pharmacology, has paved the door for fresh approaches to drug discovery and development. Developing and analyzing drug-gene-disease interaction networks (L.), predicting disease-related genes through networks, identifying drug targets, forecasting drug functionalities for specific conditions, and creating networks for herbal medicine are some of the basic techniques used in network pharmacology to find new targets and clarify molecular mechanisms (Zhao *et al.,* 2023). FAA is a medicinal plant that has demonstrated various pharmacological properties, including anticancer activity, particularly in the treatment of hepatocellular carcinoma (HCC) and hepatitis (Erdenebileg *et al.,* 2024; Guo *et al.,* 2024; Lee, Yoon & Choi, 2025). For the first time, the pharmacological mechanism of bioactive FAA components in HCC treatment was evaluated in this work using network pharmacology and molecular docking. Based on their drug-likeness (DL) of > 0.18 and oral bioavailability (OB) of ≥ 30%, nine FAA compounds were selected from the TCMP database. An internet program was used to decide their objectives. There were 500 FAA probable targets in all. A total of 19677 potential HCC targets were found using online databases. Additionally, 277 overlapping targets between HCC-related targets and the possible target of FAA active components were found.

Figure 3B shows the PPI network analysis for intersecting targets as well as the degree of connection between the top 100 targets. Figure 3C shows the top ten key targets implicated in the anti-HCC activities of FAA's bioactive chemicals, which include AKT1, SRC, EGFR, PPARG, ESR1, BCL2, PTGS2, HSP90AA1, HIF1A, and MAPK3. These genes contribute significantly to HCC cell proliferation, migration, and apoptosis. Upregulated expression of AKT1 in HCC has been shown to enhance cell proliferation and migration (Mroweh *et al.,* 2021). Overexpression of SRC has been linked to the development and metastasis of HCC (Zhao *et al.,* 2015). Similarly, prior studies have revealed that EGFR overexpression plays a role in HCC development, with its activation leading to HCC cells' main resistance to sorafenib (Sueangoen, Tantiwetrueangdet & Panvichian, 2020). Furthermore, increased PARG expression has been linked to a poor prognosis in HCC, increasing tumour development and metastasis through DDB1-dependent regulation of c-Myc (Yu *et al.,* 2022). Additionally, elevated HIF1A protein levels are strongly linked to the onset, progression, and poor prognosis of HCC (Chu *et al.,* 2022). The Estrogen Receptor (ER), a protein produced by the ESR1 gene, is well known for stimulating cancer cell proliferation and metastasis in breast and ovarian malignancies. In contrast to ESR2's ERβ, ERα protects the liver and prevents cancer (O'Brien *et al.,* 2021). It is also proven that ERα expression is decreased in primary HCC tissues relative to normal liver or surrounding tissues, supporting its suppressive role in HCC. (Meng & Liu, 2022). Many investigations must be conducted to clarify the function. Furthermore, extensive data demonstrates that cancer cells avoid apoptosis and resist therapeutic treatments by upregulating anti-apoptotic proteins in the Bcl-2 family (Hafezi & Rahmani, 2021). MAPK3 is also overexpressed in HCC and influences medication sensitivity and resistance in cancer treatment (Lee, Rauch & Kolch, 2020). HSP90AA1 expression has been linked to a considerably worse disease-free survival rate and a significantly increased risk of tumour recurrence in HCC patients (Xiang, You & Li, 2018). A recent research found that ketoconazole suppresses the development of HCC cells by accelerating mitophagy through PINK1-PRKN-mediated pathways, which is caused by the inhibition of PTGS2, leading to mitochondrial malfunction and consequent demise (Chen *et al.,* 2019). On the other hand, the GEPIA2 database demonstrated that all ten key targets were differentially expressed between HCC and normal samples, and that overexpression of these targets is associated with a poor prognosis, overall survival, and disease-free survival in HCC patients (Figures 10 and 11). All of these findings led us to firmly conclude that these main targets play an important role in the evolution of HCC and constitute attractive therapeutic targets for HCC therapy with FAA components.

The FAA target genes for HCC treatment were leveraged to construct an enrichment map of GO and KEGG pathways using the ShinyGO online tool (Figure 4). FAA revealed its anti-HCC technique by modulating gene targets involved in BP, such as programmed cell death control, apoptotic process regulation, protein phosphorylation, and so on, as indicated in Figure 4. By altering protein phosphorylation within intracellular signaling pathways and influencing the functions and characteristics of liver cells, phosphatases have been implicated in liver diseases and the development of HCC, according to recent research (Yoon & Lee, 2022). There may be two sides to cell death in HCC. In advanced HCC, it can be a viable therapeutic target to restrict tumor development, even if it can promote inflammation, fibrosis, and angiogenesis processes that are tightly regulated by varied resident and invading host cells (García-Pras *et al.,* 2022). Additionally, Phosphatidylinositol 3-Kinase (PI3K) class IA, PI3K I, receptor complex, nucleoplasm, endoplasmic reticulum, and other CC were linked to the target associated with FAA's anti-HCC. Controlling endoplasmic reticulum stress, which mediates cell death and has been related to different phases of liver damage, shows great potential for treating liver injury and HCC, according to studies (Zhang *et al.,* 2022). Moreover, the results showed that the target is involved in a number of MF, including as kinase activity, protein serine/threonine/tyrosine kinase activity, phosphotransferase activity, and others. The involvement of kinases such as EGFR and PI3KCA in cellular transformation, tumor genesis, survival, and HCC cell proliferation are intricate and interconnected. In recent years, the therapy of cancer has been significantly impacted by targeting these kinases.

EGFR tyrosine kinase inhibitor resistance, the PI3K-AKT signaling pathway, the RAS signaling system, cancer pathways, and proteoglycans were the main metabolic pathways supporting the anti-HCC actions of FAA active components, per the KEGG enrichment analysis. Many cellular processes depend on the phosphatidylinositol 3-kinase (PI3K)-AKT pathway, which becomes overactive in cancers and promotes the growth and development of tumors (He *et al.,* 2021). Research indicates that The PI3K/AKT and MAPK signaling pathways play pivotal roles in HCC progression by regulating cell survival, proliferation, metabolism, cell cycle progression, inflammation, and migration. Hyperactivation of these pathways is frequently observed in HCC, leading to uncontrolled tumor growth, resistance to apoptosis, and enhanced metastatic potential. Therefore, compounds targeting these pathways hold significant promise in attenuating tumor growth and preventing metastasis (Guo *et al.,* 2024; Tian, Smit & Jücker, 2023). It has been also that proteoglycans are common structural and functional constituents of the extracellular matrix. Dysregulated proteoglycan expression and distribution cause extracellular matrix dysfunction and structural instability (Wei *et al.,* 2020). Heparan sulphate proteoglycans, found on the surface of liver cells, are reported to be overproduced in the case of HCC as a result of cell damage, indicating that they might be a feasible target for treating HCC (Baghy *et al.,* 2016). Furthermore, RAS proteins have important roles in cellular networks that control cell growth, proliferation, survival, differentiation, adhesion, cytoskeletal dynamics, and motility (Delire & Stärkel 2015). The RAS pathway is often active in 50–100% of human HCC cases, according to research, and blocking this pathway has been shown to improve prognosis (Murugan, Grieco & Tsuchida, 2019). Moreover, EGFR tyrosine kinase inhibitors (EGFR-TKIs) have been effectively utilized in the treatment of a number of cancers after it was discovered that epidermal growth factor receptor (EGFR) mutations are the cause of non-small cell lung cancer (NSCLC). Patients may surely acquire treatment resistance owing to a multitude of factors. Examples include histological changes, shedding of ATP-binding cassette (ABC) transporters, aberrations in downstream pathways, secondary mutations, activation of alternative pathways, and anomalies in the EGFR-TKI-mediated apoptotic pathway (Huang & Fu 2015). However, it has been demonstrated that lenvatinib and the EGFR inhibitor gefitinib together have significant anti-proliferative effects in vitro in HCC cell lines that express EGFR. Additionally, this combination works well in vivo in immunocompetent animal models, xenografted HCC cell lines, and patient-derived HCC tumors in mice (Jin *et al.,* 2021). Importantly, the selection of the five primary KEGG pathways led to the discovery of eight of the primary core targets: AKT1, SRC, EGFR, ESR1, BCL2, PPARG, HIF1A, and MAPK3. This result clearly suggested that by altering the expression of these key targets, the five pathways could contribute significantly to FAA's anti-HCC actions. AKT1, a serine/threonine kinase, is a crucial downstream effector in the PI3K pathway. When overexpressed or

aberrantly activated, it contributes to tumor development, angiogenesis, and chemoresistance in cancer (Degan & Gelman 2021). Similarly, SRC, a non-receptor tyrosine kinase, plays a significant role in cancer pathogenesis. Its activation enhances cell motility, invasion, and resistance to apoptosis, thereby promoting tumor progression and metastasis (Codenotti *et al.,* 2024; Hon *et al.,* 2025).

To verify the anticancer effect of FAA on HCC, its inhibitory capacity against significant anti-HCC targets was evaluated using molecular docking. Particularly, docking experiments were conducted between the four main compounds (R)-naringenin, quercetin, mandenol, and ethyl oleate, and the five main targets: AKT1, SRC, EGFR, ESR1, and PPARG. The findings showed high binding affinities between the proteins and inhibitors, with the exception of the SRC-mandenol and SRC-ethyl oleate interactions, which exhibited binding energies of -2.49 and -2.67 kcal/mol, respectively. Previous studies have shown that compounds from FAA exhibit strong bioactivities and can inhibit proteins involved in cancer progression mechanisms (Guo *et al.,* 2024; L. P. Tang *et al.,* 2024). Additionally, the stability of the protein-ligand complexes was found. However, the AKT1-mandenol, PPARG-ethyl oleate, and AKT1-ethyl oleate complexes did not exhibit hydrogen bonding. Many studies emphasis that hydrogen bonds are widely regarded as essential for molecular recognition and have a major role in the stability of protein-ligand complexes (Hubbard & Haider, 2010).

The identification of FAA compounds interacting with key targets and pathways in hepatocellular carcinoma (HCC) provides promising insights into novel therapeutic strategies. Unlike conventional chemotherapy, FAA-derived compounds offer multi-targeted actions, lower toxicity, and potential for combination therapies. The ability of these bioactive compounds to modulate major oncogenic pathways highlights their strong translational potential from computational predictions to preclinical and, eventually, clinical applications.

The strength of this study lies in the fact that it is the first to propose the potential mechanisms by which FAA may contribute to cancer treatment. Nevertheless, the current findings are primarily based on in silico analysis and lack experimental validation.

Future studies should involve both in vitro and in vivo experiments to further elucidate the anticancer mechanisms of FAA compounds. Additionally, it is essential to evaluate the impact of FAA treatment on the expression and activity of all relevant interacting proteins.

Conclusion

The primary goal of this study was to evaluate the ways in which Folium Artemisiae Argyi (FAA) suppresses the proliferation of HCC cells by concentrating on certain compounds. FAA's therapeutic effects encompass a wide range of biological processes and signaling pathways, including the PI3K-AKT signaling system, the RAS signaling circuit, cancer-related pathways, proteoglycans in cancer, and EGFR tyrosine kinase inhibitor resistance. Cell growth, proliferation, survival, and treatment resistance are all regulated by these routes and activities. Furthermore, it was shown that overexpression of important HCC-related targets is linked to poor prognosis and survival in HCC patients, and that these targets showed differential expression between HCC and normal samples. In summary, it is clear that FAA supports HCC treatment through a number of targets and pathways. However, further clinical, in vivo, and in vitro studies are required to confirm FAA's clinical application in HCC treatment.

Conflict of Interest

There are no conflicting interests to disclose, according to the authors.

Acknowledgement

All the authors express their gratitude to the EuroMed Research Centre at the EuroMed University of Fes for their exceptional support in enabling this investigation.

References

Baghy, K., Tátrai, P., Regős, E., & Kovalszky, I. (2016). Proteoglycans in liver cancer. *World Journal of Gastroenterology, 22(*1), 379–393. https://doi.org/10.3748/wjg.v22.i1.379

Balogh, J., Victor III, D., Asham, E. H., Burroughs, S. G., Boktour, M., Saharia, A., ... & Monsour Jr, H. P. (2016). Hepatocellular carcinoma: a review. *Journal of Hepatocellular Carcinoma*, 41-53. https://doi.org/10.2147/JHC.S61146

Casadei-Gardini, A., Scartozzi, M., Tada, T., Yoo, C., Shimose, S., Masi, G., ... & Kawata, K. (2021). Lenvatinib versus Sorafenib in first-line treatment of unresectable hepatocellular carcinoma: an inverse probability of treatment weighting analysis. *Liver International*, *41*(6), 1389-1397. https://doi.org/10.1111/liv.14817

Chen, H. N., Chen, Y., Zhou, Z. G., Wei, Y., & Huang, C. (2019). A novel role for ketoconazole in hepatocellular carcinoma treatment: linking PTGS2 to mitophagy machinery. *Autophagy*, *15*(4), 733-734. https://doi.org/10.1080/15548627.2019.1569934

Chu, Q., Gu, X., Zheng, Q., & Zhu, H. (2022). Regulatory mechanism of HIF-1α and its role in liver diseases: a narrative review. *Annals of translational medicine*, *10*(2). https://doi.org/10.21037/atm-21-4222

Codenotti, S., Sandrini, L., Mandracchia, D., Lorenzi, L., Corsetti, G., Poli, M., ... & Fanzani, A. (2024). Statin-sensitive Akt1/Src/caveolin-1 signaling enhances oxidative stress resistance in rhabdomyosarcoma. *Cancers, 16*(5), 853.https://doi.org/10.3390/cancers16050853

Dai, W., Chen, C., Dong, G., Li, G., Peng, W., Liu, X., ... & Hu, X. (2022). Alleviation of Fufang Fanshiliu decoction on type II diabetes mellitus by reducing insulin resistance: A comprehensive network prediction and experimental validation. *Journal of Ethnopharmacology*, *294*. https://doi.org/10.1016/j.jep.2022.115338

Degan, S. E., & Gelman, I. H. (2021). Emerging roles for AKT isoform preference in cancer progression pathways. *Molecular Cancer Research*, *19*(8), 1251-1257. https://doi.org/10.1158/1541-7786.MCR-20-1066

Delire, B., & Stärkel, P. (2015). The Ras/MAPK pathway and hepatocarcinoma: pathogenesis and therapeutic implications. *European Journal of Clinical Investigation*, *45*(6), 609-623.https://doi.org/10.1111/eci.12441

El-Serag, H. B. (2011). Hepatocellular Carcinoma. *The New England Journal of Medicine 365*(12): 1118–27. https://doi.org/10.1056/NEJMra1001683

Erdenebileg, S., Kim, M., Nam, Y., Cha, K. H., Le, T. T., Jung, S. H., & Nho, C. W. (2024). Artemisia argyi ethanol extract ameliorates nonalcoholic steatohepatitis-induced liver fibrosis by modulating gut microbiota and hepatic signaling. *Journal of Ethnopharmacology*, *333*. https://doi.org/10.1016/j.jep.2024.118415

García-Pras, E., Fernández-Iglesias, A., Gracia-Sancho, J., & Pérez-del-Pulgar, S. (2021). Cell death in hepatocellular carcinoma: pathogenesis and therapeutic opportunities. *Cancers*, *14*(1). https://doi.org/10.3390/cancers14010048

Ge, S. X., Jung, D., & Yao, R. (2020). ShinyGO: a graphical gene-set enrichment tool for animals and plants. *Bioinformatics*, *36*(8), 2628-2629. https://doi.org/10.1093/bioinformatics/btz931

Gu, L., Wang, X., Shao, X., Ding, Y., & Li, Y. (2022). Study on chemical constituents of Folium Artemisiae argyi Carbonisatum, toxicity evaluation on zebrafish and intestinal hemostasis. *Saudi Pharmaceutical Journal*, *30*(5), 532-543. https://doi.org/10.1016/j.jsps.2022.02.018

Guan, X., Ge, D., Li, S., Huang, K., Liu, J., & Li, F. (2019). Chemical composition and antimicrobial activities of Artemisia argyi Lévl. et Vant essential oils extracted by simultaneous distillation-extraction, subcritical extraction and hydrodistillation. *Molecules*, *24*(3). https://doi.org/10.3390/molecules24030483

Guo, J., Yan, W., Duan, H., Wang, D., Zhou, Y., Feng, D., ... & Qin, X. (2024). Therapeutic effects of natural products on liver cancer and their potential mechanisms. *Nutrients*, *16*(11). https://doi.org/10.3390/nu16111642

Hafezi, S., & Rahmani, M. (2021). Targeting BCL-2 in cancer: advances, challenges, and perspectives. *Cancers*, *13*(6). https://doi.org/10.3390/cancers13061292

Hassanipour, S., Vali, M., Gaffari-Fam, S., Nikbakht, H. A., Abdzadeh, E., Joukar, F., ... & Mansour-Ghanaei, F. (2020). The survival rate of hepatocellular carcinoma in Asian countries: a systematic review and meta-analysis. *EXCLI Journal*, *19*, 108–130. https://doi.org/10.17179/excli2019-1842

He, Y., Sun, M. M., Zhang, G. G., Yang, J., Chen, K. S., Xu, W. W., & Li, B. (2021). Targeting PI3K/Akt signal transduction for cancer therapy. *Signal Transduction and Targeted Therapy*, *6*(1). https://doi.org/10.1038/s41392-021-00828-5

Hilmi, M., Vienot, A., Rousseau, B., & Neuzillet, C. (2019). Immune therapy for liver cancers. *Cancers*, *12*(1). https://doi.org/10.3390/cancers12010077

Hon, K. W., Nag, S., Stany, B. K., Mishra, S., & Naidu, R. (2025). Identification of SRC, AKT1 and MAPK3 as therapeutic targets of apigenin and luteolin in colorectal and colon carcinoma through network pharmacology. *Food Bioscience*, *67*. https://doi.org/10.1016/j.fbio.2025.106313

Huang, L., & Fu, L. (2015). Mechanisms of resistance to EGFR tyrosine kinase inhibitors. *Acta Pharmaceutica Sinica B*, *5*(5), 390-401. https://doi.org/10.1016/j.apsb.2015.07.001

Hubbard, R. E., & Haider, M. K. (2010). Hydrogen bonds in proteins: role and strength. *Encyclopedia of Life Sciences*, *1*, 1-6. https://doi.org/10.1038/npg.els.0003011

Jin, F. J., Hu, S., Wang, B. T., & Jin, L. (2021). Advances in genetic engineering technology and its application in the industrial fungus Aspergillus oryzae. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.644404

Lee, S., Rauch, J., & Kolch, W. (2020). Targeting MAPK signaling in cancer: mechanisms of drug resistance and sensitivity. *International Journal of Molecular Sciences*, *21*(3). https://doi.org/10.3390/ijms21031102

Lee, Y., Yoon, Y., & Choi, K. H. (2025). Correlation of periodontitis with hepatic and intestinal inflammation and glycemic control, and effects of bioconverted Artemisia herba-alba by Lactiplantibacillus plantarum SMFM2016-RK. *Journal of Oral Microbiology*, *17*(1). https://doi.org/10.1080/20002297.2025.2473246

Liu, R., Zhao, J., He, K., Zhang, X., Chang, L., & Xiang, G. (2018). Determination of Eupatilin in Folium artemisiae Argyi and its inhibitory effect on hepatoma cells. *Pharmacognosy Magazine, 14*(53). https://doi.org/10.4103/pm.pm_472_16

Lv, J. L., Duan, J. A., Shen, B., & Yin, Y. Y. (2013). Caffeic acid esters from Artemisia argyi and their antioxidant activities. *Chemistry of Natural Compounds*, *49*, 8-11. https://doi.org/10.1007/s10600-013-0492-5

Majeed H., & Gupta V. (2025). Adverse Effects of Radiation Therapy. [Updated 2023 Aug 14]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; Available at: https://www.ncbi.nlm.nih.gov/books/NBK563259/

Meng, X., & Liu, X. (2021). Therapeutic value of estrogen receptor α in hepatocellular carcinoma based on molecular mechanisms. *Journal of Clinical and Translational Hepatology*, *10*(1). https://doi.org/10.14218/JCTH.2021.00224

Mittal, S., & El-Serag, H. B. (2013). Epidemiology of hepatocellular carcinoma: consider the population. *Journal of Clinical Gastroenterology*, *47*, S2-S6. https://doi.org/10.1097/MCG.0b013e3182872f29

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*, *30*(16), 2785-2791. https://doi.org/10.1002/jcc.21256

Mroweh, M., Roth, G., Decaens, T., Marche, P. N., Lerat, H., & Macek Jílková, Z. (2021). Targeting Akt in hepatocellular carcinoma and its tumor microenvironment. *International Journal of Molecular Sciences*, *22*(4). https://doi.org/10.3390/ijms22041794

Murugan, A. K., Grieco, M., & Tsuchida, N. (2019). RAS mutations in human cancers: Roles in precision medicine. In *Seminars in Cancer Biology* (Vol. 59, pp. 23-35). Academic Press. https://doi.org/10.1016/j.semcancer.2019.06.007

Nosengo, Nicola. 2016. "Can You Teach Old Drugs New Tricks?" *Nature 534*(7607): 314–316. https://doi.org/10.1038/534314a

O'Brien, M. H., Pitot, H. C., Chung, S. H., Lambert, P. F., Drinkwater, N. R., & Bilger, A. (2021). Estrogen receptor-α suppresses liver carcinogenesis and establishes sex-specific gene expression. *Cancers*, *13*(10). https://doi.org/10.3390/cancers13102355

Pawar, S. S., & Rohane, S. H. (2021). Review on discovery studio: An important tool for molecular docking. *Asian Journal of Research in Chemistry 14*(1), 86-88. https://doi.org/10.5958/0974-4150.2021.00014.6

Pfab, C., Schnobrich, L., Eldnasoury, S., Gessner, A., & El-Najjar, N. (2021). Repurposing of antimicrobial agents for cancer therapy: what do we know?. *Cancers*, *13*(13). https://doi.org/10.3390/cancers13133193

Pinzi, L., & Rastelli, G. (2019). Molecular docking: shifting paradigms in drug discovery. *International Journal of Molecular Sciences*, *20*(18), https://doi.org/10.3390/ijms20184331

Pushpakom, S., Iorio, F., Eyers, P. A., Escott, K. J., Hopper, S., Wells, A., ... & Pirmohamed, M. (2019). Drug repurposing: progress, challenges and recommendations. *Nature Reviews Drug Discovery*, *18*(1), 41-58. https://doi.org/10.1038/nrd.2018.168

Ru, J., Li, P., Wang, J., Zhou, W., Li, B., Huang, C., ... & Yang, L. (2014). TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. *Journal of Cheminformatics*, *6*, 1-6. https://doi.org/10.1186/1758-2946-6-13

Rumgay, H., Arnold, M., Ferlay, J., Lesi, O., Cabasag, C. J., Vignat, J., ... & Soerjomataram, I. (2022). Global burden of primary liver cancer in 2020 and predictions to 2040. *Journal of Hepatology*, *77*(6), 1598-1606. https://doi.org/10.1016/j.jhep.2022.08.021

Rumgay, H., Ferlay, J., de Martel, C., Georges, D., Ibrahim, A. S., Zheng, R., ... & Soerjomataram, I. (2022). Global, regional and national burden of primary liver cancer by subtype. *European Journal of Cancer*, *161*, 108-118. https://doi.org/10.1016/j.ejca.2021.11.023

Song, X., Wen, X., He, J., Zhao, H., Li, S., & Wang, M. (2019). Phytochemical components and biological activities of Artemisia argyi. *Journal of Functional Foods*, *52*, 648-662. https://doi.org/10.1016/j.jff.2018.11.029

Sueangoen, N., Tantiwetrueangdet, A., & Panvichian, R. (2020). HCC-derived EGFR mutants are functioning, EGF-dependent, and erlotinib-resistant. *Cell & Bioscience*, *10*(1). https://doi.org/10.1186/s13578-020-00407-1

Szklarczyk, D., Gable, A. L., Nastou, K. C., Lyon, D., Kirsch, R., Pyysalo, S., ... & von Mering, C. (2021). The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic acids research*, *49*(D1), D605-D612. https://doi.org/10.1093/nar/gkaa1074

Tang, L. P., Liu, T., Han, X. Y., Li, B., Liu, H. D., & Gao, X. M. (2024). Unlocking the power of sesquiterpenoids: phytochemistry and bioactivities in Artemisia (2017–2023). *Phytochemistry Reviews*, 1-85. https://doi.org/10.1007/s11101-024-10040-2

Tang, Y., Sun, L., Wei, J., Sun, C., Gan, C., Xie, X., ... & Huang, Y. (2022). Network pharmacology identification and in Vivo validation of key pharmacological pathways of Phyllanthus reticulatus (Euphorbiaceae) leaf extract in liver cancer treatment. *Journal of Ethnopharmacology*, *297*. https://doi.org/10.1016/j.jep.2022.115479

Terashima, T., Yamashita, T., Takata, N., Toyama, T., Shimakami, T., Takatori, H., ... & Kaneko, S. (2020). Comparative analysis of liver functional reserve during lenvatinib and sorafenib for advanced hepatocellular carcinoma. *Hepatology Research*, *50*(7), 871-884. https://doi.org/10.1111/hepr.13505

Tian, L. Y., Smit, D. J., & Jücker, M. (2023). The role of PI3K/AKT/mTOR signaling in hepatocellular carcinoma metabolism. *International Journal of Molecular Sciences*, *24*(3). https://doi.org/10.3390/ijms24032652

Wei, J., Hu, M., Huang, K., Lin, S., & Du, H. (2020). Roles of proteoglycans and glycosaminoglycans in cancer development and progression. *International Journal of Molecular Sciences*, *21*(17). https://doi.org/10.3390/ijms21175983

Xia, J. X., Zhao, B. B., Zan, J. F., Wang, P., & Chen, L. L. (2019). Simultaneous determination of phenolic acids and flavonoids in Artemisiae Argyi Folium by HPLC-MS/MS and discovery of antioxidant ingredients based on relevance analysis. *Journal of Pharmaceutical and Biomedical Analysis*, *175*. https://doi.org/10.1016/j.jpba.2019.06.031

Xiang, X., You, X. M., & Li, L. Q. (2018). Expression of HSP90AA1/HSPA8 in hepatocellular carcinoma patients with depression. *OncoTargets and Therapy*, 3013-3023. https://doi.org/10.2147/OTT.S159432

Xiao, J. Q., Liu, W. Y., Sun, H. P., Li, W., Koike, K., Kikuchi, T., ... & Zhang, J. (2019). Bioactivity-based analysis and chemical characterization of hypoglycemic and antioxidant components from Artemisia argyi. *Bioorganic Chemistry*, *92*. https://doi.org/10.1016/j.bioorg.2019.103268

Yoon, J. S., & Lee, C. W. (2022). Protein phosphatases regulate the liver microenvironment in the development of hepatocellular carcinoma. *Experimental & Molecular Medicine*, *54*(11), 1799-1813. https://doi.org/10.1038/s12276-022-00883-0

Yu, M., Chen, Z., Zhou, Q., Zhang, B., Huang, J., Jin, L., ... & Ye, Q. (2022). PARG inhibition limits HCC progression and potentiates the efficacy of immune checkpoint therapy. *Journal of Hepatology*, *77*(1), 140-151. https://doi.org/10.1016/j.jhep.2022.01.026

Zhang, B., Shi, H., & Wang, H. (2023). Machine learning and AI in cancer prognosis, prediction, and treatment selection: a critical approach. *Journal of Multidisciplinary Healthcare*, 1779-1791. https://doi.org/10.2147/JMDH.S410301

Zhang, J., Guo, J., Yang, N., Huang, Y., Hu, T., & Rao, C. (2022). Endoplasmic reticulum stress-mediated cell death in liver injury. *Cell Death & Disease*, *13*(12). https://doi.org/10.1038/s41419-022-05444-x

Zhang, L. B., Lv, J. L., Chen, H. L., Yan, X. Q., & Duan, J. A. (2013). Chemical constituents from Artemisia argyi and their chemotaxonomic significance. *Biochemical Systematics and Ecology*, *50*, 455-458. https://doi.org/10.1016/j.bse.2013.06.010

Zhao, L., Zhang, H., Li, N., Chen, J., Xu, H., Wang, Y., & Liang, Q. (2023). Network pharmacology, a promising approach to reveal the pharmacology mechanism of Chinese medicine formula. *Journal of Ethnopharmacology*, *309*. https://doi.org/10.1016/j.jep.2023.116306

Zhao, R., Wu, Y., Wang, T., Zhang, Y., Kong, D., Zhang, L., ... & Zhang, F. (2015). Elevated Src expression associated with hepatocellular carcinoma metastasis in northern Chinese patients. *Oncology Letters, 10*(5), 3026-3034. https://doi.org/10.3892/ol.2015.3706